Published in final edited form as:

Mol Psychiatry. 2016 October ; 21(10): 1333–1341. doi:10.1038/mp.2016.134.

Novel Roles of Amyloid-Beta Protein Precursor Metabolites in Fragile X Syndrome and Autism

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Abstract

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and is associated with up to 5% of autism cases. Several promising drugs are in preclinical testing for FXS; however, bench-to-bedside plans for the clinic are severely limited due to lack of validated biomarkers and outcome measures. Published work from our laboratories has demonstrated altered levels of amyloid-beta (Aβ) protein precursor (APP) and its metabolites in FXS and idiopathic autism. Westmark and colleagues have focused on β-secretase (amyloidogenic) processing and the accumulation of Aβ peptides in adult FXS models while Lahiri and Sokol have studied α-secretase (nonamyloidogenic or anabolic) processing and altered levels of sAPPα and Aβ in pediatric autism and FXS. Thus, our groups have hypothesized a pivotal role for these Alzheimer's disease (AD)-related proteins in the neurodevelopmental disorders of FXS and autism. In this review, we discuss the contribution of APP metabolites to FXS and autism pathogenesis as well as the potential use of these metabolites as blood-based biomarkers and therapeutic targets. Our future focus is to identify key underlying mechanisms through which APP metabolites contribute to FXS and autism condition-to-disease pathology. Positive outcomes will support utilizing APP metabolites as blood-based biomarkers in clinical trials as well as testing drugs that modulate APP processing as potential disease therapeutics. Our studies to understand the role of APP metabolites in developmental conditions such as FXS and autism are a quantum leap for the neuroscience field, which has traditionally restricted any role of APP to AD and aging.

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Financial Disclosures

CJW: none

DKS: none

DKL: reports grants as a Principal Investigator from the National Institute on Aging, National Institute of Health, USA; Member, Scientific Advisory Board, QR Pharma, Inc., Berwyn, PA, USA; Member, Scientific Advisory Board, Yuma Therapeutics, Boston, MA; Member, Scientific Advisory Board, Entia Biosciences, Inc., Sherwood, OR; Member, International Advisory Board of the Drug Discovery and Therapy World Congress, Boston, MA; and Editor-in-Chief, "Current Alzheimer Research", Bentham Sciences Publishers.

Keywords

APP; fragile X syndrome; autism; biomarker; seizure; macrocephaly

1. Introduction

No validated blood-based biomarkers or behavioral outcome measures currently exist for fragile X syndrome (FXS), which is impeding bench-to-bedside clinical efforts. Several recent mega-sequencing studies suggest a strong molecular link between FXS and autism.[1–3] Elucidation of shared and contrasting neurobiologies of these neurodevelopmental conditions is expected to identify novel disease biomarkers and therapeutic targets, which are critical needs for the field. Research from our laboratories has demonstrated amyloid-β (Aβ) protein precursor (APP) and its metabolites are dysregulated in FXS and autism. APP is a large membrane-spanning glycoprotein that undergoes proteolytic cleavage by secretases to form either Aβ peptides found in the extracellular cerebral amyloid plaques of Alzheimer's disease (AD) and neurotoxic secreted APPβ (sAPPβ) or non-toxic, non-amyloidogenic p3 peptide ($Aβ_{17–40/42}$) and secreted APP alpha (sAPPα) believed to have neurotrophic properties. The localization of APP at dendritic synapses and its roles in neurogenesis and cell adhesion suggest that this protein and its metabolites play pivotal roles in development. Our central hypothesis is that APP metabolite profiles are viable biomarkers for disease severity in FXS and autism. To date, there is a dearth of available data regarding APP metabolite profiles as a function of age, potential alterations in proteolytic processing with development, or associations between APP metabolite levels and FXS and autism traits. This lack of knowledge is a significant problem because it prohibits broader understanding of how APP metabolites are connected to neuronal function and behavior as well as their potential use as biomarkers for disease severity and drug efficacy testing. Our hypothesis is formulated, in a large part, based on published data from our laboratories, which addresses this lack of knowledge from opposing but complementary directions in studying amyloidogenic (β-secretase) versus non-amyloidogenic (α-secretase) APP processing and in working with samples from adult FXS versus pediatric autism subjects. Herein, we meld our findings with those from the Bagni laboratory exploring the role of sAPPα in FXS,[4] the Wegiel laboratory demonstrating $Aβ_{17–40/42}$ (also known as p3 peptide, generated by the a-secretase pathway) accumulation in autistic brain,[5] and the Erickson laboratory regarding the use of APP metabolites as drug responsive biomarkers[6] to generate a model of how APP metabolites contribute to FXS and autism pathogenesis as well as their potential use as blood-based biomarkers and drug targets.

