The Human Amyloid Precursor Protein Adapter Protein X11α Enhances Amyloidosis in a Mouse Model of Alzheimer's Disease

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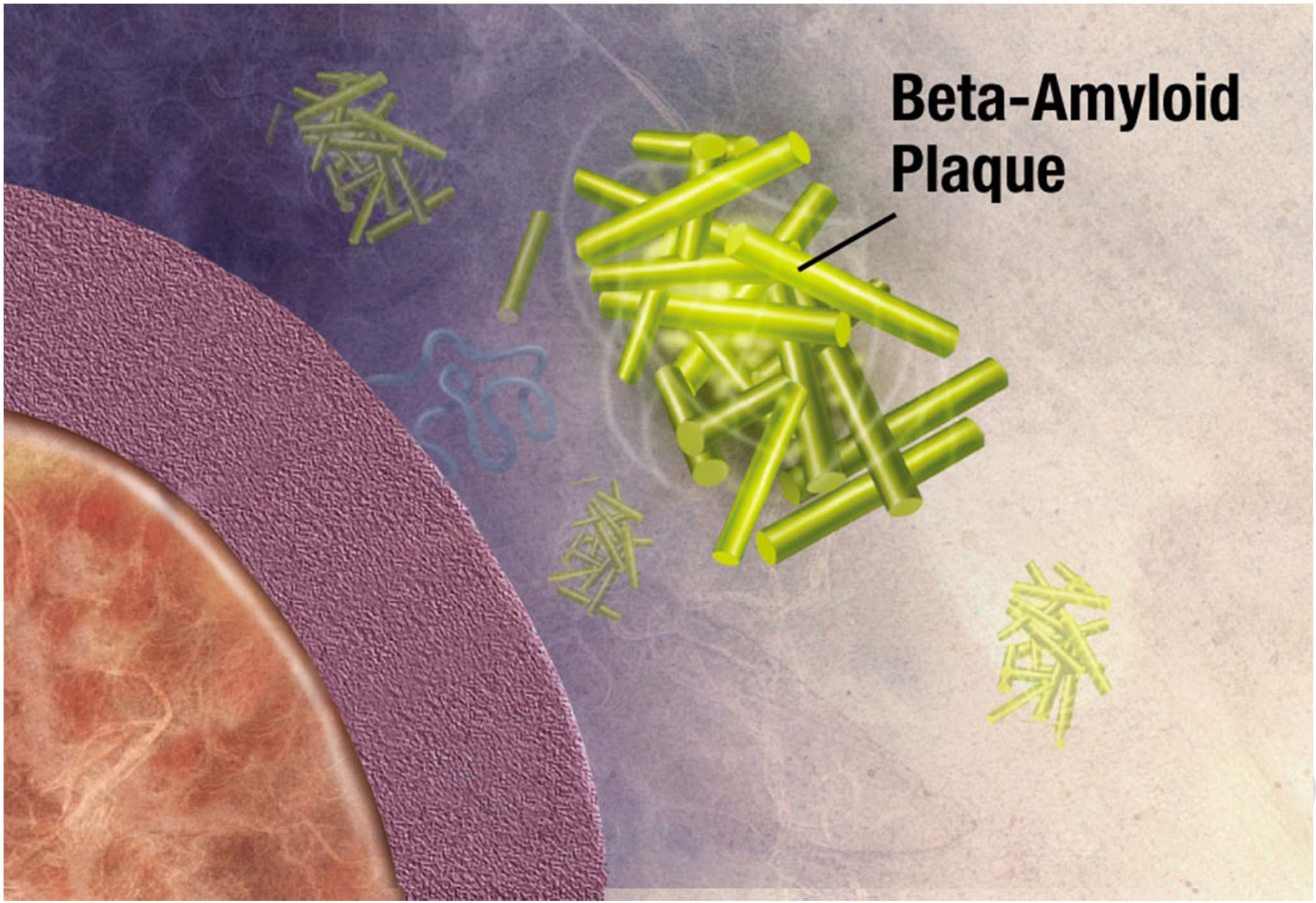
Research Overview

Alzheimer's disease (AD) is a major contributor to the global burden of disease. It affects:

- 25 million as of 2008; expected to rise to 81 million by 2040
- 1 in 8 persons over the age of 65; 1 in 2 persons over the age of 85

- X11α has been found to have the ability to bind to the Amyloid Precursor Protein (APP) which is the origin of the pathogenesis of AD
  - As such, X11α has the potential to affect the pathology of AD

Pathogenesis of Alzheimer's Disease



Graphic from wikipedia.org

X11 Family of Adaptor Proteins

(Tanahashi et al., 1999; Rogelj et al., 2006; Okamoto et al., 2004)

