2024 PWS Research Symposium Abstract Booklet

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SPEAKER ABSTRACTS MORNING SESSION 1

Learning and memory in Prader-Willi Syndrome through the lens of *snord116* in catecholaminergic cells

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Abstract:
Prader-Willi Syndrome (PWS) results from genetic deletion of paternal alleles on chromosome 15 and is characterized by altered learning and memory. Of the paternal alleles deleted, knocking-out the non-coding RNA *snord116* in mice disrupts learning and memory. However, the effects of knocking out the paternal allele of *snord116* in specific neuronal populations remains unexplored. Catecholaminergic cells are an ideal population within which to investigate contributions of *snord116* to learning and memory because of their involvement in learning and memory and their region-specific organization throughout the brain. We initially examined the influence of knocking-out the paternal allele of *snord116* in all catecholaminergic cells of the mouse brain on learning and memory. Next, we targeted the catecholaminergic-dense locus coeruleus (LC), a brain region involved in learning and memory. Using a CRE-LoxP approach and an elaborate breeding strategy in mice, we first knocked-out paternal *snord116* in all catecholaminergic cells (Global-KO) of the mouse brain and measured learning and memory, using auditory fear conditioning (AFC), extinction training, and extinction recall tests. Next, using intra-cranial infusions of CRE recombinase directly into the LC, we determined how knocking out paternal *snord116* in the LC (LC-KO) influenced learning and memory. All animals acquired fear during auditory fear conditioning and extinguished their fear during extinction training. However, both Global-KO and LC-KO animals exhibited alterations in memory recall during the extinction recall test.
Specifically, Global-KO and LC-KO males tended to freeze less compared to control males, while Global-KO and LC-KO females froze more compared to control females. Our data suggests that knocking-out the paternal allele of *snord116* in all catecholaminergic cells and more specifically, cells in the LC, results in altered learning and memory. Our work sets us on the path to uncover cell- and regional-specific neurobiological contributions to PWS-associated deficits in learning and memory.

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Reduction of cholinergic neurons in the Nucleus Basalis of Meynert of patients with Prader-Willi Syndrome

Presenting Author: Chun-Xia Yi [1,2,3]

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Abstract:

The cholinergic nucleus basalis of Meynert (NBM) plays a crucial role in fundamental brain processes such as learning, memory, awareness, mood control, and sleep, primarily via its cortical projections. Loss of acetylcholine (ACh)-producing neurons in the NBM has been associated with various neurological disorders, including Alzheimer's disease. To date, very few studies have investigated the cholinergic neurons in the NBM of patients with Prader-Willi Syndrome (PWS). In our ongoing study, using a relatively large sample of postmortem hypothalami donated by PWS patients (n=9) and comparing them with age- and sex-matched controls without PWS (n=15), we performed immunohistochemical analysis of the choline acetyltransferase (ChAT)-expressing neurons in the NBM. Our preliminary data showed a robust decrease in ChAT immunoreactive neurons in 8 out of 9 PWS individuals. Moreover, in both the PWS patients and controls, we did not observe clear hyperphosphorylated tau AT8 expression, likely due to the relatively young age of the subjects. We are currently conducting further analysis of other cells in the NBM, including microglia, astrocytes, and vasculature. Our preliminary finding of a loss of ChAT-expressing neurons suggests that the behavioral phenotype of PWS patients might be associated with a lack of acetylcholine. This raises the issue of whether measuring the cholinergic index in cerebrospinal fluid could be considered a diagnostic approach and whether administering acetylcholinesterase inhibitors could be a potential therapy to ameliorate PWS symptomatology.

Acknowledgements/Funding: N/A

Altered Hypothalamic Functional Connectivity in Adults with PWS

Presenting Author: S. Sanaz Hosseini
Additional Authors: Stephanie Brown, Tony Holland
Institution(s): University of Cambridge

Abstract: Hyperphagia is a significant challenge for individuals with Prader-Willi Syndrome (PWS) and remains an aspect of the disorder that requires further study to advance our understanding of its mechanisms and develop appropriate treatments. Previous research has shown the importance of the hypothalamus in controlling several bodily functions, including food intake. To unravel the hypothalamus network, its functional connectivity to several brain regions in healthy normal-weight and obese adults have been established [1, 2, 3]. In this study, we aimed to investigate the hypothalamic functional connectivity with other brain regions using resting-state multi-echo fMRI analysis. Data from 17 young adults with PWS diagnosis (age 19-27 years, 4 females) and 36 healthy control (age 19-25, 14 females) were analyzed. Results indicated several differences in functional connectivity between hypothalamus and other brain regions across the groups. Results for the PWS group showed reduced functional connectivity to several brain regions such as temporal, frontal, and cingulate cortices, as well as cerebellum and thalamus. On the other hand, strong functional connectivity between the hypothalamus and bilateral hippocampus and amygdala was noted in both PWS and control groups. However, the activation clusters in the right hemisphere seem to be more prominent compared to the left hemisphere in the PWS. The co-activated areas of the amygdala are located further anterior in the PWS group compared to the control, suggesting altered connectivity to sub-regions of the amygdala in the PWS. Previous studies suggest that the amygdala plays a role in food consumption behaviors, and distinctions between functional roles of the left and right amygdala have also been proposed. Our results also stress the need for further investigation into abnormal functional connectivity of the hypothalamus and amygdala sub-regions in the PWS.

Acknowledgments/Funding: This research was funded by FPWR. Data used in this study was collected with funding from PWSA UK.

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SPEAKER ABSTRACTS MORNING SESSION 2

Targeted activation of imprinted PWS genes using CRISPR/Cas9-based epigenome editing

Presenting Author: Dahlia Rohm[1,2]

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Abstract:
Prader-Willi Syndrome (PWS) is an imprinting disorder that arises from a loss of expression of genes on paternally inherited chromosome 15, most commonly through a large deletion spanning 5-6 Mbp. In all cases of PWS, the maternally inherited allele is intact but epigenetically silenced, suggesting the possibility of restoring expression of the missing genes from the maternal copy of chromosome 15 to study and potentially treat PWS. We sought to restore expression of the maternal locus through targeted epigenetic modification using CRISPR/Cas9-based epigenome editors.
CRISPR/Cas9 is a powerful tool for manipulating gene expression in mammalian cells. Nuclease-deactivated Cas9 (dCas9) fusions with transcriptional activators or repressors have been used to program epigenetic states by recruiting transcriptional machinery or chromatin modifying enzymes and/or by directly depositing chromatin marks.
Using dCas9-based epigenome modifiers, we sought to identify regulatory regions of the PWS locus and specifically activate PWS genes in human induced pluripotent stem cells (hiPSCs) and hiPSC-derived neurons. We first used CRISPR interference and activation screens to identify regulatory elements of the PWS gene *SNRPN* in hiPSCs. To do so, we generated reporter cell lines in which the paternal or maternal *SNRPN* gene is specifically tagged with *2A-GFP*. These high-throughput screens in the paternal and maternal *SNRPN-GFP* hiPSCs revealed regions within the PWS locus that abate paternal SNRPN-GFP expression or activate maternal SNRPN-GFP, respectively. We hypothesized that these regions may be involved in maintaining the epigenetic state of the PWS locus. Furthermore, we show that transcriptional activator ^{VP64}dCas9^{VP64} and DNA demethylase ^{Tet1}dCas9 can each activate maternal *SNRPN* and downstream PWS transcripts, including the critical *SNORD116* cluster, in hiPSCs harboring a PWS deletion. ^{VP64}dCas9^{VP64} and ^{Tet1}dCas9 function at unique regions and preferentially activate different *SNRPN* transcript variants. Using ATAC-seq and targeted bisulphite sequencing, we show that both activators increase chromatin accessibility in a region surrounding the *SNRPN* gene, but unlike ^{Tet1}dCas9, ^{VP64}dCas9^{VP64} does not alter DNA methylation at the PWS imprinting center, indicating these two epigenome editors are

functioning at the PWS locus through distinct mechanisms. We also find that transient delivery of the demethylase ^{Tet1}dCas9 and a single gRNA leads to stable, long-term maternal *SNRPN* expression in hiPSCs. Our studies implement CRISPR screens to identify regulatory regions of the PWS locus and offer insight into the epigenetic modifications that are sufficient to stably activate *SNRPN* and other PWS-associated transcripts in human cells.