2. FXS and APP Metabolites

FXS is the most common form of inherited intellectual disability with a frequency of 1 in 2,500 births, and is associated with up to 5% of autism cases.[7–9] It is clinically characterized by highly variable cognitive function (overall IQ<70), autistic-like behaviors, seizures, macrocephaly and macroorchidism.[10] FXS results from a mutation in a single gene on the X-chromosome, *FMR1*, which codes for fragile X mental retardation protein (FMRP). FMRP expression is absent or greatly reduced in FXS and many disease phenotypes are

manifested in *Fmr1*^{KO} mice, which lack expression of FMRP. FMRP is a multi-functional RNA binding protein that is ubiquitously expressed throughout the body with significantly higher levels in young animals.[11] It is involved in the transport, localization and translational regulation of mRNA ligands and is required for normal dendrite development. In aggregate, over 500 mRNA ligands for FMRP have been identified, many with the potential to influence synaptic formation and plasticity.[12–14]

The prevailing theory regarding the pathogenic mechanism underlying FXS is that uncontrolled metabotropic glutamate receptor 5 ($mGluR_5$) signaling causes exaggerated protein synthesis in the absence of the translational repressor FMRP.[15] Exaggerated translation at synapses underlies abnormal dendritic spine morphology, seizure activity and FXS behaviors. There has been an intense effort to identify mRNAs that are translationally repressed by FMRP as the proteins they code for are potential disease biomarkers and therapeutic targets. We identified *App* mRNA as a FMRP synaptic target.[16] Using the UV crosslinking-immunoprecipitation (CLIP) assay developed by the Darnell laboratory, Westmark and colleagues demonstrated that FMRP binds directly to a guanine-rich region in the coding region of *App* mRNA;[16] this important finding was reproduced by Myriam Gorospe's laboratory.[17] Stimulation of cortical synaptoneurosomes with (S)-3,5-dihydroxyphenylglycine (DHPG), a group 1 mGluR agonist, releases FMRP from *App* mRNA leading to APP synthesis or regulated translation. In synaptoneurosomes and primary cultured neurons prepared from *Fmr1*^{KO} mice, basal APP levels are increased and do not change in response to DHPG suggesting that constitutive translation occurs in FXS.[16] Elevated APP levels provide more target for secretase processing consistent with the finding that Aβ is elevated in older *Fmr1*^{KO} mice[16] (Figure 1).

Fmr1^{KO} mice exhibit features shared with FXS patients such as increased susceptibility to seizures, altered anxiety, and dendritic spine phenotypes.[10,18–26] In order to establish if APP directly contributed to FXS pathogenesis, Westmark and colleagues modulated APP expression in *Fmr1*^{KO} mice by creating mice that express only one *App* allele (*Fmr1*^{KO}/*APP*^{HET}).[27] Western blot analyses confirmed APP levels were reduced by 50% in *Fmr1*^{KO}/*APP*^{HET}. Animals were evaluated for seizure susceptibility (audiogenic-induced seizures, AGS), repetitive behavior (marble burying), anxiety (open field), mGluR-LTD, and dendritic spine phenotypes. All of these phenotypes were attenuated in *Fmr1*^{KO}/*APP*^{HET} (Table 1).[27]

Differential expression and processing of APP throughout development is expected to affect APP metabolite profiles and FXS phenotypes. Both amyloidogenic and non-amyloidogenic secretases are expressed during embryonic, postnatal and adult development.[28] Recent work from Claudia Bagni's laboratory elegantly demonstrates the role of sAPPα in mediating immature spine, mRNA translation and mGluR-LTD phenotypes in postnatal *Fmr1*^{KO} mice.[4] Others have shown that sAPPα, but not sAPPβ, rescues LTP and dendritic spine density in adult *App*^{KO}.[29–30] Thus, sAPPα plays important roles in synaptic plasticity at multiple stages of development. These data strongly support the hypotheses that modest over-expression of APP in the context of the *Fmr1*^{KO} contributes to many pathological phenotypes observed in FXS.

The aforementioned mouse studies suggest that APP metabolites are a potential disease biomarker for FXS. Westmark and colleagues evaluated APP/sAPPα and Aβ levels in blood plasma from full-mutation adult FXS subjects and found $A\beta_{42}$ was significantly lower in the FXS group (2.1–fold decrease, $P<0.004$).[27] APP/sAPPα and $A\beta_{40}$ levels were equivalent between FXS males and controls. A reduced $A\beta_{42}/A\beta_{40}$ blood plasma ratio as seen for FXS is also a putative biomarker for AD[31], autism[32], and Down syndrome.[33] The prevailing theory in AD and Down syndrome is that the brain acts as a sink for $A\beta_{42}$ and a lower blood plasma level indicates increased brain deposition. APP/sAPPα and Aβ were measured in a limited number of adult control and FXS autopsy brain samples and there was a trend toward increased Aβ with a reciprocal decrease in sAPPα levels.[27] These studies are in agreement with those in *Fmr1*^{KO} mice, which exhibit elevated Aβ in the brain,[16] and support a model of increased β-secretase processing in adult FXS associated with Aβ accumulation in the brain and clearance from the blood consistent with the AD "brain sink model". Lahiri's group recently found increased $A\beta_{42}$ ($P<0.001$) and equivalent $A\beta_{40}$ levels in FXS pediatric plasma versus control samples. sAPPα ($P=0.015$) and total sAPP ($P<0.001$) were both increased in FXS.[34] These data suggest increased APP expression possibly accompanied by increases in α- and β-secretase processing in pediatric FXS.

