**Lauren Laboissonniere**  
Iowa State University, Graduate Student

**Title**  
USING CRISPR-CAS TO ENGINEER ALS RELATED MUTATIONS IN ZEBRAFISH

**Abstract**  
Genome editing is a useful tool for the characterization of gene function as well as the creation of disease-related mutations. Through the use of clustered regularly interspaced short palindromic repeats (CRISPR), we have successfully induced mutations in zebrafish for the study of four genes known to cause Amyotrophic Lateral Sclerosis (ALS) in humans. The use of genome editing to affect the production of protein allows for the observation of phenotypes related to gene loss. We will examine the development of motor neurons in these zebrafish by injecting the guide RNA into Mnx1:GFP zebrafish which will allow visualization of these cells throughout the lifetime of our fish. Swimming assays in collaboration with Dr. Johanna Kowalko will also allow for the behavioral phenotype to be observed. In the future we plan to use homologous recombination in accordance with CRISPR technology to introduce specific mutations known to cause ALS and observe the cellular defects of these fish.

**Dr. Li Lian Yuan**  
Des Moines University, Faculty

**Title**  
Antidepressant actions of glutamatergic agents

**Abstract**  
Accumulating evidence suggests dysregulation of glutamatergic transmission in the brain is linked with depressive disorders. Ketamine, an antagonist of a subtype of glutamate receptors, exhibits fast-acting and long-lasting antidepressant effects in humans and in animal models. However, limitations of ketamine use as a long-term treatment, particularly its dissociative/psychotomimetic effects and abuse potential, highlight the need for alternative glutamatergic agents. D-serine, an endogenous NMDA receptor co-agonist, also targets glutamate transmission. Their opposing actions on the shared target (NMDAR) and the highly overlapped molecular signature evoked by ketamine and D-serine raises the possibility that their co-administration may diminishes adverse side effects of ketamine without compromising its antidepressant effectiveness. Immediately following intraperitoneal injection (< 1 min), ketamine (10 mg/kg) altered states of consciousness and induces motor incoordination in mice. Co-administration of D-serine significantly reduced the level of motor incoordination as quantified by the number of falls induced by ketamine. However, the antidepressant effects of ketamine did not appear to be compromised by D-serine as assessed in the forced swim test. In parallel with its behavioral influences, D-serine addition did not interfere with ketamine-activated signaling pathways; it actually enhanced ketamine’s effects on key synaptic proteins. These results support the notion that
ketamine and D-serine may represent a more effective combination therapy than either one alone.

| Name (University & Position) | Matthew Neal  
Iowa State University, Graduate Student |
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<tr>
<td>Title</td>
<td>Prokineticin-2 gene therapy shows anti-inflammatory and anti-apoptotic effects in preclinical cell culture and animal models of Parkinson's disease</td>
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<td>Abstract</td>
<td>Prokineticin-2 (PK2) is a small secretory peptide with diverse biological functions. We recently identified that dopaminergic neurons upregulate PK2 in response to neurotoxic stress. To better understand the functional role of PK2 upregulation, we created stable PK2-overexpressing dopaminergic cells by transfecting the PK2 gene into mouse dopaminergic MN9D cells. PK2-overexpressing cells exposed to the Parkinsonian neurotoxicant MPP+ showed significant protection against neurotoxicity relative to vector control cells. The neuroprotection mediated by PK2 against MPP+-induced toxicity was associated with increased Bcl2 gene expression and reduced apoptotic cell death. We then adopted adeno-associated viral (AAV2) vector technology to develop a PK2-GFP gene construct delivery system, and we tested the efficacy of this new viral vector in mouse models. PK2 AAV gene delivery in the striatum significantly reduced basal gene expression of inflammatory cytokines, indicating an anti-inflammatory function for PK2 in the striatum. In contrast, PK2 AAV expression significantly upregulated Bcl2 and Pink1, suggesting that PK2 can upregulate cell survival signaling pathways. Initial results show that PK2 AAV expression attenuates MPTP-induced behavioral deficits and TH depletion in the C57 black mouse model. Taken together, these results demonstrate for the first time, that PK2 overexpression can protect dopaminergic neurons in both cell culture and animal models by attenuating the glial cell-mediated inflammatory response as well as by upregulating Bcl2 and Pink-1 protective signaling. Further characterization of the efficacy of PK2 gene therapy in preclinical models of Parkinson's disease may provide a viable neuroprotective strategy for the chronic neurodegenerative disease.</td>
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| Name (University & Position) | Dr. Auriel Willette  
Iowa State University, Faculty |
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<tr>
<td>Title</td>
<td>MRI, biomarkers of metabolism and inflammation, and cognition</td>
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<td>Abstract</td>
<td>Introduction: By 2050, Alzheimer’s disease (AD) is projected to affect 13.8 million Americans and cost 1.2 trillion dollars per year. Peripheral insulin resistance (IR), the progressive inability of insulin to act on its receptor, predicts progressive brain atrophy, less glucose metabolism, and neuropathology in AD patients, as well as late middle-aged,</td>
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cognitively normal (CN) participants at risk for AD. To date, however, there is no reliable biomarker of brain IR. Autotaxin, an adipose-derived enzyme, is highly correlated with IR, increases with obesity, and is higher in the frontal lobe of AD patients. The goal was to see if CSF autotaxin showed IR-like associations with AD outcomes. Methods: Mass spectrometry was used to assay CSF autotaxin in 86 CN, 135 Mild Cognitive Impairment (MCI), and 66 AD participants. Frontal glucose metabolism and cortical thickness (CT) were assessed, as well as CSF amyloid and tau, which are AD hallmarks. Executive function and Odd Ratios (OR) for being MCI or AD relative to CN were also gauged.