- All X11s binds with N-terminus of APP via PTB
- X11γ binds to APP through its PDZ2 domain
- The mechanisms by which X11s bind to APP are not yet clear
- The X11-APP reactivity has lead the scientific community to treat X11s as a potential therapeutic target for AD

X11A

- Differentiated effects were reported for X11α and X11ß; X11α can inhibit the generation of both $Aß_{40}$ and $Aß_{42}$, whereas X11ß can only inhibit $Aß_{40}$ (Rogelj et al., 2006)
- Most *in vivo* studies of X11-APP interactions conclude that expression of X11 inhibits the deposition of Aß plaques (Sano et al., 2006; Lee et al., 2003; Saluja et al., 2009)
- Two recent studies claim the opposite: the deletion of X11α protein decreases amyloid production (Ho et al., 2008; Xie et al, 2005).

RESEARCH OBJECTIVES

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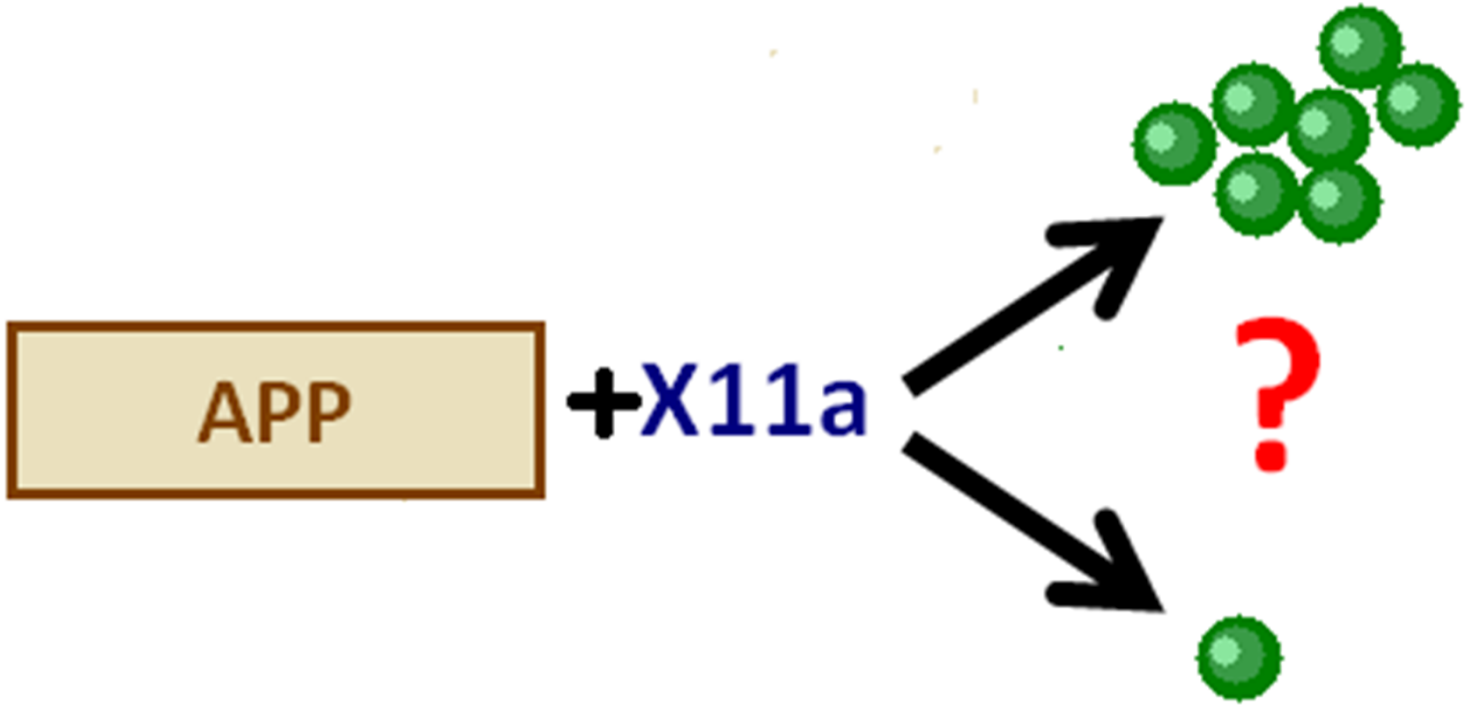

Null

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amyloid deposition between APP23 mice without X11α overexpression and APP23 mice overexpressing X11α.

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METHODS

Transgenic mice

- 31 Brains of 12 month APP23, X11α, APP23/X11α, and WT mice were harvested by post docforates in lab prior to 10/15/2008.
- Tissue was embedded in formalin and paraffin media for storage.