3. Autism and APP Metabolites

Autism is a cluster of complex neurobiological symptoms, autism spectrum disorder (ASD), that normally present in the second or third years of life. The core features include impairments in social interaction and communication, and repetitive stereotyped behavior. Many autistic children exhibit intellectual disability and marked delay in motor milestones. ASD are estimated to occur in 1 in 68 children with prevalence 4.5-fold higher in males via conventional epidemiological estimates,[35] with total-population sample studies proposing rates as high as 1 in 38.[36] The etiology of autism is unknown but genetic, epigenetic and environmental factors likely affect symptom severity.[37–39] FXS shows high comorbidity with autism (although not vice versa).[40–41] Recent mega-sequencing studies suggest a strong molecular link between these disorders. In particular, many of the protein-interacting partners of FMRP harbor autism-associated common variants,[1] there is an enrichment of autism rare *de novo* variations in FMRP targets,[2] and there is an enrichment of FMRP targets in neuronal gene expression modules in autism brain.[3] Autism is, nevertheless, distinct from FXS in several neuroanatomical and behavioral aspects.[42–48] Seminal work from the Sokol and Lahiri laboratory demonstrated significantly elevated sAPPα with reduction of sAPPβ and Aβ peptides in plasma from severely autistic children.[49–51] Most of these subjects exhibited intellectual disability and seizures. Two subjects were comorbid for FXS with autism and exhibited the highest levels of sAPPα[49] Importantly, they observed a negative correlation between sAPPα levels and age. In a larger study, they repeated the finding of reduced Aβ in plasma and brains of idiopathic autism subjects, independent of severity.[34] No significant correlation between sAPPa levels and age was found, but they did report a significant positive relationship between α-secretase and age in non-FXS autism brains samples. Two independent laboratories have replicated portions of this work with different patient cohorts.[32,52] Bailey and colleagues found significantly increased sAPPα in 60% of known autistic children.[52] Al-Ayadhi and colleagues found significantly lower $A\beta_{40}$

and $A\beta_{42}$ and a lower $A\beta_{40/42}$ ratio in Saudi autistic children.[32] Fatemi and colleagues found increased $mGluR_5$ in the superior frontal cortex of children with autism versus healthy controls[53] and a corresponding increase in APP.[54] They found decreased APP in the vermis of adult subjects with autism. Overall, these findings support abnormal levels of $mGluR_5$ and APP in autism and support a model of increased α-secretase processing in severe childhood autism. It remains to be determined how extensively APP processing changes with aging and how altered metabolite levels contribute to ongoing disease pathology (Figure 2), as there are currently no reported studies of sAPPα levels in adults with autism, although a significant positive relationship has been reported between age and ADAM17 (the primary α-secretase) in idiopathic autistic brain versus controls (P=0.011).[34] There have been limited studies to examine APP metabolite levels or function in mouse models of autism other than in *Fmr1*^{KO} and TgsAPPα mice. The TgsAPPα mice developed by Bailey and colleagues exhibit hypoactivity, impaired sociability, increased brain glial fibrillary protein (GFAP), and altered Notch1 and IL-6 levels.[55–56] Longitudinal studies in Tg-sAPPa mice could identify age-related effects of sAPPa.

Ferreira and Klein elegantly reviewed the numerous synaptic activities of Aβ-derived diffusible ligands (ADDLs) and proposed that the molecular and synaptic similarities between AD, FXS and autism may be sufficient to regard AD, in principle, as a type of ASD that shows very late onset and to view FXS as an early manifestation of Aβ oligomer-induced disease.[57] Indeed, microarray analysis of cerebellar samples demonstrated altered expression of 40% of AD-related genes in autistic subjects.[58] Validation of this theory awaits further experimentation including a thorough analysis of APP metabolite profiles throughout the lifespan and determination of their contribution to the behavioral and cognitive phenotypes of the aforementioned disorders. Notably, certain APP metabolites are implicated in seizure propensity and macrocephaly.

Epileptic seizures may drive the development of autism in neurodevelopmental disorders.[59–60] Epilepsy is highly comorbid in autism with a 21.4% prevalence in autistic subjects with intellectual disability and 8% in subjects without intellectual disability.[61] EEG abnormalities were found in 31% of children with ASD.[62] Seizures and EEG abnormalities are also found in FXS[63] and AD.[64–65] These disorders are all characterized by abnormal levels of APP metabolites, albeit each with potentially distinct profiles.[66] Genetic suppression of transgenic APP in mice rescued epileptiform activity suggesting that abnormal APP levels are a potential cause of seizure activity.[67] Abnormal EEG discharges in the Born study were independent of plaque load and reduction of Aβ levels implicating full-length APP or another metabolite in seizure propensity.[67] Other studies indicate that Aβ oligomers induce dynamic redistribution of $mGluR_5$ receptors to synapses,[68] facilitate mGluR-LTD,[69] and induce intrinsic excitability in CA1 pyramidal neurons.[70] Thus, multiple APP fragments or the ratio of various fragments may contribute to seizure activity.

Macrocephaly in autism is a disputed topic.[71–81] An NIH study has questioned early brain overgrowth in autism as detected by head circumference measurements, which may represent norm bias rather than an autism-specific biomarker.[71] Despite the controversy regarding dramatic brain overgrowth in autism during the first year of life, there is a possible subtle divergence in head circumference during the second year.[71] Brain overgrowth is a

feature of several genetic syndromes that are comorbid with autism including FXS;[82] however, autism can also be comorbid with several microcephalic syndromes such as Down syndrome, which is trisomic for the *APP* gene. In a sample of children ages four and older, Wegiel and colleagues conducted a stereological study of nuclear and cytoplasmic volumes of neurons in 16 brain structures of autistic and control subjects.[83] They found significant deficits of neuronal soma and nuclear volumes in 13 of the 16 brain regions examined in pediatric autism samples and increased nuclear volumes in 8 of 16 structures in autistic teenagers and young adults. Their findings suggest global abnormalities in brain development in autism. Enlargement in white, rather than gray matter, likely accounts for macrocephaly.[74] White matter abnormalities have also been observed in FXS brain and implicated in the *Fmr1*^{KO}.[84–86] The secreted sAPPα fragment of APP, which has known neurotrophic properties, is elevated in both brain and plasma of autistic patients[34], particularly those with severe autism.[49–51] It remains to be determined if sAPPα localizes to white matter and contributes to brain overgrowth and how this may relate to autism severity, particularly since brain overgrowth, while less common than previously thought, may be predictive of more severe autism.[87]

