Biochemical Evidence for the Dysregulation of Alzheimer’s Amyloid Precursor Protein (APP) Expression and Metabolism in Fragile X Syndrome (FXS) and Severe Autism

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Abstract

BACKGROUND: FXS and autism are distinct neurodevelopmental disorders with pathological similarities (e.g. macrocephaly). We have demonstrated that plasma levels of secreted amyloid precursor protein-α (sAPPα), derived from o-secretase cleavage of APP, are elevated in severely autistic patients with regression. Fragile X mental retardation protein (FMRP) expression, which is inhibited in FXS, represses translation of APP transcript. Therefore, sAPPα levels should be elevated in FXS. We predict neurotrophic sAPPα is elevated in these disorders and contributes to disease etiology, including macrocephaly. Further, amyloidogenic products of APP metabolism (e.g. sAPPβ and Aβ) may be altered. Herein, we analyze plasma APP metabolite levels in a larger cohort of autism and FXS patients.

METHODS: Following IRB approval, plasma samples were collected from control (n=18), autistic (n=21), and FXS (n=18) patients. Plasma samples were depleted of human serum albumin prior to some analyses. Levels of total sAPPα, sAPPβ, Aβ40, and Aβ42 peptides were assessed by ELISA and immunoblotting. Levels of sAPPα, sAPPβ, and Aβ were also significantly elevated in FXS patients vs. controls. Levels of Aβ were also significantly elevated in severely autistic patients (not mild-to-moderate) vs. controls, however, sAPPβ and Aβ42 levels were decreased vs. controls.

CONCLUSIONS: Secreted APP metabolites are broadly increased in FXS plasma, supporting elevated holo-APP expression in response to decreased FMRP. Notably, secreted APP metabolites are altered in autism plasma in directions that suggest APP is differentially processed by o-secretase. Therefore, sAPPα appears to be peripherally dysregulated in both FXS and autism, a phenomenon, evidenced by a novel biomarker.

Materials and Methods

• Plasma samples were obtained from 18 control, 21 autism and 18 FXS subjects
• Severity of autism was determined by CARs scale. CARs scores up to 36.5 were considered mild-to-moderate and more than 37 was considered severe.
• Highly abundant brain-to-serum (B/S) ratio was removed from plasma using a spin column chromatographic purification technique (Pharmacia, CA, USA). HSA-depleted plasma was used for Western immunoblotting.
• sAPPβ, BSNP (Aβ(1-42), Aβ(1-40)) assays were carried out with sensitive and specific ELISAs, which were validated in plasma. For all ELISAs, linearity of signals was established by analyzing different volumes of raw plasma.
• sAPPβ was measured in HSA-depleted plasma by specific ELISA.

All data are presented as mean ± SEM. Further, ANOVA or ANCOVA analyses were performed. Post-hoc multiple comparisons were assessed using Tukey’s test or Sidak’s multiple comparison test and the α threshold was set to 0.05 for determining statistical significance in all cases. 

Plasma levels of sAPPβ in Autism and FXS Subjects

Plasma Levels of sAPPβ in Autism and FXS Subjects

Plasma Levels of Aβ (1-40) in Autism and FXS Subjects

Plasma Levels of Aβ (1-42) in Autism and FXS Subjects

Plasma Levels of BDNF in Autistic Individuals

Plasma Levels of BDNF in Autistic Individuals

Plasma Levels of sAPPα in Autism and FXS Subjects

Plasma Levels of sAPPα in Autism and FXS Subjects

Plasma Levels of BDNF in Autistic Individuals

Plasma Levels of BDNF in Autistic Individuals

Patients’ Demography

Introduction

• FXS and autism are distinct neurodevelopmental disorders with behavioral phenotypic similarities. Approximate incidence rate of FXS and autism in the United States are in 1 in 100 and 1 in 150 respectively.

Both disorders share some common pathological findings, including enlarged brain volume (macrocephaly)1). We previously demonstrated that levels of the alpha isoform of secreted amyloid precursor protein (sAPPα), which is derived from o-secretase cleavage of Alzheimer’s APP, were elevated in the plasma of severely autistic patients exhibiting aggressive behaviors.

Recent studies have also demonstrated that FXS was a mental retardation disorder (FXS) interacts with the APP transcript and represses translation. Elmination of FMRP expression as occurs in FXS results in elevated APP levels in synaptosomes and animal models. Therefore, levels of secreted APP metabolites are expected to also be elevated in FXS.

Given the neurotrophic properties of sAPPα, we hypothesized that elevated sAPPα in these disorders may cause autistic behavior, including stereotypies and echolalia (as seen in autism). We also measured brain derived neurotrophic factor (BDNF) levels in the same plasma samples.

We further postulate that amyloidogenic products of APP processing, such as sAPPβ and Amyloid β (Aβ) peptides, may also be altered. To further elucidate whether APP expression or metabolism is dysregulated in autism and FXS, we have characterized plasma samples from a larger cohort of autism and FXS patients.

APP Processing Pathways

APP is cleaved by two different proteolytic pathways. The α-secretase (left of the figure) cleaves APP within its Aβ domain to produce sAPPα. This is a non-amyloidogenic pathway. The β-secretase (right of the figure) cleaves APP in the presence of an amyloidogenic Aβ peptide to produce the cleaved fragment Aβ. The Aβ peptides derived from APP cleavage are β-secretase (S) secreted (sAPPα), Aβ, and Aβ(1-42) as detected in CSP and plasma by highly sensitive and specific ELISA procedures. These ELISAs are validated to detect low levels of proteins in plasma.

Determination of sAPPα was carried out in raw plasma using sensitive and specific ELISA. Obtained sAPPα levels were normalized to the total protein content of the plasma samples and expressed as “% Control.”

Conclusions

• Secreted APP metabolites are broadly increased in FXS plasma, which is in agreement with in vitro and animal model studies demonstrating elevated APP expression in response to decreased FMRP. Notably, secreted APP metabolites are altered in autism plasma in directions that suggest APP is differentially processed by o-secretase.

• Therefore, sAPPα appears to be peripherally dysregulated in both FXS and autism, and sAPPα may be a novel biomarker.

• Further, Aβ levels in the plasma (particularly Aβ 1-42) can have potential diagnostic value in FXS.

• Further studies are required to understand the consequence(s) of raised Aβ levels in FXS individuals.

References


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