Published in final edited form as:

Neurobiol Dis. 2009 October ; 36(1): 162–168. doi:10.1016/j.nbd.2009.07.006.

X11 α haploinsufficiency enhances A β amyloid deposition in Alzheimer's disease transgenic mice

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Abstract

The neuronal adaptor protein X11 α /mint-1/APBA-1 binds to the cytoplasmic domain of the amyloid precursor protein (APP) to modulate its trafficking and metabolism. We investigated the consequences of reducing X11 α in a mouse model of Alzheimer's disease (AD). We crossed hAPP^{swe}/PS-1 Δ E9 transgenic (AD tg) mice with X11 α heterozygous knockout mice in which X11 α expression is reduced by approximately 50%. The APP C-terminal fragments C99 and C83, as well as soluble A β 40 and A β 42, were increased significantly in brain of X11 α haploinsufficient mice. A β /amyloid plaque burden also increased significantly in the hippocampus and cortex of one year old AD tg/X11 α (+/−) mice compared to AD tg mice. In contrast, the levels of sAPP α and sAPP β were not altered significantly in AD tg/X11 α (+/−) mice. The increased neuropathological indices of AD in mice expressing reduced X11 α suggest a normal suppressor role for X11 α on CNS A β /amyloid deposition.

Keywords

Amyloid precursor protein; A β ; amyloid; X11 α ; mint-1; APBA-1; Alzheimer's disease; knockout mice; transgenic mice

The progressive accumulation and deposition of A β /amyloid plays a central role in Alzheimer's disease (AD) pathogenesis (Selkoe and Schenk, 2003). Accordingly, the metabolism and processing of the amyloid precursor protein (APP) is critically important in AD (reviewed in Turner, 2006). Cytoplasmic adaptor proteins, including those in the X11 and Fe65 families, regulate APP catabolism *in vitro* and *in vivo* (King and Turner, 2004; Miller et al., 2006). The X11 family constitute multidomain adaptor proteins composed of a PTB domain and two C-terminal PDZ domains involved in forming multi-protein complexes. For example, the PTB domain of X11 α /mint-1 (munc18-1-interacting protein)/APBA-1 (Amyloid Precursor Binding protein A-1)), binds to the conserved – YENPTY – motif in the cytoplasmic domain of APP to modulate its trafficking and metabolism (Borg et al., 1998a; King et al., 2003; Saluja et al., 2006). Overexpression of X11 α or X11 β *in vitro* inhibits APP catabolism, increases the half-life of APP, and reduces the levels of secreted APP(sAPP) and A β (Borg et al., 1998b; Sastre et al., 1998; McLoughlin et al., 1999; Mueller et al., 2000; Biederer et al., 2002; Saluja et al., 2006). Overexpression of X11 α or X11 β *in vivo* reduces A β /amyloid plaque deposition in AD tg mice (Lee et al., 2003, 2004). X11 α does not inhibit the catabolism of C-terminal fragments

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of APP in a cell-free *in vitro* system, suggesting that X11 α inhibits A β generation from APP by impairing its trafficking to subcellular sites of active γ -secretase complexes (King et al., 2004).

X11 α and X11 β are expressed primarily in neurons (Okamoto et al., 2000), while X11 γ is ubiquitously expressed. X11 proteins are implicated in neuronal signaling and plasticity, protein targeting and tethering, and trafficking of protein complexes. They may be involved in both pre-synaptic and post-synaptic functions (King and Turner., 2004). In presynaptic neurons, X11 α interacts with munc — a protein essential for neurotransmitter vesicle docking and exocytosis (Okamoto and Sudhof., 1997; Ho et al., 2006). In the post-synaptic region, X11 α is a component of protein complexes mediating glutamate receptor trafficking (Rongo et al., 1998; Hong and Hsueh., 2006).