How could APP metabolites, in particular sAPPα, facilitate white matter brain overgrowth? Evidence associating sAPPα with brain overgrowth was found by Bailey and colleagues in sAPPα-overexpressing transgenic mice, which produce an abundance of astrocytes, GFAP and brain CD8 T cells.[55–56] Convergent, albeit indirect evidence for the role of sAPPα in aberrant brain growth was reported by Zeidan-Chulia et al who found anabolic upregulation of GRIN1, NMDA glutamate receptors, and MAP3K1, known activators of ERK pathways, in cerebellar samples from autistic individuals.[88] They hypothesized that deregulated glutamatergic synaptic transmission/plasticity, caused by increased GRIN1 with resultant increased density of NMDA receptors, favors the nonamyloidogenic pathway and production of sAPPα through ERK mediated α-secretase activity. sAPPα, then may facilitate proliferation by activating the P13K/Akt/mTOR pathway. They also showed altered expression of genes in the AD and WNT pathways in autism. The WNT–β -catenin adhesion pathway is abnormal in autism.[89] Disruption of brain cell adhesion would favor brain overgrowth; further, APP has been associated with downregulation of α-catenin.[90]

Unlike AD individuals who show confusion and visual memory disturbance, autism subjects exhibit hypervigilance and often excellent visual memory.[91] Could a derangement in APP metabolites within white matter account for this? Although AD is considered a gray matter disease, there is evidence that oligodendrocytes and myelin are targeted via Aβ peptides before signs of amyloid deposition and Tau aggregates.[92] Desai et al speculate that early derangement in myelin might make the affected axon vulnerable to inflammation or oxidative stress. No studies to date of sAPPα in white matter exist; however, tumor necrosis factor alpha (TNF) converting enzyme (TACE or ADAM17) was found essential for oligodendroglia development and CNS myelination in a nonpathological mouse model.[93] Therefore, by proxy sAPPα may localize to oligodendrocytes. In contrast, Wegiel et al[5] reported signs of elevated a-secretase activity, specifically increased $Aβ_{17–40/42}$, in the presence of microcephaly in individuals with chromosome 15q11.2–q13 duplications (Dup(15)) suggesting that α-secretase is not associated with brain overgrowth. Up to 80% of individuals with Dup(15) acquire microcephaly by age 2,[94] unlike congenital microcephaly

that exists at birth and in opposition to the prevalence of macrocephaly in other syndromic forms of autism (e.g., FXS with autism) and in up to 20% of idiopathic autism.[95] The Dup(15) ubiquitin protein ligase E3A (UBE3A), associated with Angelman's syndrome, interacts with the primary microcephaly protein ASPM.[96] This association may override an effect of sAPPa in contributing to macrocephaly. In any case, APP has been associated with the ubiquitin proteosome machinery that degrades and clears proteins, and underlies UBE3A.[97] Furthermore, APP has been shown to modulate β-catenin degradation which occurs via ubiquitinylation and proteosome degradation.[90]

4. The APP Metabolite Profile as a Blood-Based Biomarker

Currently, FXS and autism therapy development efforts range from early translational studies to human clinical trials. As promising candidate therapies move forward, there is a critical need for sensitive, reliable and valid biomarkers in model organisms, particularly measures that can predict efficacy in human trials and can be used in titrating and monitoring therapies. The recent discontinuation of clinical trials by Novartis and Roche of promising $mGluR_5$ antagonists in FXS subjects highlights the compelling need to identify and validate physiologic measures that successfully bridge pre-clinical and clinical studies in this disorder.[98–99]

Published data from our laboratories present a complex picture that may show significant age-related changes in the molecular neurobiology of FXS and autism. Our groups demonstrated reduced Aβ in blood plasma and brains from autistic children, but increased levels in FXS children.[34,50] Full-mutation FXS adults, on the other hand, had reduced blood Aβ versus controls.[27] Our results differ in showing elevated sAPPα in blood plasma from autistic and FXS children[34,49–50] but no difference in sAPPα in adult FXS.[27] These observations create important gaps in the literature regarding: (1) differential APP processing as a function of tissue, age and disease status; (2) methodological differences in blood collection protocols that could contribute to APP processing post-sample collection; and (3) utility of using APP metabolites as disease biomarkers and drug targets for FXS and autism. Toward addressing the first aforementioned gap, Lahiri and colleagues measured APP metabolites in brain tissue of autistic and FXS children compared to age-matched controls. In left temporal lobe samples from the Autism Tissue Program, they found a similar APP profile as in plasma, i.e. non-significant elevation in sAPPα for autism ($P > 0.05$) (n=7) compared to controls but significant elevation in FXS vs. controls ($P = 0.01$) and decreased $Aβ_{40}$ ($P = 0.001$) in autism but not FXS vs. controls ($P > 0.05$).[34] Studies from the Wegiel laboratory demonstrate abnormal intracellular accumulation and extracellular deposition of $Aβ_{17–40/42}$ in idiopathic and Dup(15) autism brains in both adults and children.[5] A recent study showed high levels of sAPPα in children with autism (n=6) in insular cortex gray matter,[56] consistent with the Lahiri laboratory temporal lobe findings. In total, these data suggest a preponderance of α-secretase processing in idiopathic autism. The Lahiri laboratory also evaluated APP/sAPP and Aβ levels in blood plasma from FXS children (n=18) and found elevated total sAPP, sAPPα, sAPPβ, $Aβ_{40}$ and $Aβ_{42}$.[34] These data in conjunction with the adult FXS and pediatric autism studies suggest both elevated catabolic and anabolic APP processing in pediatric FXS with a shift toward decreased α-secretase activity with aging.