Acknowledgements/Funding:
FPWR, NIH, NSF GRFP

In vivo and *in vitro* evidence that targeting SMCHD1 may be an epigenetic therapy for PWS

Presenting Author: Marnie Blewitt

Additional Authors: Megan Iminitoff, Tamara Beck, Andrew Keniry, Anna LeFevre, Caleb Chew, Hannah Vanyai, James Murphy, Christian Schaaf, Merlin Thomas

Institution(s):
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Abstract:

One potential therapy for PWS is to activate the maternal allele of the PWS genes by removing their epigenetic silencing. Our prior work has shown that the epigenetic regulator SMCHD1 binds and silences the PWS locus in the mouse, suggesting targeting SMCHD1 might achieve such a goal. I will provide an update on our mouse *in vivo* and *in vitro* PWS patient-derived cell work, that show that removing SMCHD1 at a therapeutically relevant time and in therapeutically relevant cells and tissues, results in activation of the PWS locus, without major changes genome-wide. While we do not observe activation to the level of the paternal allele, we observe it has a positive effect on PWS phenotypes in our pilot cohort of animals. However, the question of what degree of activation may be sufficient in patients for therapeutic benefit remains open. I will discuss our data on factors that may collaborate with SMCHD1 in silencing the PWS locus, that may be targeted in combination.

Acknowledgements/Funding:
FPWR, Pamela and Lorenzo Galli Foundation, Brian M Davis Charitable Trust, Prader Willi Research Foundation Australia, WEHI

Transcriptome-wide identification of snoRNA-binding targets reveals non-canonical mode of snoRNA action and novel targets of *SNORD115/116*

Presenting Author: Bei Liu

Additional Authors: Tao Pan, Chuan He

Institution(s): University of Chicago

Abstract:
Clusters of box C/D small nucleolar RNA (*SNORD115/116*) genes have been identified as the principal genetic determinant for PWS. The canonical function of SNORDs is to install 2'-O-methylation (Nm) on ribosomal RNAs. However, *SNORD115/116* lack apparent sequence complementarity to canonical targets, hindering progress in understanding their roles in PWS due to a lack of tools to directly identify their targets. Here we develop a chemical crosslinking-based approach that comprehensively detects cellular RNA targets of snoRNAs, yielding thousands of previously unrecognized snoRNA-mRNA interactions in human cells and mouse brain tissues, including ~500 *SNORD115/116* targets. Many interactions occur outside of snoRNA-guided RNA modification sites, hinting at non-canonical functions beyond RNA modification. Notably, one of these snoRNAs, *SNORA73*, targets mRNAs that encode secretory proteins and membrane proteins. *SNORA73* also interacts with *7SL* RNA, part of the signal recognition particle (SRP) required for protein secretion. The mRNA-*SNORA73*-*7SL* RNA interactions enhance the association of the *SNORA73*-target mRNAs with SRP, thereby facilitating the secretion of encoded proteins. This RNA triad represents a novel mode of snoRNA action and underscores the efficacy of snoKARR-seq in unveiling new functional interactions. Additionally, we observed interactions between *SNORD115/116* and chromatin-associated RNAs, including retrotransposon elements, as well as significant interactions between *SNORD116* and snRNAs. These findings suggest potential non-canonical functions for *SNORD115/116*.

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Identification of hypothalamic miRNAs targeting the Prader-Willi-related gene *Magel2* in a rat model of altered puberty induced by early overfeeding

Presenting Author: Álvaro Aranda Torrecillas

Additional Authors: Elvira Rodríguez-Vázquez, María López-Sancho, Marta Navarro Torrente, Esperanza Uceda, Ana Belen Rodríguez-Sánchez, David García-Galiano, Manuel Tena-Sempere, Juan Manuel Castellano

Institution(s): Department of Cell Biology, Physiology and Immunology, University of Cordoba (Spain) & Maimonides Research Institute of Cordoba (IMIBIC, Spain)

Abstract:
Alterations in the timing of puberty are common in Prader-Willi (PWS) syndrome. *Magel2* is a Prader-Willi-related gene that is highly expressed in the hypothalamus and has been associated with these alterations. However, the molecular underpinnings of its pubertal actions remain unknown. MicroRNAs (miRNAs) have recently emerged as relevant regulatory factors of pubertal timing. Nevertheless, their potential involvement in regulating *Magel2* and their contribution to alterations in the timing of puberty onset remain elusive. To address this question, we initially analyzed the hypothalamic expression of *Magel2* mRNA in a rat model of early overfeeding that leads to obesity and precocious puberty. Then, we screened direct miRNAs-*Magel2* mRNA interactions in the medial basal hypothalamus (MBH) of these animals. To this end, we employed a novel technology termed miR-eCLIP, which can detect the formation of miRNA-mRNA chimeras throughout the transcriptome by employing (i) immunoprecipitation of the AGO2 protein, (ii) RNA-RNA ligation, and (iii) and high-throughput sequencing. To analyze direct miRNAs-*Magel2* mRNA interactions, we subjected all samples to enrichment for this gene. We found that the hypothalamic *Magel2* mRNA levels significantly decreased in obese animals with precocious puberty compared to lean control animals with normal pubertal development. We also identified miR-124-3p as the miRNA that forms the most miRNA-mRNA chimeras in the whole transcriptome, including the *Magel2* gene, in both the MBH of obese rats with precocious puberty and their respective lean controls. Additionally, we found the interaction of 22 miRNAs with *Magel2* in the MBH of early overfed female rats with precocious puberty and their control animals. Specifically, we observed that the interaction of three miRNAs, miR-320-3p, miR-149-5p, and miR-143-3p, was significantly upregulated in obese animals, suggesting their potential modulatory role on *Magel2* expression. Collectively, our findings support that hypothalamic alterations in the interactions among specific miRNAs and *Magel2* might contribute to alterations in the timing of puberty onset. However, further studies are needed to validate these preliminary data.