Transgenic Mice

Histological Staining & Immunohistochemistry

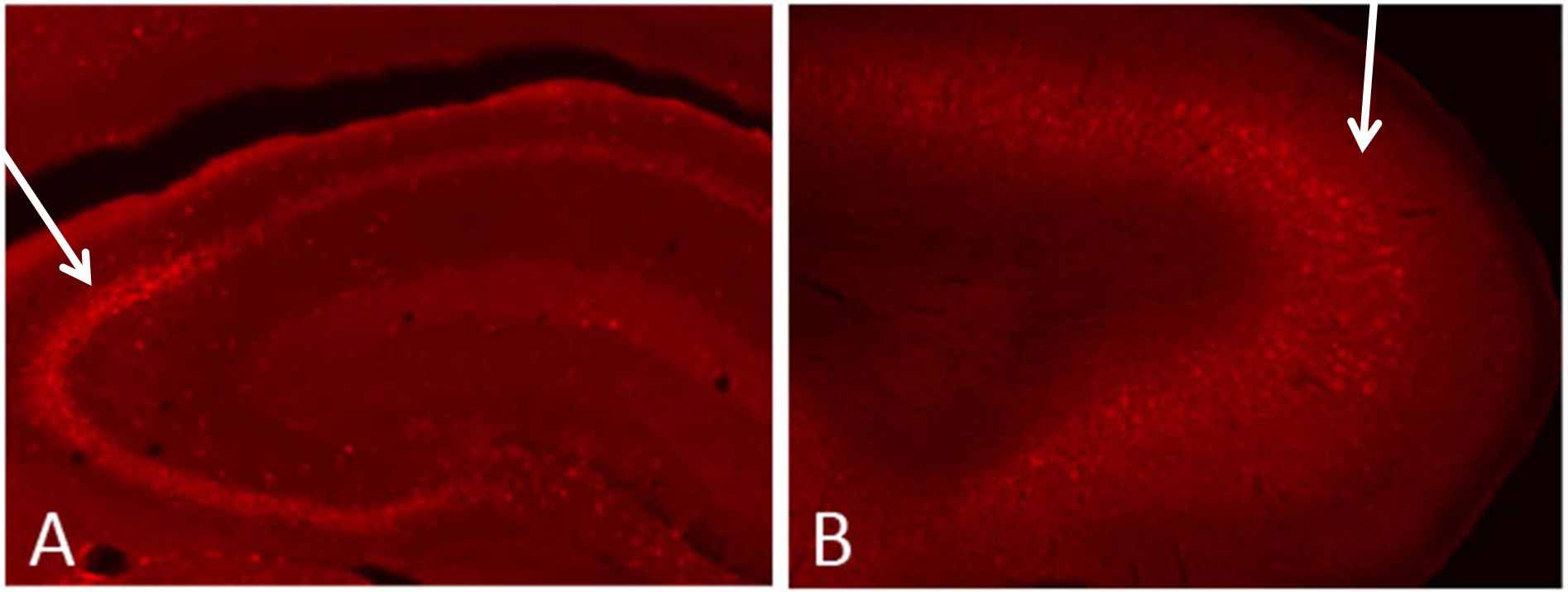
Photography & Quantification

IMMUNOHISTOCHEMISTRY: X11A, GFAP, 4G8

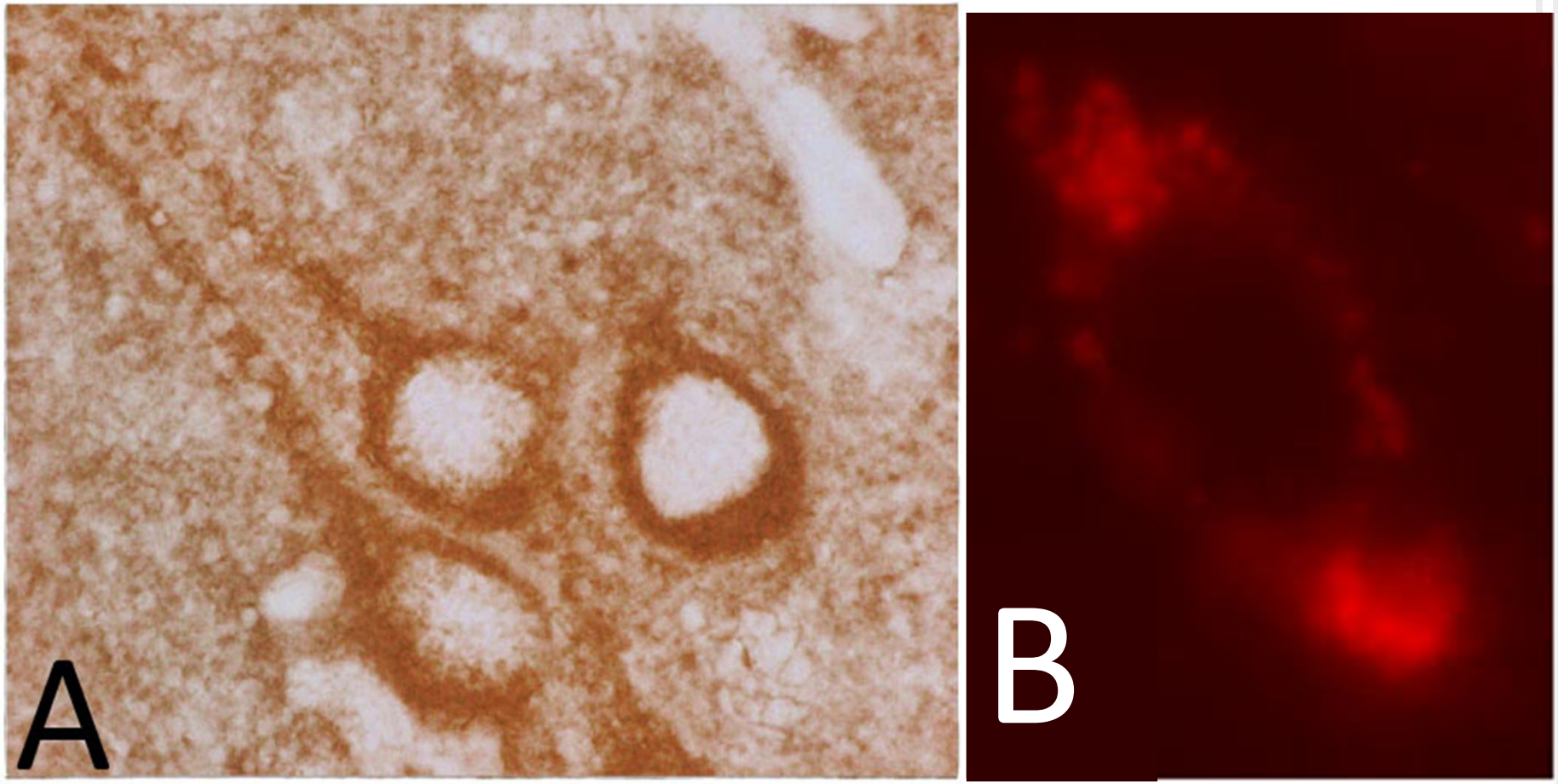
- Free floating sections were rinsed in a series of TBS followed by incubation in blocking buffer.
- Primary antibody was applied and set to incubate overnight at 4°C or for 2 hours at room temperature
- Immunoreactions were revealed by secondary antibody after 90 minute incubation
- Sections were mounted onto gelatin coated slides, preserved with Fluormount, and coverslipped for storage and observation