The major difference in blood collection protocols was the choice of anticoagulant. The adult blood samples were collected in heparin and the pediatric samples in EDTA. To determine effects of anticoagulant on APP metabolite profiles, Westmark et al collected blood samples from 5 adult control subjects splitting the blood into various anticoagulant tubes (lithium heparin, sodium heparin, EDTA, sodium citrate) and assessed APP metabolite levels.[100] With EDTA or sodium citrate, sAPPα measurements were significantly higher compared to sodium or lithium heparin. There was a significant inverse effect on $A\beta_{42}$ levels with no change in $A\beta_{40}$. These data suggest that anticoagulant affects sAPP stability post-blood collection, which could account for varied results concerning sAPPα levels, but does not account for varied results with Aβ, which may be due to subject age and disease severity. The possibility exists that EDTA chelation of calcium or other divalent ions may explain some differences in Aβ detectability.[101] Overall, these data demonstrate that FXS is associated with dysregulated post-transcriptional synthesis and processing of APP and prompt studies to assess APP metabolite levels as a function of age and disease severity under standardized blood collection protocols.

Regarding the third aforementioned gap in the literature, the utility of using APP metabolites as disease biomarkers for FXS and autism, Bailey and colleagues developed a sensitive ELISA to specifically measure sAPPα in human plasma and umbilical cord blood. They found significantly elevated levels of plasma sAPPα in 60% of autistic children and 10 of 150 human umbilical cord blood samples.[52] It remains to be determined which, if any, of the newborn babies from which cord blood was analyzed develop autism, which would support using sAPPα measurements as an early diagnostic tool. Erickson and colleagues are developing APP metabolites as blood-based biomarkers that are sensitive to drug treatment in autism and FXS clinical trials. Their team found acamprosate was associated with a significant reduction in plasma sAPP and sAPPα with no change in $A\beta_{40}$ or $A\beta_{42}$ in ASD youth[6] (Table 1). Furthermore, they showed that youth with FXS-associated ASD showed increased sAPPα processing compared to age-, gender- and IQ-matched youth with idiopathic ASD. Thus, APP metabolite profiles are a potential blood-based biomarker for disease severity and drug efficacy in FXS and autism.

Based on our results, we suggest that the APP biochemical pathway is dysregulated in both FXS and autism, but these two disorders differ in specific metabolite profiles (for example, low Aβ in pediatric ASD and high Aβ in pediatric FXS). Altered APP metabolite profiles could explain neuroanatomical differences between ASD and FXS. Interestingly, this observation is consistent with the amygdala being enlarged in ASD (low Aβ) and reduced in FXS (high Aβ causing atrophy). Differential Ab expression in FXS and ASD is not contradictory to the commonality of high sAPP in both disorders. How is that possible mechanistically? APP cleavage by β-site APP-cleaving enzyme (BACE1, aka β-secretase) depends on the availability of APP in the endosome where cleavage occurs[102]. That is, unless both APP and BACE1 meet in the endosomal compartment, there is no direct interaction and no Aβ formation. Circumstantial evidence suggests that clathrin-coated endosomes are deficient in ASD, which might lead to reduced cleavage of APP by BACE1 and thus decreased Aβ formation in ASD but not in FXS. What is interesting is that an endosomal mechanism can disrupt APP processing toward the anabolic pathway even when

levels of APP, BACE1, and ADAM proteins are normal. This is a testable hypothesis, which can be verified by analyzing endosomal structure in ASD cases versus controls.