Recent studies employing knockout mice or RNAi-mediated suppression of X11 family proteins have yielded conflicting results on the role of X11 protein family in APP metabolism and A β generation. Studies in X11 α and X11 β double KO mice showed an increase in endogenous murine APP C-terminal fragments and A β (Saino et al., 2006; Saito et al., 2008). In contrast, RNAi mediated knockdown *in vitro* of X11 α /mint-1 or X11 β /mint-2 was shown to inhibit γ -cleavage of APP-CTFs and reduce A β generation (Xie et al., 2005). And in a recent study of all X11 family members (Ho et al., 2008), either a decrease or (less frequently) increase in CNS amyloid plaque deposition was observed, depending on which X11/mint protein or combination of X11/mint proteins has been deleted, the age of the mouse analyzed, and the brain regions examined (hippocampus or cortex). Because X11 α is viewed as a potential disease modifier in AD, we sought to explore the consequences of X11 α reduction in AD transgenic mice expressing both the hAPP^{swe} and PS-1 Δ E9 familial AD mutations. Despite interest among AD researchers in the role played by X11 proteins in AD pathogenesis, the effects of X11 α haploinsufficiency (~50% reduction) in AD double tg mice have not been reported. Thus, we crossed hAPP^{swe}/hPS-1 Δ E9 (AD tg) mice with X11 α haploinsufficient (+/-) mice and assessed the influence of X11 α on the appearance of neuropathological hallmarks of disease. Our findings suggest a suppressor role for X11 α on cleavage of APP and its C-terminal fragments C99 and C83, and on CNS A β /amyloid deposition *in vivo*.

Materials and methods

Transgenic and knockout Mice

The hAPP^{swe}/hPS-1 Δ E9 double transgenic mice harboring familial AD mutations were obtained from Jackson Laboratories (Bar Harbor, Maine). X11 α knockout mice (Mori et al., 2002) were a generous gift of Dr. K. Okuyama (Otsuka GEN Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10, Kagasuno Kawauchi-cho, Tokushima 771-0192, Japan). All mice were maintained in the C57BL/6J genetic background. The mice were back crossed for seven generations to C57BL/6J; all mice used in the experiments were littermates. Mice were housed under specific pathogen free conditions with a 12-hour light-dark cycle and food and water provided *ad libitum*. Animal care was in compliance with institutional guidelines and with the Guide for the Care and Use of Laboratory Animals as adopted by the NIH. Housing facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care. Prior approval of this research was obtained from the University Committee on Use and Care of Animals.

For genotyping of mice, genomic DNA was extracted from tail biopsies by a DNeasy tissue kit (Qiagen). Five μ l of purified genomic DNA was used in a 50 μ l PCR reaction and transgene integration was determined by PCR. The hAPP^{swe} transgene (primer pair sequences: 5'-GAC TGA CCA CTC GAC CAG GTT CTG-3' and 5'-CTT GTA AGT TGG ATT CTC ATA TCC G-3') and co-segregating hPS-1 Δ E9 (5'-AAT AGA GAA CGG CAG GAG CA-3' and 5'-GCC

ATG AGG GCA CTA ATC AT-3') were detected in genomic DNA by 466 and 608 base pair PCR products, respectively. The X11 α knockout mice also revealed an 800 base pair PCR product with primer pairs 5'-TCAGAAGAACTCGTCAAGAAGGC-3' and 5'-ACAAGATGGATTGCACGCAGG-3' (Mori et al., 2002). Female X11 α (+/-) mice were crossed with male hAPPswe/hPS-1 Δ E9 mice to generate wt, X11 α +/- (heterozygous, +/-), hAPPswe/hPS-1 Δ E9 (AD tg), and hAPPswe/hPS-1 Δ E9/X11 α +/- (AD tg/X11 α +/-) mice.

A β 40 and A β 42 ELISA of brain homogenates

We measured soluble A β 40 and A β 42 by sensitive and specific ELISA of proteins extracted from mouse (12 months old) brain homogenates. After removing the brain, the tissue was homogenized in a buffer containing 50 mM Tris HCl, 150 mM Sodium chloride, 5mM EDTA, 0.1 % SDS, 0.25 % Triton X100, and protease inhibitors (Complete Protease Cocktail, Roche Diagnostics GmbH, Mannheim, Germany). The homogenates were centrifuged at 20,000 $\times$ g and supernates removed for assay of soluble A β 40 and A β 42 levels. A β concentrations were determined in the supernates after dilution of the homogenate 10 times to reduce any potential inhibitory effect of SDS. Extracts were analyzed with human A β 40 and A β 42 Colorimetric Immunoassay ELISA kits (BioSource International, Inc., Camarillo, California). Each sample was analyzed in triplicate and each experiment was repeated four times. The A β concentration was calculated using the linear phase of the standard curve. Standard curves were generated with serial dilutions of synthetic human A β 40 and A β 42 as provided in the ELISA kit. Absorbance was measured by a plate reader at 450 nm (PerkinElmer Life and Analytical Sciences, Shelton, CT) and data expressed as pg/mg total protein.

