



2023 PWS Research Symposium Abstract Booklet

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Table of Contents

Table of Contents	2
October 5 th , 2023	5
SPEAKER ABSTRACTS MORNING SESSION I	5
Characterizing cognitive function in <i>Magel2</i> null mice	6
Investigation into the genetic factors of phenotypic variability in Prader-Willi syndrome.....	7
<i>Magel2</i> Role in the Stress Response to Fasting: From Hypothalamic Single-cell Dynamics to Hormonal Balance Throughout the Body	8
SPEAKER ABSTRACTS MORNING SESSION II	9
Investigation of Oxytocin Neurons and Oxytocinergic Projections in a Rat Model of Schaaf-Yang syndrome	10
Dissecting the molecular and electrophysiological phenotypes in human cortical organoids derived from Prader-Willi Syndrome patients	12
Investigating the role of glial cells in the pathophysiology of Prader-Willi syndrome	14
AAV-BDNF gene therapy ameliorates a hypothalamic neuroinflammatory signature in the <i>Magel2</i> - null mouse model of Prader-Willi syndrome	15
SPEAKER ABSTRACTS AFTERNOON SESSION I	16
Cellular and Molecular Basis for Obesity in PWS	17
Role of DMH MC3R in defending against weight gain	18
Pharmacological inhibition of cholesterol esterification alleviates adiposity and food intake in the <i>Magel2</i> -null mouse model of Prader-Willi Syndrome	19
Rationale for and Results from a Randomized Withdrawal Period Following Long-Term Administration of Diazoxide Choline Extended-Release Tablets to People with Prader-Willi Syndrome	20
Heightened response to the anorexigenic peptide CART in a rat model of Prader-Willi Syndrome..	21
SPEAKER ABSTRACTS AFTERNOON SESSION II	22
The role of non-canonical SMC protein SMCHD1 in regulating the Prader Willi cluster.....	23
Phenotypic Characterization of a Novel Mouse Model of Prader-Willi Syndrome with Genetic Invalidation of both <i>Magel2</i> and <i>Necdin</i>	24
Cardiac phenotypes of the <i>Magel2</i> KO mouse, an animal model of Schaaf-Yang syndrome and PWS	25
Determining beloranib's mechanism of action to inform novel drug targets for Prader-Willi syndrome	26
Inhibition of FKBP51 signaling partially reversed impaired behavioral and metabolic phenotypes displayed in <i>Magel2</i> -null mice	27
POSTER SESSION	28

Role of mRNA Translational Regulation in Hormone Secretion Deficit in MAGE-L2 KO Mouse Model	29
Molecular Subtyping of Prader-Willi Syndrome by Long-Read Genome Sequencing with Simultaneous Methylation Detection	30
Identifying Key Proteins in Prader-Willi Syndrome through Graph Learning on a Protein-Protein Interaction Network	31
Swallowing function, chokings and aspirations in infants with Prader-Willi Syndrome: data from the Global Prader-Willi Syndrome Registry	33
Glucose Homeostasis in Patients with PWS	34
Exploring the Role of Social Cognition in Autism Behaviours within Prader-Willi Syndrome	35
Development of a Conjugated Linoleic Acid Weight Loss Food Product for PWS patients.....	36
Investigating the Epigenetic Regulator Smchd1 as a Potential Therapeutic Target for the Treatment of PWS	37
Needs Assessment Survey to Study the Knowledge of Parents of Children with Prader-Willi Syndrome in Tertiary Care Centre in Southern India	38
Metabolic and Bariatric Surgery for Obesity in Prader-Willi Syndrome: Systematic Review and Meta-Analysis.....	40
Comprehensive Cardiovascular Risk Assessment in Children and Adolescents with Prader-Willi Syndrome Compared to Lean Controls	41
Magel2 Deficient Mice Exhibit a Diminished Hedonic Drive During Adolescence	42
Reactivation of <i>SNRPN</i> Gene via Reduction of Repressive H3K9me2 Histone Marker and Demethylation in Prader-Willi Syndrome Using <i>In Vitro</i> Model	43
The Role of <i>NPAP1</i> Gene Variants in Prader-Willi Syndrome Pathophysiology	44
Characteristics of Baseline Polysomnograms and Resulting Interventions in Patients with Prader-Willi Syndrome Prior to Initiation of Growth Hormone Therapy	45
Comprehensive Assessment of Body Composition, Physical Activity, and Health Markers in Pediatric Prader-Willi Syndrome	47
Secondary Outcomes From a Phase 2 Double-Blind, Placebo-Controlled Signal-Detection, Proof-of-Concept Study of Pitolisant in Prader-Willi Syndrome	48
Effects of Dietary Fibers on Hyperphagia, Metabolic Markers, and the Gut Microbiota in Individuals with Prader–Willi Syndrome: Preliminary Findings.....	49
Psychiatric Medication Use in Patients with PWS	50
Experiences and Support Needs of Unaffected Siblings and Parents of Individuals with Prader-Willi Syndrome- Findings from a Two-Stage Qualitative Study	51
Treating Sleep Problems in Children with Prader-Willi Syndrome.....	52
PWS research in Australia – contributing to the global effort!	53

A Patient-Centered Pilot Study of Whole Genome Sequence and Pharmacogenomic Analysis of Participants in the Global PWS Registry: Preliminary Findings	54
Noninvasive Neuromodulation of a Novel Cerebellar Satiety Circuit in PWS: Design and Methodology of a Pilot Clinical Trial	55
October 6 th , 2023	56
SPEAKER ABSTRACTS MORNING SESSION IA.....	56
Assessment of a Screening Assay for use in Newborn Screening for Prader-Willi Syndrome and Other Chromosome 15 Imprinting Disorders.....	57
Increased numbers of arginine-vasopressin neurons and enhanced glymphatic system activity in the suprachiasmatic nucleus of Prader-Willi Syndrome Individuals.....	58
Aberrant Behavior Checklist Scores in Youth with Prader-Willi Syndrome.....	59
Using the Food Attentional Bias Task to measure hyperphagia in Prader-Willi Syndrome	60
SPEAKER ABSTRACTS MORNING SESSION IB.....	61
Identifying key underlying regulatory networks and predicting targets of orphan C/D box <i>SNORD116</i> snoRNAs in Prader-Willi Syndrome	62
Assessing <i>Dgkk</i> as a <i>Snord116</i> target in the pathogenesis of PWS	63
Roles of SNORD115 and SNORD116 ncRNA clusters in neuronal differentiatin	64
Loss of <i>Snord116</i> has lasting consequences for neuronal and microglial morphology in mice	65
SPEAKER ABSTRACTS MORNING SESSION IIA.....	66
Primary Efficacy and Safety Results of a Phase 2 Double-Blind, Placebo-Controlled, Signal-Detection, Proof-of-Concept Study of Pitolisant in Prader-Willi Syndrome	67
A Novel Approach to Regulating the Impaired Gut-Brain Axis of Hunger Control in PWS using ARD-101	68
A Double Blind, Placebo-Controlled, Fixed-Flexible Dose, Randomized Clinical Trial of Guanfacine Extended Release for the Reduction of Aggression and Self-injurious Behavior Associated with Prader-Willi Syndrome	69
Project WellCAST: What we are learning about PWS caregiver mental health before and after telemental health treatments	70
SPEAKER ABSTRACTS MORNING SESSION IIB.....	72
Co-localization of <i>Snhg14</i> and <i>Nhlh2</i> in mouse brain tissue	73
Analysis of Circadian Rhythm Defects in Prader-Willi and Schaaf-Yang Syndrome Neurons.....	74
Defining Transcriptional Signatures in Blood and Brain Related to Severity of Prader-Willi Syndrome	75
Established a computational platform for the Pre-Clinical Animal Network (PCAN) Initiative	76
PWS Research Resources	77

October 5th, 2023
SPEAKER ABSTRACTS MORNING
SESSION I

Characterizing cognitive function in Magel2 null mice

Presenting Author: Elisabeth Saraceno

Additional Authors: Rachel A Ross

Institution(s): Albert Einstein College of Medicine

Abstract:

Prader-Willi Syndrome (PWS) is a rare, paternally inherited genetic disorder that can cause a variety of developmental, endocrine, and cognitive dysfunctions such as delayed learning and difficulty inhibiting emotional behavior. The Magel2-null mouse model for PWS is a relatively consistent model for PWS-like phenotypes with early-life hypotonia, later-life hyperphagia, and endocrine dysfunction, however, the cognitive impairment of this model has yet to be fully established. In order to characterize the cognitive function of the Magel2-null mouse, we completed a series of behavioral tests using an automated behavior system (Med Associates). To assess associative learning, we tracked the time it took animals to learn to press a lever or to nosepoke to receive a food reward. To study complex task learning, we added a second operation to the sequence of tasks that the animal had to perform to achieve a food reward. To assess motivated behavior, we used a progressive-ratio task, where the number of lever presses needed to obtain a food reward continues to increase over the time of testing. Motivation is evaluated by the breakpoint, which is the maximum number of required lever presses reached during testing. We found that Magel2-null mice took approximately 1.5x longer to learn to nosepoke for a food reward, compared to their control littermates. When the second operation was added to a task sequence to obtain the food reward, Magel2-null mice took almost 1.75x longer than their control littermates to learn this new behavior. Additionally, a two-way t-test shows that Magel2-null mice took significantly more time ($M = 7.4$ days) to learn to lever press for a food reward than their control littermates ($M = 5.6$ days; $t(24) = 2.64$, $p = .014$). Lastly, the progressive ratio task revealed there was no difference in motivation between the Magel2-null and control mice, as there was no difference between the groups' breakpoint values. Taken together, we have found that Magel2-null mice display a delay in learning which is not attributed to motivation, which is consistent with the clinical symptoms of PWS. We next plan to use a cognitive behavior task that will allow us to measure behavior inhibition, which will represent the cognitive dysfunction of inflexibility and emotional outbursts seen in people with PWS. These findings will provide a baseline for the Magel2-null cognitive function, so that future studies can test new treatments to help this aspect of PWS.

Acknowledgements/Funding: Foundation for Prader Willi Research

Investigation into the genetic factors of phenotypic variability in Prader-Willi syndrome

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Abstract:

Prader-Willi Syndrome (PWS) is a neurodevelopmental disorder due to the loss of expression of multiple genes in the Prader-Willi critical region on chromosome 15q11-13. Significant heterogeneity among the clinical phenotypes has been observed in PWS patients, this has been largely speculated to be linked to PWS genotype-phenotype correlation. Specifically, the genetic subtypes (deletion and non-deletion) have consistently shown statistical differences in the frequency and severity of some clinical features.

In a large PWS cohort collection (n=155) with dense phenotyping, combined with genotyping data, we set out to characterise the genetic factors behind the phenotypic complexity in PWS. We explored thirty-nine clinical features and measurements across this cohort and report descriptive statistics for these 39 phenotypes, including their phenotypic correlation as well as detailed comparisons across genetic subtypes. Several clinical phenotypes showed convincing PWS genotype-phenotype correlation. We found that individuals with the non-deletion subtype scored higher than the deletion ones in the following traits: depression, social relating scores, and weight (kg); whereas deletion patients showed a high level of glycosylated haemoglobin (HbA1c %). In addition, we found the deletion genetic subtype (Type I) was more susceptible to obesity with high mean values of body composition and body fat mass. In our study, behaviour issues were more prevalent in non-deletion patients compared to the deletion group. We also performed phenotypic correlation between the traits and observed some expected correlations, as well as unexplored associations, for example links between HQCT score and certain psychiatric traits. This analysis might potentially enable the identification of effective targeted therapeutic interventions in PWS.

Acknowledgements/Funding: N/A

Magel2 Role in the Stress Response to Fasting: From Hypothalamic Single-cell Dynamics to Hormonal Balance Throughout the Body

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Introduction: The dysregulation of hormonal secretion has emerged as a key molecular mechanism underlying various symptoms of Prader-Willi Syndrome (PWS). Notably, studies using mouse models with *Magel2* loss have implicated the requirement of this protein for regulated secretion in the hypothalamus. However, the specific reasons why MAGEL2 is crucial for the secretory process in the hypothalamus, and which other molecular pathways are regulated by MAGEL2 under different conditions, remain poorly understood. Therefore, our objective is to gain mechanistic insights into the endocrine deficiencies resulting from the loss of MAGEL2. We comprehensively investigate MAGEL2-driven neuroendocrine defects at the organismal, cellular, and molecular levels, both under normal conditions and in response to stress. The hypothalamus is situated at the core of animal body homeostasis and enables adaptation to environmental changes, including fluctuations in food availability. We propose the hypothesis that *Magel2* has evolved in mammals as a hypothalamic rheostat to finely tune the adaptive responses to dynamic environmental cues. **Methodology:** To gain an unbiased understanding of *Magel2*'s role in the hypothalamus under stress conditions, wild-type, and *Magel2*^{pΔ/m+} mice were subjected to a 24-hour fasting period. We are currently analyzing serum and different tissues, including performing single-nuclei RNA sequencing on the hypothalamic tissue and polysome profiling of the hypothalamus and pituitary. **Results, Conclusions, and Future Directions:** Our initial findings demonstrate a significant increase in corticosterone levels following 24-hour fasting. We are currently performing diverse physiological, molecular, and single-cell analyses to elucidate the underlying mechanisms involved in behavioral and endocrine regulation. Additionally, by single-cell analysis, we seek to identify the specific molecular strategies employed by different cell populations in the hypothalamus to adapt to homeostatic perturbations, which are disrupted in the absence of *Magel2*. The insights gained from our studies will be presented, which may provide novel perspectives on the pathogenesis of Prader-Willi Syndrome, potentially leading to the discovery of innovative therapeutic interventions for affected patients.

Acknowledgments/Funding: This work was supported by the Foundation for Prader-Willi Syndrome Research Grant 22-0321 and 23-0447 (to K.F.T. and Z.K.), Texas Tech University start-up (to K.F.T.), and Cancer Prevention and Research Institute of Texas Scholar Award RR200059 (to K.F.T.).

SPEAKER ABSTRACTS MORNING SESSION II

Investigation of Oxytocin Neurons and Oxytocinergic Projections in a Rat Model of Schaaf-Yang syndrome

Presenting Author: Tim Schubert

Additional Authors: Moritz Wimmer, Laura Dötsch, Yuval Hazor, Quirin Krabichler, Colleen Wagner, Gina Yosten, Valery Grinevich, Christian P. Schaaf, Ferdinand Althammer

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Abstract:

Specialized neuroendocrine command systems originating from the hypothalamus intricately regulate a plethora of physiological functions in vertebrates. Despite its diminutive size, this area orchestrates processes ranging from metabolism, energy expenditure and sleep-wake cycles to reproductive and social behavior.

Some of these functions are impaired in individuals with Prader-Willi Syndrome (PWS) and the related Schaaf-Yang syndrome (SYS), often necessitating the administration of growth hormone. While PWS commonly manifests with hyperphagia and obesity, SYS patients show a higher prevalence of Autism Spectrum Disorder (ASD) (75 - 85%).

The hypothalamic neuropeptide oxytocin (OT), known for its pleiotropic effects, is increasingly being associated with ASD pathophysiology. Parvocellular OT neurons are particularly interesting since they project to several extrahypothalamic brain regions to modulate complex mechanisms like pain processing, fear response and social behavior.

Humans and mice with *MAGEL2* deficiency produce less bioactive hormones like oxytocin, probably due to impaired retromer activity. Our own work also showed that *Mage/2* knockout mice have a reduction in the total number of OT neurons.

Notably, *Mage/2* knockout mice display unique behavioral patterns (e.g., lack of social discrimination and altered social interaction). These deficits bear remarkable resemblance to those in OTR-deficient mice used in ASD research, and could be partially mitigated by daily oxytocin injections.

Prior investigations employed mouse models with *Mage/2* deletion, partially emulating the larger chromosomal deletions of the paternal 15q11-q13 region in PWS patients. Recently however, a novel rat model with truncating *Mage/2* mutation was generated to better resemble SYS-causing mutations. These rats displayed altered anxiety-like and perseverative behaviors as well as changed social processes.

Given that rats naturally show more complex behavioral patterns than mice, this model is positioned to be an invaluable resource for studying intellectual disability and ASD in SYS. Accordingly, we present a comprehensive characterization of the OT network in the new *Mage/2* rat model. We performed automated quantification of the entire population of OT neurons in the rats' hypothalami using Imaris software. Furthermore, we analyzed neuron morphology and projections of parvocellular OT neurons to brain regions implicated in the observed behavioral changes.

Surprisingly, we detected a marked increase in OT neuron numbers which contrasts previous findings in *Mage/2* knockout mice. The most pronounced effect was observed in male pups

where the mean number of OT neurons in heterozygous animals ($M = 7153$, $SD = 334.4$) exceeded the mean number in wildtype animals ($M = 5666$, $SD = 527.7$) by 26 percent; $t(6) = 4.762$, $p = 0.0031$.

This work lays the foundation for utilizing *Mage12* rats in elucidating pathogenic pathways which underly complex behavioral changes manifesting as ASD. Additionally, it serves as a basis for the development and testing of therapeutic strategies targeting the OT system for the benefit of patients with SYS, PWS and ASD.

Acknowledgements/Funding:

This work was supported by the Foundation for Prader-Willi Research (to Christian P. Schaaf) and the DFG (AL 2466/2-1 to Ferdinand Althammer).

Dissecting the molecular and electrophysiological phenotypes in human cortical organoids derived from Prader-Willi Syndrome patients

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Abstract:

Prader-Willi syndrome is a neurodevelopment disorder characterised by a complex spectrum of clinical manifestations, including impaired satiety, severe childhood obesity, sleep abnormalities in addition to intellectual disabilities and social learning deficits. Deletion, uniparental disomy (UPD), or imprinting defects of paternally expressed genes in the human chromosome 15 (15q11-13 locus) are known to cause profound endocrine dysfunctions and abnormal functional connectivity among different brain regions of PWS patients, including the cerebral cortex and subcortical structures. While the genetic architecture of the disorder has been identified, our understanding of the mechanisms underlying the complex spectrum of clinical phenotypes associated with PWS remains still largely elusive. The PWS locus is evolutionarily conserved and several animal models for PWS have been generated over the past decades, providing instrumental insights in PWS research. Although PWS mouse models are important for dissecting PWS phenotypic mechanisms, the emerging species-specific genetic and neurodevelopmental differences motivate our work to develop innovative experimental systems to address PWS pathophysiology and explore effective therapies. We have generated human 3D cortical organoids (cOrg) from induced pluripotent stem cells (iPSC) derived from multiple PWS patients, bearing the distinctive patient genetic traits (PWS 1-2 maternal uniparental disomy, PWS 2-9 small atypical deletion), and control iPSC and hES lines from healthy donors. These organoids serve as a valuable human model for investigating the molecular and electrophysiological alterations occurring early in development in PWS patients, with the aim to identify novel disease-associated pathways.

Molecular profiling, at single cell resolution, of PWS and CTRL-derived cOrgs has revealed cortical differentiation defects and the generation of PWS-specific neuronal cell types. Furthermore, PWS-derived cOrgs present an impairment in neuronal differentiation and the acquisition of neuronal identity, and an altered gliogenic phenotype with an increased amount of glia cells produced compared to CTRLs. Ongoing analysis aims at further dissecting the common and unique molecular pathways associated with different PWS genetic background. In order to assess the functional capabilities of cOrg neurons to establish efficient networks, we performed electrophysiological recordings using a 4,096-microelectrode array device. A robust network activity of CTRL cOrgs was reported after 4 months of in vitro differentiation. CTRL

electrophysiological profile will be compared to PWS 1-2 and PWS 2-9 derived cOrgs in order to identify affected neuronal population.