- Negative controls included substitution of the primary antibody with mouse serum.
- Paraffin embedded sections' experiments were identical save for the inclusion of a 5 minute incubation in DAB solution

- Sections were photographed using Carl Zeiss Axioscope 2 microscope.
- Images were captured with the Mosaic function, a compiled figure of individual 10x microscope images
- All pixels were counted with imaging software and quantified in Microsoft Excel

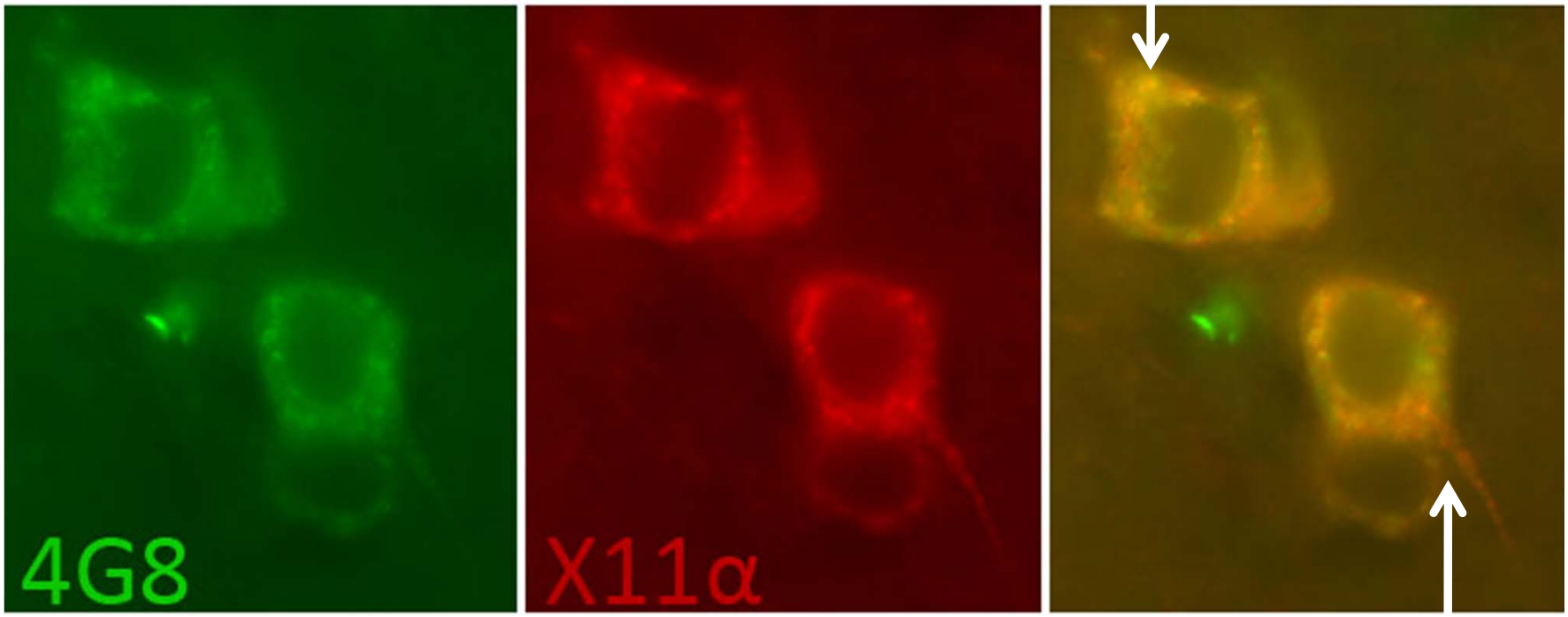
Elevated levels of X11a in CA3 region and sensory motor cortex