Immunoblot analyses

After sacrifice, brains were dissected, homogenized, and lysed in homogenization buffer (0.1 M NaCl, 0.01 M Tris-HCl, 0.01 M EDTA, complete protease inhibitor tablets (Roche Diagnostics), and 100 μ g/ml phenylmethylsulfonyl fluoride). Total protein concentration was measured using the BCA Protein Assay kit according to the manufacturer's protocol (Pierce). Equal volumes of homogenized brain and 2 $\times$ SDS sample buffer (with 0.03 g/ml dithiothreitol) were mixed and sonicated for 30 seconds. Proteins were separated by SDS-PAGE and transferred to PVDF membranes. To detect APP, PVDF membranes were incubated with the monoclonal antibody 22C11 (Chemicon) raised to an N-terminal domain of APP (residues 61–88). A rabbit polyclonal antibody (Calbiochem, Cat. No. 171610) raised against the synthetic peptide (C) KMQQNGYENPTYKFFEQMQN was used to detect C-terminal fragments of hAPP. This antibody recognizes full-length APP and C-terminal soluble products. Results were confirmed with a second antibody (Sigma-A8717), a synthetic peptide corresponding to the C-terminal region of human APP695 (amino acids 676–695) conjugated to KLH as immunogen. sAPP α was detected by 6E10 (Covance) and antibody from IBL (Japan) specific for sAPPswe β was used to detect sAPP β levels. To confirm reduced expression in X11 α +/- mice, murine X11 α was detected with anti-Mint-1 antibody (BD Biosciences) or with anti-X11 α antibody (Santa Cruz). X11 β and X11 γ were detected by specific Mint-2 and Mint-3 antibodies (BD Biosciences). Horseradish peroxidase-conjugated goat anti-mouse/anti-rabbit antibodies were used as the secondary antibodies. Bands were visualized by enhanced chemiluminescence (ECL detection kit; GE Biosciences). All blots were stripped and re-probed for β -actin (Calbiochem) as a loading control for semi-quantitative analyses. Protein bands were quantitated with a STORM Scanner using ImageQuant software (Molecular Dynamics, Sunnyvale, CA) and confirmed with Quanti1 software (Biorad).

Stereology

Stereological counts of amyloid plaques in cortex and hippocampus were obtained from silver-stained brain sections of ADtg transgenic versus ADtg/X11 α +/- mice at 12 months of age

using StereoInvestigator software (MicroBrightField, Colchester, VT). One half of the sagittally-bisected mouse brain was immersion fixed in 4% paraformaldehyde and embedded in paraffin. The brains were sectioned at 8 μ m and sections were selected at 5-section intervals for analysis using a light microscope interfaced with StereoInvestigator. The optical fractionator method was used to generate an estimate of amyloid plaques counted in an unbiased selection of serial sections in a defined volume of cortex and hippocampus. Cortical and hippocampus borders were delineated on a silver stained section by reference to a mouse brain atlas (Paxinos and Franklin, 2001). The cortex and hippocampus volumes were reconstructed by the StereoInvestigator software using the Cavalieri principle. Serially cut sagittal tissue sections (every fifth section) were analyzed for one entire hemisphere of animals in each genotype cohort ($n = 4$ per group). The total number of plaques was calculated for both groups.

Statistical analyses

Significant differences between means were determined by multiple analyses of variance using a two-tailed *t*-test.

Results and Discussion

Expression of X11 α , X11 β , and X11 γ in AD tg/X11 α +/- mice

Immunoblot analyses of proteins extracted from mouse brain homogenates confirmed a reduced level of X11 α protein in the AD tg/X11 α +/- mice compared to AD tg mice. Semi-quantitative analyses of immunoblots revealed that the relative band intensity of X11 α in haploinsufficient mice was reduced to 54% of the level found in AD tg mice. In contrast, the levels of X11 β and X11 γ were not significantly altered in brains of X11 α +/- mice (Fig. 1, A, B, C, and D). This suggests that there were no compensatory changes in X11 β and X11 γ levels caused by the reduction of X11 α protein. Analyses of Western blots for presenilin-1 (PS-1) also showed no changes in the protein levels of PS-1 in the X11 α haploinsufficient mice compared to ADtg mice (data not shown).