Results: Autotaxin levels increased in a stepwise manner from CN to AD. Higher autotaxin conferred a mean OR of 3.5 (95% CI=1.1-11.4) and 4.9 (95% CI=1.1-21.4) for having MCI or AD respectively. Among AD participants, higher autotaxin predicted less frontal glucose metabolism (R-Squared=0.294), less CT in frontal regions like medial orbitofrontal cortex (R-Squared=0.272), a greater amyloid:tau ratio (R-Squared=0.018), and worse executive function (R-Squared=0.048).

Conclusions: Autotaxin predicts AD outcomes similar in direction and effect size to peripheral IR. Autotaxin may be a useful brain biomarker of IR.

**Name**
Naveen Kondru
Iowa State University, Graduate Student

**Title**
Development and Characterization of an Ex-vivo Brain slice Culture Model of Chronic Wasting Disease

**Abstract**
Prion diseases have long incubation times in vivo, therefore, modeling the diseases ex-vivo will advance the development of rationale-based therapeutic strategies. An organotypic slice culture assay (POSCA) was recently developed for scrapie prions by inoculating mouse cerebellar brain slices with prions. However, efforts to develop a POSCA model for chronic wasting disease (CWD) have failed due to the species barrier between mice and cervids. To overcome this difficulty, we adopted a transgenic cervidized (Tg12) mouse model and successfully developed an ex-vivo brain slice culture model for CWD. We incubated 350-µm cerebellar slices from Tg12 mouse pups with CWD brain homogenate and washed them thoroughly. With the genotypes of the pups blinded, the cultures were grown over 42-48 days before being tested for CWD infectivity using the recently developed RT-QuIC assay. Slices from Tg12 cervidized pups with PrP+/− genotype tested positive but slices from the Tg12 PrP−/− genotype did not. Infectivity was present in 11 out of 11 Tg12 PrP+−, whereas 0 out of 10 Tg12 PrP−/−. We have successfully cultured POSCA brain slices infected with RML scrapie as confirmed by RT-QuIC and PK digestion assays. Biochemical studies revealed degenerative changes and oxidative damage in the prion infected slice cultures. Our results demonstrate that combining the brain slice culture
model of prion diseases with the RT-QuIC assay offers a promising platform for studying the mechanisms of prion proteinopathies as well as for screening anti-prion therapeutics. (ISU Presidential Wildlife initiative, ISU-CVM Diagnostic lab and NIH ES19267 and NS074443).

| Name (University & Position) | Staci Solin  
Iowa State University, Graduate Student |
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<td>Title</td>
<td>Rapid tumor induction in zebrafish by TALEN-mediated somatic inactivation of the retinoblastoma1 tumor suppressor rb1</td>
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<td>Abstract</td>
<td>Staci L. Solin, Heather R. Shive, Kevin D. Woolard, Jeffrey J. Essner, Maura McGrail Investigating the in vivo role of tumor suppressor genes in cancer is technically challenging due to their essential requirement during early animal development. To address this bottleneck, we generated genetic mosaic adult zebrafish using TALEN genome editing and demonstrate somatic inactivation of the tumor suppressor retinoblastoma1 (rb1) induces tumorigenesis at high frequency. 11–33% of 1-cell stage embryos injected with TALEN mRNAs targeting rb1 exon 2 or 3 develop tumors beginning as early as 3.5 months of age. Lesions predominantly arise in the brain and show features of neuroectodermal-like and glial-like tumors. Regions of high mitotic index and variable expression of brain tumor marker s100 were detected in most tumors. Mutant allele analysis is consistent with tumor initiation due to somatic inactivation of rb1, revealing a conserved role for rb1 in tumor suppression across vertebrates. In contrast to genetic mosaics, heterozygous rb1−/+ adults show no evidence of neoplasia, while homozygous mutant rb1−/− are larval lethal. This is the first demonstration that somatic inactivation of a tumor suppressor causes cancer in zebrafish, and highlights the utility of site-specific nucleases to create genetic mosaic zebrafish for tumor suppressor gene discovery. Current studies involve the characterization of the molecular signature of the zebrafish tumors using RNA-seq.</td>
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