5. APP Metabolites as Drug Targets for FXS and Autism

Therapies directed at modulating APP metabolites, as studied for AD, may be applicable to FXS and autism. For example, drugs that modulate secretase activity could be repurposed.[103–104] β- and γ-secretase inhibitors are currently in the preclinical stage of investigation and could provide a means to reduce the production of Aβ. BACE1 cleaves at the amino-terminus of Aβ and is the rate-limiting step in Aβ peptide generation. BACE1 inhibitor design has proven difficult due to the large size of the enzyme catalytic pocket.[105] It is also difficult for BACE1 inhibitors to reach the central nervous system. Nevertheless, GRL-8234 is a blood-brain barrier permeable compound that potently blocks BACE1 activity[106]. A single dose of GRL-8234 reduced plasma levels of Aβ by 50–65%[106–107] and brain interstitial fluid levels by 50% within 3 hr in Tg2576 AD mice[107]. *Fmr1*^{KO} mice exhibit elevated brain Aβ expression but not to the same extent as Tg2576. Thus, optimized pharmacokinetic treatment conditions could be determined to normalize Aβ levels in *Fmr1*^{KO}. Another option to reduce production of carboxyl-terminal fragments (CTF) of APP is γ-secretase inhibitors, which should decrease Aβ and g-CTF but increase b-CTF, which may also be neurotoxic. The problem associated with the use of γ-secretase inhibitors is that these drugs inhibit proteolytic processing of other proteins such as Notch that are critical for cellular function.[108] Supplementary Table 1 summarizes the numerous Aβ-modulating drugs in clinical trials for AD.[109–110] In addition, new approaches that modulate the expression rather than the catalytic activity of BACE1 are actively being sought. The Puglielli laboratory has identified novel biochemical inhibitors of acetyltransferases that significantly reduce the levels of BACE1 and the generation of Aβ in cellular systems.[111–113] Alternatively, the disintegrins proteins such as ADAM10 and ADAM17 possess α-secretase activity.[114] Cleavage by α-secretase increases sAPPα as well as carboxyl-terminal metabolites of APP. sAPPα has neuroprotective properties, but as previously discussed, elevated levels in autism and FXS may be contributing to macrocephaly. Data from the Bagni laboratory suggest that pharmacological inhibition of ADAM10 may be a viable therapeutic option for FXS (Table 1). Considering the complexity of APP processing and the potential feedback mechanisms associated with various APP metabolites, it may be necessary to concurrently modulate α- and β-secretase processing as well as APP synthesis to attain APP homeostatic conditions.

Despite decades of research, the exact roles of APP and Aβ in AD are still surrounded by inconsistencies and controversies.[115] Our work complicates matters by adding FXS and autism to the list of disorders that display abnormal APP metabolite profiles. We found altered levels of APP and Aβ in FXS mice as well as altered levels of $Aβ_{42}$ in plasma samples from adult FXS males. We found higher levels of total APP, sAPPα and lower levels of Aβ peptide in plasma from children with autism with the same pattern in brain tissue specimens from autistic patients. In part, these results have been replicated by independent laboratories. Furthermore, transgenic mouse models link the overexpression of varied APP metabolites with FXS phenotypes, autistic features, increased abundance of brain astrocytes and seizures. Recently, APP metabolites were reported as potentially useful

biomarkers in a FXS drug study. These studies highlight the importance of future endeavors to understand the role of various APP metabolites in the development and pathology of FXS and autism and to develop these proteins as disease biomarkers and therapeutic targets. The goal is to repurpose decades of AD drug-related research to provide therapeutics for FXS and autism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

CJW has received funding from FRAXA Research Foundation, NIH (R21AG044714 and R03HD075881), the University of Wisconsin (UW)-Madison Alzheimer's Disease Research Center (NIA P50AG033514), the UW-Madison Clinical and Translational Science Award (CTSA) program (NCATS UL1TR000427), Lundbeck USA, Merz Pharmaceuticals and Pierre Fabre.

DKS has received funding from the Riley Memorial Foundation, Mental Health Association for the Advancement of Mental Health Research and Education, Clarian Health Partners, Indiana University Collaborative Research Grant (22-140-29).

DKL has received funding from NIH (R01AG051086, R21AG4687100, P30AG010133), Alzheimer's Association, Indianan Clinical and Translational Sciences Institute (ICTSI), and ISDH Spinal Cord and Brain Injury Board.

Funding agencies played no role in the preparation of this publication. We thank the reviewers for their careful and insightful critiques, which greatly improved the manuscript.

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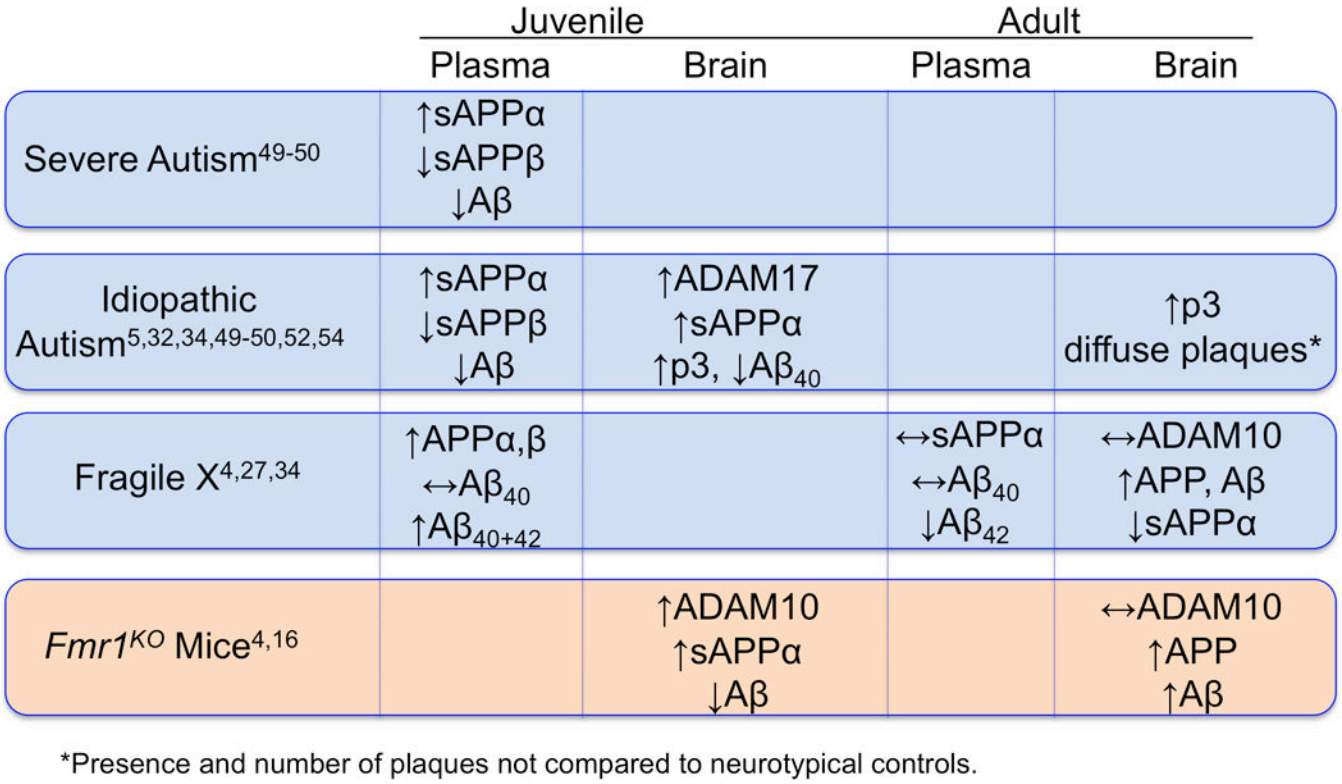