Our multimodal approach will track phenotypic characteristics of PWS in vitro, providing the opportunity of designing and testing novel therapeutic strategies and identifying susceptible window of action. Overall, this study bears the potential for the development of personalized strategies to treat the PWS.

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Investigating the role of glial cells in the pathophysiology of Prader-Willi syndrome

Presenting Author:

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Additional Authors:

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Abstract:

The role of glial cells such as microglia, astrocytes and oligodendrocytes in the onset and pathophysiology of Prader-Willi syndrome (PWS) has not yet been systemically addressed. To fill in this gap and to better understand the respective role of glial cells in PWS, we analyzed microglia morphology, microglia-vessel and microglia-oligodendrocyte interactions in *Magel2* KO mice, performed scRNA sequencing of hypothalamic tissue samples of *Magel2* KO mice and revisited existing datasets on PWS patients to study genetic alterations to glial cells. Microglia, the immune cells residing in the brain, exhibit highly dynamic movements and variable morphology that strongly correlates with their respective functional state. Previous studies have shown that individuals with PWS display accelerated brain aging and increased low-grade systemic inflammation, suggesting potential activation of microglia and systemic inflammation. However, the impact of PWS on the morphology of microglia in mouse models has not been investigated. To this end, we utilized our previously developed microglia morphometric profiler, implemented through Imaris software, to examine microglial surface and filaments in different brain regions of both wildtype and *Magel2* KO mice. By employing this semi-automated pipeline, we successfully reconstructed over 7000 microglia per animal, enabling detailed analysis of their morphology. Intriguingly, we observed significant changes in microglial morphology in adult *Magel2* KO mice, some of which were already present as early as P8. While blood-brain barrier integrity and microglia-vessel interactions were unaltered, *Magel2* KO mice displayed impaired microglia-oligodendrocyte interactions. Analysis of hypothalamic samples of *Magel2* KO mice via scRNA sequencing revealed drastic genetic changes in astrocytes, microglia and oligodendrocytes. Finally, we revisited existing bulk RNA transcriptomic datasets of hypothalamic samples from PWS patients to gain insights into altered gene expression and impaired intracellular pathways in various glial cells.

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AAV-BDNF gene therapy ameliorates a hypothalamic neuroinflammatory signature in the *Mage12*-null mouse model of Prader-Willi syndrome

Presenting Author: Lei Cao

Additional Authors: Nicholas Queen, Xunchang Zou, Wei Huang, Xiaokui Mo

Institution(s): Dept Cancer Biology and Genetics, College of Medicine, The Ohio State University

Abstract:

Individuals with Prader-Willi syndrome (PWS) exhibit several metabolic and behavioral abnormalities associated with excessive food-seeking activity. PWS is thought to be driven in part by dysfunctional hypothalamic circuitry and blunted responses to peripheral signals of satiety. Previous work described a hypothalamic transcriptomic signature of individuals with PWS. Notably, PWS patients exhibited downregulation of genes involved in neuronal development and an upregulation of neuroinflammatory genes. Deficiencies of brain-derived neurotrophic factor (BDNF) and its receptor were identified as potential drivers of PWS phenotypes. Our group recently published a preclinical study assessing the efficacy and safety of an adeno-associated viral (AAV)-based gene therapy in a PWS mouse model *Mage12*-null mice. The proof-of-concept study has shown that hypothalamic AAV-BDNF gene therapy normalized metabolic and behavioral abnormalities in the *Mage12*-null mice. However, it remained unclear how AAV-BDNF was influencing the hypothalamic microenvironment and how its therapeutic effect was mediated. In the current study, we hypothalamically injected AAV-BDNF to wild type and *Mage12*-null mice and performed mRNA sequencing on hypothalamic tissue to assess genotype- and gene therapy-driven alterations in hypothalamic transcriptome. We report that (1) *Mage12*-null mice recapitulate transcriptomic signatures that were found in PWS patients such as heightened neuroinflammation and activation of microglial cells within the hypothalamus and (2) AAV-BDNF gene therapy induced a reversal of the *Mage12*-null neuroinflammatory signature, and several genes involved in microglial activation and function. These new findings shed lights on potential mechanisms of action of the hypothalamic BDNF gene therapy, which is important for assessing the translational potential of this gene therapy for PWS, a devastating rare disease lacking effective therapeutic interventions.

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SPEAKER ABSTRACTS AFTERNOON SESSION I

Cellular and Molecular Basis for Obesity in PWS

Presenting Author: Han L. Tan

Additional Authors: Yi Zhou, Niklas Vieweg, Jessica Ivanov, Jeffrey M. Friedman

Institution(s): Laboratory of Molecular Genetics, Howard Hughes Medical Institute, The Rockefeller University, New York. NY. USA

Abstract:

Prader-Willi Syndrome (PWS) is a complex genetic disorder characterized by severe obesity and uncontrollable hyperphagia, along with other associated abnormalities. Despite its significant health burden, there are currently no approved treatments for this condition. The hypothalamus, a key regulator of food intake and energy expenditure, has been implicated in the hyperphagia and obesity observed in PWS patients. However, due to the heterogeneity of cell types in this brain region with numerous distinct cell types, the specific cells and molecular mechanisms underlying the eating disorder in PWS patients remain unclear. To address this, we used multiplexing single nuclei RNA sequencing (snRNA-seq) to compare gene expression in hypothalamic neurons at the single-cell level in wild type and *Magel2* knockout mice. *Magel2* is located within the human PWS deletion region and knockout mice lacking *Magel2* exhibit several features similar to those seen in individuals with PWS, although not all. We also conducted a comprehensive characterization of feeding- and metabolism-related phenotypes that are disrupted in *Magel2* knockout mice and observed development-dependent alterations in feeding, body weight, and composition. This systematic phenotypic analysis provides a foundation for correlating these phenotypes with changes in cell types and gene expression in these knockout animals. Employing multiplexing snRNA-seq, we examined cell-specific transcriptomic changes induced by *Magel2* knockout in various regions of the hypothalamus, including ARC, PVH, DMH, and VMH. A total of 30,678 neurons, approximately evenly distributed among the regions and *Magel2* genotypes, were analyzed. While no changes in cell types were observed between WT and *Magel2* knockout mice, we identified significant differences in the abundance of specific cell types in *Magel2* knockout mice. These included *Lef1*-expressing neurons in ARC, *Bdnf*-expressing neurons and *Oxt*-expressing neurons in PVH, and *Rorb*-expressing neurons in DMH. Moreover, we observed significant transcriptional changes across individual populations in *Magel2* knockout mice, with neurons in ARC and PVH exhibiting more extensive and pronounced changes compared to neurons in DMH and VMH. Notably, we identified primary changes occurring in neurons crucial for energy balance regulation, such as *Agrp* neurons in ARC. Furthermore, *Magel2* knockout induced distinct gene expression programs in different clusters of neurons. In summary, our study provided unprecedented spatiotemporal resolution of the cellular and molecular changes induced by *Magel2* deletion, shedding light on key targets that may contribute to hyperphagia and obesity in PWS. These findings enhance our understanding of the pathogenesis of PWS and may guide future therapeutic approaches for this challenging disorder.

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Role of DMH MC3R in defending against weight gain

Presenting Author: Ingrid Camila Possa-Paranhos¹

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Abstract:

Obesity is a chronic disease that can be caused by several factors, such as genetics, which is the case in Prader Willi Syndrome. In response to high fat diet feeding, mammals gradually gain weight leading to the development of obesity. The pathways promoting weight gain in response to high fat diet are incompletely understood, although the leptin-melanocortin pathway is believed to play a critical role. This pathway consists of leptin (LEP), a hormone secreted in proportion to body fat, which indirectly leads to the synthesis of α -melanocyte stimulating hormone (α -MSH), an agonist of melanocortin-4 receptor (MC4R) and melanocortin-3 receptor (MC3R), signaling satiety. Although the role of the MC4R in central melanocortin signaling is well established, the specific role of MC3R in energy homeostasis is not well understood.

The MC3R is densely expressed throughout the medial-basal hypothalamus (MBH), a region critical for normal regulation of energy homeostasis, which includes the dorsal-medial hypothalamus (DMH). Prior work in global MC3R knockout mice indicates a unique metabolic phenotype characterized by normal feeding when fed a regular chow (RC) diet, and a hyperphagic and obese phenotypes when mice are fed a high fat diet (HFD). Thus, MC3R plays an important role in food intake and body weight regulation by controlling the magnitude and duration of response to orexigenic stimuli, such as exposure to a high fat diet. However, the neuroanatomical and molecular mechanism(s) mediating the role of MC3R in high fat diet induced weight gain is unknown. Thus, in this study we sought to map the specific regions of the brain mediating the role of MC3R in high fat diet feeding by using adeno associated viral (AAV) injections of Cre recombinase in the MBH in MC3R floxed mice, allowing for deletion of MC3R in discrete hypothalamic nuclei in adult female and male mice.

Selective deletion of MC3R in MBH recapitulated the increased weight gain on HFD observed in global MC3R KO mice, indicating that this phenotype is mediated by adult hypothalamic MC3R. Next, to identify the specific region(s) of MBH causing this phenotype, we targeted AAV-Cre virus specifically to DMH in male and female MC3R floxed and WT mice. The DMH MC3R-KO mice presented the same phenotype as observed in the adult MBH KO and the global MC3R KO mice, where male mice were hyperphagic in response to HFD with a normal metabolic response on a regular chow diet. These data indicate that DMH deletion of MC3R results in a hyperphagic response to HFD, and that the hyperphagia observed in the global MC3R-KO is not of developmental origin and is mediated by loss of MC3R signaling in adults. Thus, future work targeting MC3R in DMH may elucidate novel approaches for preventing weight gain in dietary and genetic forms of obesity.

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Pharmacological inhibition of cholesterol esterification alleviates adiposity and food intake in the Magel2-null mouse model of Prader-Willi Syndrome

Presenting Author: Kee-Hong Kim

Additional Authors: Jeonghoon Kim and Mulim Choi

Institution(s): Purdue University (KHK) and EFIL BioScience Inc. (JK and MC)

Abstract:

Individuals with Prader-Willi Syndrome (PWS) exhibit excessive hunger, obesity, developmental delays, cognitive impairment and behavioral abnormalities. However, current PWS treatment is largely limited to growth hormone therapy, underscoring the need for new therapeutic strategies. Acyl-coenzyme A:cholesterol acyltransferases (ACATs) catalyze the formation of cholesteryl ester (CE) from free cholesterol to regulate intracellular cholesterol homeostasis. Overly activated ACAT1 has been proposed to be associated with various diseases, such as prostate cancer, pancreatic ductal adenocarcinoma, hepatocellular carcinoma, chronic myelogenous leukemia, and Alzheimer's disease. Recently, we demonstrated the therapeutic potential of pharmacological ACAT1 blockage in obesity and its-associated hyperphagia. Here, we present our findings on the effects of avasimibe (AVA), a safe ACAT1 inhibitor, on high-fat diet (HF) obese Magel2-null mice. Two to three weeks of subcutaneous administration of AVA at a dosage of 20 mg/kg body weight to obese Magel 2-null mice resulted in a significant reduction in body weight, adipose tissue, and food intake. Furthermore, the mice that received AVA exhibited lowered levels of blood glucose, insulin, and total cholesterol. To assess avasimibe's weight-reducing capabilities, we compared it with beloranib, an irreversible inhibitor of methionine aminopeptidase 2 (METAP2), known for its efficacy in combating obesity PWS in preclinical animal models. Our results demonstrate that AVA exhibited a weight loss effect similar to or even greater than beloranib in HFD-fed obese Magel 2-null mice. Collectively, our findings indicate the potential of avasimibe as a promising candidate for addressing obesity-related issues in animal models of PWS.

Acknowledgements/Funding:

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Rationale for and Results from a Randomized Withdrawal Period Following Long-Term Administration of Diazoxide Choline Extended-Release Tablets to People with Prader-Willi Syndrome

Presenting Author: Dr. Jennifer Miller¹

Additional Authors: Dr. Nicola Bridges², Dr. Evelien Gevers³, Dr. Tony Holland⁴, Dr. M. Guftar Shaikh⁵, Dr. Verghese Matthew⁶, Dr. M. Jennifer Abuzzahab⁷, Dr. Lynne Bird⁸, Dr. Parisa Salehi⁹, Dr. Ashley Shoemaker¹⁰, Dr. Amy Fleishman¹¹, Dr. David Stevenson¹², Dr. Jorge Mejia-Corletto¹³, Dr. David Viskochil¹⁴, Dr. Jack A. Yanovski¹⁵, Dr. Katherine S. Obrynba¹⁶, Dr. Virginia Kimonis¹⁷, Dr. Eric Felner¹⁸, Dr. Melissa Lah¹⁹, Dr. Elizabeth Littlejohn²⁰, Dr. Katerina Harwood²¹, Dr. Heidi C. Shea²², Kristen Yen²³, Patricia Hirano²³, Dr. Neil Cowen²³ and Dr. Anish Bhatnagar²³

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Abstract:

DESTINY PWS (C601) was an international, placebo-controlled, Phase 3 study of DCCR (diazoxide choline) extended-release tablet in participants with PWS, age 4 and older with hyperphagia. C602 was a long-term, open-label extension to C601. DCCR administration to people with PWS in these studies resulted in significant improvements in multiple PWS associated behaviors, body composition and metabolic markers. In order to further establish the effects of DCCR administration on hyperphagia and other endpoints in a controlled setting, a randomized withdrawal (RW) period was initiated for participants enrolled in C602. All these participants had received DCCR for more than 2 years. The goal of the RW period is to generate further controlled data in people with PWS to supplement the currently available data in support of a regulatory submission. Input into the design, conduct and analysis of the RW period was sought from patients and caregivers, clinicians and from the FDA. Seventy-seven participants who continued in C602 were randomized 1:1 to DCCR or placebo for the 16-week RW period. Following completion of the RW period, participants are eligible to enroll in a new open-label study (C614). The primary endpoint of the RW period is change from baseline in Hyperphagia Questionnaire for Clinical Trials total score. Secondary endpoints include the Clinical Global Impression of Improvement (CGI-I), and the Clinical Global Impression of Severity (CGI-S). Additional endpoints include changes in other PWS-associated behaviors as assessed by the Prader-Willi Syndrome Profile Questionnaire, weight and BMI. The Baseline and 16-week visits are in-clinic, all others are conducted by telemedicine. Safety monitoring is conducted as it was in C602. Topline results from the randomized withdrawal period will be presented.

Acknowledgements/Funding: Clinical Study C602 is sponsored by Soleno Therapeutics. Each Institution received funding for the conduct of the study from Soleno.

Heightened response to the anorexigenic peptide CART in a rat model of Prader-Willi Syndrome

Presenting Author: Colleen Bocke

Additional Authors: Willis K Samson, PhD; Gina LC Yosten, PhD

Institution(s): Saint Louis University School of Medicine Department of Pharmacology and Physiology

Abstract:

Disruptions in expression of *Magel2*, such as the deletion that is seen in Prader-Willi Syndrome (PWS) can contribute to reduced production, processing, and secretion of important neural peptides, including those that are important in contributing to feeding behavior. The insatiable hunger and hyperphagia that are hallmark traits of PWS are likely the result of the disruption of multiple signaling pathways, many of which have been investigated as potential therapeutic targets. One potential contributor to the interruption of satiety signaling is cocaine and amphetamine regulated transcript (CART), an anorexigenic peptide hormone that is processed into its bioactive form by the prohormone convertase PC1/3. PC1/3 is a secretory granule enzyme that is reduced in individuals with PWS and in rodent models of PWS. Because of this reduction of PC1/3 in PWS patients, we believe that bioactive CART levels may also be reduced thereby reducing normal satiety signaling that happens during a meal. Using a rat model of PWS that harbors a truncating deletion in the paternal allele of the *Magel2* gene, we are investigating the potential contributions of CART and its receptor, GPR160, to differences in feeding behavior. Using BioDAQ continuous food and water intake monitoring cages, we have shown that *Magel2* truncation alone is insufficient to produce hyperphagic behavior in rats, but does change meal patterning behavior, especially during the light phase. Exogenous administration of CART directly to the 4th ventricle has been shown to reduce cumulative food intake in wild type rats, and we have shown that this effect is magnified in *Magel2* rats. The food intake of *Magel2* rats is reduced immediately after administration of CART, and this reduction of food intake is not compensated for in the 24 hours following injection, while in WT rats the effect of CART is short-lived, and compensatory feeding occurs during the same feeding cycle as administration. Here, we investigate the potential mechanism for this heightened response to CART administration on feeding behavior. Using open-field behavior testing, we sought to determine if reduction in food intake was related to an overall reduction in ambulatory behavior. In addition to this, we collected tissue from the brainstem and hypothalamus of both WT and *Magel2* animals and performed ELISA to determine if PC1/3 levels are reduced, thereby potentially contributing to a reduction in bioactive CART levels. Finally, we have performed an immunohistochemical analysis of the hypothalamus and brainstem of these animals to investigate differences in the expression and localization of GPR160 in the feeding centers of the brain. We anticipate that changes to the CART/GPR160 system contributes to the modulation of satiety signaling and feeding behavior in PWS patients and may provide a potential therapeutic target for PWS-associated hyperphagia.

Acknowledgements/Funding:

This work has been funded through a grant from the Foundation for Prader-Willi Research.

SPEAKER ABSTRACTS AFTERNOON SESSION II

The role of non-canonical SMC protein SMCHD1 in regulating the Prader Willi cluster

Presenting Author: Marnie Blewitt

Additional Authors: Megan Iminoff^{1,2}, Tamara Beck^{1,2}, Merlin Thomas³, Christian Schaaf⁴, Stormy Chamberlain⁵, Quentin Gouil^{1,2}, Andrew Keniry^{1,2}

1. Institution(s): The Walter and Eliza Hall Institute of Medical Research

2. The University of Melbourne

3. Monash University

4. The University of Heidelberg

5. Roche/UConn

Abstract:

We are working on the SMC protein SMCHD1, which we have previously shown silences several clustered gene families spread throughout the genome including the PWS cluster, alongside the inactive X chromosome in female mice. I will present our current evidence from human studies relating to SMCHD1 as a drug target for Prader Willi syndrome. While SMCHD1 is essential for gene silencing at all its targets during early embryonic development, we have found that removing SMCHD1 after this stage, has very limited effects on gene expression. Importantly, the PWS cluster of genes remains sensitive to removal of SMCHD1 in Prader-Willi patient iPSC cell-derived neural progenitors. Our genomic data suggest that both the protein coding maternally silenced genes and *SNRPN* rely on SMCHD1 to maintain their silent state in the neural system. Importantly, we have obtained similar findings in mouse models using neural-lineage specific deletion of *Smchd1*. The effect on *SNRPN* is neural-specific and so we are now working to understand the neural-specific imprints that may mediate this effect, which could reveal more about the mechanism of SMCHD1 silencing at this locus, with relevance to understanding epigenetic mechanisms more broadly.

Acknowledgements/Funding: We appreciate funding from FPWR, Prader Willi Research Foundation Australia, The Pamela and Lorenzo Galli Foundation and WEHI in support of this program of research.