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SPEAKER ABSTRACTS AFTERNOON SESSION 1

Disrupted hypothalamic and oxytocin system development in rats with truncating *Magel2* mutation

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Abstract:
Background: The hypothalamus, a crucial region for neuroendocrine function where MAGEL2 is highly expressed, plays a central role in maintaining organismal homeostasis and adapting to environmental changes. Hypothalamic dysfunction is a common feature in Prader-Willi syndrome (PWS) and Schaaf-Yang syndrome (SYS). SYS, resulting from pathogenic variants in MAGEL2, is characterized by joint contractures and a high prevalence (75-85%) of autism spectrum disorder (ASD). Oxytocin (OT) levels and the OT system's function around birth are vital for stimulating and coordinating the maturation of neuronal networks, which in turn influence food intake, cognition, and behavior. Early disruptions in OT system functioning can impair neuronal circuit development, leading to ASD and other related symptoms. Clinical trials of OT treatment for PWS and ASD have yielded mixed results, indicating that OT's effectiveness may depend on administration during critical developmental periods. *Objective and Methods:* This study aims to elucidate the effects of Magel2 truncation on hypothalamic and OT system development, understand underlying pathomechanisms, and develop rationales for effective therapies. We utilized a rat model with truncating *Magel2* mutation. Using our semi-automated pipeline for immunohistochemistry, whole slide imaging, and three-dimensional tissue analysis, we conducted the most comprehensive quantification of OT neurons and OTergic projections across hypothalamic development (day 0, week 8, week 15) to date. In addition, we performed multi-omics studies (RNA sequencing and TMT-based mass spectrometry) on hypothalamic tissue. Given the role of Magel2 in ubiquitination and RNA metabolism, we extracted both RNA and protein for each sample and compared transcriptomic and proteomic profiles to understand whether changes on either level influence the other. Further validation was performed using nCounter® gene expression analysis. *Results:* Magel2-deficient rats exhibited a transient increase in oxytocinergic neurons during newborn and adolescent stages, which largely resolved in adulthood. Female rats showed significant reductions in OTergic projections to the nucleus accumbens, a region essential for social motivation and reward. Preliminary transcriptomic data implicate several genes involved in transcription, transcriptional regulation, and CNS development. Notably, *Sonic hedgehog protein*, an essential morphogen during the early patterning of the hypothalamus, was downregulated in Magel2-deficient animals. *Conclusion:* Our study provides detailed accounts of the OT system across brain development in rats, supporting the notion of a critical period for effective OT treatment. We offer novel findings on the impact of Magel2 truncation on various biological pathways at both transcriptomic and proteomic levels, highlighting potential pathomechanisms and therapeutic targets.
Acknowledgements/Funding: This work was supported by the FPWR (to Christian P. Schaaf) and the DFG (AL 2466/2-1 to Ferdinand Althammer).

Orexin agonism failed to rescue the diminished hedonic drive observed in Magel2 null mice

Presenting Author: Richard M. O'Connor
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Abstract:
Prader-Willi Syndrome (PWS) is a neurodevelopmental disorder, characterized by a spectrum of symptoms including insatiable hyperphagia leading to morbid obesity. Research has isolated a silencing of the Magel2 gene product as the main molecular pathology underpinning PWS linked metabolic disruptions including obesity. Magel2 silencing in mice results in dramatic decreases in the levels of the hypothalamic neuropeptide orexin and a corresponding decrease in the levels of orexin in the cerebrospinal fluid of individuals with PWS has been reported. The potential of simultaneously targeting orexin receptor type 1 (OX1R) and type 2 (OX2R) for treating PWS remains unexplored. We hypothesized pharmacological stimulation of the orexin system would rescue any observed feeding disruptions. Wild-type and Magel2 null mice underwent mild food restriction for 24 hours, followed by measurement of standard laboratory chow consumption over a 180-minute period. The rate of chow refeeding was similar between both groups. When we measure hedonic feeding, sated Magel2 null mice consumed significantly less palatable food compared to their wild-type counterparts, suggesting a disruption in hedonic feeding systems. When granted free access to both standard chow and palatable food in their home cages we unexpectedly found Magel2 null mice gained more weight than their wild-type counterparts despite consuming the same number of total calories. However, magel2 null mice obtained a larger portion of their calories from the palatable food source compared to wild-type mice. We delivered a dual OX1R and OX2R agonist intraperitoneally 30 minutes prior to the onset of homeostatic and hedonic feeding to wild-type and Magel2 null mice. We found an unexpected injection stress effect in Magel2 null mice that resulted in reduced levels of chow intake compared to wild-type mice. Moreover, we also surprisingly found Orexin receptor agonism reduced chow intake during refeeding in wild-type mice while not altering feeding in Magel2 null mice. This may reflect profound deficits in orexin signaling infrastructure in Magel2 null mice rendering pharmacological agonism ineffective. Orexin agonism had no effect in in wild-type mice on hedonically driven palatable food intake and failed to rescue the observed deficit to hedonic drive in Magel2 null mice. Thus, a lack of Magel 2 expression leads to distinct change to food-related motivation dependent on the palatability of the food source. Dual orexin receptor agonism failed to rescue these deficits. We will further characterize the potential of orexin pharmacology to restore normal feeding behavior in Magel2 null mice by increasing sample size, recording homecage food intake and including additional assessments of reward-related behavior. Moreover, we will also establish if an underlying deficit to orexin function may preclude the use of orexin based pharmacological tools to restore normal feeding behavior.
Acknowledgements/Funding: Foundation for Prader-Willi Research

Investigating the Transcriptional and Translational Effects of MAGEL2 Loss in Fed and Fasted Mice Using Single Nucleus RNA Sequencing and Polysome Profiling

Presenting Authors: Tara Bayat, MS, and Denis Stepihar, MS

Additional Authors: Maria Camila Hoyos Sanchez[1,2], Elena B Tikhonova[3], Cristian Camilo Rodriguez Almonacid[3,4], Farzana Popy[1,2], Stephanie Myers[1], Andrey L. Karamyshev[3], Zemfira N. Karamysheva[3], Christian Bustamante[3], Isabel Castro[3], Sambantham Shanmugam[3], Jorge Riano Diaz[1,2], Igor Ponomarev[3], and Klementina Fon Tacer[1,2]

Institution(s): [1] School of Veterinary Medicine, Texas Tech University, 7671 Evans Dr., Amarillo, TX; [2]Texas Center for Comparative Cancer Research (TC3R), 7671 Evans Dr., Amarillo, TX; [3]Texas Tech University Health Sciences Center, 3601 4th St, Lubbock, TX

Abstracts:

Introduction: Translational regulation of gene expression occurs on polysomes and is in particularly important in secretory cells, including neuroendocrine cells of the hypothalamus, to achieve high protein synthesis levels without triggering the protein stress response. Dysregulation of mRNA translation contributes to several neurodevelopmental diseases. However, its contribution to the pathogenesis of Prader-Willi syndrome (PWS) is not known. PWS is a neurodevelopmental disorder, whose hallmark is improper hypothalamic neuroendocrine function. PWS is caused by the loss of several genes, including melanoma antigen L2 (*MAGEL2*), which was recently shown to be critical for the regulated secretion of the hypothalamus. We hypothesize that MAGEL2 contributes to proper hypothalamic neuroendocrine function also on the translational level. **Methods:** To investigate the potential role of MAGEL2 in translational regulation, we performed polysome profiling of the hypothalamus and pituitary of 16 wild-type and *Magel2*$^{p\Delta/m+}$ mice. Hypothalamic and pituitary lysates were loaded on the top of a sucrose gradient, and ultracentrifugation was performed to separate mRNAs associated with different numbers of ribosomes, including monosomes, light polysomes, and heavy polysomes which correspond to the translation efficiency from low to high, respectively. To evaluate the role of Magel2 on translatome, we extracted RNA from each group and performed RNA sequencing. **Results:** RNA sequencing data were analyzed to determine differentially translated genes in KO vs. normal tissues. We found that several mRNAs were differentially present in the *Magel2*$^{p\Delta/m+}$ hypothalamus and pituitary monosomes, light polysomes, and heavy polysomes fractions compared to the wild-type animals; specifically, in the pituitary, we found 402 differentially present genes in monosomes, 404 genes in light polysomes, and 198 genes in heavy polysomes. This suggests that Magel2 contributes to the translation efficiency of several mRNAs in the pituitary and hypothalamus. **Conclusion**: Our data provide novel insight into the potential role of MAGEL2 in protein synthesis and hormone secretion with implications for symptom development in patients with PWS. Future analyses are warranted to determine the underlying molecular mechanism and potential therapeutic potential.