Figure 1.

Comparison of APP metabolite levels in human autism and FXS and in *Fmr1*^{KO} mice as a function of age (juvenile versus adult) and tissue (plasma and brain). APP is dysregulated in both FXS and ASD. While APP dysregulation seems to be persistent in life (young and adult/old), its processing changes during development. In young individuals and juvenile mice, the upregulated APP is processed by the a-secretase (non-amyloidogenic pathway) liberating sAPPa (human blood and mouse brain). In aged individuals and mice, the upregulated APP is processed by β-secretase liberating Aβ (human blood and mouse brain). Thus, there is a switch in the processing of APP during aging and the majority of the papers published report consistent findings in that young patients with FXS and ASD have an excess of sAPPa (possibly due to increased ADAM10 during that specific developmental window as shown in mice) while there is an increase in Aβ with age (possibly due to increased BACE1 activity).

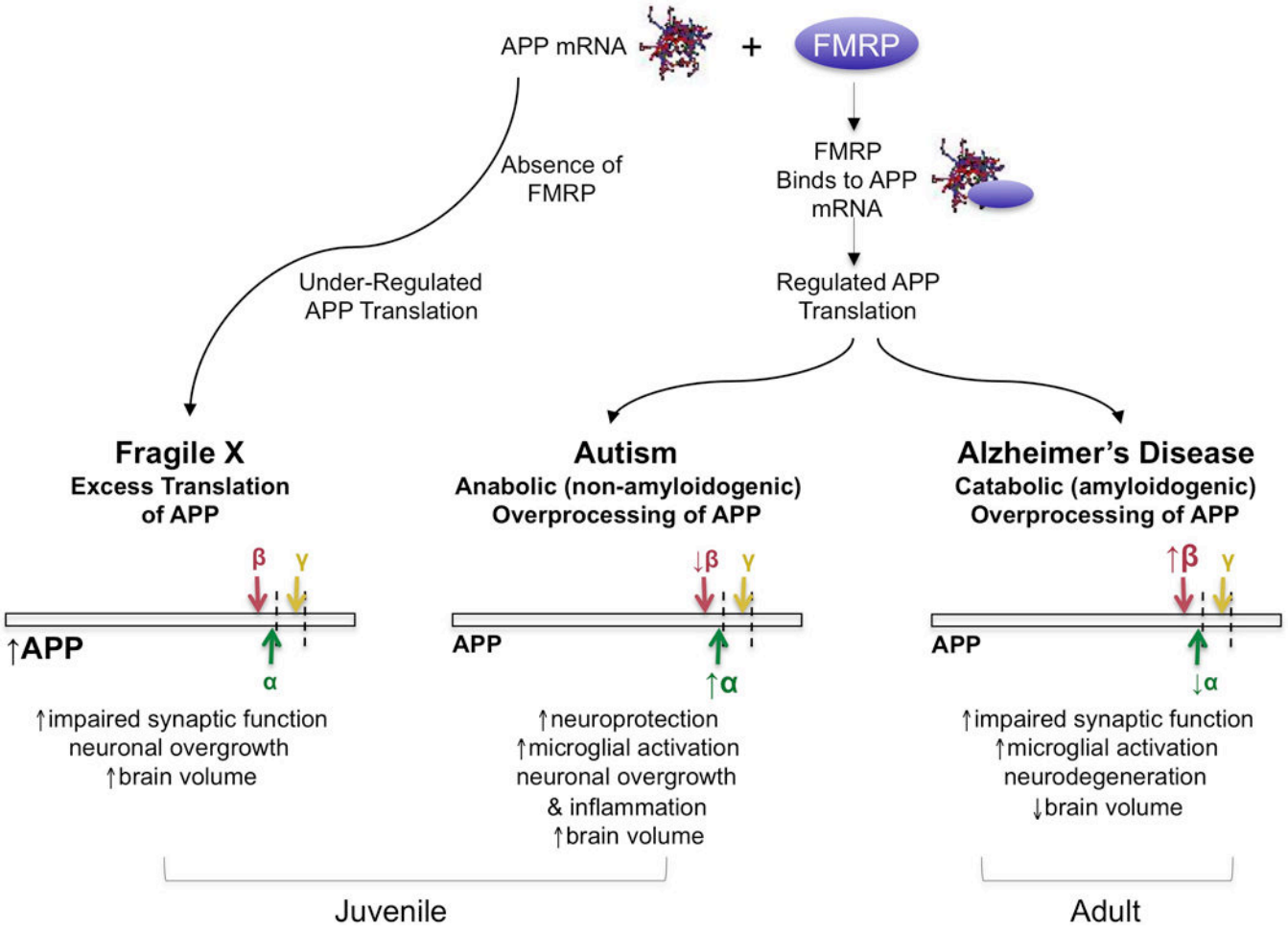

Figure 2.

APP expression and processing contrasted among FXS, autism, and Alzheimer's disease. At the level of mRNA translation, with a normal *FMR1* gene, FMRP binds to (among other targets) *APP* mRNA and inhibits translation resulting in regulated APP synthesis. In FXS, loss of the translational repressor FMRP leads to exaggerated protein synthesis resulting in elevated APP levels. At the level of protein processing, excess APP provides more target for both anabolic and catabolic secretase processing. In the case of FXS, APP processing may change with age such that exaggerated anabolic processing in childhood leads to neuronal overgrowth followed by increased catabolic processing in adulthood both accompanied by associated outcomes. In the case of autism, α-secretase processing is increased resulting in increased levels of anabolic/neurotrophic sAPPα. By comparison, relative levels of catabolic products (e.g., Aβ) are insufficient to compensate, resulting in neuronal overgrowth and associated outcomes. In Alzheimer's disease (normally a geriatric condition), excess catabolic processing by β-secretase, possibly accompanied by insufficient anabolic processing, results in inflammation, neurodegeneration, and loss of brain volume.

Table 1

Summary of FXS and Autism Phenotypes Rescued by Manipulation of APP Metabolites