C-terminal fragments of APP are increased in brains of AD tg/X11 α +/- mice

Immunoblot of proteins extracted from brains of AD tg versus AD tg/X11 α +/- mice revealed that total APP levels did not differ between the two genotypes. Immunoblots also revealed two discrete bands corresponding to the APP C-terminal fragments, C99 (CTF β) and C83 (CTF α). The levels of C83 and C99 were increased in ADtg/X11 α +/- mice compared to ADtg mice. The relative band intensities of these CTFs were determined and normalized to β -actin levels as a loading control. Semi-quantitative analyses revealed a significant increase in both C83 (Fig. 2C) and C99 (Fig. 2D) in AD tg/X11 α +/- mice compared to ADtg mice. The C83 intensity increased 2.6 fold ($p < 0.01$) while the C99 intensity increased 1.5 fold ($p < 0.05$). To determine if sAPP α and sAPP β levels correspondingly changed with this increase in the CTF C83 and C99 levels, we performed Western blots for sAPP α and sAPP β . Analyses of the immunoblots revealed statistically nonsignificant decrease in sAPP α and sAPP β levels in AD tg/X11 α +/- mice compared to AD tg mice (Fig 3, A–C).

Increased soluble A β and A β /amyloid plaque count in X11 α +/- mice

The levels of soluble A β 40, A β 42, and total A β extracted from brain homogenates of AD tg/X11 α +/- mice were significantly increased compared to those in AD tg mice (Fig. 4, A–C). Although the A β 42/A β 40 ratio showed a trend towards a decrease in the AD tg/X11 α +/- mice, the difference in ratios did not reach statistical significance (Fig. 4D). Correspondingly, the density of A β /amyloid plaques in silver-stained sections from cortex and hippocampus of 12 month old AD tg/X11 α +/- mice was increased compared to AD tg mice (Fig. 5A). As expected, A β /amyloid plaques were restricted to cortical and hippocampal regions in both genotypes.

Serially cut sagittal sections (every fifth section) were analyzed for one entire hemisphere of mice in each genotype cohort. Plaque counts in both cortex and hippocampus of AD tg mice were significantly increased ($p < 0.05$) in the setting of X11 α haploinsufficiency (Fig. 5, B–C). The larger effect size apparent in hippocampus compared to cortex may reflect the higher level of expression of X11 α in the limbic system, including hippocampus (Nakajima et al., 2001), or difference in neuronal population expressing X11 α in hippocampus versus cortex (Ho et al 2008).

Discussion

To establish a physiological role for X11 α in APP metabolism and A β deposition, we explored the consequences of X11 α reduction in AD tg mice that express both the hAPP^{swe} and the PS-1 Δ E9 familial AD mutations. Our major findings are that X11 α reduction leads to: 1) increase in APP CTFs (C99 and C83); 2) increase in soluble A β 40 and A β 42 levels without a significant change in APP, sAPP α , or sAPP β ; and 3) increase in amyloid plaque density in hippocampus and cortex of ADtg/X11 α (+/–) mice compared to AD tg mice. Our results suggest an intrinsic suppressor role for X11 α on cleavage of APP and its C-terminal fragments C99 and C83, and on CNS A β /amyloid deposition *in vivo*.

The possible mechanisms by which X11 α haploinsufficiency alters hAPP^{swe} metabolism include: 1) altered formation of multiprotein complexes that are mediated by the specific protein interaction domains of X11 α ; 2) altered trafficking of APP to intracellular sites where functional α -, β - and/or γ -secretases are present; and 3) impaired trafficking of APP in secretory and endocytic cellular compartments. These are not mutually exclusive possibilities and their full elucidation will require further experimental analysis. Indeed, the mechanisms by which adaptor proteins such as X11 α regulate APP metabolism are likely multifaceted (Parisiadou and Efthimiopoulos, 2007).

We found a significant increase in the APP C-terminal fragments C83 (CTF α) and C99 (CTF β) in brains of one year old AD tg/X11 α +/– mice, while holoAPP levels and sAPP levels were unchanged. These data are consistent with recent findings showing unaltered levels of murine APP when X11 α is deleted (Sano et al, 2006) or X11 α and X11 β are both deleted (Saito et al, 2008). APP processing is sequential: the cleavage of APP by either α - or β -secretase is a prerequisite for γ -secretase processing. Although one would expect that increased levels of C83 and C99 in AD tg/X11(+/–) mice might be associated with a corresponding increase in sAPP α and sAPP β levels, we did not detect a statistically significant change in these fragments. X11 proteins may more readily affect the steady-state levels of APP catabolic fragments with an intact cytoplasmic domain, which is essential for X11 interaction. Regulatory effects of X11 proteins on holo APP levels may be diluted by the high levels of APP transprotein expressed in brain.