The MAGE family protein MAGEL2 is coded within the critical region of chromosome 15 (15q11-q13) and is key in the occurrence of Prader-Willi syndrome (PWS), a rare, genetic, multisystemic, neurodevelopmental disorder. While the deletion of the whole chromosomal region causes PWS, the mutations in the gene *MAGEL2* represent the underlying cause of a similar disorder called Schaaf-Yang syndrome (SYS). On a cellular level, deletion, and

mutations of MAGEL2 were recently associated with the reduction in proteins associated with secretory granules, due to MAGEL2 regulating retromer-dependent endosomal trafficking, suggesting MAGEL2's critical role in hypothalamic neuroendocrine function. Recent data indicate that the MAGE gene family has evolved to protect cells from environmental stress, such as nutritional deprivation, facilitating stress adaptation, but the role of MAGEL2 during stress is not known.

To address this question, we exposed wild-type and *Magel2*$^{p\Delta/m+}$ male mice to 24h-fasting-induced stress and measured serum corticosterone levels. Then we performed single nucleus RNA sequencing (snRNAseq) on hypothalami from 16 mice from 4 different groups, 4 mice per group: 1 - wild-type/control, 2 - wild-type/24h fasting, 3 - *Magel2*$^{p\Delta/m+}$/control and 4 - *Magel2*$^{p\Delta/m+}$/24h fasting group. For snRNAseq library generation, 2 animals per group were pooled and 2 libraries per group were analyzed. Initial quality control (QC), alignment, and quantification were done with CellRanger (v7.2.0), and data was filtered by the percentage of mitochondrial content, number of counts, and number of detected genes. Normalization, dimension reduction, clustering, and differential expression were done with Seurat (v5.0.3). After quality control, we obtained 130,000 nuclei per library, which were subsequently classified into 23 cell types based on established cell type markers, including nonneuronal (astrocytes, oligodendrocytes, microglia, tanycytes, ependymal and endothelial cells) and neuronal cells (several excitatory and inhibitory neurons). Then, we analyzed cellular composition among 4 major treatment groups and found that the ratio of cell types was not significantly changed between different groups, except oligodendrocytes. Then we performed differential gene expression (DEG) analysis in each cell type to determine the effects of fasting and the Magel2 knockout. The cell types that express the highest number of DEGs upon starvation are endothelial cells, microglia, and excitatory neurons. The cell types with the highest DEG upon Magel2 depletion are microglia, endothelial cells, and oligodendrocyte precursor cells. These findings suggested that Magel2 affects several cell types in the hypothalamus in a fed and fasted state. With ongoing gene ontology analysis, we aim to get the first insights into the molecular mechanisms regulated by Magel2 in specific cell types, which may lead to a new understanding of Magel2's role in hypothalamic function under nutritional stress and in the pathogenesis of PWS and SYS.

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Characterizing Adrenal Insufficiency in a Rat Model of Prader-Willi Syndrome: Insights into Hypothalamic-Pituitary-Adrenal Axis Dysregulation

Presenting Author: Colleen Bocke

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

Institution(s): Saint Louis University School of Medicine

Abstract:
Prader-Willi Syndrome (PWS) is a complex genetic disorder with distinct developmental stages. In the first stage, PWS manifests as failure to thrive, hypotonia and feeding difficulty in infants. This is followed by hyperphagia, hypogonadism, and behavioral challenges which characterize the later stage of this disease. Hypothalamic-pituitary dysregulation is believed to be responsible for many aspects of the PWS phenotype, potentially contributing to several of the hallmark characteristics of this disorder. Changes in the ability of the central nervous system to respond to stressors by integrating and responding to insults to the homeostatic state are well documented in PWS, and adrenal insufficiency may further complicate endocrine dysfunction in PWS. The inability of PWS patients to respond to stress may be due to alterations at any or all three levels of the hypothalamo-pituitary-adrenal (HPA) axis. In this study, we sought to investigate the hypothesis that adrenal insufficiency in Magel2-deficient rats, a model of PWS, is at least in part due to loss of pituitary secretory capacity, since disruption of Magel2 expression, as is observed in PWS patients, is known to affect vesicular trafficking and secretion. To address this hypothesis, anterior pituitaries collected from Magel2-deficient and wild type rats were dispersed and aliquoted. The following day, cells were treated with either 0.1, 1.0, 10, 100, or 1000 nM of corticotrophin-releasing hormone (CRH). Following a 60 minute incubation, media were collected for the assessment of adrenocorticotropin (ACTH) by radioimmunoassay. We also collected pituitary cells for measurement of intracellular content of ACTH. Our results showed that cells from Magel2-deficient rats had decreased basal ACTH secretion, but remained responsive to CRH stimulation, albeit to a lesser degree than cells isolated from wild type animals. This suggests a pituitary deficiency in the response to hypothalamic factors (e.g. CRH) that normally control ACTH release. It is also possible that the other two levels of the HPA axis are altered in PWS. We now are investigating potential alterations in CRH prohormone processing or release (i.e. hypothalamic deficiencies) and responsiveness of the adrenal gland itself to ACTH administration. Furthermore, we have investigated circulating levels of cortisol in stressed and non-stressed animals to determine if the Magel2 rat model recapitulates the aberrant stress response observed in PWS patients. Through this investigation of Magel2's potential contributions to adrenal insufficiency, we hope to advance the understanding of PWS pathophysiology and lay the groundwork for developing new treatment strategies.

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Neonatal lethality due to hypoglycemia and pancreatic islet deficits in a Prader-Willi Syndrome imprinting defect mouse model and approaches to therapeutic rescue

Presenting Author: Haley L. Crable1

Additional Authors: Krishna Prasadan2, Tina Zhang2, Erik A. Koppes1,3, James L. Resnick4, George K. Gittes2, Robert D. Nicholls1

Institution(s): 1Division of Genetic and Genomic Medicine, Dept. of Pediatrics, and 2Division of Pediatric Surgery, Dept. of Surgery, UPMC Children's Hospital of Pittsburgh and University of Pittsburgh; 3Magee-Womens Research Institute, University of Pittsburgh; 4University of Florida College of Medicine.

Abstract:
Prader-Willi syndrome (PWS) displays neonatal failure to thrive (FTT), hypotonia, hyperphagia, obesity, endocrine and metabolic deficits, with behavioral and cognitive impairments. Growth hormone improves height, muscle function, and body composition, but is not a cure and new therapeutics are critical to develop. Two dogmata haunt an understanding of PWS. First, that PWS has solely a hypothalamic etiology, but mechanisms are unknown for endocrine and metabolic dysfunction in PWS. Second, that PWS is due to one or two major genes, yet evidence from mouse and human studies suggests a multigene hypothesis for PWS. Previously, using a mouse model (TgPWS) with deletion of all PWS-imprinted genes, we demonstrated severe FTT, growth retardation, low plasma insulin and glucagon, deficits in pancreatic islet development and insulin secretion, and neonatal lethality due to postnatal onset of severe progressive hypoglycemia. Subsequently, hypoglycemia was found clinically in PWS infants. Further, by use of a genome-edited *in vitro* model we found that PWS β-cells display a cell-autonomous deficit in insulin secretion, with proteome and transcriptome studies identifying reduced levels of secreted peptides (insulin, IAPP, NPY) and numerous endoplasmic reticulum (ER) chaperones including GRP78 and GRP94, the two major chaperones in folding and trafficking of secreted peptides.