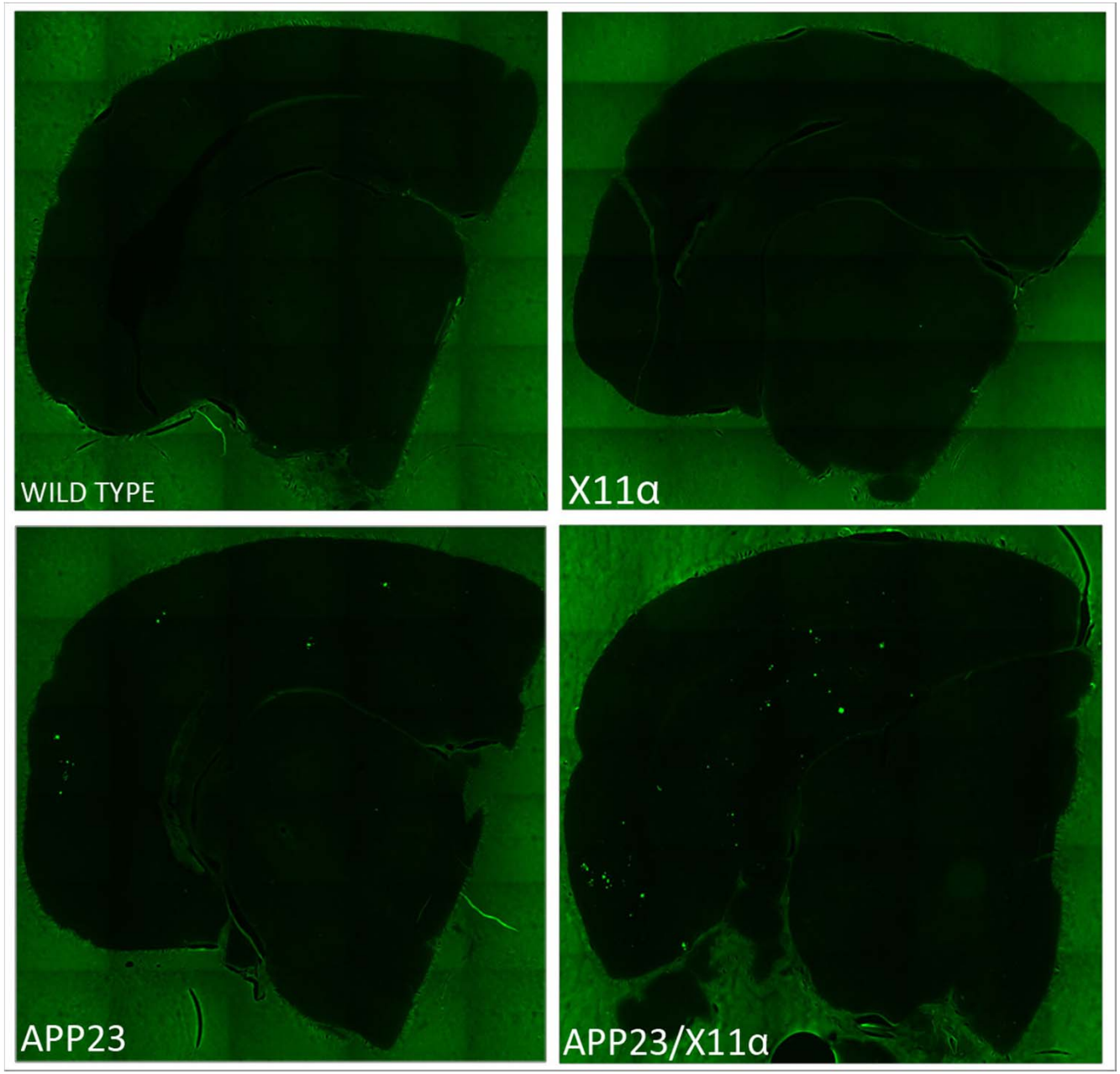
Intracellular X11a expression



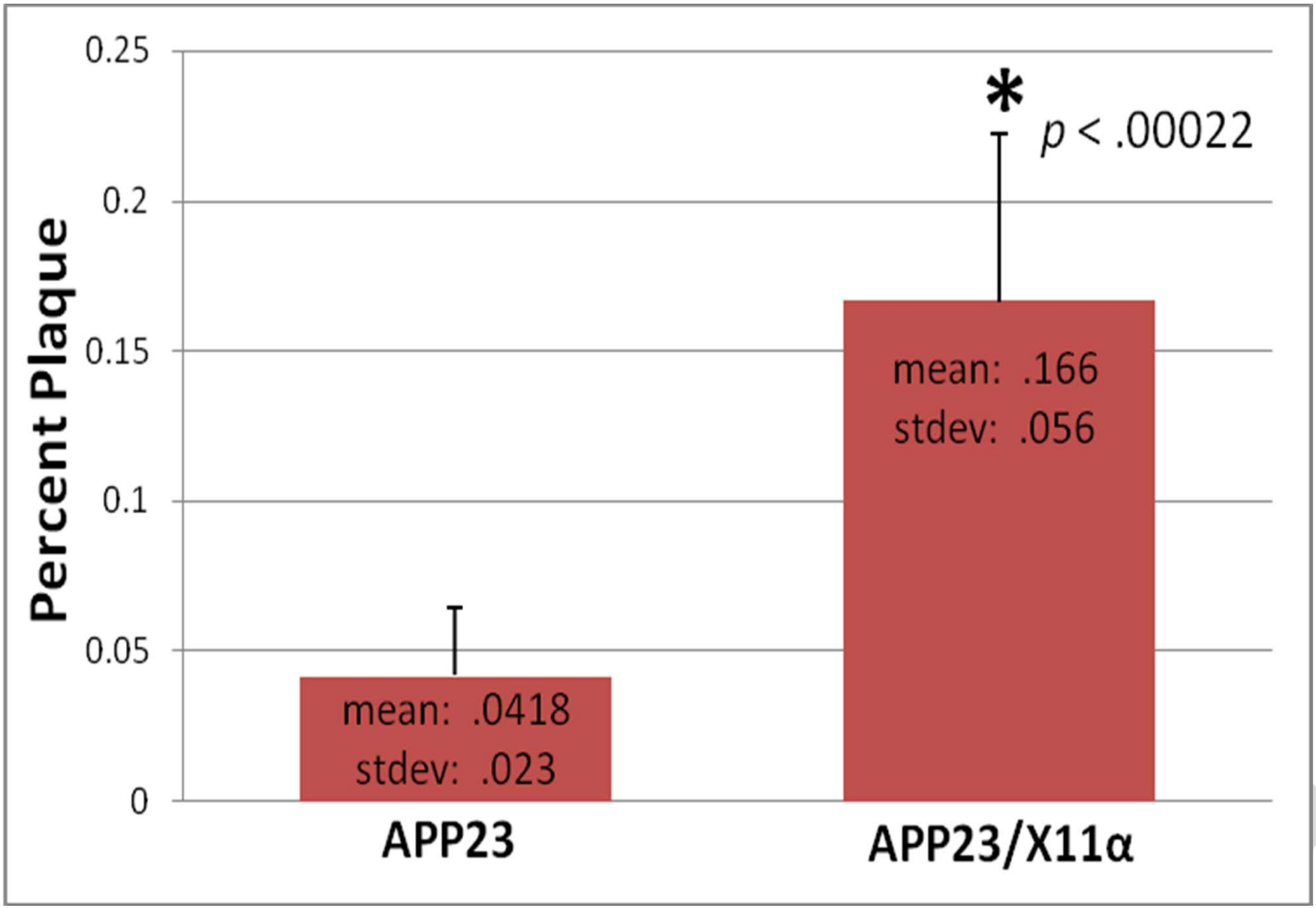
X11A COLOCALIZES WITH APP



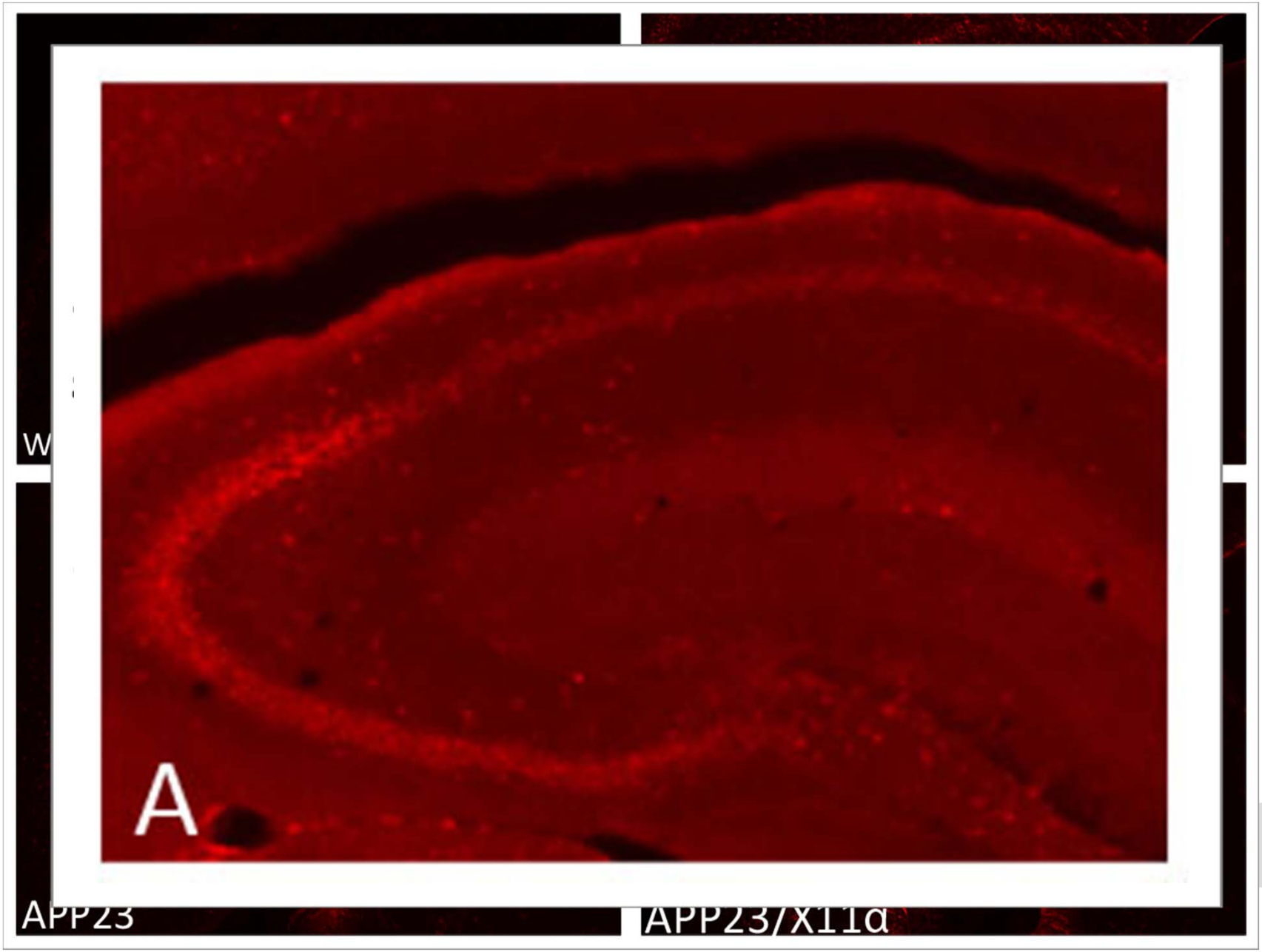
RESULTS



Statistical Analyses



GLIOSES REACTIONS



Major Findings

- X11α overexpression in APP23 mice significantly increases amyloid plaque deposition compared to the control
- X11α also increases GFAP response in X11α mice as compared to Wildtype, and in X11α/APP23 mice as compared to APP23
  - This signifies an increased stress response by brain cells to increased levels of X11α

Future Research

Because X11α overexpression yields greater amyloidosis:

- increased X11α expression may be considered a minor risk factor for the development of AD

As such, future research should:

- look into the mechanisms by which X11α modulates APP
- possible methods of inhibiting these mechanisms as a potential therapeutic strategy
- Utilize a greater variety of scientific methods, such as Western blots and ELISAs, methods not used in presented research due to constraints effected by an insufficiency of genetic material

BIBLIOGRAPHY

Alzheimer's Association. (2009) 2009 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, 5: 234-270.

Alzheimer's Disease Education and Referral (ADEAR) Center. February 17, 2009. National Institute on Aging. August 12, 2009.<http://www.nia.nih.govA/lzheimers/Publications/medicationsfs.htm>

Blennow, K., de Leon, Mony., Zetterberg, H. (2006) Alzheimer's disease. *Lancet*, 368: 387-403.