Phenotypic Characterization of a Novel Mouse Model of Prader-Willi Syndrome with Genetic Invalidation of both *Magel2* and *Necdin*

Presenting Authors: Sebastien G Bouret¹ & Françoise Muscatelli²

Additional Authors: Pierre-Yves Barelle^{1*}, Alicia Sacardi^{1*}, Fabienne Schaller^{2*}, Felix Omnes², Emilie Caron¹, Vincent Prevot¹ [**co-first authors*]

Institution(s): ¹ *Inserm, Laboratory of Development and Plasticity of the Neuroendocrine Brain, Lille Neuroscience & Cognition Research Center, UMR-S 1172, Lille, 59000, France*; ² *University of Lille, FHU 1,000 Days for Health, Lille, 59000, France*; ³ *Institut de Neurobiologie de la Méditerranée, INSERM, U1249, Aix Marseille University, Marseille, France*

Abstract:

Prader-Willi syndrome (PWS) is a multigenic disorder caused by the loss of seven contiguous paternally-inherited genes located in the human 15q11-q13 region (chromosome 7 in mice). Mouse models with inactivation of all PWS genes display 100% lethality within the first postnatal week and have, therefore, not been very helpful in understanding the postnatal physiopathology of this syndrome. Mouse knockout (KO) models for each candidate gene were then generated. Among them, *Necdin*- and *Magel2*-KO mouse models display distinct phenotypes mimicking part of the PWS clinical features. However, none of these single KO mice recapitulate all the symptoms observed in PWS patients. However, this is expected since the PWS phenotype is likely the result of multiple genes that are lost and usually interact with each other at the cellular and molecular levels. For example, there is a genetic interaction between *Necdin* and *Magel2*: when *Necdin* is deleted, *Magel2* expression is over-expressed and *vice versa*. In the present study, we used a newly generated double KO (DKO) mouse model to explore the effect of a combined deletion of *Magel2* and *Necdin*, therefore avoiding the putative functional interaction and redundancy between these two genes. Neonatal lethality (likely due to respiratory and/or suckling defects) could be observed in DKO pups depending on the sanitary status of the animal facility (*i.e.*, conventional *versus* EOPS), reflecting the respiratory distress and suckling deficit observed in *Necdin* and *Magel2* KO mice, respectively. DKO mice exhibit a lower body weight at birth that persists in adulthood. DKO mice also have a lower fat mass, an elevated lean mass, and an altered glucose tolerance. A delayed onset of puberty is also observed in DKO mice. We are currently examining the effect of the combined loss of *Magel2* and *Necdin* on various behavioral outcomes (*e.g.*, motor and sensory activity, feeding behavior, social behavior, and cognition) during infancy and adulthood. Together, these data indicate that mice with a genetic deletion of both *Magel2* and *Necdin* in the C57Bl6/J background display a phenotype that is the sum of single KO mice and still does not recapitulate the obesity of patients with PWS.

Acknowledgements/Funding:

This project was supported by a grant from the Foundation for Prader-Willi Research (to FM and SGB)

Cardiac phenotypes of the *Magel2* KO mouse, an animal model of Schaaf-Yang syndrome and PWS

Presenting Author:

Henning Fröhlich¹

Additional Authors:

Laura Dötsch¹, Felix Marbach¹, Susanne Theiß¹, Tobias Thiemann², Florian Leuschner², Leo Weirauch², Constanze Schmidt², Christian P. Schaaf¹

Institution(s):

¹Institute of Human Genetics, Heidelberg University, Heidelberg, Germany

²Heidelberg University Hospital, Internal Medicine III: Heart, Vascular and Lung, Heidelberg, Germany

Abstract:

Schaaf-Yang syndrome (SYS) is a rare genetic disorder caused by truncating variants of the paternal *MAGEL2* copy within the imprinted Prader-Willi region at 15q11-q13. It is characterized by hypotonia, feeding problems in infancy, global developmental delay, intellectual disability, and sleep apnea. In addition, affected individuals manifest a high prevalence of autism spectrum disorder and joint contractures.

Previously published data suggested overexpression of mTOR and overactive mTOR signaling in *Magel2* KO mice, an animal model of SYS. Therefore, we investigated whether mTOR inhibition with rapamycin has a beneficial effect on the phenotype of these animals. To our surprise, our studies were not able to recapitulate the overexpression of mTOR, and both long-term and short-term treatment with rapamycin did not attenuate the existing behavioral changes and decreased grip strength. However, long-term treatment significantly improved the reduced endurance of *Magel2* KO animals in the treadmill test.

Rapamycin is known to extend the lifespan of mice, be cardioprotective, and protect several organs, including skeletal muscle from age-related loss of function. Therefore, we investigated both *Magel2* expression in skeletal and heart muscle tissue. In contrast to previously published results, *Magel2* is abundant not only in skeletal and cardiac muscle in young animals but also in adults, albeit at a very low level compared to the brain. Interestingly, transthoracic echocardiography revealed cardiac phenotypes in *Magel2* KO animals suggestive of heart failure with preserved ejection fraction (HFpEF). Further investigations shall clarify to what extent the increased endurance of treated *Magel2* KO mice is due to improved heart and muscle function. In addition, we are currently exploring the extent to which cardiac dysfunction is directly attributable to the lack of *Magel2* or if obesity and possibly diabetes in *Magel2*-deficient animals are relevant contributing causes. The results of these studies promise to be relevant for management of individuals with SYS and possibly PWS, as they might provide an opportunity for secondary prevention of related phenotypes.

Acknowledgements/Funding:

Foundation for Prader-Willi-Research grant to Dr. Christian Schaaf

Determining beloranib's mechanism of action to inform novel drug targets for Prader-Willi syndrome

Presenting Author: Michael R MacArthur

Additional Authors: Charlotte G Mann, Sarah J Mitchell

Institution(s): Princeton University

Abstract:

The fumagillin derivative beloranib has demonstrated striking efficacy in reducing body weight and compulsive eating behaviors in individuals with Prader-Willi syndrome (PWS). Unfortunately, clinical development of beloranib was halted in 2015 due to risk of pulmonary embolism. Since then, no drug has come close to achieving the efficacy seen with beloranib in PWS. Our group has worked extensively over the past 5 years to characterize the mechanism of action of beloranib derivatives to identify new drug targets for PWS. We have previously confirmed that beloranib's physiologic mechanism of weight loss is through reduced food intake. We have demonstrated that brain-specific administration of a single dose is sufficient to reduce food intake in mice after one hour. On a molecular level, RNA sequencing identified a key requirement for p53 for the anorectic and anti-obesity effects of the beloranib derivative, ZGN1062. Interestingly, the p53 target Gdf15 was among the top three increased transcripts at multiple timepoints from our RNASeq data. *In vivo*, p53 KO mice did not lose weight in response to ZGN1062 treatment or decreased food intake. However recent work in both GDF15 and GFRAL KO mice showed both weight loss and reduced food intake with ZGN1062, pointing to an alternative downstream mediator of these effects. To further understand mechanisms of p53 regulation, we performed genome wide CRISPR KO screens and identified multiple components of the Hippo signaling pathway that were required for p53 activation by ZGN1062. These data suggest that activation of Hippo signaling is required for p53 activation by ZGN1062 and may be required for the anorectic effects of ZGN1062. Surprisingly, we have found that the putative target of beloranib, MetAP2, is dispensable for p53 activation *in vitro* and anorectic responses *in vivo*, excitingly suggesting an alternative target of beloranib. Our current work in collaboration with the Chemistry Department at Princeton University is focused on developing photocatalyst-tagged beloranib derivatives to identify potential alternative binding targets. If successful we hope to identify PWS therapeutics that leverage the mechanism of beloranib to achieve similar efficacy without thrombotic effects.

Acknowledgements/Funding: We gratefully acknowledge the support of the grant program from the Foundation for Prader-Willi Research for funding our work.

Inhibition of FKBP51 signaling partially reversed impaired behavioral and metabolic phenotypes displayed in *Mage/2*-null mice

Presenting Author: Hui Yu¹

Additional Authors: Angelika Chiang¹, Felix Hausch², Vanessa Buffa², Malcolm J. Low¹

Institution(s): ¹Department of Molecular and Integrative Physiology, University of Michigan, USA; ²Department of Biochemistry, Technical University of Darmstadt, DE

Abstract:

The mechanism on how loss of *Mage/2* expression leads to clinical features of Prader-Willi syndrome (PWS) is poorly understood. In our preliminary investigation, we utilized single nucleus RNA sequencing to profile hypothalamic cells obtained from *Mage/2*-deficient mice. Our findings indicate that the absence of *Mage/2* leads to an elevated expression of numerous genes, including *Fkbp5*, across multiple cell clusters. Notably, previous studies have reported an increase in *Fkbp5* expression in postmortem hypothalamic tissue from PWS patients and in the hypothalamus of mice lacking *Snord116*, one of the small nucleolar RNAs located in the PWS genetic locus. Based on the function of *Fkbp5* in regulating neuroendocrine stress responses, body weight and corticosterone levels, the current study aimed to investigate whether pharmacological inhibition of FKBP51 (the protein encoded by *Fkbp5*) signaling can reverse the altered behavioral, metabolic, and disrupted stress response phenotypes resulting from the absence of *Mage/2*. *Mage/2*-wt and *Mage/2*-null mice were injected i.p. with SAFit2, a selective FKBP51 inhibitor, or vehicle twice per day for five weeks. Our results suggest that chronic inhibition of FKBP51 significantly altered anxiety-related behaviors. Specifically, in the Elevated Plus Maze, treatment of *Mage/2*-null mice with SAFit2 significantly reduced the number of entries in the open arms and increased the duration of time spent in the closed arms. In the Open Field Assay, SAFit2 treatment greatly reduced the time that *Mage/2*-null mice spent in the center zone. Injection of SAFit2 didn't change the corticosterone levels under basal, stress or insulin stimulation, however, it significantly reduced circulating corticosterone after CRH injection in both *Mage/2*-wt and *Mage/2*-null animals. A previous study reported significantly decreased food intake in *Mage/2*-null mice after 48 hours fasting. Injection of SAFit2 increased food intake by 16 % in *Mage/2*-null mice after a 20-hour fasting period. In addition, a previous study found that *Mage/2*-null mice exhibited defective counter regulation of hypoglycemia, while our results suggested that injection of SAFit2 significantly promoted glucose recovery. After 120 mins of insulin injection, the blood glucose in *Mage/2*-null mice with SAFit2 treatment was 1.5-fold higher than *Mage/2*-null mice administered vehicle. In conclusion, our study suggested that FKBP51 contributes to PWS development and inhibition of FKBP51 could partially rescue disrupted phenotypes in *Mage/2*-null mice.

Acknowledgements/Funding:

The authors thank the Foundation for Prader-Willi Research (FPWR) grants, Dr. Mathias Schmidt for the insightful discussion and the Mouse Metabolic Phenotyping Center at Michigan for conducting metabolic related studies (U2CDK110768).

POSTER SESSION

Role of mRNA Translational Regulation in Hormone Secretion Deficit in MAGE-L2 KO Mouse Model

Presenting Author: Tara Bayat^{1,2}

Additional Authors: Elena B Tikhonova³, Maria Camila Hoyos Sanchez^{1,2}, Farzana Popy^{1,2}, Hanin Diab¹, Jon Thompson¹, Andrey L. Karamyshev³, Zemfira N. Karamysheva⁴, and Klementina Fon Tacer^{1,2}

Institution(s): ¹ School of Veterinary Medicine, Texas Tech University, 7671 Evans Dr., Amarillo, TX

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Abstract:

Introduction: We aim to determine whether melanoma antigen L2 (Magel2) contributes to protein translation regulation in the hypothalamus and pituitary of the Prader-Willi syndrome (PWS) mouse model and by doing so contributes to proper hypothalamic endocrine function, particularly during stress. Translational control of gene expression occurs on polysomes which are made of mRNAs, ribosomes, proteins, and non-coding RNAs and is critical for proper cellular stress response.

Dysregulation of protein translation contributes to human diseases, however, if and how it contributes to PWS pathogenesis, is not known. Several PWS clinical symptoms suggest hypothalamic endocrine dysfunction. Our previous work eluted that Magel2 is critical for proper hormonal secretion of several hypothalamic hormones, including oxytocin, however, the underlying mechanisms are still enigmatic. **Methodology:** In this project, we aim to perform polysome profiling analysis of hypothalamic and pituitary translome of wild type and *Magel2*^{pΔ/m+} mice exposed to 24h-fasting-induced stress to determine genes whose translation depends on Magel2 and normal hormonal output. We first optimized the polysome fractionation of the hypothalamus and pituitary tissues, the stress response readouts upon 24h-fasting, and oxytocin measurements by LC-MS, and will perform the analysis of tissues from 10 animals per group. **Results, conclusions, and future directions:** Our data showed that fasting induced strong induction of stress response in mice, reflected by serum corticosterone levels. With translome analysis, we will provide the first insights into translational regulation in PWS with potential therapeutic implications for PWS children.

Acknowledgments/Funding:

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Molecular Subtyping of Prader-Willi Syndrome by Long-Read Genome Sequencing with Simultaneous Methylation Detection

Presenting Author: Anita E. Beck, MD, PhD

Additional Authors: Cate R. Paschal, PhD, Bri Dingmann, MS, CGC, Miranda Galey, MS, Madelyn A Gillentine, PhD, Parisa Salehi, MD and Danny E. Miller, MD, PhD

Institution(s): University of Washington and Seattle Children's Hospital

Abstract:

BACKGROUND: Methylation testing of the SNRPN locus at chr15q11.2 detects >99% of Prader-Willi syndrome (PWS) but cannot determine the underlying molecular etiology. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) of the wider PWS/Angelman syndrome (AS) locus can determine methylation status and if a deletion is present, but cannot confirm uniparental disomy (mUPD15), an imprinting center deletion, or an epimutation. A single test to determine methylation and molecular subtype would improve the identification and understanding of PWS.

METHODS: Genome-wide, long-read sequencing (LRS) with detection of differentially methylated DNA regions was used to assay DNA samples from individuals with PWS seen at the Seattle Children's Hospital multidisciplinary PWS clinic and historical samples from the Seattle Children's Hospital laboratory. These included individuals with typical deletions (BP1-BP3 and BP2-BP3), atypical deletions, uniparental disomy and failure of erasure of imprinting. Differential methylation was called genome-wide using our newly developed program MeOW (Methylation Optimization Wizard).

RESULTS: The molecular etiology for individuals with PWS in our multidisciplinary clinic include 50% with deletions, 47% with mUPD15, and 3% with epimutations. We performed LRS on twelve individuals with PWS (and nine individuals with Angelman syndrome), identifying methylation status across the entire chr15q11.2q13.1 region, precise breakpoints in deletion cases, and chromosome 15 regions of loss-of-heterozygosity. Three independent analysts blinded to the diagnosis and molecular etiology arrived at the same correct molecular diagnosis for all cases.

DISCUSSION: LRS is unique in that it can be used to identify single nucleotide variants (SNVs), insertion or deletion variants (indels), structural variants (SVs), and differences in methylation from a single data source. While many tools exist for the identification and analysis of SNVs, indels, and SVs, there are few, if any, able to perform genome-wide evaluation for differentially methylated regions (DMRs) that may contribute to disease. Our LRS project determined methylation status across the entire PWS region, precise deletion size, identified mUPD (if a region of isodisomy is present on chromosome 15), and detected failure of erasure of imprinting. LRS with methylation calling would also detect a homozygous pathogenic variant within a region of isodisomy or a deletion at the PWS imprinting center. Validation of LRS as a clinical assay for specific imprinting disorders is one step towards being able to detect imprinting disorders and many other types of genomic alterations using LRS without the clinician needing to specify a single genetic condition of interest.

Acknowledgements/Funding:

To all the families of the Seattle Children's Hospital (SCH) multidisciplinary PWS clinic, and the hardworking clinicians that care for these families. Drs. Beck, Paschal, and Miller are supported in this research endeavor by an SCH Academic Enrichment Fund award.

Identifying Key Proteins in Prader-Willi Syndrome through Graph Learning on a Protein-Protein Interaction Network

Presenting Author: Kunal Bham¹

Additional Authors: Manju Anandakrishnan², Cathy H. Wu^{2,3}, and Karen E. Ross³

Institution(s): ¹Thomas Jefferson High School for Science and Technology, Alexandria, VA, USA; ²University of Delaware, Newark, DE, USA; ³Georgetown University Medical Center, Washington, DC, USA

Abstract:

Purpose. Despite the abundance of research on Prader-Willi Syndrome (PWS), there is a lack of understanding of the molecular mechanisms underlying the disease. The goal of this research is to deepen our molecular understanding of PWS, uncover novel insights into its mechanisms, and potentially guide the development of targeted interventions and therapies.

Background. PWS is a rare genetic disease associated with endocrine disruption, developmental and neurological problems, physical challenges, and intellectual and behavioral dysfunction. Symptoms include hyperphagia, obesity, cognitive delay, growth hormone deficiency, hypogonadism, and skeletal abnormalities. Although there is no cure, treatments are available for symptom mitigation. PWS is caused by genetic defects, usually deletion or uniparental disomy (UPD), in an imprinted, paternally expressed region of chromosome 15, q11.2-q13. This region contains a cluster of snoRNAs (SNORD116) that has been implicated in PWS. snoRNAs can have wide-ranging effects on protein expression; however, in this case, the specific targets are unknown. Moreover, because proteins interact in a complex network, changes in the expression or activity of one protein can have far-reaching and unexpected effects even on indirectly connected proteins. Graph learning techniques that calculate vectorized embeddings for proteins in a network based on the structure and strength of their connections can provide insight into these effects. Here, we utilize graph learning to analyze the effects of changes in gene expression in PWS on a human protein-protein interaction network.

Methods. Our approach is an adaptation of the GeneEMBED method (PMID:36268052). Publicly available gene expression data on neurons derived from dental pulp stem cells taken from control and PWS subjects (GEO ID: GSE178687; PMID: 34776864) was represented as edge weights in a human protein-protein interaction network from STRING. The edge weights were calculated as the sum of the mean and minimum gene expressions of the two proteins. Four networks were constructed: control, PWS-deletion, and PWS-UPD with and without Autism Spectrum Disorder (ASD). Utilizing the GraphWave algorithm, deterministic embeddings were generated for the protein nodes within the networks. To effectively remove noise, Principal Component Analysis (PCA) was subsequently employed on the control and PWS-subgroup embedding pairs, reducing their dimensions from 100D to 2D. Furthermore, to pinpoint the proteins most significantly impacted by the PWS subgroups, cosine difference tests were conducted between the control

and PWS subgroups embeddings. From the cosine differences, a list of the 10 most impacted proteins for each PWS subgroup was compiled. The function of these proteins was investigated by a literature search.

Results. There were 23 unique proteins among the top 10 most impacted proteins in the three PWS subgroups (7 proteins were identified in two subgroups). Two proteins have a known connection to PWS: the peptidase PCSK2, whose expression is decreased in PWS, and TMEM184B, whose gene shows reduced methylation in PWS. Sixteen proteins are implicated in obesity, adipogenesis, and/or related metabolic dysfunction, which are hallmark phenotypes of PWS. Finally, we identified three proteins involved in synthesis or processing of N- and O-linked glycans. Disrupted protein glycosylation has not been associated with PWS to date, so it is an intriguing area for follow-up studies.

Conclusions. The findings of this study provide novel insights into the molecular mechanisms underlying PWS by highlighting specific proteins that are significantly impacted in individuals with the syndrome. By deepening our understanding of the key proteins associated with PWS, this research contributes to the broader goal of advancing personalized interventions and therapies for individuals affected by this complex genetic disorder. Further studies are warranted to elucidate the precise roles of these proteins in PWS pathogenesis and to explore their potential as therapeutic targets.

