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Identification of candidate sudden arrhythmic death - causing variants in a spontaneous animal model

Sian Durward-Akhurst, Joy Stock, Freya Stein, Christopher Stauthammer and Samuel Dudley
University of Minnesota

OBJECTIVES/GOALS: 1. Identify candidate AN-SAD-causing variants. 2. Estimate the variant effect size of and genotypic relative risk for arrhythmias and AN-SAD. METHODS/STUDY POPULATION: We performed whole genome sequencing (WGS) on 59 Thoroughbred AN-SAD cases and 58 controls. WGS was mapped and variants identified using a modified version of the Genome Analysis Toolkit best practices. Variants will be selected based on case-control analysis using SnpSift and presence in candidate genes. The top 400 candidate AN-SAD-causing variants will be selected based on being common in cases and rare or absent in controls, and uncommon (allele frequency less than 10%) in a catalog of genetic variation for the horse. The 400 variants will be genotyped in our cohort of 1,200 racehorses to determine variant effect size and genotypic relative risk. RESULTS/ANTICIPATED RESULTS: 17,182,003 variants were identified. 230 variants had significantly different allele frequencies (AF) between cases and controls (SnpSift). 723 high and 4,824 moderate impact variants were identified in 1,072 candidate genes. 3,681 variants were present at an AF 10% in the equine variant catalog. Variant effect prediction is ongoing to select the final 400 variants. Cardiac phenotyping (cardiac auscultation and ECG before, during, and after exercise) was performed on 790 racehorses and we will have 1,200 racehorses with cardiac phenotypes and/or AN-SAD. We will genotype the top 400 candidate AN-SAD-causing variants in these 1,200 horses to identify variants of large effect size. DISCUSSION/SIGNIFICANCE: Identification of candidate AN-SAD variants in a spontaneous animal model can facilitate interpretation of candidate variants in humans and horses. This project will provide further support for the racehorse AN-SAD model and will support future work exploring the genetic and environmental risk factors contributing to AN-SAD in this animal model.

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Ketamine Promotes Sustained Brain-Derived Neurotrophic Factor Levels in the Corticoaccumbens Circuit

Holly L. Chapman and Noelle C. Anastasio
The University of Texas Medical Branch at Galveston

OBJECTIVES/GOALS: Neuropsychiatric disorders classified as synaptopathies are marked by a glutamate-associated hypofrontality which impacts decision making and impulsivity. We hypothesized that behavioral efficacy of the psychoplastogen ketamine is mediated in part through lasting promotion of markers of synaptic strength in corticoaccumbens circuit. METHODS/STUDY POPULATION: Male, Sprague-Dawley rats received an intraperitoneal (i.p.) injection of saline, single ketamine (10 mg/kg; 1x/day), or repeated ketamine (10 mg/kg; 1x/day for three days). Twenty-four hrs following the dosing regimen, animals were euthanized, and brains dissected to harvest corticoaccumbens structures including the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc). mRNA was extracted and converted to cDNA. Levels of brain derived neurotrophic factor (BDNF) exon II mRNA were quantified using reverse transcriptase polymerase chain reaction (RT-PCR); cyclophilin A (PPIA) was used as a loading control. Gene expression differences in ketamine-treated rats were identified versus saline-treated rats. BDNF protein levels were quantified using capillary-electrophoresis immunoblotting. RESULTS/ANTICIPATED RESULTS: Repeated, but not single, ketamine administration decreased mPFC, but increased NAc, BDNF exon II mRNA levels versus saline ($p<0.05$). Single and repeated ketamine administration increased NAC BDNF protein ($p<0.05$), while both dosing paradigms induced a trend towards an increase in mPFC BDNF levels. DISCUSSION/SIGNIFICANCE: We discovered a dosing regimen-dependent and sustained effects of ketamine administration on BDNF levels in the rodent brain. Taken together, ketamine-mediated BDNF levels may sustain synaptic strengthening mechanisms supporting future investigation into the utility of ketamine for diseases characterized by synaptopathies.

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The Role of bHLHe40 in Systemic Sclerosis-associated Pulmonary Fibrosis

Adegboyega "Tim" Adewale and Carol Feghali-Bostwick
Medical University of South Carolina

OBJECTIVES/GOALS: The dominant complication of Systemic Sclerosis (SSc) is clinically severe and commonly fatal pulmonary fibrosis (PF). We sought to determine the downstream regulatory role of the basic Helix-Loop-Helix protein 40 (bHLHe40), in response to Insulin-like Growth Factor II (IGF-II) on Pro-Lysyl Oxidase cleavage products. METHODS/STUDY POPULATION: We examined the response of primary pulmonary fibroblasts cultured from the lungs of control donors and SSc lung explants to IGF-II as well as human recombinant Lysyl Oxidase Propeptide (LOX-PP). In addition, we utilized an experimentally-induced model of lung fibrosis with intratracheal bleomycin administration. We used qPCR and immunoblotting to quantify mRNA and protein levels, respectively. We used sequence-specific small-interfering RNA to silence targeted genes. Immunoblots were quantified in ImageJ (NIH) and statistical analyses were performed in GraphPad Prism. RESULTS/ANTICIPATED RESULTS: IGF-II regulates levels of Pro-LOX, active LOX, and LOX-PP, as well as isoforms of proteases Bone Morphogenetic Protein 1 (BMP1) and Tolloid-like 1 (TLL1). The transcription factorbHLHe40 localizes to the nucleus in response to IGF-II. bHLHe40 silencing downregulated TLL1, abrogating the enzymatic cleavage of Pro-LOX. SSc lungs have higher baseline levels of the total (N-glycosylated/unglycosylated) LOX-PP than normal lung tissues, and baseline levels of LOX-PP correlated with TLL1 Isoform 2 in SSc lungs. LOX-PP contributes to the development and progression of SSc-PF by mediating changes consistent with the extracellular matrix deregulation implicated in SSc-PF: elevated levels of Collagen 3A1 (COL3A1), Fibronectin-1 (FN1), and Plasminogen Activator Inhibitor-1 (PAI1). DISCUSSION/SIGNIFICANCE: Our findings indicate that bHLHe40, TLL1, and LOX-PP may serve as targets of therapeutic intervention to stop the progression of SSc-PF. Since activation of common fibrotic pathways are involved in different diseases characterized by lung fibrosis such as IPF, our findings may have wider implications for lung fibrosis associated with other diseases.

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