Borg, J.P., Ooi, J., Levy, E., Margolis, B. (1996) The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. *Molecular Cellular Biology*, 16(11): 6229-41.

Borg, J.P., Yang, Y., De Taddéo-Borg, M., Margolis, B., Turner, R.S. (1998) The X11alpha protein slows cellular amyloid precursor protein processing and reduces Aβ 40 and Aβ 42 secretion. *Journal of Biological Chemistry*. 273(24):14761-14766.

Ho, A., Liu, X., and Sudhof, T. C. (2008) Deletion of Mint Proteins Decreases Amyloid Production in Transgenic Mouse Models of Alzheimer's Disease. *Neurobiology of Disease*, 28(53): 14392-14400.

King, G. D., and Turner, R. S. (2004) Adaptor protein interactions: modulators of amyloid precursor protein metabolism and Alzheimer's disease risk? *Experimental Neurology*, 185: 208-219.

King, G.D., Perez, R.G., Steinhilb, M.L., Gaut, J.R., and Turner, R. S. (2003) X11α modulates secretory and endocytic trafficking and metabolism of amyloid Precursor Protein: Mutational analysis of the YENPTY sequence *Neuroscience*, 120: 143-154.

Lee, J-H, Lau, K-F, Perkinton, M.S., Standen, C. L., Shemilt, S.J.A., Mercken, L., Cooper, J.D., McLoughling, D.M., Miller, C.C.J. (2003) The Neuronal Adaptor Protein X11α Reduces Aβ Levels in the Brains of Alzheimer's APPswer Tg2576 Mice. *The Journal of Biological Chemistry*, 278(47): 47025-46029.

Miller, C. C. J., McLoughlin, D. M., Lau, K., Tennant M. E., and Rogelj, B. (2006) The X11 proteins, Aβ production and Alzheimer's disease. *TRENDS in Neurosciences*, 29(5): 280-284.

Mueller, H.T., Borg, J-P, Margolis, B., and Turner, R.S. (2000) Modulation of Amyloid Precursor Protein Metabolism by X11α/Mint-1. *Journal of Biological Chemistry*, 275(50): 39302-39306.

Nakajima, Y., Okamoto, M., Nishimura, H., Obata, K., Kitano, H., Sugita, M., and Matsuyama, T., FirstInitial. (YEAR) Neuronal expression of mint1 and mint2, novel multimodular proteins, in adult murine brain. *Molecular Brain Research*, 92(2): 27-42.

Rogelj, B., Mitchell, J. C., Miller, C. C. J., and McLoughlin, D. M. (2006) The X11/Mint Family of adaptor proteins. *Brain Research Reviews*, 52(2), 305-315.

Saito, Y., Sano, Y., Vassar, R., Gandy, S., Nakaya, T., Yamamoto, T., and Suzuki, T. (2008) X11 Proteins Regulate the Translocation oof Amyloid β-Protein Precursor (APP) into Detergent-resistant Membrane and Suppress the Amyloidogenic Cleavage of APP by β-Site-cleaving Enzyme in Brain. *The Journal of Biological Chemistry*, 283(51): 35763-35771.

Saluja, I., Paulson, H., Gupta, A., Turner, R. S. (2009) X11α haploinsufficiency enhances Aβ deposition in Alzheimer's disease transgenic mice. *Neurobiology of Disease*, 36(1): 162-168

Sano, Y., Syuzo-Takabatake, A., Nakaya, T., Saito, Y., Tomita, S., Itohara, S., and Suzuki, T. (2006) Enhanced Amyloidogenic Metabolism of the Amyloid β-protein Precursor in the X11L-deficient Mouse Brain. *The Journal of Biological Chemistry*, 281(49): 37853-37860.

Sastre, M., Turner, R. S., and Levy, E. (1998) X11 Interaction with β-Amyloid Precursor Protein Modulates its Cellular Stabilization and Reduces Amyloid β-Protein Secretion. *The Journal of Biological Chemistry*, 273(35): 22351-22357.

Sumioka, A., Saito, Y., Sakuma, M., Araki, Y., Yamamoto, T., Suzuki, T. (2008) The X11L/X11β/MINT2 and X11L2/X11γ/MINT3 scaffold proteins shuttle between the nucleus and cytoplasm. *Experimental Cell Research*, 314(5): 1155-1162.

Xie, Z., Romano, D.M., and Tanzi, R.E. (2005) RNA Interference-mediated Silencing of X11α and X11β Attenuates Amyloid β-Protein Levels via Differential Effects on β-Amyloid Precursor Protein Processing. *Journal of Biological Chemistry*, 280(15): 15413-15421.

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Questions?