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Swallowing function, chokings and aspirations in infants with Prader-Willi Syndrome: data from the Global Prader-Willi Syndrome Registry

Presenting Author: Pascale Borensztein¹

Additional Authors: Jessica E. Bohonowych², Theresa V. Strong², Caroline Richard¹, Camille Banh¹

Institution(s): ¹OT4B SAS, 26 rue Vignon, 75009 Paris France; ²Foundation for Prader-Willi Research, California, United States

Abstract:

Infants with Prader-Willi syndrome display severe poor motor oral skills that may cause life-threatening complications such as aspiration pneumonia and choking. Using data from the Global PWS Registry, we analyzed the links between the results of swallow studies and the risk of aspiration or choking.

Methods: Data, reported by caregivers who completed the Pulmonary Survey in the Global PWS Registry between May 2015 and February 2023, were analyzed using descriptive statistics. All analyses were focused on infants aged [0-2[years.

Results: There were 116 patients aged 0-2 years at the time the Pulmonary Survey was filled in. Among these patients, 54.3 % had a swallow study which was performed before the age of 1 year in 76.2 % of cases. The swallow study was reported as abnormal in 57.1% of the cases (N=36). When the swallow study was abnormal, 50.0% of children displayed episodes of choking or aspiration. When the swallow study was normal (N=24), only 8.3 % of infants had episodes of choking or aspiration. When clinical symptoms of choking or aspiration were reported, this was associated with an abnormal swallow study in 85.7% of cases.

Conclusion: These data confirm the high prevalence of sucking and swallowing disorders in infants with PWS aged 0-2 years and highlight the importance of performing swallow study in this population. They show the high proportion of “subclinical dysphagia” i.e. without evident clinical symptoms whereas displaying clinical symptoms is highly associated with abnormal swallow study.

Acknowledgements/Funding: NA

Glucose Homeostasis in Patients with PWS

Presenting Author: Neil M. Cowen

Additional Authors: Ava Peyton, Kristen Yen, Patricia Hirano

Institution(s): Soleno Therapeutics

Abstract:

Introduction: Patients with Prader-Willi syndrome (PWS) may be at a higher risk for developing Type II Diabetes Mellitus. Determination of fasting insulin and glucose levels is a significant challenge if the patient with PWS has developed hyperphagia. Instead, the use of glycosylated hemoglobin (HbA1c), which effectively assesses the patient's average glucose level over a three-month period leading up to the measurement, is a reliable measurement for glycemic status that does not require a fasting blood sample.

Methods: Glycemic status of individuals who were screened for inclusion in the DESTINY PWS clinical study (C601) was assessed using glycosylated hemoglobin (HbA1c). The American Diabetes Association has specified ranges of HbA1c values associated with three glycemic status categories: normoglycemia, prediabetes and diabetes. Using these ranges, data will be classified for all screened participants into one of these three categories and summarized by key demographic and baseline characteristics. In addition, a literature review will be conducted to identify studies about altered insulin and glucose activity in people with PWS.

Results: Glucose homeostasis (HbA1c values) in patients with PWS screened for the DESTINY PWS study will be summarized in relation to the patients' age, PWS genetic subtype, baseline diabetic status, and medications, particularly psychiatric medications.

Acknowledgements/Funding:

The authors thank the DESTINY PWS program participants and their caregivers, the study investigators, and their study staff for their contributions to the study data analyzed. The authors would also like to acknowledge Jing Gong for her support.

Funding for this study was provided by Soleno Therapeutics.

Exploring the Role of Social Cognition in Autism Behaviours within Prader-Willi Syndrome

Presenting Author: Sarah-Marie Feighan¹

Additional Authors: Ciara Molloy¹, Megan Young¹, Áine McNicholas¹, Linda Lisanti, , Clare Kelly², Eleisa Heron¹, Edna Roche³, Louise Gallagher^{4,5,6}

Institution(s): ¹ Department of Psychiatry, Trinity College Dublin, Ireland; ² Trinity Institute of Neuroscience, Trinity College Dublin, Ireland, ³ School of Medicine, Trinity College Dublin, Ireland., ⁵ The Hospital for SickKids, Toronto, Canada; ⁶ The Centre for Addiction and Mental Health, Toronto, Canada; ⁷ Dept. Of Psychiatry, Temerty Faculty of Medicine, University of Toronto, Canada

Abstract:

Background: The behavioural phenotype of individuals with PWS encompasses autism-like behaviours and challenges in social functioning (Bennet et al., 2015). However, the underlying nature of autism behaviours within the context of PWS remains poorly understood, and the link between social cognition and social functioning in PWS has not been thoroughly investigated. This study aimed to assess social motivation in individuals with PWS and explore its relationship with autism behaviours using a well established eye-tracking paradigm, the Face Pop Task (Gliga et al., 2009), that has been used in consortium studies investigating stratification biomarkers in autism (Loth et al., 2016).

Methods: Autism traits and diagnosis were assessed using the Autism Diagnostic Observation Schedule 2 (ADOS-II), Autism Diagnostic Interview–Revised (ADI-R) and the Socialisation scale from the Vineland Adaptive Behaviour Scales (VABS-II) in the PWS cohort. Social attention was investigated using the Face Pop Task, which tests whether participants automatically orient to faces and whether they prefer to look at faces compared to non-social stimuli. A linear mixed-effects model was used to investigate the effect of group (PWS vs COM), stimulus category (face, bird, phone, noise, car) and their interaction on the dependent variable, proportion of dwell time, i.e. the relative time spent looking at a stimulus.

Results: The interaction between the stimulus category and group was significant, $F(4, 1533) = 25.4$, $p < .001$. with posthoc analyses of the interaction effect revealing that the PWS group spent significantly more time looking at face stimuli than the COM group ($M_{diff} = -.778$, $p = .014$, 95% CI $[-1.397, -.160]$). ADOS severity scores in the PWS group were significantly negatively correlated with time spent maintaining attention to face stimuli ($r_s = -.50$ $p = 0.015$), suggesting PWS participants with higher autism severity scores looked at face stimuli less.

Discussion: Participants with PWS spent relatively more time looking at faces than COM participants. However, PWS participants with higher levels of autism severity looked at faces less, suggesting reduced social attention may have a role in autism behaviours within PWS.

Acknowledgements/Funding: Thank you to FPWR for providing the funding that made this research possible. Thank you to the Prader-Willi Syndrome Association of Ireland for all their support and encouragement. Thank you, especially to the families and individuals with PWS who gave us their valuable time participating in this study.

Development of a Conjugated Linoleic Acid Weight Loss Food Product for PWS patients

Presenting Author: ¹Deborah J. Good, Ph.D.

Additional Authors: ²Sean O'Keefe, Ph.D., ¹Shadi Ariyanfar, M.S., and Daniel Miglia

Institution(s): ¹Department of Human Nutrition, Foods, and Exercise, and ²Department of Food Science, Virginia Tech

Abstract: Conjugated Linoleic Acids (CLA) are naturally occurring omega-6 derived fatty acids which previously have been shown to have weight loss and muscle building capacity in both mice and humans. We recently showed that the mouse model of Prader-Willi syndrome, lacking the *Snord116* gene also responds to CLA, even while eating a high fat diet. Additionally, a mouse model lacking the gene *Nhlh2*, which whose expression levels are reduced in human pluripotent stem cells lacking *Snord116*, shows both weight loss and non-statistical improvement in testicular development. These research findings are the basis for two US and one international patent. While CLA pills are available over the counter, they are difficult to swallow because of their large size and require 4-5 pills per day for effective weight maintenance. Using funding from the Center for New Ventures at Virginia Tech, we are developing a food product that will contain 4-5 grams of CLAs per serving. Customer interviews found that most individuals would prefer a breakfast, snack, or pre-/post-workout product in a bar or other convenient form. Interviews with medical personnel, especially those who treat patients with PWS, concluded that total calorie count is important, as are taste, and convenience. Medical personnel stated they would recommend scientifically proven products to their patients. Two FPWR board members who were interviewed suggested that adult patients with PWS might be better targets for the food product, but either way, parents tend to decide on food for their children. Development of the food product is underway with plans to have samples available at the conference for testing by parents and their children. Future work includes taste and food sensory studies, with surveys within the Virginia Tech food Sensory Evaluation laboratory, as well as continuing the basic research studies in mice to understand more about the mechanisms of weight loss and testicular development by CLA.

Acknowledgements/Funding: This original work was funded by generous grant funding from BASF Corporation (Ludwigshafen am Rhein, Germany) and more recently by LAUNCH (Center for New Ventures, Virginia Tech), and the CALS Strategic Plan Advancement Seed Award (College of Agriculture and Life Sciences, Virginia Tech).

Investigating the Epigenetic Regulator Smchd1 as a Potential Therapeutic Target for the Treatment of PWS

Presenting Author: Megan Iminoff^{1,2}

Additional Authors: Tamara Beck^{1,2}, Andrew Keniry^{1,2}, Kelsey Breslin^{1,2}, James Murphy^{1,2}, Marnie Blewitt^{1,2}

Institution(s):

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²The Department of Medical Biology, The University of Melbourne, VIC, Australia

Abstract:

Smchd1 is an epigenetic repressor, targetable by small molecules and known to play a role in silencing PWS cluster genes on the maternal allele in mice. We are working on proof-of-concept data for gene reactivation using SMCHD1 as a target for potential therapy. Removal of *Smchd1* *in vitro* has been shown to result in activation of the silent maternally inherited copy of PWS genes at the proximal end of the cluster. Using a reporter mouse model for *Magel2* expression we have shown that in line with previous data deletion of *Smchd1* can result in reactivation of maternally inherited PWS genes *in vivo* in the brain, specifically in the hypothalamus. Furthermore, in hypothalamic tissue we see reactivation of PWS genes not only from the proximal end of the cluster but from the distal end as well, with seemingly no effect on *Ube3a* expression. To determine whether the level of reactivation we observe is sufficient to rescue disease phenotypes in the *Magel2* paternal-null model we have begun behavioural and motor testing using mice that have had *Smchd1* deleted in the brain, looking at features of PWS within the cohort. From this we aim to increase understanding of the molecular mechanisms at work within the PWS cluster and to confirm viability of SMCHD1 as a target for epigenetic therapy of PWS.

Acknowledgements/Funding: Lorenzo and Pamela Galli Medical Research Trust, FPWR, PWRFA, WEHI

Needs Assessment Survey to Study the Knowledge of Parents of Children with Prader-Willi Syndrome in Tertiary Care Centre in Southern India

Presenting Author: Dr Jahnvi Muralikrishnan

Additional Authors: Dr N Kavitha Bhat

Institution(s): Aster CMI Multispeciality hospital, Bengaluru, India

Abstract

Background: Prader Willi syndrome (PWS) is a rare multisystem disorder. The complexity of the disorder makes multidisciplinary care along with patient education essential for effective management¹. There is a lack of structured Indian specific patient education resources in view of the rarity of the condition. To prepare effective patient education materials, it is important to assess the level of knowledge and determine the needs of the patients.

Study methods: A 49-item needs assessment survey was conducted to understand the needs of the caregivers of children with PWS. The forms were made available through the Google Forms platform. The primary caregivers of the children aged between 1- 18 years with a confirmed genetic diagnosis of PWS were included in the survey. A descriptive analysis of the responses was done.

Results: Caregivers of 15 children with Prader Willi Syndrome participated in the survey. The questionnaire was answered by either parent for each child. The survey consisted of 8 sections. The mean age at diagnosis was 35 months. All the respondents were aware that PWS is a genetic disorder. The salient results are depicted below:

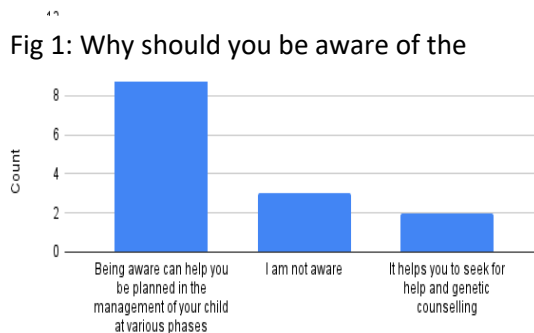


Fig 2: How will you handle skin picking issues?

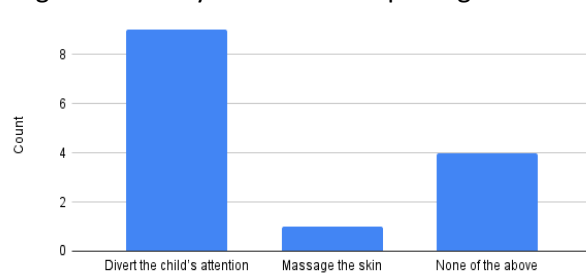


Fig 3: PWS spreads through family

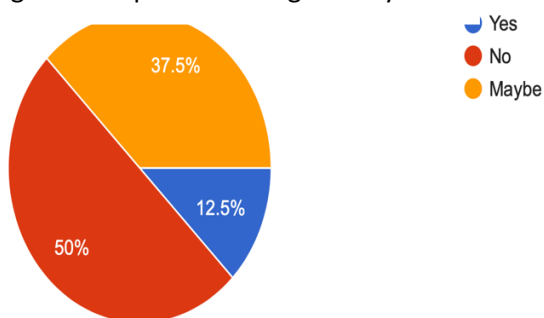


Fig 4: Weight gain in PWS is inevitable

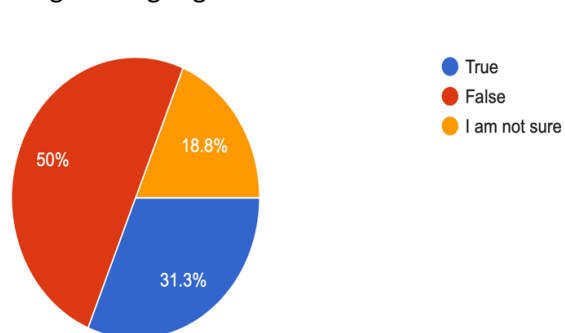
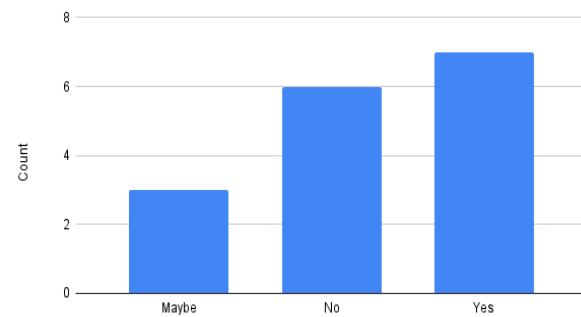
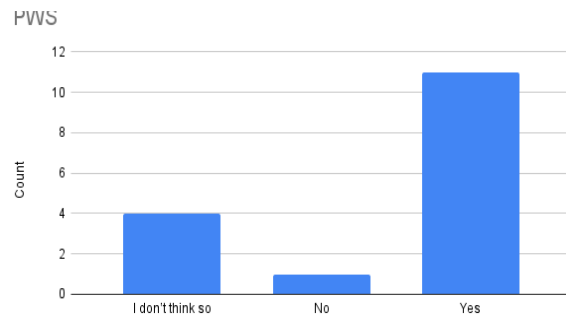


Fig 5: Growth hormone should be started in PWS

Fig 6: Do you know about food security measures?



Conclusion: Through this survey, the key areas identified are practical tips for management of feeding issues in infancy, techniques to improve the muscle strength, ways to control the excessive eating and obesity and behavior management issues.

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Acknowledgements/Funding: N/A

Metabolic and Bariatric Surgery for Obesity in Prader-Willi Syndrome: Systematic Review and Meta-Analysis

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Institution(s):

Drexel University College of Medicine, St. Christopher's Hospital for Children, Reading Hospital, Tower Health

Abstract:

Background

Obesity is the leading cause of morbidity and mortality in patients with Prader-Willi Syndrome (PWS).

Objectives

To compare changes in body mass index (BMI) after metabolic and bariatric surgery (MBS) for the treatment of obesity (BMI ≥ 35 kg/m²) in PWS.

Methods

A systematic review of MBS in PWS was performed using PubMed, Embase and Cochrane Central identifying 254 citations. Sixty-seven patients from 22 articles met criteria for inclusion into the meta-analysis. Patients were organized into three groups: laparoscopic sleeve gastrectomy (LSG), gastric bypass (GB), and biliopancreatic diversion (BPD).

Results

No mortality within one year was reported in any of the three groups after a primary MBS operation. All groups experienced a significant decrease in BMI at 1 year with a mean reduction in BMI of 14.7 kg/m² (p-value <0.001). The LSG groups (n=26) showed significant change from baseline in years 1, 2, and 3 (p-value at year three=0.002) but did not show significance in years 5, 7, and 10. The GB group (n=10) showed a significant reduction in BMI of 12.1 kg/m² in the first two years (p-value=0.001). The BPD group (n=28) had a significant reduction in BMI through seven years with the average reduction of 10.7 kg/m² (p-value=0.02) at year seven.

Conclusion

Individuals with PWS who underwent MBS had significant BMI reduction sustained in the LSG, GB, and BPD groups for three, two, and 7 years respectively. No deaths within one year of these primary MBS operations were reported in this study or any other publication.

Acknowledgements: The authors would like to acknowledge Alexandra Short for her assistance in the librarian mediated search, Tom Wasser, PhD for statistical support, and Lisa Baro RN, CSN for acquisition of MBSAQIP 2020 data.

Funding: None

Comprehensive Cardiovascular Risk Assessment in Children and Adolescents with Prader-Willi Syndrome Compared to Lean Controls

Presenting Author: Kathryn Obrynba MD

Additional Authors: Ayse Bulan, MD, Robert Hoffman MD, Kathryn Anglin RN MSN, Andrew Tran MD,

Institution(s): Nationwide Children's Hospital, Columbus, Ohio

Abstract:

Objective:

Prader Willi Syndrome (PWS) is the most common cause of syndromic obesity (OB) in humans which affects 1/10,000- 1/30,000 live births. Adults with PWS have chronic low-grade inflammation associated with obesity evidenced by increased plasma pro-inflammatory cytokines accompanied by microvascular and macrovascular dysfunction. However there has not been a comprehensive vascular risk assessment in children and adolescents with PWS to determine whether this association is present earlier in life. The objective of this study is to conduct a comprehensive vascular risk assessment of children and adolescents with PWS compared to lean, non-PWS controls.

Design and Subjects:

In this cross-sectional study, thirteen subjects with genetically confirmed PWS, 13.6±3.1 years (mean ± SD), body mass index (BMI) 23.7±7.2 kg/m² were compared with ten age- and gender-matched lean controls, 13.7±2.1 years, BMI 20.8±2.8 kg/m². To assess vascular function, arterial stiffness (Augmentation Index, AI) was examined. Pro-inflammatory markers (hs-CRP and IL-6), adiponectin, and lipid profile were obtained. In addition, truncal and total fat mass determined by DXA were correlated with measures of vascular function, proinflammatory markers, adiponectin, and lipid profile. Mann-Whitney U Test was used to compare results in two groups. Spearman correlation coefficient was used to determine relationships between variables.

Results:

Arterial stiffness (AI) was higher in subjects with PWS 15.5 (-12.00-60.00), median (IQR), vs controls 0.5 (-4.5-10), p=0.050. Subjects with PWS had significantly elevated CRP 2.38 mg/dl (1.56-4.91) and LDL 103 mg/dl (63-134) vs controls 0.3 mg/dL (0.3-0.44) and 69.5 mg/dL (57-86.5), p=0.000, p=0.008. PWS subjects had higher total body fat 49.1% (40.2-54) and elevated truncal fat 45.3% (35.2-51.1) vs controls 33.4% (25-34.1) and 28.5% (20.6-29.8), p=0.002, p=0.004, despite no significant difference in BMI. Adiponectin was inversely related to truncal fat (r=-0.722, p=0.002) and total body fat (r=-0.650, p=0.005) in PWS subjects.