Genetic Reduction of *App* in *Fmr1*^{KO} Mice (Westmark et al[27])		
Phenotype	**Rescue**	**Rescue with *Fmr1*^{KO}/*APP*^{HET}**
1. APP expression (western blot)	YES	↓50% (equal to WT levels; n=3 mice per cohort, ANOVA $P<0.0001$)
2. Seizures (AGS)	PARTIAL	↓54% (intermediate between WT and *Fmr1*^{KO}; n=23, Fisher Exact $P<0.05$)
3. Perseverant behavior – marble assay	YES	100% (equal to WT levels; n=8–10 mice per cohort, ANOVA $P=0.03$)
4. Anxiety (open field)	YES	100% (equal to WT levels; n=14–18 mice per cohort, ANOVA $P<0.0001$)
5. Percent mature spines (diI labeling)	YES	100% (equal to WT levels; n=2 individual neuronal cell preps, neurons from 2–6 coverslips per prep analyzed, 2–12 dendrites analyzed per coverslip, minimum of 746 spines analyzed per cohort)
6. Dendritic spine length (diI labeling)	PARTIAL	11% (intermediated between WT and *Fmr1*^{KO}; n=2 individual neuronal cell preps, neurons from 2–6 coverslips per prep analyzed, 2–12 dendrites analyzed per coverslip, minimum of 746 spines analyzed per cohort, ANOVA $P<0.0001$)
7. mGluR-LTD (field recordings)	YES	100+% (decreased LTD compared to WT; n=3 mice per cohort, n=10–13 slices per cohort, ANOVA $P<0.0002$)
Reduction of ADAM10 in *Fmr1*^{KO} mice (Pasciuto et al[4])		
Phenotype	**Rescue**	**Rescue with TAT-Pro Peptide**
1. APP α-cleavage (western blot)	YES	100% (equal to WT levels; n=5 mice per cohort, ANOVA $P<0.01$)
2. mGluR-LTD (field recordings)	YES	100% (equal to WT; n=4–6 mice per cohort, n=8–9 slices per cohort, ANOVA $P<0.0002$)
3. Biomarker expression (ARC, APP, ADAM10, STEP) (western blot)	YES	100% (equal to WT levels; n=5 per cohort, ANOVA $P<0.05$)
4. Distance & speed (open field)	YES	100% (equal to WT; n=11–17 mice per cohort, ANOVA $P<0.05$)
5. Preference test for novel arm (T-maze)	NO	100+% (increased preference for novel arm compared to WT; n=9–12 mice per cohort, Chi square $P<0.001$)
6. Nest building	YES	100% (equal to WT; n=5–8 mice per cohort, ANOVA $P<0.001$)
Pharmacological Rescue of APP Metabolites in FXS and Autism (Erickson et al[6])		
Phenotype	**Rescue**	**Rescue with Acamprosate**
1. APP expression (ELISA)	YES	Significant rescue in plasma sAPP (total) and sAPPα levels (n=9 FXS/ASD, n=6 ASD, Hedge's *g*, $P<0.05$)
2. Aβ expression (ELISA)	NO	No change in $A\beta_{40}$ or $A\beta_{42}$ levels (Hedge's *g*)