An imprinting control (IC) region, the PWS-IC, establishes epigenetic marks that activate or silence the paternal and maternal alleles, respectively.

We are using PWS-ICdel imprinting defect mice to further investigate postnatal phenotypes in PWS mice. On the C57BL/6J (B6) inbred strain, PWS-ICdel pups have severe FTT and survive < 24 hours after birth, with significant reductions in body weight and blood glucose levels *vs.* their WT littermates. In addition, by using immunohistochemistry, PWS mice have a decrease in the total number of endocrine cells and of both β (insulin) and α (glucagon) cells within the islet. At the F4 generation after outcrossing to CD-1, PWS-ICdel pups survive up to 4.5 days after birth, however, have FTT, reduced body weight, and progressive hypoglycemia. As a potential therapeutic, we are testing the effects of the small molecule BiP inducer X (BiX), which upregulates GRP78 and other ER chaperones that have decreased levels in PWS β-cells. At a dose of 10 mg/kg, we injected BiX intraperitoneally (i.p.) in pregnant B6 mothers during early, mid, and late gestation. Preliminary results from 3 litters suggest potential rescue of blood glucose levels in ~ 55% of PWS pups. These studies are continuing with both B6 and CD-1 strains and to assess dose amount, delivery (i.p. *vs.* amniotic injection), timing, and postnatal delivery (to pups and lactating females, or orally). Taken together, PWS-genes have critical endocrine and metabolic roles for transition from the fetal to newborn environment.

Acknowledgements/Funding: Supported by 1) National Institutes of Health/NICHD, R21-HD108695; and 2) the Storr Family Foundation through PWSA.

SPEAKER ABSTRACTS AFTERNOON SESSION 2

Assessment of a Screening Assay for use in Newborn Screening for Prader-Willi, Angelman and Dup15q Syndromes

Presenting Author: Brooke Migliore[1]

Additional Authors: Elizabeth Jalazo[2], Anne Wheeler[1, 3], Katerina S. Kucera[1]

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Abstract:
Prader-Willi syndrome (PWS), Angelman syndrome (AS), and Dup15q syndrome are largely caused by copy number variants and aberrant inheritance of imprinting markers at the 15q11-13 locus, resulting in inappropriate DNA methylation patterns and disruption of gene regulation. Newborn screening (NBS) provides the only population-based strategy to identify infants 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 PWS, AS and Dup15q has been a limitation. We evaluated a single molecular test that detects the characteristic aberrant DNA methylation signatures in the *SNRPN* promoter at the 15q11-13 locus. Identification of individuals with Dup15q syndrome may also be possible with this test, though less reliable due to the more complex underlying genetic changes. The three conditions have different etiologies but share diagnostic options, are targets for emerging therapeutics, and have been identified as candidates for universal NBS by public health systems. 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. Since detection of methylation changes requires additional procedural steps prior to molecular testing, demonstrating the feasibility of these procedures, in addition to evaluating the performance of the assay, to the current molecular NBS workflows will be essential for implementing state-wide screening. Early Check, an ongoing expanded NBS study currently taking place in North Carolina, provides an opportunity to generate data on early identification of individuals with rare disorders and inform public policy.
We will present our assay validation results of a candidate screening assay [Godler et al., 2022 PMID 34982160]. The quantitative melt analysis (MS-QMA) is a low-cost methylation-specific first-tier test for use with dried blood spots (DBS) to detect abnormal levels of SNRPN promoter methylation. The test involves sample lysis to release DNA, automated bisulfite conversion, real-time PCR detection, and methylation ratio analysis to detect methylation signatures specific to each disorder. We utilized a variety of Quality Control materials from condition positive individuals and unaffected individuals to perform Precision, Carryover, Accuracy and Analytical Sensitivity studies. We also identified reference ranges in the North Carolina newborn population from evaluation of 3000 deidentified residual newborn screening dried blood spot specimens obtained from the North Carolina State Laboratory of Public Health.
We plan to add the MS-QMA test to Early Check in the future as a consented pilot study offered to parents of babies born in North Carolina. Current Early Check newborn screening includes genome sequencing for two panels of monogenic conditions and risk for type 1 diabetes (T1D) using a polygenic risk score. Panel 1 includes 178 gene-condition pairs with high actionability

and early age of intervention initiation (≤ 2 years). Panel 2 offers screening for an additional 29 gene-condition pairs that have emerging potential for actionability (e.g., conditions for which there are therapies in clinical trials). AS and PWS have been added to these gene panels that will continue to grow over time. The implementation of the methylation-based NBS test for AS, PWS, and Dup15q in parallel with genome sequencing can assess the clinical sensitivity and specificity of both methods. The Early Check prospective pilot studies generate essential data for the evaluation of the feasibility and acceptability of NBS on a large-scale and to maximize benefits and reduce harm to the screened population.

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

Utilizing megabase-scale deletion models to explore allelic differences in the chromosome 15q11-q13 locus

Presenting Authors: Rachel B. Gilmore

Additional Authors: Michael Chung, MD, PhD; Jennifer VanOudenhove, PhD; Stormy J. Chamberlain, PhD; Justin Cotney, PhD

Institution(s): UConn Health

Abstract:
Prader-Willi syndrome (PWS) and Angelman syndrome (AS) result primarily from deletion of the paternal or maternal 15q11-q13 allele, respectively. Much effort has been made towards understanding alteration in gene expression at the chr15q11-q13 locus in the context of these disorders. However, our understanding of the higher order chromatin structure, epigenetic modifications, and overall regulation at this locus is still lacking. To examine this in a rigorous manner, we have generated isogenic human embryonic stem cell (hESC) lines harboring megabase-scale deletions to model common deletions found in PWS and AS patients. We targeted chromosome 15 specific repeats with CRIPSR/Cas genome editing technologies to generate these large deletions, without causing off-target effects. We used these novel isogenic hESC models and patient-derived induced pluripotent stem cells (iPSCs) to interrogate allele-specific differences in the chr15q11-q13 locus through multiple molecular techniques including chromatin immunoprecipitation followed by sequencing (ChIP-seq) and Hi-C. ChIP-seq showed a clear allelic difference in histone modifications at the chr15q11-q13 locus. The H3K9me3 modification, commonly associated with heterochromatin, is enriched across the imprinted domain of the repressed maternal allele. The H3K4me3 mark, commonly associated with active gene promoters, is enriched at promoters on the active paternal allele. Hi-C, a method used to capture the three-dimensional conformation of the genome, showed differences in chromatin conformation following a deletion of either the maternal or paternal allele in the chr15q11-q13 locus. Our results led us to hypothesize about a model of regulation occurring at this locus in which the maternal chr15q11-q13 allele is tethered to the nucleolar space which permits accumulation of H3K9me3 to reinforce the silent state of the maternal allele. The active transcription occurring on the paternal allele prevents it from becoming tethered to the nucleolus, resulting in a lack of H3K9me3 deposition. These results highlight the inherent differences between the two parental alleles in this locus, which could have ramifications for development of therapeutics targeting this region.