Conclusion:

Children and adolescents with PWS have increased cardiovascular risk as demonstrated by increased inflammation, arterial stiffness and LDL compared to control subjects, even without differences in BMI. This may be due to an abnormal body composition, with increased fat mass and lower lean mass seen in PWS individuals.

Acknowledgements/Funding: The Research Institute at Nationwide Children's Hospital

Magel2 Deficient Mice Exhibit a Diminished Hedonic Drive During Adolescence

Presenting Author: Richard M. O'Connor

Additional Authors: Piya Pillai and Elley Ishikawa

Institution(s): Nash Family Department of Neuroscience, Icahn School of Medicine at Mount Sinai

Prader-Willi Syndrome (PWS) is a neurodevelopmental disorder that is recognized as the most common cause of life-threatening childhood obesity. Initially, individuals with PWS show a failure to thrive in the neonatal period resulting in impaired growth. This is followed by insatiable hyperphagia typically emerging between 3-8 years of age. Food-seeking behaviors can become so extreme that caregivers cite a lack of effective therapeutics to attenuate this behavior as an urgent unmet medical need and a priority endpoint for any future efforts for medications development. PWS arises from dysregulated silencing to several genes on a portion of human chromosome 15. The loss of any single gene does not recapitulate the entire spectrum of PWS symptoms, suggesting that each affected gene makes a discrete contribution. Obesity-related symptoms such as hyperphagia, weight gain, increased fat mass and reduced levels of voluntary activity have been linked specifically to silencing of the *Magel2* gene. However, the impact of *Magel2* absence on food-related motivation is still not fully understood. Homeostatic and hedonic feeding are driven by distinct yet overlapping brain circuits, with the lateral hypothalamus (LH) a key node for both. *Magel2* absence profoundly alters the release and function of neuropeptides involved in feeding, including orexin and oxytocin. As such we hypothesized that mice lacking functional expression of *magel2* would show disruptions to homeostatic and hedonic feeding behaviors.

To assess homeostatic feeding, we food-restricted *Magel2* null mice and their wild-type littermates for 24 hours before allowing them to freely consume standard laboratory chow. The amount of food consumed was periodically assessed over a 3-hour period. To measure food-related hedonic drive we allowed the same mice to freely consume calorically dense palatable food items while fully sated. Tests were performed ~PND35 which approximately corresponds to adolescence in humans. We found *Magel2* null mice weighed significantly less than their wild-type counterparts on PND35. *Magel2* null mice consumed similar amounts of laboratory chow during the refeeding session indicating homeostatic feeding remained intact in these animals. However, *Magel2* null mice consumed significantly less calorically dense palatable food compared to wild-type animals suggesting impaired hedonic drive during this adolescent period. Thus, *Magel2* expression leads to distinct change to food-related motivation that is dependent on the palatability of the food source. Future research aims include continuing to assess homeostatic and hedonic drive in *Magel2* mice at different stages of development and record weight gain during unrestricted access to calorically dense food items. We further aim to establish the underlying hypothalamic disruptions emerging from a lack of *Magel2* expression that drive such altered food motivation and whether pharmacological tools can reverse such pathology.

Acknowledgements/Funding: Foundation for Prader-Willi Research

Reactivation of *SNRPN* Gene via Reduction of Repressive H3K9me2 Histone Marker and Demethylation in Prader-Willi Syndrome Using *In Vitro* Model

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Additional Author: Balachandar Vellingiri^{1,3}, Dhivya Venkatesan¹

Institutions

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3. Stem Cell and Regenerative Medicine, Translation Research, Department of Zoology, School of Basic Sciences, Central University of Punjab (CUPB), Bathinda- 151 401, Punjab, India.

Abstract

Prader - Willi syndrome (PWS) is a neurogenetic disorder, which is mostly caused by loss of expression of in the paternally imprinted genes in chromosome 15q11-q13 region. Nevertheless, there has been no clear report of the epigenetic mechanism for cause of PWS. The aim of the present study is to investigate the chromosome alterations and to find whether the histone markers are associated with the gene silencing within chromosome 15q11-q13, and could reduce repressive histone marker which could activate silent gene expression in PWS. To address this challenge, treatment of PWS lymphoblastoid cells (LCLs) with 5-Aza-2'-deoxycytidine, and then bisulfite sequence using, CHIP assay was performed using specific histone markers for H3K9me2 and H3K4me2. Furthermore, RT-PCR was carried out for study of gene expression. study clearly indicates the reduce methylation of the CpG site at exon 1 *SNRPN* gene with inhibitor of 5 aza-dC. The ChIP assay results disclosed that no amplification was detected for *SNRPN* at exon 1 with H3k4me2 immunoprecipitated DNA, whereas clear amplification was detected for *SNRPN* at exon1 with H3K9me2 immunoprecipitated DNA in the untreated cells of PWS and amplification was also found in the positive control of GAPDH. Further, in the present study, the RT-PCR results showed that *SNRPN* gene was highly expressed at exon 1 after the treatment with 5-aza-dc. In conclusion, the present study suggests that the both methylation and histone methylation play a significant role in gene silencing in 15q11-q13 and that silenced genes in PWS can be reactivated by drug treatment of 5-aza-dc.

Acknowledgments

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Funding: None

The Role of *NPAP1* Gene Variants in Prader-Willi Syndrome Pathophysiology

Presenting Author: Alessia Polito^{1,2}

Additional Authors: Hanako Tsushima^{1,4}, Angelo Serani¹, Gianluca Como^{1,3}, Alice Melloni^{1,2}, Alessio Vizzoca¹, Nunzia Colonna Romano¹, Valter Tucci^{1*}

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Abstract: We identified a new evolutionary mechanism involving the human-specific nuclear pore complex-associated protein 1 (NPAP1) in lipid accumulation and circadian rhythms alteration in Prader-Willi Syndrome (PWS). Since *NPAP1* is a paternally- expressed gene (PEG) with no orthologue in the mouse genome, we overexpressed the modern variant WQT and the ancient variant WET in mouse cells lacking paternal *Snord116*. The overexpression revealed that the ancient haplotype may have a role in adipogenesis and lipids uptake pathways and in altering the circadian oscillation clock genes. On the other hand, the modern WQT variant reduced lipid accumulation. 3D protein modeling suggested that conformational changes of the WET NPAP1 may alter the interaction with other molecular determinants and its role in regulating lipid accumulation. Looking at the functional mechanism behind the *Snord116*-*NPAP1* relation, we analyzed the level of *NPAP1* after the transfection of several *Snord116* variants in human cells, and only the *Snord116-6* downregulated the endogenous *NPAP1* mRNA expression. Interestingly, we found that none of the *Snord116* we tested altered the expression of a reporter gene driven by the promoter or the 3'UTR sequence, suggesting that the coding portion of the mRNA is the putative target of the *Snord116* regulation. Since the NPAP1 functions are poorly characterized, we investigated the protein complexes in which it participates by co-immunoprecipitation followed by mass spectrometry. Moreover, mouse embryonic stem cells in which WQT NPAP1 was cloned in the human orthologue region revealed the deregulation of a representative list of PEGs and MEGs. These mESCs were used to generate a novel humanized mouse model. A preliminary characterization revealed that heterozygous *NPAP1* mice exhibit no significant changes in weight and size. They present with regular viability and normal locomotor activity. However, there was a notably low frequency of *NPAP1*^{+/+} mice compared to the heterozygous mice, and the female mice were capable of mating and becoming pregnant but unable to deliver their pups at full term. Finally, to correct the *NPAP1* expression in our *in vivo* and *in vitro* models, we are currently testing a novel epigenetic writer. This new CRISPR-based tool can modify the methylation of the imprinting control region of the PWS locus in the *NPAP1*-mESCs. This epigenetic writer can potentially revert the phenotypes induced by the *NPAP1*/*Snord116* imbalance in our animal models. Furthermore, it may assist in the development of a therapeutic strategy for PWS patients.

Acknowledgements/Funding: Italian Institute of Technology (IIT)

Characteristics of Baseline Polysomnograms and Resulting Interventions in Patients with Prader-Willi Syndrome Prior to Initiation of Growth Hormone Therapy

Presenting Author: Sani M. Roy MD

Additional Authors: Amy Trejo BS, Deborah Rafferty MSc, Keisha Shaheed DO

Institution(s): Cook Children's Medical Center, Fort Worth, TX

Abstract:

Background: Early initiation of growth hormone (GH) in Prader-Willi syndrome (PWS) is widely endorsed due to its positive benefits on growth, tone, and strength. However, there are known risks of GH in PWS, including reports of childhood death, related to respiratory complications; thus, general consensus recommendations have been to obtain a baseline polysomnogram (PSG) prior to starting GH. PSGs are time-intensive, resource-heavy, costly, and difficult to expedite, particularly in infants < age 6 months, which can lead to delays in starting GH. A critical review is needed of the characteristics of baseline PSGs and resulting interventions in order to determine the utility of baseline PSGs prior to starting GH in PWS.

Methods: The current study is a retrospective chart review of patients with PWS who have been treated at Cook Children's Medical Center in Fort Worth, Texas since March 1, 2018. Patients were included in the study if they had a baseline PSG performed at age ≤ 24 months prior to the initiation of GH. Demographics, baseline PSG characteristics, sleep quality symptoms, and recommended interventions were assessed. Differences in PSG results and symptoms noted prior to baseline PSG were compared based on the post-PSG intervention. Due to unequal variance, Welch's *t*-Tests were performed to assess for significance. Chi-Squared Tests of Independence using the likelihood ratio were used to compare sleep quality symptoms between intervention groups. Data was collected and stored in REDCap, and analyses were performed using Excel and SPSS. The study was approved by the Cook Children's IRB.

Results: Eighteen patients were included in the current study (57.9% female; 38.9% Hispanic White, 27.8% Black and 27.8% non-Hispanic White; 50.0% with PWS due to paternal deletion). On average, patients were 8.08 months old (range: 1.58 – 22.13) at the time of the baseline PSG. As a result of baseline PSG outcomes, 44.4% ($n = 8$) of patients received oxygen (O₂) therapy and 55.6% ($n = 10$) received no intervention. No patients received pressure support. When comparing patients who received O₂ therapy to those who did not, the O₂ therapy group had a significantly higher obstructive apnea hypopnea index (oAHI; $m = 10.6$ vs. $m = 2.67$, $t(7.29) = 2.38$, $p = 0.048$), number of desaturations $>3\%$ ($m = 136.1$ vs. $m = 36.6$, $t(7.38) = 2.50$, $p = 0.039$), and desaturation index ($m = 20.6$ vs. $m = 5.1$, $t(7.26) = 2.47$, $p = 0.042$). There were no significant differences in other PSG indices when comparing infants who received O₂ therapy compared to those who did not ($ps > .073$). Patients who received O₂ therapy were more likely to snore, $\chi^2(1, N = 18) = 5.83$, $p = .016$, and have apnea, $\chi^2(1, N = 18) = 7.98$, $p = .005$. Most ($n = 17$, 94.4%) patients started GH following the baseline PSG at a mean age of 10.93 months (median: 6.41 months, range: 2.01 – 32.35 months).

Discussion: The only notable intervention following baseline PSG in children with PWS ≤ 24 months of age and prior to GH initiation was oxygen therapy. No infants received an ENT diagnostic or surgical intervention, and no infants were placed on pressure support based on baseline PSG prior to starting GH. Snoring and witnessed apnea may be key symptoms that

predict a higher oAHI and the need for oxygen intervention. Notably, our data found a higher mean oAHI than the few other studies investigating infant PSG data in PWS. Future studies are being planned to investigate for trends across multiple sites and with detailed comparison of baseline PSG to follow-up PSG following GH initiation. As such, these studies will provide further insights into the utility of baseline PSG in the first 24 months of life as a prerequisite to starting GH therapy.

Comprehensive Assessment of Body Composition, Physical Activity, and Health Markers in Pediatric Prader-Willi Syndrome

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Institution(s):

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Abstract:

Despite the well-documented clinical manifestations of Prader-Willi Syndrome (PWS), there remains a lack of comprehensive research focusing on body composition, physical activity, and associated health markers specifically in pediatric individuals with PWS. This study aims to address the significant scientific gap by examining body composition, physical activity levels, and health markers in pediatric PWS, thereby providing valuable insights to optimize management strategies for this population. A holistic assessment will be conducted including children diagnosed with PWS and utilizing state-of-the-art technology, such as InBody® scanner, Fitbit® activity trackers, and digital skinfold calipers. Health markers, including metabolic parameters, will be thoroughly evaluated. By analyzing the collected data, using descriptive statistics, correlation analyses, and regression models, this study intends to reveal the relationships between body composition, physical activity, and health markers in pediatric PWS. Findings from this study will help develop management strategies to optimize outcomes and quality of life in individuals with PWS. The preliminary results of this study will be reported and discussed.

Acknowledgments/Funding: This research project was funded by the Nexus Hope Foundation.

Secondary Outcomes From a Phase 2 Double-Blind, Placebo-Controlled Signal-Detection, Proof-of-Concept Study of Pitolisant in Prader-Willi Syndrome

Presenting Author: David Seiden, MD¹

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Abstract:

Introduction: Prader-Willi Syndrome (PWS) is a complex genetic disorder with symptoms that include hyperphagia, behavioral disturbance, and excessive daytime sleepiness (EDS). There is an association between EDS and emotional/behavioral disturbances in children and young people with PWS. Pitolisant, a histamine 3 receptor antagonist/inverse agonist approved for the treatment of EDS or cataplexy in adults with narcolepsy, is being investigated as a treatment for EDS in patients with PWS.

Methods: In this phase 2, exploratory, proof-of-concept study (which was not powered to show statistical significance), patients with a confirmed diagnosis of PWS with EDS were randomized to receive higher-dose pitolisant, lower-dose pitolisant, or placebo (dose based on age group). The 11-week double-blind treatment period consisted of a 3-week titration phase and an 8-week stable-dose phase. Secondary/exploratory endpoints included change from baseline in Caregiver Global Impression of Severity (CaGI-S) for EDS, behavioral disturbance (Aberrant Behavior Checklist-2 [ABC-2]), and hyperphagia (Hyperphagia Questionnaire for Clinical Trials [HQ-CT] in conjunction with the Food Safe Zone Questionnaire [FSZQ]). The primary time frame of interest was from baseline to Week 11.

Results: Among the 65 enrolled patients (50.8% male), 34 (52.3%) were children (6 to <12 years), 19 (29.2%) were adolescents (12 to <18 years), and 12 (18.5%) were adults (≥18 years). Results are presented here for the youngest age group (additional age groups will be shown in the poster). Mean (SD) change on the CaGI-S was -1.1 (1.1) in the higher-dose pitolisant group, -1.0 (1.2) in the lower-dose pitolisant group, and -0.5 (1.2) in the placebo group. Mean reduction was greater for higher-dose pitolisant compared with placebo on all ABC-2 domains (irritability, social withdrawal, hyperactivity/noncompliance, inappropriate speech, stereotypic behavior). Mean (SD) HQ-CT score decreased with higher-dose pitolisant (-2.0 [2.8]) and lower-dose pitolisant (-2.5 [6.2]) and increased slightly with placebo (0.1 [3.4]). The most common adverse events in pitolisant-treated patients (doses pooled) were irritability (17.4%) and anxiety (13.0%).

Conclusions: In children with PWS, pitolisant reduced EDS severity (as rated by caregivers), behavioral disturbances, and hyperphagia. Improvement was also observed on the primary endpoint, change from baseline in the score on the parent/caregiver version of the Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD), which is reported separately. An upcoming phase 3 clinical trial will further evaluate the efficacy and safety of pitolisant in patients with PWS and EDS aged 6 years and older.

Acknowledgements/Funding: This study was sponsored by Harmony Biosciences.

Effects of Dietary Fibers on Hyperphagia, Metabolic Markers, and the Gut Microbiota in Individuals with Prader–Willi Syndrome: Preliminary Findings

Presenting Author: Qiming Tan

Additional Authors: Flavio T. Vieira, Edward C. Deehan, Mohammadreza Pakseresht, Peng Ye, Hein Min Tun, Karen Madsen, Catherine Field, Andrea M. Haqq

Institution(s): University of Alberta

Abstract:

Background: Children with Prader–Willi syndrome (PWS) have a distinct gut microbiota profile. The observed microbial alterations in PWS have been associated with hyperphagia and metabolic outcomes. Purified dietary fibers may induce satiety and promote beneficial changes in the gut microbiota. The objectives of this study were to investigate the effects of a high-dose fiber supplement mix on hyperphagia, metabolic markers, and gut microbiota in individuals with PWS.

Methods: In a cross-over trial, participants were randomly assigned to receive a daily 35g fiber mix (oligofructose, resistant maltodextrin, acacia gum, and resistant starch type 2) or placebo for 3 weeks, followed by a 4-week washout and the alternate treatment for another 3 weeks. Hyperphagia was measured by the hyperphagia questionnaire for clinical trials (HQ-CT). Fasting glucose, insulin, glucagon-like peptide-1 (GLP-1), and high sensitive-C reactive protein (hs-CRP) were assessed. Homeostatic model for assessment of insulin resistance (HOMA-IR) was calculated. Fecal samples were used to determine change in the relative abundance of *Bifidobacterium*. Data were preliminarily analyzed using Paired-Samples T-test or Wilcoxon test based on the results of Shapiro-Wilk normality test.

Results: We enrolled 13 participants in the study (7 females; median age 12.0 years). Group-level responses to the intervention showed no differences in HQ-CT, HOMA-IR, GLP-1, and hs-CRP. However, at the individual level, there was an average reduction of 44.4% in the HQ-CT total score ($n = 7$) and an average reduction of 25.0% in the HOMA-IR ($n = 6$) following the fiber supplementation. The relative abundance of *Bifidobacterium* increased from 3.17% (IQR 0.85–6.45%) to 10.33% (IQR 4.87–18.05%) at week 3.

Conclusions: No differences were detected in hyperphagia, insulin resistance, satiety hormone, and inflammation at the group level post-fiber intervention in PWS. However, the response was highly individualized, with some participants experiencing improvements in hyperphagia and insulin resistance. The fiber intervention stimulated the growth of *Bifidobacterium*, gut microbes shown to play an important role in maintaining gut homeostasis and health. Ongoing analyses are exploring the effect of fiber supplementation on other health-related markers and gut microbiota features, as well as features to explain the personalized responses.

Keywords: Prader-Willi syndrome, hyperphagia, dietary fiber, gut microbiota

Acknowledgements/Funding: This work was supported by the Weston Family Microbiome Initiative, Women and Children's Health Research Institute, and Foundation for Prader-Willi Research.

Psychiatric Medication Use in Patients with PWS

Presenting Author: Annabelle Tiller

Additional Authors: Patricia Hirano, Neil Cowen

Institution(s): Soleno Therapeutics

Abstract:

Introduction: Patients with Prader Willi syndrome (PWS) are often prescribed standard psychiatric medications to treat common behavioral manifestations of the syndrome. Many may be taking more than one psychiatric medication. While there are no currently approved medications to manage any of the behavioral symptoms associated with PWS, patients may take one or more medications that have not been evaluated in clinical trials in people with PWS. Minimal data regarding psychiatric medication use have been published for typical clinical trial participants with PWS. This assessment will summarize the psychiatric medication use data in participants who were screened for the Phase 3 DESTINY PWS study of DCCR.

Methods:

Prior medication use data from Soleno Therapeutics' Phase 3, DESTINY PWS program of DCCR will be tabulated to determine the number (%) of participants who have taken or are taking at least one psychiatric medication at the time of screening. In this sub-population, psychiatric medications will be categorized by class (antidepressants, antipsychotics, stimulants, excessive daytime sleepiness drugs, other). The data will be summarized by the number and/or percent of patients taking medications by class and individual medications per class. Additional summaries may include analyses by key demographic parameters (age at start of medication use, sex, and/or PWS genetic subtype), number of participants taking more than one medication and/or more than one medication per class by one or more demographic parameters. These may be grouped into a combined category of one or more medications or classes, where reasonable.

Results:

A total of 156 people with genetically confirmed PWS ages 4 and older and with hyperphagia were screened in the DESTINY PWS program. Prior psychiatric medication use of patients with PWS randomized in DESTINY PWS will be summarized by drug class, average start age on drug, number of medications taken at once, and medications across different drug classes. Additional data will be summarized.

Acknowledgements/Funding: The authors wish to thank the DESTINY PWS program participants and their caregivers, the study investigators, and their study staff for their contributions to the study data analyzed. The authors would also like to acknowledge Jing Gong for her support. Funding for this study was provided by Soleno Therapeutics.

Experiences and Support Needs of Unaffected Siblings and Parents of Individuals with Prader-Willi Syndrome- Findings from a Two-Stage Qualitative Study

Presenting Author: Meghana Wadnerkar Kamble¹

Additional Authors: Jen Dawe^{1,2} and Karen Bunning¹

Institution(s): ¹School of Health Sciences, University of East Anglia, Norwich, Norfolk, UK;

²National Literacy Trust, London, UK

Abstract: Introduction: Healthy siblings of people with a chronic neurodevelopmental condition can experience associated stresses that impact on their personal and social development. Prader-Willi Syndrome (PWS) is a complex multi-system neurogenetic condition characterised by developmental delays, learning difficulties, life-long hyperphagia, obsession with food, and accompanying distressed behaviours that can challenge the family functioning and dynamics. Disability research exploring the unaffected sibling's perspective i.e. the sibling without PWS, has tended to rely on parental reports. This contrasts with the fact that siblings and parents have a different view on the influence that a child with learning difficulties has on their sibling. The objective of this study was to investigate the experiences and support needs of the unaffected siblings and parents of individuals with Prader-Willi syndrome. **Methods:** Unaffected siblings aged 11 years+ (N=11), and parents (N=8) from the UK were recruited using convenience snowball sampling. A combination of semi-structured online paired interviews and focus groups were used at Stage 1 to understand the experiences and support needs of unaffected siblings. Thematic discourse analysis was used to understand how the siblings and parents talked about their experiences in the context of their families. Nominal Group technique was used at Stage 2 for the online consensus meeting to prioritise the support needs. **Findings:** At Stage 1 several group specific and cross group themes emerged that centered around family and relationships, sharing experiences, relationship with food, awareness and understanding, advocacy, and accommodation and adjustments. Stage 2 findings, which were mainly based on the older siblings and parents focused on the practical aspects of support. Findings highlight the specific ways that the unaffected siblings' experience family life with a sibling with PWS and what support they would like. **Conclusions:** Findings can potentially inform the designing of appropriate interventions for sibling support and for supporting the family system. This novel study highlights the importance of family-centeredness in the context of PWS.

Acknowledgements/Funding: This research was funded by the Prader-Willi Syndrome Association UK (PWSA UK)

Treating Sleep Problems in Children with Prader-Willi Syndrome

Presenting Author: Aliana Weavers

Additional Authors: Laura Kruse, Kasey Bedard

Institution(s): The Chicago School of Professional Psychology

Abstract:

The purpose of the present study was to investigate the effects of a sleep treatment package on the frequency of night awakenings, bedtime resistance, and parent stress with the parents/caregivers of three children diagnosed with Prader-Willi syndrome (PWS) using a multiple baseline across subjects design. During baseline, participants were not exposed to any procedure. During intervention, caregivers implemented a treatment package including graduated extinction, bedtime routines, and bedtime fading without response cost. All interventions were previously successful with children diagnosed with autism spectrum disorder but had not been attempted with this population. Correct responding was defined as zero instances of night awakenings, zero instances of bedtime resistance (different for each child participant) and decreases in parent-reported stress. In addition, social validity was assessed using a Likert-style questionnaire. The results show that the treatment package had varying success across all participants, with the most success being for reducing behaviors at bedtime. These results provide further understanding of the affect behavior-analytic approaches can have on children diagnosed with Prader-Willi syndrome, increasing the number of applications of applied behavior analysis and the supports available for parents of children diagnosed with PWS.

Keywords: Prader-Willi syndrome (PWS), applied behavior analysis (ABA), sleep, children

Acknowledgements/Funding:

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PWS research in Australia – contributing to the global effort!

Presenting Author: Diane Webster

Additional Authors: Kathlene Jones

Institution(s): Prader-Willi Research Foundation of Australia

Abstract:

The Prader-Willi Research Foundation Australia (PWRFA) exists to improve clinical outcomes and deliver better treatments for people living with Prader-Willi Syndrome (PWS). We fund cutting-edge research which will help people with PWS live independent lives, free from the most debilitating aspects of the condition.

Our 2023 PWS Research Roadmap is structured around four key elements:

- **Care** – Develop innovative models to deliver best practice clinical care and clinical trials
- **Treat** – Accelerate research into treatments that target the most debilitating symptoms
- **Transform** – Accelerate research to transform life by reactivating the silent PWS genes
- **Connect** - Promote funding, awareness and collaboration to support PWS research and clinical trials

This program of work is delivered by creative and innovative researchers working in world leading institutions and hospitals across Australia. Drawing from a deep expertise in neuroscience, behavioural psychology, epigenetics & gene regulation, transcriptomics & bioinformatics, sleep & respiratory medicine, and exercise & community engagement - Australian researchers are contributing to the global effort to improve outcomes for people with PWS.

This presentation will provide an overview of the PWS research ecosystem in Australia, including basic science, health care delivery and clinical trials. We'll highlight why Australia is an excellent location for clinical trial sites, and the many opportunities for collaboration across the whole pipeline of research and development.

Acknowledgements/Funding: *The PWS community of Australia, particularly the people with PWS who participate in research and clinical trials, and the families who support them and raise funds for PWRFA.*

A Patient-Centered Pilot Study of Whole Genome Sequence and Pharmacogenomic Analysis of Participants in the Global PWS Registry: Preliminary Findings

Presenting Author: Elizabeth A Worthey¹

Additional Authors: Brandon Wilk¹, Caroline Vrana-Diaz², Donna Brown¹, Manavalan Gajapathy¹, Jessica Bohonowych¹, Yael Bar-Peled³, Jessica Denton³, Jaimie Richards¹, Theresa V Strong^{2,3}

Institution(s): ¹Center for Computational Genomics and Data Science, Department of Pediatrics, Marnix E. Heersink School of Medicine, University of Alabama at Birmingham (UAB)
²Foundation for Prader-Willi Research, Covina, CA, ³Department of Genetics, UAB, Birmingham, AL

The clinical and behavioral symptoms associated with Prader-Willi syndrome (PWS) vary widely in frequency and severity, making individualized risk difficult to predict. To better understand the contribution of genetic variants outside the PWS region to the individual risk for PWS-associated characteristics, we initiated a pilot study of whole genome sequence (WGS) of individuals with PWS, paired with de-identified phenotypic data gathered in the Global PWS Registry. This study also prioritizes the responsible return of actionable genetic information that is of interest to families. The entire project is being performed remotely, with independent IRB oversight.

Participants and/or their legally authorized representative participated in a videoconference to provide consent, and received a 'finger prick' blood collection kit, as well as a buccal swab kit in their home. Blood spots from a finger prick were sent directly to PerkinElmer Genetics for WGS, and buccal swabs were sent directly to Kailos Genetics, Inc for confirmatory pharmacogenomics (PGx). Participants have the option to receive secondary findings as recommended by the American College of Medical Genetics (ACMG SF v3.0), with access to My Gene Counsel, a digital health company providing an updating genetic counseling report. In addition, participants could choose to receive a PGx report, developed with input from a PWS parent advisory board. The PGx report provides information on DNA variants impacting drug safety and metabolism, according to the guidelines of the US Food and Drug Administration (FDA) and the Clinical Pharmacogenetics Implementation Consortium (CPIC). The participants' attitudes towards PGx testing and the utility of their results is being assessed by surveys administered before and after (1 month and 6 months) receiving their PGx report. The PGx testing panel included high-risk variants in the prothrombin (F2) and factor V (Leiden mutation) genes, which predispose individuals to blood clots. This is of particular interest since the PWS population is already at high risk for thrombosis.

The study enrolled 50 participants, with 49 WGS completed to date and currently being analyzed (1 sequence was not completed due to inadequate sample). We have also completed 48 PGx analyses with 1 sample having an inadequate sample and one participant opting out of receiving PGx results. Two individuals were identified as having a DNA variant that increases the risk of blood clots and two additional individuals have actionable secondary findings unrelated to PWS, which will be confirmed by Sanger sequencing. All genomes are being analyzed individually for DNA variants/structural variants of interest and regions of homozygosity, and as part of a cohort for genome-wide associations and polygenic risk scores. Almost half of the deletion cases show an unexpectedly complex structure. Overall, these initial findings suggest WGS may provide additional insights into the complexity of PWS and can provide important information impacting the individual care of persons with PWS.

Noninvasive Neuromodulation of a Novel Cerebellar Satiety Circuit in PWS: Design and Methodology of a Pilot Clinical Trial

Presenting Author: Laura Holsen

Additional Authors: Mark Halko, Roscoe Brady, Lillian Hacsí, Emma Joncas, Emily Payne

Institution(s): Brigham and Women's Hospital; McLean Hospital; Harvard Medical School

Abstract: Among the phenotypic characteristics of PWS, hyperphagia remains one of the most challenging factors to cope with for individuals with PWS and their families. Severity of hyperphagia in PWS is associated with greater caregiver burden, poorer overall quality of life, and greater morbidity and mortality. Behavior, emergent from brain function, likely governs several primary sources of hyperphagia in PWS, yet therapeutic intervention on known brain circuits have not yielded dramatic success, suggesting a need to examine alternative neural circuits associated with appetite and food intake. Our recent reverse-translational work identified the novel involvement of the cerebellum in regulating food-related responsivity in PWS, with preclinical data suggesting cerebellar-mediated attenuation of food reward related activity in the VS. These findings suggest cerebellar neuromodulation would directly impact food reward and satiation in PWS. Our group's work in neuromodulation provides evidence that cerebellar TMS can selectively modulate network function in cerebral cortex and cerebellum, and when applied therapeutically can reduce symptom severity in patient populations. Although these empirical data support the use of TMS in modulating cerebellar pathways linked to clinical outcomes, data are lacking on the feasibility and tolerability of cerebellar TMS and impact on cerebellar-VS circuitry function and food reward behaviors and consumption in PWS. In this study, we will assess the feasibility of implementing a 5-day course of cerebellar TMS, and examine its impact on the satiety response to palatable food in neural systems and behavioral endpoints using an unblinded, single-arm trial involving cerebellar TMS neuromodulation in 12 adults with PWS. The approach will include functional MRI to ascertain neural reactivity to high-palatable food in the cerebellum and VS, as measures of target engagement. Food-related behavior will be measured using objective and subjective assessments at pre- and post-TMS in-person study visits and during a 2-week remote follow-up phase. This poster will detail the rationale and design of the trial, including methodological and safety considerations. Findings from the completed study will provide mechanistic understanding of neuromodulatory effects of cerebellar TMS in PWS at neural and behavioral levels and critical insight into development of cerebellar TMS as a standalone or add-on treatment for PWS.

Acknowledgements/Funding: This study is funded by the Foundation for Prader-Willi Research.

October 6th, 2023
SPEAKER ABSTRACTS MORNING
SESSION IA

Assessment of a Screening Assay for use in Newborn Screening for Prader-Willi Syndrome and Other Chromosome 15 Imprinting Disorders

Presenting Author: Brooke Migliore

Additional Authors: Elizabeth Jalazo, Anne Wheeler, Katerina Kucera

Institution(s): RTI International, Raleigh, NC, RTI International, Carolina Institute for Developmental Disabilities, UNC Chapel Hill, UNC School of Medicine, UNC Chapel Hill

Abstract:

Newborn screening (NBS) provides the only population based strategy to identify newborns who could benefit from early disease detection and treatment, however, the lack of an affordable and accurate screening test for presymptomatic identification of babies with Prader-Willi syndrome has been a limitation for NBS. Prader-Willi syndrome (PWS), along with Angelman syndrome (AS), and Dup15q syndrome are known as Chromosome 15 imprinting conditions and arise due to copy number variants at the 15q11-13 locus on Chromosome 15. The variants cause changes in DNA methylation patterns and lead to a disruption of imprinted gene regulation. The conditions have different phenotypes but share diagnostic options and are targets for emerging therapeutics. The average age of diagnosis ranged from 6 months for infants with PWS to 3 years for children with Dup15q, and much later for cases of nondeletion subtypes of PWS or AS.

A major challenge in adding screening for PWS, AS and Dup15q to states' NBS panels will be the establishment of a new testing methodology in state laboratories to detect the specific methylation patterns at the *SNRPN/UBE3A* locus that are the molecular signatures of these conditions. Detection of methylation changes requires additional procedural steps prior to molecular testing that are currently not established in NBS laboratories. Demonstrating the feasibility of adding these procedures to the current molecular NBS workflows, in addition to evaluating the performance of the assay, will be essential for implementing state-wide screening.

We assessed a candidate NBS assay [Godler et al., 2022 PMID [34982160](#)] that quantifies the DNA methylation ratio in dried blood spots (DBS) in the Newborn Screening and Genetics Laboratory at RTI International. The assay utilizes lab equipment commonly used in NBS laboratories. In addition to DNA extraction and PCR, the processes include bisulfite conversion and methylation specific quantitative melt analysis of the PCR product. This presentation will discuss the laboratory set up, evaluation of the test with quality control materials from condition-specific patients and healthy newborns, assay trial and optimization, and preliminary results obtained from the test assessment. We will also discuss the next steps to validate the assay for clinical testing purposes, obtain a population-based screening cut off, and the path required to implement the assay as part of the Early Check newborn screening program.

Acknowledgements/Funding: This work was funded by The Angelman Syndrome Foundation, Foundation for Angelman Syndrome Therapeutics, Foundation for Prader-Willi Research, and the Dup15q Alliance

Increased numbers of arginine-vasopressin neurons and enhanced glymphatic system activity in the suprachiasmatic nucleus of Prader-Willi Syndrome Individuals

Presenting Author: Chun-Xia Yi^{1,2}

Additional Authors: Felipe Correa da Silva^{1,2}, Margje Sinnema³, Connie T. R. M. Schrandt-Stumpel⁴, Leopold M.G. Curfs⁴, Andries Kalsbeek^{1,2}, Alberto M. Pereira¹, Eric Fliers¹, Dick F. Swaab²

Institutions: 1. Department of Endocrinology and Metabolism, Amsterdam University Medical Center, University of Amsterdam, Netherlands; 2. Netherlands Institute for Neuroscience, Amsterdam, Netherlands; 3. Policlinic Clinical Genetics, Maastricht University Medical Center, Netherlands; 4. Faculty of Health, Medicine and Life Sciences, Maastricht University Medical Center; the Netherlands

Abstract: The hypothalamic suprachiasmatic nucleus (SCN) hosts the central pacemaker of the circadian system and regulates various physiological rhythms. Within the SCN, the circadian clock is primarily governed by arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP)-expressing neurons as well as astrocytes. Individuals with Prader-Willi Syndrome (PWS) commonly experience circadian disruptions, which are particularly evident in their sleep patterns. Interestingly, earlier research has shown that orexin-expressing neurons in the hypothalamus, which are responsible for sleep coordination, appear unaffected in PWS subjects. In this study, we investigated the relationship between neurons and neighboring glial cells in the SCN using the postmortem hypothalami donated by PWS patients (n=9) and age- and sex-matched controls without PWS (n=15). Our analysis revealed an increased number and relative intensity of AVP-expressing neurons in the PWS group compared to the controls, while VIP-expressing cells remained unaltered. Additionally, we examined microglial and astroglial populations to gain insight into glial involvement in neuropathological changes in the SCN of PWS. Evaluation of microglial presence and functionality using ionized calcium binding molecule 1 (Iba1) as a pan microglia identifier, along with the relative intensity of NADPH oxidase 2 (Nox2) as a measure of microglial activity, demonstrated comparable markers between controls and PWS subjects. Furthermore, we assessed astrocyte numbers through the immunoreactivity of glial fibrillary acid protein (GFAP-ir) and aquaporin 4 (Aq4-ir). Overall, GFAP-ir particles were significantly lower in PWS, suggesting a reduced involvement of astrocytes in controlling the master clock. Interestingly, the Aq4-ir subpopulation of astrocytes, which constitute the glymphatic system responsible for waste clearance, exhibited an increase in PWS, indicating the potential accumulation of metabolic waste and neurotoxic factors in the SCN. To further investigate this, we examined the presence of advanced glycation end products (AGEs) within the SCN. Increased AGEs levels were observed in the PWS group, suggesting disruption of neuronal homeostasis and activity. Collectively, our findings provide crucial insights into the neuroanatomical basis of circadian misalignment in PWS, focusing on AVP-expressing neurons in the SCN. In conjunction with altered numbers and accumulation of neural metabolic waste, we observed augmented glymphatic system activity and astrocyte dysfunction. These findings suggest the potential utility of circadian-based therapies in ameliorating PWS symptomatology.

Acknowledgements/Funding: AMC PhD Scholarship (FCS, Amsterdam UMC, 2019)

Aberrant Behavior Checklist Scores in Youth with Prader-Willi Syndrome

Presenting Author: Bridget McNulty, MD, MS

Additional Authors: Waylon J. Howard, PhD; Lydia Kim, MD; Soo-Jeong Kim, MD; Parisa Salehi, MD

Institution(s): University of Washington School of Medicine; Seattle Children's Hospital

Abstract:

Background: Prader-Willi Syndrome (PWS) is a rare genetic disorder caused by the loss of expression of paternal genes on chromosome 15, resulting in an array of physical, cognitive, and behavioral disturbances. The Aberrant Behavior Checklist (ABC) is a standardized rating scale assessing maladaptive behaviors with established use in populations with developmental disabilities. Although used in the PWS population, changes in ABC scores and how they relate to hyperphagia (HP) and quality of life (QL) have not been analyzed. **Objective:** To describe ABC scores in youths with PWS, changes in scores over time, and how they relate to HP (measured by the Hyperphagia Questionnaire [HQ]) and QL (measured by Quality-of-Life questionnaire [QoL]). **Design/Methods:** A retrospective review of patients (5-22) seen at the SCH PWS clinic (2014-2022). Questionnaires obtained as part of clinical visits. Only completed checklists were analyzed. Scores analyzed by age ranges (5-8, 9-12, 13-16, 17-22) and described as mean $\pm$ standard deviation (SD). Multivariate multilevel models evaluated the change of ABC, HQ, and QoL scores over time. Variables assessed include sex at birth and genetic subtype (deletion [D] vs non-deletion [ND]). **Results:** Analysis included 69 subjects (50 F/19 M; 24 D/40 ND/5 undetermined) with 322 completed ABC questionnaires. ABC scores rose in early childhood and decreased during teen years. Average HQ and QoL scores did not change significantly over time. In D vs. ND subtypes, D subtypes showed less improvement in ABC, HQ, and QoL over time. The higher the total ABC score at 13 years, the more significant the improvement in ABC noted by 20 years. Higher ABC Total scores (higher maladaptive behaviors) were associated with lower QoL scores (worse QL) and a higher HQ (worse health) at 13 years. At age 13, higher QoL (better QL) was related to a lower HQ score (better health). HQ changes were negatively related to change in QoL ($r = -0.841$, $p < .001$) and in ABC Total ($r = -0.696$, $p < .001$) such that a slower decrease in HQ per year (worse health) corresponds with a slower increase in QoL scores (worse QL) and faster increase in ABC Total (higher maladaptive behaviors). **Conclusion(s):**

Individuals with PWS and their families face many challenges related to maladaptive behavior. Our study is the first to describe changes in ABC scores in youth with PWS across ages to evaluate maladaptive behaviors and how they relate to HP and QL. This study suggests relationships among maladaptive behaviors, H and QL in individuals with PWS. The ABC, HQ, and QoL may be used together to assess clinical improvement with intervention.

Acknowledgements/Funding:

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Using the Food Attentional Bias Task to measure hyperphagia in Prader-Willi Syndrome

Presenting Author: Sarah-Marie Feighan¹

Additional Authors: Megan Young¹, Ciara Molloy¹, Clare Kelly², Eleisa Heron¹, Edna Roche³, Sasha Key^{4,5}, Anastasia Dimitropoulos⁶, Louise Gallagher^{7,8,9}

Institution(s): ¹ Department of Psychiatry, Trinity College Dublin, Ireland; ² Trinity Institute of Neuroscience, Trinity College Dublin, Ireland, ³ School of Medicine, Trinity College Dublin, Ireland. ⁴ Vanderbilt Kennedy Center for Research on Human Development, ⁵ Department of Hearing and Speech Sciences, Vanderbilt University Medical Center, ⁶ Department of Psychological Sciences, Case Western Reserve University, Cleveland, USA ⁷ The Hospital for SickKids, Toronto, Canada; ⁸ The Centre for Addiction and Mental Health, Toronto, Canada; ⁹ Dept. Of Psychiatry, Temerty Faculty of Medicine, University of Toronto, Canada

Background: Hyperphagia in Prader-Willi Syndrome (PWS) presents as an intense, persistent sensation of hunger accompanied by food preoccupations, an extreme drive to consume food, food-related behaviour problems, and a lack of normal satiety (Schwartz et al., 2021). A lack of biomarkers of hyperphagia for PWS is a barrier to evaluating potentially life-altering drug therapies. Previous work from our lab involved the development of a free-viewing eye-tracking paradigm, the food attention bias task. Using the FAB task, we showed that attentional bias to food stimuli was reduced in healthy participants after consumption of a standardised meal, i.e. there was a decline in the motivational significance of food after eating. Evaluating attentional bias towards food in individuals with PWS may offer an objective and reliable measure of the absence of satiety seen in hyperphagia.

Methods: This study aimed to compare performance on the FAB task protocol between individuals with PWS (n = 23, mean age = 17.9 years, SD = 8.2; 15 female) and a typically developing comparison group (COM; n = 23, mean age = 16.6 years, SD = 7.2; 15 female). We hypothesised that (1) the COM group would show a reduction in AB to food stimuli after eating a meal and (2) the PWS group would not change AB to food across meal conditions. Proportional dwell time, the relative time a participant spent looking at food stimuli compared to all other stimuli, was used to measure attentional bias. A linear mixed model was used to investigate the effects of group (COM/PWS), meal condition (pre/post) and their interaction on proportional dwell time to food stimuli.

Results: A significant interaction effect of group and meal interaction was observed $F(1, 498) = 5.05$, $p < .025$, indicating distinct patterns of change in the relative duration of gaze towards food AOs from premeal to postmeal conditions for the PWS and COM groups. Specifically, the COM group showed decreased gaze duration towards food AOs after eating, while the PWS group displayed an increased gaze duration for food afterwards.

Discussion: A lack of reduction in attentional bias to food may be a potential marker of the absence of satiety in hyperphagia. The results indicate that eye tracking may be a promising objective marker of hyperphagia that could be further investigated as a clinical endpoint in future clinical trials.

Acknowledgements/Funding: Thank-you to FPWR for providing the funding that made this research possible. Thank you to the Prader-Willi Syndrome Association of Ireland for all their support and encouragement. Thank you, especially to the families and individuals with PWS who gave us their valuable time participating in this study.

SPEAKER ABSTRACTS MORNING SESSION IB

Identifying key underlying regulatory networks and predicting targets of orphan C/D box *SNORD116* snoRNAs in Prader-Willi Syndrome

Presenting Author: Rachel B. Gilmore¹

Additional Authors: Yaling Liu¹, Gordon Carmichael¹, Justin L. Cotney^{1,2}

Institution(s): ¹Department of Genetics and Genome Sciences, UConn Health Center, Farmington, CT

²Institute for Systems Genomics, University of Connecticut, Storrs, CT

Abstract:

It has long been understood that Prader-Willi Syndrome (PWS) is caused by loss of paternal expression of the imprinted region on chromosome 15q11-q13. While most PWS cases exhibit megabase-scale deletions, several PWS patients have been identified harboring a much smaller deletion encompassing primarily *SNORD116*. This finding suggests *SNORD116* is a direct driver of PWS phenotypes. The *SNORD116* gene cluster is composed of 30 copies of individual *SNORD116* C/D box small nucleolar RNAs (snoRNAs). Many C/D box snoRNAs have been shown to guide chemical modifications of other RNA molecules, often ribosomal RNA (rRNA). However, *SNORD116* snoRNAs are termed 'orphans' because no verified targets have been identified and their sequences show no significant complementarity to rRNA. It is crucial to identify the targets and functions of *SNORD116* snoRNAs because all reported PWS cases lack their expression.

To begin addressing this, we engineered two different deletions modelling PWS in two distinct human embryonic stem cell (hESC) lines to control for effects of genetic background. We also utilized an inducible Neurogenin-2 (NGN2) expression system to enable quick, reproducible differentiation of these lines into neurons. Performing bulk RNA-sequencing on resulting neurons allowed us to identify a novel list of ~40 genes transcriptionally dysregulated in our PWS-like systems. Importantly, our results showed it is critical to use multiple isogenic cell line pairs as this eliminated many spuriously differentially expressed genes. Employing the recently described computational tool snoGloBe, we discovered these dysregulated genes are significantly enriched for predicted *SNORD116* targeting versus multiple control analyses. Our results indicate a novel gene regulatory network controlled by *SNORD116* is likely perturbed in PWS patients.

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Assessing *Dgkk* as a *Snord116* target in the pathogenesis of PWS

Presenting Author: Yiying Zhang, PhD

Additional Authors: Kaiying Guo, Daniel Carolan, Rudolph L. Leibel

Institution(s): Department of Pediatrics, Division of Molecular Genetics, Columbia University Irving Medical Center, New York, New York

Abstract:

SNORD116 is a C/D box small nucleolar RNA (SNORD) located in the minimal PWS critical interval. SNORDs typically interact with their target RNAs by base pairing to modulate pre-mRNA splicing and maturation, mRNA stability and/or translation. The RNA targets of *SNORD116* and the role of *SNORD116* deficiency in the pathogenesis of PWS are poorly understood. We have previously shown that the mRNA levels of diacylglycerol kinase kappa (*Dgkk*), a master regulator of lipid signaling that participates in neuronal function, are elevated in the hypothalamus of *Snord116* null mice. *In silico* analyses predicted strong RNA:RNA interactions between *Snord116* and *Dgkk* mRNA, which is fully conserved in mouse and human. We investigated effects of *Snord116* overexpression or knockdown/knockout on *Dgkk* mRNA and protein levels and used a modified RNAscope technique to identify brain regions and cell types in which *Snord116* and *Dgkk* are co-expressed. In human neuronal SH-SY5Y cells, overexpression of *Snord116* decreased *Dgkk* mRNA levels while ASO-mediated knockdown (KD) of *Snord116* increased them. The decrease in *Dgkk* mRNA level was associated with increased *Dgkk* mRNA stability. These manipulations of *Snord116* levels in SH-SY5Y cells affected *Dgkk* mRNA and protein levels in the opposite directions. DGKK protein levels were also trended lower in the hypothalamus of *Snord116* KO mice relative to the wild type controls. To assess the molecular mechanisms by which *Snord116* modulates *Dgkk* mRNA/protein levels, we examined A-to-I editing and 2'-O-methylation near the putative *Snord116*-interacting site of mouse *Dgkk* mRNA. No A-to-I editing or 2'-O-methylation was detected in the wild type or *Snord116* KO mice. The known A-to-I editing sites on the serotonin receptor 2a (*Htr2a*) mRNA were not affected by *Snord116* KO. Both *Snord116* and *Dgkk* are expressed in adult mouse neurons; *Snord116* expression is widespread in the brain while *Dgkk* expression is much more restricted. We found that *Snord116* and *Dgkk* were co-expressed in several brain regions that have been implicated in feeding behavior and energy balance, including the arcuate and lateral nuclei of the hypothalamus. *Snord116* signals appeared to be associated with subnuclear bodies. *Dgkk* mRNA signals were detected mostly in the cytoplasm. Given the known role of *Dgkk* in regulating neurite growth/stability, these results support the hypothesis that *Snord116* interacts with *Dgkk* pre-RNA/mRNA to regulate its expression and that dysregulation of *Dgkk* may contribute to the neuro-molecular pathophysiology of PWS.

Acknowledgements/Funding: This work was supported in part by a research grant from the Foundation for Prader-Willi Research and the New York Nutrition & Obesity Research Center (DK26687).

Roles of SNORD115 and SNORD116 ncRNA clusters in neuronal differentiation

Presenting Author: David Tollervey

Additional Authors: Aleksandra Helwak, Tomasz Turowski, Christos Spanos

Institution: Wellcome Centre for Cell Biology, The University of Edinburgh, Scotland

Abstract:

Prader-Willi syndrome shows features linked to brain development and hypothalamus-related endocrine abnormalities. The smallest clinical deletions fall within the large (~650Kb) SNHG14 gene, removing 29 consecutive introns that each generate SNORD116. SNHG14 also includes 48 tandem introns encoding SNORD115 and generates multiple, extended snoRNA-related species. SNORD115 and SNORD116 resemble box C/D small nucleolar RNAs (snoRNAs) but lack known targets. Both snoRNAs strongly accumulated during neuronal differentiation. SNORD116 accumulation apparently reflected stabilization, potentially linked to the appearance of FBLL1, a homologue of the ubiquitous snoRNA-associated protein Fibrillarin (FBL). In contrast, SNORD115 was selectively transcribed, apparently due to regulated termination. For functional characterization we created cell lines lacking only the expressed, paternal, SNORD115 or SNORD116 cluster. Analyses during neuronal development indicated changes in RNA stability and protein synthesis. Altered mRNAs included *MAGEL2*, mutation of which causes the PWS-like disorder Schaaf-Yang syndrome. Comparison of SNORD115 and SNORD116 mutants indicated overlapping or interacting functions. Most changes in mRNA and protein abundance appeared relatively late in development, with roles including cytoskeleton formation, extracellular matrix, neuronal arborization. Comparison with human embryonic midbrain development indicated that the effect of SNORD116 loss is to advance developmental timing. It might be envisaged that many defects in cellular metabolism could delay developmental progression, perhaps by limiting gene expression. In contrast, advances in developmental timing seem likely to reflect more specific changes. Subtle impairment of relative neuronal maturation during development, might generate the clinical phenotypes.

Acknowledgements/Funding: AH was supported by the Foundation for Prader-Willi Research; TWT was supported by the Polish Ministry of Science and Higher Education Mobility Plus program; CS was supported by the Wellcome Centre core grant; DT was supported by a Wellcome Principal Research Fellowship.

Loss of *Snord116* has lasting consequences for neuronal and microglial morphology in mice

Presenting Author: Jack Reddaway

Additional Authors: Anna Pavlova, Jessica Stoneman, Peter Richardson, Wiktoria Stefaniak, Sean Wyatt, Timothy Wells

Institution(s): Cardiff University

Abstract:

Among the complex phenotypic characteristics of Prader-Willi syndrome (PWS), the mechanisms of cognitive impairment, remain poorly understood. Since cognitive performance is largely determined by cortical and hippocampal complexity, we have quantified neuronal morphology in *Snord116*^{p-/m+} (*Snord116*^{del}) mice, a model for PWS displaying impaired cognition. Furthermore, as microglia, the innate immune cells in the CNS, are essential for neuronal development and refinement, we also quantified microglial activity in this model of PWS.

Cortical pyramidal neurons of 10-day old (P10) *Snord116*^{del} mice exhibit 20% fewer branch points and a 15% reduction in mean dendritic length in basal dendrites. These deficits, which are seen in males and females are confined to layer V of the cortex and become more exaggerated by P15, the number of branch points being halved. In contrast, while pyramidal neurons in the CA1 subregion of the hippocampus show a 55% reduction in dendritic length reduced in P10 mice, branch point number and mean bifurcation angle were increased by 50% and 65% respectively (resulting in a broader, more complex dendritic arbor). Similar morphological profiles were observed at P15. Preliminary data suggests that these alterations in neuronal morphology are retained in adult *Snord116*^{del} mice and are accompanied by robust changes in cortical microglial morphometrics (20-30% increases in territory, number of endpoints and branch length).

Since basal and apical dendrites receive information from neighboring neurons, our findings imply that the integrational capacity of layer V cortical pyramidal cells in *Snord116*^{del} mice may be compromised by restricted connectivity with layer VI and sub-cortical inputs. Based on our observations, these changes are unlikely to be driven by the reduced pruning activity of cortical microglia. Additionally, the spatial, contextual and emotional mapping of hippocampal CA1 neurons may be modified by the reshaped arborization among the Schaffer collaterals.

Our research indicates that *Snord116* is a crucial determinant of the developing environment of the cortical and hippocampal neuropil. Thus, our work is beginning to characterize the mechanisms of cognitive impairment in PWS and the delineation of these processes will ultimately predicate therapeutic strategies for alleviating this distressing aspect of human PWS.

Acknowledgements/Funding:

Foundation for Prader-Willi Research

Cardiff University's Future Leaders in Neuroscience and Mental Health Initiative

SPEAKER ABSTRACTS MORNING SESSION IIA

Primary Efficacy and Safety Results of a Phase 2 Double-Blind, Placebo-Controlled, Signal-Detection, Proof-of-Concept Study of Pitolisant in Prader-Willi Syndrome

Presenting Author: Ameet Revana, DO¹

Additional Authors: Rakesh Bhattacharjee, MD²; Jennifer L. Miller, MD³; Priya Khanna, MD⁴; Ann O. Scheimann, MD, MBA⁵; Aaron Chidekel, MD⁶; Shawn E. McCandless, MD⁷; Althea Robinson Shelton, MD, MPH⁸; Haramandeep Singh, MD⁹; Daniel Norman, MD¹⁰; Tarak Patel, MD¹¹; James P. Maynard, MD¹²; Casey Burg, MD¹³; Sarayu Ratnam, PhD⁴; David Seiden, MD¹⁴; Krystle Davis Rapchak, MS¹⁴; Eric Bauer, BS¹⁴; Grant Runyan, PhD¹⁴; Kumar Budur, MD, MS¹⁴

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Abstract:

Introduction: Prader-Willi Syndrome (PWS) is a genetic, neurodevelopmental disorder characterized by global hypothalamic dysfunction leading to a variety of symptoms including hyperphagia, behavioral disturbance, and excessive daytime sleepiness (EDS). EDS can lead to worsening behaviors and impact daily function at school and work. Pitolisant, a histamine 3 receptor antagonist/inverse agonist, is approved for the treatment of EDS or cataplexy in adults with narcolepsy. The aim of this phase 2, proof-of-concept study was to evaluate the safety and efficacy of pitolisant in patients with PWS.

Methods: In this phase 2, exploratory, proof-of-concept study (which was not powered to show statistical significance), patients aged 6-65 years with a confirmed diagnosis of PWS and a score ≥ 12 on the parent/caregiver version of the Epworth Sleepiness Scale for Children and Adolescents (ESS-CHAD) were randomized to receive higher-dose pitolisant, lower-dose pitolisant, or placebo (dose based on age group). The 11-week double-blind treatment period included a 3-week titration phase and an 8-week stable-dose phase. The primary efficacy endpoint was change from baseline at Week 11 in ESS-CHAD score. In a separate analysis, treatment response was defined as an ESS-CHAD score reduction of ≥ 3 points from baseline or a Week 11 score of ≤ 10 .

Results: Of the 65 enrolled patients (50.8% male; age range, 6-28 years), 59 patients (90.8%) completed the study. Across the 3 treatment groups, mean baseline ESS-CHAD scores ranged from 15 to 16 (scores ≥ 16 indicate severe EDS). Mean (SE) change in ESS-CHAD score at Week 11 was greater in the higher-dose pitolisant group (-4.9 [1.23]) and lower-dose pitolisant group (-4.1 [1.06]) compared with the placebo group (-3.7 [1.13]). The rate of treatment response at Week 11 was 70.0% in the higher-dose pitolisant group, 55.6% in the lower-dose pitolisant group, and 52.6% in the placebo group. The most common adverse events were anxiety (11.9% with pitolisant vs 8.7% with placebo), irritability (11.9% vs 4.3%), and headache (11.9% vs 8.7%). No serious adverse events were reported in the pitolisant groups.

Conclusions: Pitolisant reduced EDS in patients with PWS, and a dose-response relationship was observed, with higher-dose pitolisant showing a stronger efficacy signal. The safety and tolerability of pitolisant in patients with PWS was consistent with the known safety profile. Based on the positive signals from this phase 2 study, a phase 3 clinical trial is being planned to enroll patients with PWS aged 6 years and older.

Acknowledgements/Funding: This study was sponsored by Harmony Biosciences.

A Novel Approach to Regulating the Impaired Gut-Brain Axis of Hunger Control in PWS using ARD-101

Presenting Author: Diane Stafford, MD¹

Additional Authors: Shawn McCandless, MD², Andreas Niethammer, MD, PhD³, Zhenhuan Zheng, PhD³, Alexa Warner, BSc³, Tien Lee, MD³

Institution(s):

¹ Stanford Medicine Children's Health, Palo Alto CA

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Abstract:

The hyperphagia of Prader-Willi Syndrome (PWS) is an intense, persistent hunger, postulated to result from an impaired gut-brain signaling. It is defined as the neurologically driven *need* to eat, differentiated from appetite which is the psychological *desire* to eat. Studies over the past three decades have indicated that a possible mechanism of action for hyperphagia in PWS includes inadequate secretion of the 'satiety hormone' cholecystokinin (CCK) by enteroendocrine I-cells, and abnormally elevated circulating levels of the 'hunger hormone' ghrelin. These findings identified CCK and ghrelin as potential therapeutic targets. Prior pharmaceutical attempts to regulate hunger using systemic CCK receptor agonists were discontinued due to severe off-target toxicities (e.g., pancreatitis). Treatment of PWS patients with an inhibitor of ghrelin conversion to its active metabolite acyl-ghrelin significantly increased circulating ghrelin levels and did not reduce the hunger score.

ARD-101 (denatonium acetate monohydrate), a bitter taste receptor TAS2R pan-agonist, stimulates the secretion of intestinal CCK with associated reduction of circulating ghrelin levels and reduced hunger. Early findings of an ongoing Phase 2a trial of oral ARD-101 in young adults with PWS confirmed safety and provided early evidence of reduced hyperphagia and improved food-seeking behavior in several treated individuals. In another randomized placebo-controlled Phase 2 study in obese patients, circulating ghrelin levels were significantly reduced in ARD-101 vs placebo treated patients. It is postulated that the oral administration of ARD-101 (which is substantially gut restricted) also acted locally to stimulate local secretion of CCK at the intestinal level, which acts in an autocrine/paracrine manner to activate gut-brain signaling for hunger control. This local action of gut CCK contrasts with previous system administration of CCK analogs to avoid off-target toxicities. The key findings will be discussed.

Acknowledgements/Funding: The research was funded by Aardvark Therapeutics

A Double Blind, Placebo-Controlled, Fixed-Flexible Dose, Randomized Clinical Trial of Guanfacine Extended Release for the Reduction of Aggression and Self-injurious Behavior Associated with Prader-Willi Syndrome

Presenting Author: Deepan Singh, MD

Additional Authors: Theresa Jacob, PhD, MPH; Michael Silver, MS

Institution(s): Maimonides Medical Center, Brooklyn, NY

Abstract:

Background: Aggressive and self-injurious behaviors (skin and rectal picking) pose significant challenges for individuals with PWS and their families. The limited treatment options for these complex behavioral problems present unique risks to persons with PWS. Guanfacine Extended Release (GXR) has shown promising results in reducing aggression and improving behavior in other neurodevelopmental disorders. This study aimed to investigate the efficacy and safety of GXR in reducing aggression and self-injurious behavior in patients with PWS.

Methods: This double-blind, placebo-controlled fixed-flexible dose, randomized clinical trial enrolled patients with PWS who exhibited moderate to severe aggression or self-injury. In Phase I, eligible subjects were randomly assigned in a 1:1 ratio to receive either GXR or placebo for 8 weeks. Following the blinded trial, an open-labeled continuation phase (Phase II) was pursued to further assess the efficacy and tolerability of GXR. Validated behavioral assessment scales were used to measure the reduction of aggression and self-injury. Clinical Global Impression- Improvement scale was utilized to evaluate overall improvement.

Results: A total of 17 subjects (3 female, 14 male; age range 6-34 years) were consented for the study. Out of that 2 subjects were screened out from participation. 3 participants dropped out during phase I of the study. There were no serious adverse events reported. The most commonly reported adverse effect was sedation. Efficacy data were analyzed and outcomes will be presented.

Conclusion: The management of behavioral problems, including aggression and self-injury, in individuals with PWS remains a significant challenge. This randomized clinical trial evaluated the efficacy and safety of GXR as a potential treatment option for these behavioral manifestations. The results of this study will be presented. The authors hope that findings from the study will contribute to the recognition of GXR as a safe and effective option for addressing some of the behavioral problems associated with PWS.

Acknowledgements/Funding:

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Project WellCAST: What we are learning about PWS caregiver mental health before and after telemental health treatments

Presenting Author: Bridgette Kelleher, Ph.D.

Additional Authors: Veronika Vozka, Kaleb Emerson, Riley Naughton, Dan Foti

Institution(s): Purdue University

Abstract:

Caregivers of individuals with Prader Willi Syndrome (PWS) and other rare neurogenetic conditions often lack access to effective, appropriate, and accessible supports for both their mental health and parenting needs. To address this gap, we will share data from Project WellCAST (Supporting the Well-being of CAregiverS via Telehealth), a community-academic partnership to deploy telemental health treatments to caregivers of people with rare disorders. This project has included three phases. Here, we focus on WellCAST 2.0, a feasibility trial that enrolled caregivers of individuals with PWS and Williams Syndrome (WS), and WellCAST 3.0, a recently launched NIH-funded clinical trial open to these and other rare disorder communities. We will share final data regarding the feasibility, preliminary efficacy, and acceptability of Project Well-CAST 2.0, including whether outcomes varied across syndrome community and treatment type. We will also share initial information about Well-CAST 3.0, including the project design and opportunities for PWS caregivers.

Method: Participants in WellCAST 2.0 were parents or siblings of people with WS (n=36) or PWS (n=44). Caregivers received one of three evidence-based mental health treatments between May and September 2022. Treatments varied in content and format: Acceptance and Commitment Therapy (ACT; Hayes et al., 2006; individual, 1x/week for 60 minutes), Dialectical Behavior Therapy (DBT; Drossel et al., 2011; group, 1x/week for 120 minutes), or Integrative Behavioral Couples Therapy (IBCT; Wittenborn et al., 2019, couples, 1x/week for 60 minutes). Eighty-three cycles of treatment were initiated, however three caregivers participated in two treatment types at separate times. Across the 80 unique caregivers who enrolled, most identified as female (75%; 25% male), Caucasian (81%; 10% Asian, 1% Black, 8% Multiracial), and non-Hispanic (93%; 4% Hispanic, 4% not reported). Median income was high (\$120,000, range \$12,000-\$1,500,000), and most (86%) completed at least 4 years of higher education.

Clinicians were master's-level clinical psychology trainees who virtually deployed treatments across a standardized 12-week cycle under the supervision of a licensed psychologist. Unique to this project, we employed a participatory community framework whereby rare disorder caregivers provided input on project design and met virtually with student clinicians to provide education about their communities prior to trainees meeting with participants. Primary outcome measures included the Depression, Anxiety, and Stress Scale (DASS-21; Henry et al, 2005) and Parenting Sense of Competency scale (PSOC; Gilmore & Cuskelly, 2008). Participants also completed daily ecological momentary assessments on their smartphones via brief surveys that were "pinged" to them 4 times per day across treatment. Items varied across surveys and included measures of psychosocial well-being, stress, daily activities, health behaviors, and caregiving; primary outcomes were momentary ratings of stress, positive affect, and negative

affect. Participants and clinicians also rated acceptability and feasibility of the program during and after treatment.

Results: Seventy-eight percent of caregivers completed WellCAST 2.0 (77% PWS, 81% WS). All participants reported access to WiFi and did not request HotSpots, however 15 (18%) preferred to use study technology rather than their own and were provided a tablet to facilitate participation. Participants who later dropped out of the study were not more likely to report connectivity issues (Fisher's $P = .394$, $p > .999$) or request to borrow technology for the study ($P = .225$, $p = .730$). Drop-out rates did not differ by syndrome group, Fisher's $P=.195$, $p=.790$, or by treatment type, Fisher's $P=.043$, $p=.743$. Similarly, Median session completion rate did not differ across PWS (83%) or WS (83%), Kruskal-Wallis $X^2(1)=0.30$, $p=.585$ or across ACT (86%), DBT (92%), or IBCT (83%), Kruskal-Wallis $X^2(2)=1.06$, $p=.590$. Participants reported mild to moderate symptoms at baseline; 31% were elevated on the DASS-21 for each depression and stress. Compared to baseline, we observed significant improvements in depression (DBT only), anxiety (ACT and IBCT only), and stress (all treatments), with effect sizes between Cohen's $d=.47$ and $d=.85$. Effects were observed in both syndrome groups but were generally larger among PWS caregivers. Parenting competency did not improve with treatment. Mixed effects models using smartphone based assessments indicated that although stress did not decrease "in the moment" as caregivers progressed through treatment, several aspects of caregiver affect and behavior improved.

Discussion. Our initial data support Project Well-CAST as a feasible and potentially efficacious community participatory framework for deploying evidence-based telemental health treatment to rare disorder communities. WellCAST 3.0, which was funded by NIH and is now enrolling up to 1,000 caregivers, will expand this work by identifying the degree to which various treatment programs are best suited to specific caregiver needs. We will discuss this new project and how results might inform the development of scalable programs to support the mental health and well-being of PWS caregivers.

Acknowledgements/Funding:

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**SPEAKER ABSTRACTS MORNING
SESSION IIB**

Co-localization of *Snhg14* and *Nhlh2* in mouse brain tissue

Presenting Author: Shadi Ariyanfar¹

Additional Authors: Dr. Deborah Good¹, Dr. Christopher K. Thompson², Dr. Stuart Tobet³

Institution(s): ¹Department of Human Nutrition, Foods, and Exercise, and ²School of Neuroscience, Virginia Tech, and ³School of Biomedical Engineering, Colorado State University

Abstract:

Prader-Willi syndrome (PWS) is a multisystemic neurodevelopmental disorder, characterized by biphasic symptoms, including diminished weight gain during development, along with neonatal hypotonia and failure to thrive, with later onset hyperphagia, severe weight gain, and morbid obesity. PWS syndrome arises from the deletion or inactivation of paternally inherited genes, with the smallest region encompassing a group of 30 small nucleolar RNAs 'snoRNAs' known as the *SNORD116@* locus. *SNORD116@* expression is driven from a larger long non-coding gene, *SNHG14*, using the same promoter for expression. Whole-body deletion of *Nhlh2* in mice results in PWS-like phenotypes, such as later onset weight gain, and delayed puberty. We previously have shown that overexpression of *Snord116-3* in a mouse hypothalamic cell line improves the stability of *Nhlh2* mRNA, likely through a motif in the 3' untranslated region of the *Nhlh2* mRNA. This motif can be disrupted by the presence of a single nucleotide polymorphism, confirming the mechanism of action. Nevertheless, to date, there are no published studies showing the *in vivo* co-localization between *Nhlh2* and *Snord116* (or its host gene, *Snhg14*), and the question of where and when *Snord116* snoRNAs interact with target mRNA *Nhlh2* remained unanswered. To fill this gap, we mapped simultaneous expression of *Snhg14* and *Nhlh2* throughout the adult perfused mice brain, using quantitative RNA multi-plex in situ hybridization "RNAScope". Our close examination with the use of commercially designed probes revealed dense labeling of both *Snhg14* and *Nhlh2* in many areas of the brain such as the hippocampus, dentate gyrus habenula, thalamus, and hypothalamus. The Gene5 spot counting module (from Agilent BioTek) was used to quantify co-localization. Using the ventromedial nucleus of the hypothalamus (VMH) as an example location, we found that out of 219 detected neurons that were captured by a 60X magnification objective (BioTek Cytation 5 Cell Imaging Multimode Reader), 51.8%, and 34.7% were positive for *Nhlh2* or *Snhg14* expression, respectively. Interestingly 28.3% of total detected signals were co-expressed in the same nuclei. To show the association between single expression rate and mutual co-expression of *Snhg14* and *Nhlh2* a logistic regression analysis was conducted. Accordingly, *Snhg14* and *Nhlh2* showed a significant positive effect on the likelihood of co-expression in the nucleus/nucleolus (odds ratio: 4.670 (95% CI: [2.631 – 8.287], P value= 0.000, odds ratio: 67.389 (95% CI: [16.277- 279.005, P value 0.000, respectively). Furthermore, Imaris imaging software (<https://imaris.oxinst.com/>) revealed overlapping nucleolar localization between *Nhlh2* and *Snhg14* transcripts. Data for other areas of the brain will be shown. Thus, based on the acquired 3D images, high magnification analysis, and the probe location we can confirm a nucleolar-specific co-localization for *Snhg14* and *Nhlh2* mRNA transcripts in critical regions of the mouse brain. It can be concluded that the *Snhg14* host transcript and *Nhlh2* interact spatially supporting the possible regulatory role for *Snord 116* snoRNAs towards *Nhlh2* mRNA stability. A database of annotated images is currently being developed using the Minerva suite of tools (<https://www.cycif.org/software/minerva>). Virginia Tech libraries will host the site as an open-access resource for both PWS researchers and families.

Acknowledgments/Funding: The Foundation for Prader Willi Research provided funding for this study.

Analysis of Circadian Rhythm Defects in Prader-Willi and Schaaf-Yang Syndrome Neurons

Presenting Author: A. Kaitlyn Victor¹

Additional Authors: Lawrence T. Reiter^{1,2,3}, Chidambaram Ramanathan⁴, Andrew C. Liu⁵, Yang Shen⁵

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Abstract:

Prader-Willi syndrome (PWS) patients are known to suffer from sleep problems, including sleep-disordered breathing and central hypersomnolence. The suprachiasmatic nucleus (SCN), within the hypothalamus, controls the circadian rhythms of many biological processes. Mouse models of PWS reflect these altered circadian rhythms, including REM sleep alterations and defects in rhythmic DNA methylation. While in cultured cells, *Mage12* was found to regulate *Bmal1* and *Per2*, both critical genes in the circadian pathway. Schaaf-Yang syndrome (SYS), a disorder with overlapping phenotypes in common with PWS, results from truncating mutations in the *MAGEL2* gene. Here, we investigated the expression of key clock gene, *Per2*, across time in dental pulp stem cell (DPSC) patient-derived neurons from PWS, SYS and neurotypical control subjects. We have established the largest collection of DPSC lines from the deciduous teeth of PWS subjects (46 total). We regularly differentiate these stem cells into cortical-like neurons for molecular and functional studies. To investigate the circadian rhythms of PWS and SYS patients, we established DPSC cell lines harboring a *Per2* promoter-driven luciferase reporter (*Per2:Luc*) to assess *in vitro* circadian bioluminescence rhythm. The *Per2:Luc* DPSC lines were differentiated for 4-weeks to achieve a mature neuronal reporter line. By measuring luciferase activity across time, we could observe circadian bioluminescence rhythms in these patient-derived neurons across several days. Here, we show for the first time, circadian period length differences in primary neurons from both PWS and SYS subjects. We found that neuronal differentiation is required for rhythmic *Per2* cycling and that in both PWS and neurotypical control neurons, the transcription translation feedback loop regulating *Bmal1* and *Per2* in the circadian pathway oscillates as expected. Importantly, we found two distinct period length phenotypes in PWS neurons, long period and short period. These differences in period length were significantly different from control neuron period lengths. We also observed discordant *Per2* cycling in SYS neurons from different individuals and among replicates from the same individual. Establishing this assay and observing circadian rhythm defects in PWS and SYS neurons paves the way for future drug discovery to rescue circadian cycling defects in PWS and SYS.

Acknowledgements/Funding:

Pilot Research Awards from the Foundation for Prader-Willi Research

Defining Transcriptional Signatures in Blood and Brain Related to Severity of Prader-Willi Syndrome

Presenting Author: David E. Godler^{1,2}

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Single-nucleus RNA-sequencing (snRNA-seq) was used to examine 8,338 long non-coding RNAs and 17,079 protein-coding genes across different cell types in the prefrontal cortex (PFC) of 8 donors affected with Prader-Willi Syndrome (PWS) (4 15q11-q13 deletion and 4 non-deletion subgroups) and to compare these to 4 age and sex matched neurotypical controls. Differentially expressed genes (DEGs) were shortlisted from these comparisons, if conserved between all cell types. The shortlisted DEGs were then confirmed using droplet digital PCR (ddPCR) in the same tissues. DEGs showing the greatest difference from PFC controls were included in genotype-phenotype studies utilizing ddPCR analyses of blood leukocytes from an independent cohort of 35 individuals with PWS (19 deletion and 16 non-deletion), 1 to 45 years of age. The mRNA levels of these genes were related to: (i) intellectual functioning assessed using the Mullen Scales of Early Learning or an age-appropriate Wechsler Scales; and (ii) challenging behaviors assessed using the PWS Behavioral Questionnaire. The PFC tissues from the PWS group showed 2,364 DEGs specific to oligodendrocytes and microglia - the highest number from comparisons to the control group of all cell types examined. Gene Ontology enrichment analysis for the DEGs, identified 53 genes and related pathways consistently dysregulated across all cell types in the PFC of the PWS group compared to controls. These included gliogenesis, nervous-system development, ribosome and cytoplasmic pathways. Genes encoded in the 15q11-q13 region (*SNRPN*, *PWRN1*, *PWRN6* and *MKRN3*) were most downregulated across all comparisons (deletion and/or non-deletion vs controls). Only 2 protein coding genes [*RPS18* and *NEFM*] from this gene list were upregulated in PWS PFC. *RPS18* was the only gene selected for the follow-up studies as *NEFM* is not expressed in blood. ddPCR testing in blood of 12 children (<13 years of age) with PWS in the non-deletion subgroup showed that expression of *RPS18* was correlated with intellectual functioning (Performance IQ (PIQ): $R=-0.86$, $p<0.0001$, $N=12$; Full Scale IQ (FSIQ): $R=-0.77$, $p=0.002$, $N=12$), and challenging behaviors (body related behaviors: $R=0.63$, $p=0.026$). For the 18 children of the combined PWS cohort (deletion and non-deletion) these relationships remained significant for PIQ ($R=-0.6$, $p<0.0088$) and challenging behaviors ($R=0.6$, $p=0.0127$). In conclusion, 53 DEGs and pathways were identified to be consistently dysregulated between cell types in the PFC of donors with PWS, with *RPS18* expression associated with severity of PWS. If confirmed in other brain areas and in larger independent cohorts, these observations may lead to development of new prognostic markers and targets for novel therapeutics based on omics-driven medical care.

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Established a computational platform for the Pre-Clinical Animal Network (PCAN) Initiative

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Abstract: Preclinical models play a crucial role in modern medical research. To advance in this direction, the Foundation for Prader-Willi Research (FPWR) has launched the Pre-clinical Animal Network (FPWR-PCAN) project. Its primary objective is to establish standardized animal models and methodically assess them using various phenotyping criteria. The development phase of the new PCAN lines is nearing completion at the MRC Mary Lyon Centre in Harwell. Among the original six lines, targeting *Magel2*, *Ndn*, *Magel2/Ndn*, and *Snord116*, four have already been successfully generated. The transformation of these efforts into a valuable translational platform for the scientific community involved in PWS research is of utmost importance.

Currently, we are engaged in an extensive, multi-step, and high-level analysis of these phenotypes, meticulously investigating all facets of the phenotypic spectrum. The ultimate aim is to provide a thorough and comprehensive examination of all phenotypes. This involves employing cutting-edge artificial intelligence (AI)-guided computational methodologies to extract meaningful biomedical characteristics and construct predictive models based on the underlying genotypes. Furthermore, the project seeks to construct virtual 'Digital Twins' of the PCAN lines, facilitating independent analysis decoupled from the physical mouse models.

PWS Research Resources

For more information about these resources, please contact the FPWR Research Team.



www.pwsregistry.org

Powered by NORD's "IAMRARE" Registry Platform, the Global PWS Registry is a secure database that supports natural history studies, endpoint development and clinical trials recruitment. The Registry currently includes more than 2,000 PWS participants from across the world.



www.pwsctc.org

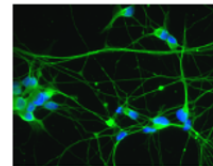
The **International PWS Clinical Trial Consortium** leverages the expertise from industry, academia, and patient organizations to address the unmet scientific, technical, clinical, and regulatory needs of clinical trials for PWS



Advancing research through the gift of brain donation.

www.autismbrainnet.org

FPWR has partnered with Autism Brain Net to enable valuable research, through an expert network to acquire and distribute high quality brain tissue. A program of the Simons Foundation, Autism Brain Net values transparency, sensitivity to families, and dedication to the highest quality research.



PWS Cell Biorepository

<https://www.fpwr.org/ipsc-biobank>

In collaboration with the University of Connecticut Stem Cell Institute, FPWR is building a repository of well-characterized iPS cells for distribution to the PWS research community. FPWR has also supported the development of biomaterials (RNA, protein) from PWS dental pulp stem cells, available through the Reiter lab (Ireiter@uthsc.edu).



PCAN

The Preclinical Animal Network is a collaboration of experts in PWS animal models and phenotypic analysis. The goal of the PCAN is to improve the predictive value of PWS mouse models to support drug development



PWS Clinical Investigation Collaborative

PWS – CLIC

<https://www.fpwr.org/pws-clic>

The mission of the PWS-CLIC, a collaborative group of clinical experts, is to improve the quality of clinical research and medical care for people with Prader-Willi syndrome (PWS) across the lifespan through collaborative investigation and research to support evidence-based care.