X11 α haploinsufficiency significantly increased the levels of A β 40 and A β 42 in AD tg mice. The observed increase in CTF β would favor increased A β generation via mass action. The initial cleavage of APP by β -secretase is the rate-limiting step in A β generation. The increased A β levels and A β /amyloid plaque density observed in AD tg/X11 α +/– mice likely reflect increased cleavage of the precursor CTF β (C99) by the γ -secretase complex. Gross et al. (2008) suggested that X11L (X11 β) does not affect γ -cleavage of APP, yet γ -secretase activity is known to vary depending on the substrate and tissue (Loewer et al., 2004).

Our data are consistent with X11 α deficiency resulting in altered multiprotein complexes, the effect of which is to direct APP processing towards a pathway that favors increased A β formation. This possibility is supported by studies showing that APP preferentially associates with a syntaxin 1-microdomain via a X11-Munc interaction which in turn inhibits the

interaction between APP and BACE-1. Dissociation of the X11/Munc18/syntaxin-1 complex results in switching of the association of APP from syntaxin 1 to BACE-1 thereby promoting β -cleavage (Sakurai et al., 2008). Alternatively, X11 α may simultaneously associate with APP and alcadein, forming a tripartite complex that inhibits access of APP to γ -secretase (Araki et al., 2003). If the formation of this APP/Alcadein/X11 α protein complex is reduced, APP cleavage may be enhanced.

Although the subcellular site(s) of X11 α action on APP metabolism are not completely understood, X11 α may regulate APP metabolism by slowing its normal secretory and endosomal/lysosomal trafficking in neurons *in vivo*. X11 α reduction would be expected to increase trafficking of APP in both pathways and thus increase A β generation by enhancing delivery of APP to β - and γ -secretases. In support of this possibility, X11 proteins have been shown to associate with APP outside of detergent-resistant membranes (DRM) and suppress the translocation of APP into DRM, where β -secretase and γ -secretase are both active (Saito et al., 2008). X11 α deficiency may result in increased translocation of APP into DRMs and therefore increased exposure to β -secretase activity, leading to increased substrate levels for γ -site cleavage.

Our data are consistent with recent reports of enhanced amyloidogenic metabolism of endogenous murine APP when X11 β is deleted (Sano et al., 2006) or both X11 α and X11 β are deleted (Saito et al., 2008). In these recent studies, A β /amyloid plaque deposition could not be assessed because, unlike the mutant form of APP expressed in AD tg mice, murine A β does not form plaques.

The role of X11 α and other X11 proteins in APP metabolism, however, still requires more investigation. Previous studies employing RNAi-mediated knockdown of X11 α or X11 β *in vitro* (Xie et al 2005) or overexpression of X11 proteins *in vitro* or *in vivo* (Borg et al., 1998b; Sastre et al., 1998; Mcloughlin et al., 1999; Mueller et al., 2000; Biederer et al., 2002; Lee et al., 2003, 2004; Saluja et al., 2006) paradoxically were *both* shown to inhibit APP-CTFs and A β generation. These conflicting results perhaps can be explained by the different approaches used. For example, siRNA can elicit off target effects at the level of mRNA (degradation) or protein (translational repression), while overexpressed proteins can have effects beyond simply complementing the endogenous protein.

In a recent elegant study, Ho et al (2008) investigated the role played by X11 proteins in AD pathogenesis. Their results suggest possible interplay among X11 proteins in regulating APP metabolism. The authors examined the effects of X11 proteins in AD tg mice that lacked one or more X11 proteins. Most pertinent to the current study, Ho and colleagues examined cohorts of mice that were deleted of X11 α and haploinsufficient for X11 β or were haploinsufficient for X11 α and deleted of X11 β . Depending on the specific X11 protein(s) knocked out or knocked down, the age of the tg AD mice, and the brain region examined (hippocampus or cortex), Ho et al (2008) reported decreases and even some increases in CNS amyloid plaque density. They did not, however, report results in aged AD tg mice in which X11 α was selectively reduced, as done here. The discrepancy between our results and those of Ho et al (2008) may reflect differences in the genes altered: in their AD tg mice X11 α was reduced or deleted in combination with X11 β reduction or deletion. Their additional studies of X11 α deletion were performed in a different AD mouse (APP^{swe/Ind}) that lacks the PS-1 mutation. Taken together the published data and the results presented here support *in vivo* regulatory effects of APP:X11 interactions in neurons.

Other proteins have also been reported to inhibit age-dependent AD-like phenotypes *in vivo* including the α -secretase ADAM10, the liver X receptor, SOD2, ABCA1, transthyretin, neprilysin, and the LRP ligand RAP (reviewed in Duyckaerts et al., 2008). A common theme

is that many of these proteins regulate APP trafficking and processing or directly promote A β clearance and degradation. Pharmacologic mimics of one or more of these proteins, or up-regulation of their expression *in vivo*, may serve as a therapeutic strategy for AD. A better understanding of the mechanisms by which APP is shunted into non-amyloidogenic versus amyloidogenic pathways is needed to better understand both the normal functions of APP and its catabolic fragments, as well as the role of their binding partners in brain.

Acknowledgments

This study was supported by the VA Ann Arbor Healthcare System GRECC, and by US Public Health Service Grant P50 AG08671. We thank Timothy Desmond, Lisa Bain, and Dr. Roger Albin for their assistance with stereology.

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Fig. 1. Reduced X11α protein in ADtg/X11α(+/-) mouse brain without changes in other X11 family proteins

(A) Immunoblot analysis of X11α in brain homogenates confirmed a reduced level of X11α in AD tg/X11α +/- mice (Lanes 3 and 4) compared to AD tg mice (Lanes 1 and 2), while there was no change in the levels of X11β and X11γ. (B) Semi-quantitative analyses of immunoblots revealed that the X11α band density in brains from AD tg/X11α +/- mice was significantly less (54 %) than in AD tg mice. (C–D) Semi-quantitative analyses of the band density for X11β and X11γ revealed no significant change in the levels of either protein. Error bars indicate S.E.M. ($n = 6$), and statistically significant differences are indicated (* $p < 0.01$)

Fig. 2. Increased C-terminal fragments in AD tg/X11α+/- mice

(A) Immunoblot analysis of APP and the C-terminal fragments C99 (CTFβ) and C83 (CTFα). Proteins (65 μg) from mouse brain homogenates were detected by immunoblot with an antibody to the N-terminus of APP (for detecting full length APP), to the C-terminus of APP (for detecting CTFs), or to β-actin. Protein from cell lysates transfected with C99 (Lane 1) was run as a positive control for detecting the C99 fragment in brain homogenate (B, C, D). Band intensities of total APP, C83 and C99 from AD tg and AD tg/X11α +/− mice were quantified and normalized to the levels of β-actin. X11α haploinsufficiency significantly increased the level of C83 (CTFα) (C) and C99 (CTFβ) (D) without altering the level of total APP (B). Error bars indicate S.E.M. ($n = 6$), and statistically significant differences are indicated (** $p < 0.01$ and * $p < 0.05$)

Fig. 3. Levels of sAPP α and sAPP β fragments in AD tg/X11 α (+/-) mice

Immunoblot analysis of sAPP α and sAPP β fragments is shown. Proteins (60 μ g) from mouse brain homogenates were detected by immunoblot with antibodies to sAPP α , sAPP β or to β -actin. Conditioned medium (CM) from a CHO (Chinese hamster ovary) cell line stably transfected with APP^{swe} and PS-1 Δ E9 mutation was used as a positive control to verify the specificity of the secreted product. (A) Band intensities were quantified and normalized to levels of β -actin. X11 α haploinsufficiency does not lead to a statistically significant change in the level of (B) sAPP α and (C) sAPP β . Error bars indicate S.E.M. ($n = 4$).

Fig. 4. Aβ levels in brain are increased by X11α reduction

Measurement of soluble Aβ levels in 12 month old mouse brain homogenates revealed a significant increase in Aβ40 (A), Aβ42 (B), and total Aβ (C) levels in ADtg mice on an X11α haploinsufficient background (n = 4; * indicates p < 0.01). The Aβ42/Aβ40 ratio (D) was not significantly altered.

Fig. 5. A β /amyloid plaque density is increased by X11 α reduction

(A) Representative silver-stained sections from the hippocampus and cortex of AD tg mice or of AD tg/X11 α haploinsufficient mice revealed a higher plaque density in AD tg mice on a X11 α +/– background. Higher magnification images in lower four panels represent insets from the top two panels. Scale bars 150 μ (top panels) and 50 μ (bottom four panels). (B–C) Increased plaque density in hippocampus of ADtg/X11 α depleted mice. Stereological counts of amyloid plaque density were obtained from sections from cortex and hippocampus of AD tg versus ADtg/X11 α +/– mice at 12 months of age. The total number of plaques in the cortex (B) and hippocampus (C) were calculated for both groups and expressed as total counts per section. The error bars indicate S.E.M ($n = 4$), and statistically significant differences are indicated (* $p < 0.05$).