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A Patient-Centered WGS-driven Pilot Study to Identify Symptom Associated Variation in Participants from the Global PWS Registry

Presenting Author: Brandon M. Wilk[1,2]

Additional Authors: Manavalan Gajapathy[1,2], Donna M Brown[1,2], Caroline Vrana-Diaz[3], Jessica Bohonowych[3], Yael Bar-Peled[2], Jessica Denton[3], Lisa Matesevac[3], Jaimie Richards[1,2], Theresa V Strong[3], Elizabeth A Worthey[1,2]

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2 Department of Genetics, UAB Marnix E. Heersink School of Medicine, University of Alabama at Birmingham, AL, USA;
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Abstract:
Clinical complexity and genotype to phenotype heterogeneity in PWS confounds individualized symptom risk prediction. To explore individualized risk for specific symptoms, we piloted application of whole genome sequencing (WGS) to identify molecular variation capable of influencing risk of specific symptoms. The project (including sample collection, enrollment, and return of findings) is performed remotely, with independent IRB oversight. 50 participants were enrolled from the Global PWS Registry; a database of patient/caregiver-reported clinical and behavioral symptoms, with >50 detailed surveys spanning endocrine, GI, orthopedics, pulmonary, neurology, behavior, medications, social aspects, etc. The pilot also prioritizes responsible return of actionable genetic information of interest to families.
Comprehensive genomic analyses were conducted assessing PGx from WGS, structural and copy number variation, regions of homozygosity, and small variation resulting in large effects as well as identification of more common variation acting in a polygenic manner. Unexpectedly, we discovered in 8 out of 9 type I deletions, and 3 out of 19 type II deletions multiple interspersed deletions within the chr15q11. 2-q13 region; expanding our understanding of the genotype to phenotype heterogeneity in PWS. Furthermore, we found 3 cases where the identified deletion from WGS differed from the reported deletion in the PWS registry. Two participants received ACMG recommended secondary findings, with access to counseling via MyGeneCounsel, a digital health company. Three participants received secondary findings important specifically for risk of adverse events associated with PWS. Participants also received CPIC conforming PGx reports. We also identified a number of candidate modifier variants relevant to differences in phenotypic display among participants for further follow up and evaluation. Collectively, the results from the pilot study highlight the comprehensive and multifunctional utility of WGS in identification of variation influencing risk of symptoms in PWS.

Acknowledgements/Funding: This work was supported by funding from the Foundation for Prader-Willi Research (FPWR)

Unveiling the mechanisms of psychosis in Prader-Willi syndrome: from genotype to neurophysiological phenotype

Presenting Author: Lucie Aman

Additional Authors: Chiara Avancini, April Le, Suzannah Lester, Stephanie Brown, Paul Fletcher, Tristan Beckinshtein, Anthony Holland

Institution(s): University of Cambridge, Department of Psychiatry

Abstract:
Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from the absent expression of maternally imprinted, paternally expressed genes located in the chromosomal region 15q11-13. This absence of expression is usually either due to a paternal deletion or maternal uniparental disomy (mUPD). Both genetic subtypes share the same core clinical symptoms, such as developmental delays, intellectual disability, hyperphagia, behavioural and social difficulties, and a heightened risk for anxiety and depression. However, only individuals with PWS due to mUPD, having an excess of maternally expressed genes, seem to be at particularly high risk for developing psychosis.
We have conducted literature reviews to 1) define the phenomenology of psychosis in PWS, and 2) propose possible mechanisms of psychosis in PWS (Aman et al., 2018 and Aman et al., 2024). We hypothesised that the GABA/glutamate equilibrium is disrupted in PWS due to the absence of paternal expression. In addition, the overexpression of maternal genes on chromosome 15 would cause cholinergic dysfunction in people with mUPD and further disrupt GABAergic, glutamatergic, dopaminergic and cholinergic functions, increasing the probability of psychotic symptoms. In line with the predictive processing model of psychosis, the neurotransmitter disruptions proposed would cause a reduction in the use of priors to formulate predictions, detect and generate prediction errors, and update the prediction system.
A case-control study comprising clinical, cognitive, neuroimaging, and remote behavioural testing was subsequently designed to investigate the above hypotheses. Twenty-four PWS participants (10 mUPD, 12 deletion and 2 unknown) have been recruited and tested. The EEG testing comprised a sensory gating paradigm and an oddball paradigm. EEG findings showed differences in sensory processing between PWS subtypes, and confirmed that people with mUPD do not update their prediction system as efficiently as individual with deletion, who themselves do not update their as well as healthy controls do. Also, contrary to our prediction, we did not find slower processing speed while processing stimuli changes in people with PWS compared to controls despite the presence of intellectual disability. We propose that this could reflect the fight or flight state in which people with PWS are believed to be due to foetal starvation, and dysregulation of autonomous system.

Acknowledgements/Funding: Sam foundation; FPWR; PWSA UK

Cerebellar TMS targeting a novel satiety network in PWS: Initial results of a pilot clinical trial

Presenting Author: Laura Holsen, PhD[1, 2]
Additional Authors: Lillian Hacsi, B.A.[1, 2], Emily Payne, B.S.[2, 3], Emma Joncas, B.A.[2, 3], Roscoe Brady, M.D., Ph.D.[2, 4], and Mark Halko, Ph.D.[2, 3]
Institution(s): 1Division of Women's Health and Department of Psychiatry, Brigham & Women's Hospital; 2Harvard Medical School; 3McLean Hospital; 4Beth Israel Deaconess Medical Center

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. In the current study, we are assessing the feasibility of implementing a 5-day course of cerebellar TMS, and examining 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. To date, we have screened 16 individuals with PWS, of whom 8 were initially eligible. Of these 8, n=3 participants are currently pending consent; n=2 participants were deemed eligible and completed all study procedures. From Pre- to Post-TMS, participant #1 exhibited a decrease in hyperphagic behaviors on the (4-point decrease on HQ-CT, 2-point decrease on PWS Significant Events Questionnaire), decreased brain activation to food cues in the cerebellum and VS ($p<0.01$), and a 3.9-pound weight loss. Anecdotally, the caregiver of this participant also reported decreased skin picking behaviors. Participant #2 exhibited a slight increase in hyperphagic behaviors, no differences in brain activation, and a slight increase in weight. Together, these data suggest initial evidence of feasibility for the study protocol, given that both participants successfully completed all procedures, with mixed results in terms of trial outcomes (hyperphagic behavior and brain activation) among this small case series of participants to date. Findings will be discussed with reference to confounds such as uncontrolled environments and effects on other behavioral characteristics of PWS. With data collection on additional participants expected within the next several months, results from the larger sample 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:
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POSTER SESSION

Design and Outcome Measures of COMPASS, a Phase 3 Study of Carbetocin Nasal Spray for the Treatment of Hyperphagia in Prader-Willi Syndrome

Presenting Author: Alana Salvucci[5]

Additional Authors: Elizabeth Roof[1], Theresa V. Strong[2], Diane E. J. Stafford[3], Deepan Singh[4], Dilesh Doshi[5], Di An[5], Alana Salvucci[5], James M. Youakim[5]

Institution(s):
1Vanderbilt University, Nashville, TN; 2Foundation for Prader-Willi Research, Covina, CA; 3Stanford University School of Medicine, Stanford, CA; 4Maimonides Medical Center, Brooklyn, NY; 5Acadia Pharmaceuticals Inc, San Diego, CA

Abstract:
Prader-Willi syndrome (PWS) is a rare, genetic, neurodevelopmental disorder characterized by endocrine and neuropsychiatric problems including hyperphagia (unrelenting hunger and excessive eating), anxiousness, and distress. There is currently no approved therapy to treat hyperphagia, often resulting in morbid obesity and contributing to diabetes, cardiovascular or orthopedic problems, and food- or non-food-related anxiety. As such, the control of hyperphagia and improving food-related behaviors are the most important unmet needs in PWS. Carbetocin is an analog of oxytocin and shares its anorectic properties but with greater oxytocin receptor selectivity and a longer duration of action. In the phase 3, CARE-PWS study, carbetocin (3.2 mg, three times daily [TID]) administered intranasally (to minimize systemic exposure) for 8 weeks was well tolerated and showed nominally significant improvements vs placebo in the Hyperphagia Questionnaire for Clinical Trials (HQ-CT), PWS Anxiousness and Distress Behaviors Questionnaire (PADQ), and Clinical Global Impression of change (CGI-C) scores, with improvements sustained during the 56-week follow up. Because the efficacy assessment of the 3.2 mg dose was not the primary outcome measure in CARE-PWS, the present phase 3 study (COMPASS) is being conducted to further substantiate evidence of effectiveness. COMPASS is a 12-week, multicenter (~36 global sites), randomized, double-blind study that will compare carbetocin nasal spray 3.2 mg TID with placebo in approximately 170 subjects with PWS. The primary efficacy endpoint is change in HQ-CT score from baseline at Week 12. Secondary endpoints include the change from baseline to Week 12 in the CGI-Severity (CGI-S) score for PWS, and in the CGI-S for hyperphagia in PWS score, the CGI-C for PWS score at Week 12, and the percentage of subjects with a treatment response (defined as improvement from baseline ≥8 points) based on the HQ-CT at Week 12. Exploratory endpoints include the change from baseline at Week 12 in scores for the PADQ, Food Safe Zone, Zarit Burden Interview, and PWS-specific Healthcare Resource Use and Caregiver Burden Questionnaire. Optional caregiver interviews at the end of the trial will assess satisfaction with treatment. These data may support the use of carbetocin as a treatment for hyperphagia in PWS.

Acknowledgments/Funding: Study was funded by Acadia Pharmaceuticals Inc., San Diego, CA.

Phase 3 Randomized Placebo-Controlled Trial of Pitolisant for Excessive Daytime Sleepiness in Prader-Willi Syndrome

Presenting Author: David Seiden, MD[2]
Additional Authors: Amee Revana, DO, FAASM[1], Krystle Davis Rapchak, MS[2]; Ann Adee, BA[2]; Grant Runyan, PhD[2]; George Nomikos, MD, PhD[2]
Institution(s): [1]Baylor College of Medicine, Texas Children's Hospital, Houston, TX, USA; [2]Harmony Biosciences, Plymouth Meeting, PA, USA

Abstract:
Introduction: Prader-Willi syndrome (PWS) is a genetic, neurodevelopmental disorder characterized by global hypothalamic dysfunction that results in a variety of symptoms including behavioral disturbance, hyperphagia, and excessive daytime sleepiness (EDS). Pitolisant is approved for the treatment of EDS or cataplexy in adults with narcolepsy. In a phase 2, proof-of-concept study, pitolisant showed trends of reduction in EDS relative to placebo in patients with PWS.
Methods: This is a phase 3, multicenter, randomized, double-blind, placebo-controlled, global clinical study (TEMPO) to evaluate the efficacy and safety of pitolisant in the treatment of EDS in patients with PWS. The study will enroll patients ≥6 years of age with a genetically confirmed diagnosis of PWS, and who have EDS as confirmed by the investigator. Selected exclusion criteria include patients with symptoms or diagnosis of psychosis or schizophrenia, inadequately treated sleep disordered breathing, and moderate or severe hepatic or renal impairment. Eligible patients will be randomly assigned (1:1) to receive pitolisant (weight-based dosing: 8.9-44.5 mg/d) or matching placebo tablets. The 11-week double-blind period will be followed by an optional 52-week open-label extension.
Results: It is estimated that 134 participants will be enrolled in this study. The primary endpoint will be change from baseline at Week 11 in EDS as measured by the PROMIS-SRI (Patient-Reported Outcomes Measurement Information System - Sleep Related Impairment [Parent Proxy]). The secondary endpoints will include behavioral disturbance as measured by the Aberrant Behavior Checklist-Community domains (irritability, social withdrawal, stereotypic behavior, hyperactive/noncompliance, and inappropriate speech), Caregiver Impression of Severity for EDS and irritability, Clinician Global Impression of Severity for EDS, Epworth Sleepiness Scale for Children and Adolescents, and the Hyperphagia Questionnaire for Clinical Trials in conjunction with the Food Safe Zone Questionnaire. Safety assessments will include incidence and severity of adverse events, clinical laboratory tests, vital signs, and electrocardiograms. The study is currently enrolling patients (NCT06366464).
Conclusion: This study is designed to determine whether pitolisant is efficacious in the treatment of EDS in patients with Prader-Willi syndrome and to further characterize its safety profile in this patient population.

Acknowledgements/Funding: Harmony Biosciences, LLC

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

Presenting Author: Blythe Stockton

Additional Authors: John W. Cassidy, MD, Geethanjali Ravindranathan, Devon Thomey

Institution(s): Nexus Health Systems

Abstract:

Objective:
The objective of this study is to evaluate the efficacy of a comprehensive inpatient program for pediatric Prader-Willi syndrome (PWS) at Nexus Children's Hospital, Texas, utilizing standardized fitness trackers to monitor treatment progress.

Methods:
Patients with PWS were consented and enrolled with IRB exemption. Fitbit Inspire 3 watches and an Inbody 570 scanner were used to analyze health parameters during admission. Fitbit monitored daily heart rate, sleep patterns, and activity levels, while the Inbody 570 scanner assessed changes in weight, BMI, and body composition weekly. Monthly assessments by occupational and physical therapy staff evaluated endurance, strength, and coordination.

Results:
Ten pediatric PWS patients (3 male, 7 female) participated in the study, with an average length of stay of 125 days. Significant improvements were observed, including a 9.64% decrease in weight, a 9.61% reduction in BMI, and a 7.15% decrease in body fat percentage ($p < 0.05$). Endurance, cardiovascular health, and mobility showed significant enhancements as evidenced by changes in the 6 Minute Walk Test and WeeFIM II scale ($p < 0.05$). Activity levels increased by 36.16% ($p < 0.05$), and sleep quality, particularly REM sleep, significantly improved ($p < 0.05$). Nine out of ten patients were discharged with improved quality of life and tools for continued fitness maintenance.

Conclusions:
The findings highlight the effectiveness of the comprehensive management program in improving various health metrics among pediatric PWS patients. The utilization of fitness trackers proved valuable in quantifying treatment efficacy and managing PWS effectively.

Acknowledgements/Funding: Funding provided by Nexus Health Systems and Nexus Hope Foundation.

Positive Responses to DCCR Correlate with Circulating Drug Levels

Presenting Author: Neil M. Cowen, PhD

Additional Authors: Russ Wada PhD, Hanbin Li PhD

Institution(s): Soleno Therapeutics and QuanTx

Abstract:

Population PK analysis for DCCR was conducted using the results from nine clinical studies, including two studies in healthy adults (PK008 and C605), two studies in adults with obesity (PK001 and TR002), two studies in adults with elevated triglycerides (PK015 and CT013), and three studies in participants with PWS (PC025, C601, and C602-OLE). The analysis dataset included 1,987 PK samples from 136 PWS and 121 non-PWS participants, treated with DCCR or placebo. The preliminary analysis of DCCR Exposure-Response (ER) was conducted on data from clinical study C601 and focused on exposure response relationships for body fat mass and HOMA-IR, while the subsequent exposure-efficacy analyses used predicted exposures from three studies in participants with PWS (PC025, C601, and C602). In total, the exposure-efficacy analysis included 137 participants, of which 42 received placebo in Study C601, 84 received DCCR in Study C601, and 11 received DCCR in Study PC025 and had PK parameters estimated in the PopPK analysis. Of the 42 placebo treated participants in Study C601, 41 were enrolled in Study C602 and were treated with DCCR. In total, 115 participants rolled over from C601 to C602. Nonlinear regression models, linear logistic regression models, and linear Cox-regression models were used for exposure-response analyses with continuous endpoints, binary endpoints, and time-to-event endpoints using R 4.0.5. Nonlinear mixed-effects regression models were used for longitudinal HQ-CT time-course analysis using NONMEM 7.4. The selected exposure metric was average trough concentration, calculated from the population PK model. Trough concentrations were used because virtually all pharmacokinetic samples obtained from participants with PWS were trough measurements. Exposures were evaluated at a significance level of $p<0.05$ using a likelihood objective function. Covariates were selected by a stepwise forward addition - backward elimination method at a significance level of $p<0.01$ using a likelihood objective function. Using the data from participants with PWS in clinical studies C601 and C602, there were significant linear relationships between trough plasma drug concentrations and every efficacy parameter evaluated including: Hyperphagia Questionnaire for Clinical Trials (HQ-CT) change from baseline ($p=0.003$); insulin change from baseline ($p=0.001$); leptin change from baseline ($p=0.001$); and from the preliminary ER analysis fat mass change from baseline ($p<0.001$) and HOMA-IR change from baseline ($p<0.001$) all characterized at 13 weeks. ER analysis of HQ-CT change from baseline predicted progressive improvements through 78 weeks, consistent with the observed data. Administration of DCCR for 13 weeks and long-term to participants with PWS resulted in a range of meaningful responses to treatment including reductions in HQ-CT and fat mass and improvements in multiple metabolic markers that are consistent with the mode of action, all of which show a clear, significant relationship between exposure to DCCR and response.

Acknowledgements/Funding: The analysis was conducted by QuanTx for Soleno as sponsor. All clinical studies were conducted by Soleno as sponsor or its predecessor.

Small Fiber Peripheral Neuropathy in Prader-Willi Syndrome

Presenting Author: Sydney Englehart1

Additional Authors: Kathryn S. Obrynba MD2, Jennifer L. McKinney MD3, Emily C. De Los Reyes MD2

Institution(s): 1 Ohio University Heritage College of Medicine, Dublin, Ohio. 2Division of Endocrinology, Nationwide Children's Hospital, The Ohio State University, Columbus, Ohio. 3Division of Child Neurology, Nationwide Children's Hospital, The Ohio State University, Columbus, Ohio.

Abstract: Background: Prader-Willi syndrome (PWS) is a complex genetic disease with manifestations of the endocrinologic, metabolic, and neurologic systems. The neurologic manifestations may include symptoms of sensory disturbance and autonomic nervous system dysfunction including increased pain and thermal threshold instability. Here we report a case of small-fiber peripheral neuropathy which is rarely investigated in the PWS population.
Case Report: A 16 yo female with PWS (15q11.2-q13.1 deletion) and a history of pre-diabetes presented with an acute, progressive onset of lower extremity pain and tingling following a viral illness. The patient described having stabbing pain and feelings of shakiness in her hands, right arm, legs, and dorsal aspect of her feet. She also experienced temperature sensitivity as well as demarcations with redness and purple discoloration on her feet. There was no relief despite gabapentin, and exercise exacerbated her discomfort. Elevation, acupuncture, and decreased activity provided some relief. Her motor exam was normal, but her sensory exam showed an exaggerated response to pinprick testing in her hands and feet and a gradient in her legs and arms with temperature sensation. Proprioception at the big toe was better on the left compared to the right. Nerve conduction studies and electromyography were normal and showed no evidence of large fiber neuropathy. An MRI of the brain and spine were normal. Subsequent testing showed an abnormal QSART (Quantitative Sudomotor Axon Reflex Test or sweat test) suggesting dysfunction of the small fiber nerves. The differential diagnosis for her small fiber neuropathy included autoimmune/inflammatory, infectious, metabolic, and toxic causes. Lab evaluation was notable for elevated HbA1c 6.0% (4.0-5.6%), hyperlipidemia including elevated total cholesterol 201 (<170 mg/dL), hypertriglyceridemia 133 (<90 mg/dL), and markedly elevated vitamin B6 level 660.4 (20-125 nmols/l). CSF studies were normal. Her vitamin B6 level corresponded with the recent intake of an over-the-counter immune supplement and suggested vitamin B6 toxicity. The supplement was stopped, and symptoms improved over several months.
Discussion/Conclusions: While individuals with PWS have known autonomic and somatosensory dysfunction presumed to be secondary to central dysregulation, peripheral nerve dysfunction, especially of the small fiber nerves, is less clearly understood. The etiology of the small fiber neuropathy was presumed secondary to acute vitamin B6 toxicity, superimposed on pre-existing dysglycemia and dyslipidemia, however, we cannot completely exclude peripheral autonomic nerve dysfunction inherent to PWS. This case highlights the importance of evaluating new sensory symptoms even when there is preexisting increased pain and thermal threshold instability. Additionally, it is a reminder that the metabolic derangements that occur in patients with PWS, including dysglycemia and dyslipidemia, are risk factors for peripheral neuropathy and should be monitored closely.
Acknowledgements/Funding: None

Perception of Prader Willi Syndrome Compared to Other Developmental Disabilities Based on Internet and News Portrayal

Presenting Author: Zoe Li-Khan

Additional Authors: Lillian Drocha, Sophia Chung, Eric Rubenstein

Institution(s): Rubenstein Lab, Boston University School of Public Health

Abstract:

Background: For people with intellectual and developmental disabilities, other's perceptions of them based on their condition often begin before birth and go on to impact relationships, opportunities, and self-perception across the life course. Search engine results and news media, which may portray these conditions stereotypically or in poor light, are often a key source in these perceptions. Our purpose was to understand how search engine results and available news media can shape perceptions on certain intellectual and developmental disabilities, including Prader Willi Syndrome, in comparison to other comparable disabilities.

Methods: We developed an online Likert-scale survey to measure differences in perceptions based on first available search engine results, images, and news headlines of four intellectual and developmental disabilities: Prader-Willi Syndrome, Angelman syndrome, cerebral palsy, and Down syndrome. These four conditions were selected to compare lesser-known (Prader-Willi and Angelman) and more well-known conditions (Down syndrome and cerebral palsy). Perception questions addressed general impressions and aspects of the disability experience expected to be impacted by perception from others. We recruited via multiple social media platforms, flyers posted in the Boston area, and word of mouth to local communities and friends.

Findings: 229 individuals opened the survey, and 125 responses were used in analysis. Mean responses to Prader-Willi syndrome were significantly more negative than responses to cerebral palsy, Down syndrome, and Angelman syndrome across all variables. Significant differences between conditions found when treating the data as continuous were confirmed when treating the data as ordinal.

Conclusion: Based on our findings, Prader-Willi syndrome is subject to more negative portrayal in media, leading to more negative perception, which may impact social, educational and therapeutic opportunities and quality of life. Distributions of responses to questions related to inspiration differed from those of questions related to general impression, capability, and finding images upsetting, indicating a diverse experience of disability perception that affects the lives of people with intellectual and developmental disabilities in various ways.

Acknowledgements/Funding: None

Focus Groups to inform a Feasibility Pilot for use of Wearable Devices in PWS Clinical Trials

Presenting Author: Sara M. Andrews[1]

Additional Authors: Sean N. Halpin[1], Anne Edwards[1], Abby Ragan[1], Ashley M. Thomas[2], Marie G. Gantz[1], Ann O. Scheimann[2]

Institution(s): [1]RTI International; [2]Johns Hopkins School of Medicine

Abstract

Background: Directly measured, objective outcomes are needed for Prader-Willi Syndrome (PWS) clinical trials. