## P3-171 MICRORNA RELATED COFILIN ABNORMALITY AND THE PATHOLOGICAL CONSEQUENCES IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

**Jiaqi Yao**[1], Flint Beal[1], Michael Lin[1], [1]*Weill Cornell Medical School, New York, New York, United States.*

**Background:** Rod-like structures composed of actin and the actin-binding protein cofilin are found in Alzheimer's disease (AD) patients. Recent studies in cells demonstrate that abnormal actin-cofilin rods form in response to various stressors. However, the mechanisms of rod formation are largely unknown. We hypothesize that microRNA regulated cofilin expression plays an important role in cofilin rod formation in AD. **Methods:** We used a wide range of biochemical, molecular and cellular methods to test our hypothesis. **Results:** We have shown that cofilin expression levels are increased in a transgenic mouse model of AD (Tg19959), and that rod-like inclusions, including cofilin and actin, develop in brain tissue and in neurons from these mice. Although cofilin protein levels are increased, cofilin mRNA levels are not, suggesting that the increase in cofilin protein levels maybe due to post-transcriptional regulation. We hypothesize that microRNAs regulations involved. Based on bioinformatic analysis, miR-103/107 are predicted to interact with the 3'UTR of cofilin mRNA. To confirm a functional interaction between miR-103/107 and the predicted target region in the cofilin 3'UTR, we construct a miR luciferase reporter. Our luciferase assay results suggest that miR-103/107 bind the putative target sequence in the cofilin 3'UTR and functionally repress translation of the upstream gene. Conversely, when endogenous miR-103 levels are reduced by anti-sense oligos, cofilin protein levels increased, as seen by Western blotting. Using quantitative RT-PCR, we show that decreased miR-103 and miR-107 in Tg19959 mouse brain compared to wild-type littermates. We also observe over expression of activated cofilin protein is sufficient to induce rod formation in primary neurons. In addition, our image data have shown shortened neurites and abnormal mitochondria in neurons containing cofilin rods. **Conclusions:** Taken together, our data indicates the deficiency of miR-103 and 107 increase cofilin protein level, resulting in formation of rod structure in a transgenic mouse model of AD. The rod formation has pathological consequences including neurite development, mitochondrial morphology and dynamics. These are the first observations that miRNAs involved protein regulation may contribute to formation of abnormal cytoskeletal aggregates in AD pathogenesis. Furthermore, these studies will give us fresh insights into AD pathogenesis and provide new therapeutic targets.

## P3-172 DEVELOPMENT OF A SINGLE ANTISENSE TO APP AND PS-1

**John Morley**[1], [1]*Saint Louis University Medical Center, St. Louis, Missouri, United States.*

**Background:** Previously we have shown that an antisense to amyloid precursor protein (APP) will reverse many of the defects seen in animal models of Alzheimer's disease (AD). However, clinical studies have suggested that an approach to a single etiological factor in AD is not particularly successful. For this reason we developed a novel approach to see if a single antisense can regulate more than one mRNA. **Methods:** SAMP8 mice have a natural mutation which results in age-related impairment in learning and memory, elevated Aß, oxidative tissue damage and diminished egress of Aß from the brain. These deficits are reversed by administration of an antisense to APP. As proof of the concept, we examined the effect of a cassette phosphorothionated olignucleotide to APP and PS-1 (Presenilin-1) (AO 10/10) in COS 7 cells. The COS-7 cells were transfected with cDNAs to APP and Presinilin (PS-1). The cells were treated with 0.5Âµg of olignucleotides complimentary to APP or Presenilin cRNA's or with hybrid antisense with 10 nucleotides, each complimentary, to APP and PS-1. Synthesis of APP was monitored with Western blotting at 48 hours. PS-1 expression was monitored by immune-blotting. In in-vivo experiments, SAMP8 mice were injected intracerebroventricularly with either vehicle, random antisense or the phosphorothionated AO 10/10 weekly for 3 weeks. Acquisition was tested 5 days after the third injection in an aversive T-maze. Retention was tested one week later. **Results:** The hybrid antisense with 10 nucleotides each complimentary to APP and PS-1 reduced the expression of both APP and PS-1 in the cell cultures. In the SAMP8 mice the AO 10/10 antisense took fewer trials to criterion ($8.85\pm0.75$) compared to the 12 month old SAMP8's which received random antisense ($13.70\pm1.35$; $p < 0.05$). On the one-week retention test, mice which received the AO 10/10 antisense took fewer trials to reach criterion of 5 avoidances in 6 consecutive trials ($7.90\pm0.67$) compared to mice which received the random antisense ($11.00\pm1.35$). **Conclusions:** We demonstrated for the first time that two mRNA's can be regulated by an antisense carrying complimentary olignucleolides to both. This has high potential for the treatment of Alzheimer's disease.

## P3-173 AMYLOID PRECURSOR PROTEIN REGULATES SREBP-MEDIATED NEURONAL LIPID HOMEOSTASIS IN MICE AND HUMANS

**Nathalie Pierrot**[1], [1]*Université catholique de Louvain, Brussels, Belgium.*

**Background:** Synthesis of cholesterol is controlled by sterol regulatory element binding proteins (SREPB). Following its translocation from the endoplasmic reticulum to the Golgi apparatus, cleavage of SREBP releases the mature SREBP transcription factor, which regulates the expression of genes controlling cholesterol and fatty acid biosynthesis. Despite extensive evidences that Amyloid Precursor Protein (APP) has critical implications in Alzheimer's disease, the neuronal function of APP remains unclear. **Methods:** Human APP (hAPP) was expressed in rat cultured of cortical neurons and astrocytes using recombinant adenoviruses. Neo-synthesized cholesterol was quantified after $^{14}C$ radio labelling. The transcription of *HMG-CoA reductase, SREBF1* and *SREBF2* genes were monitored by real-time PCR. The expression of SREBP in cellular and transgenic mouse models was analyzed by western blotting. Subcellular localisation of SREBP and hAPP was analyzed by immunocytochemistry and their interaction by co-immunoprecipitation assays. **Results:** Expression of hAPP decreased HMG-CoA reductase mRNA levels and neuronal cholesterol synthesis in rat cortical neurons but not in astrocytes. The interaction between hAPP and SREBP in the Golgi complex prevented the release of mature SREBP, leading to the inhibition of the transcription of SREBP target genes. Consequently, SREBP was decreased in hAPP expressing neurons. SREBP was also decreased in brain of hAPP transgenic mice, and increased in brain of APP knockout animals. In human brain, a reverse correlation was observed between SREBP and APP expression. **Conclusions:** These results argue for a role of APP in the control of neuronal lipid homeostasis both in vitro and in vivo.

## P3-174 ROLES OF SNX FAMILY MEMBERS IN NEURODEGENERATIVE DISEASES

**Yun-wu Zhang**[1], [1]*Xiamen University, Xiamen, China.*

**Background:** One major pathological hallmark of Alzheimer's disease (AD) is formation of senile plaques, whose major components are small peptides called beta-amyloid (Abeta) that are derived from beta-amyloid precursor protein (APP) through sequential cleavages by beta-secretase (BACE1) and gamma-secretase complex. APP, BACE1 and gamma-secretase complex components are transported through the secretory pathway; and Abeta can be generated at various sub cellular compartments depending on the interaction between APP and BACE1/gamma-secretase. Therefore, dysregulated trafficking of these proteins will affect Abeta generation and contribute to disease pathogenesis. Sorting nexin (SNX) family proteins are a diverse group of cellular trafficking factors that are unified by the presence of a phospholipid-binding motif - the PX domain. The ability of these proteins to bind specific phospholipids, as well as their propensity to form protein-protein complexes, points to a role for these proteins in membrane trafficking and protein sorting. However, information on regulation of AD proteins by SNX members is limited. **Methods:** We modulated the levels of different SNX family members in cell cultures and studied their effects on intracellular trafficking of APP, BACE1, and gamma-secretase components.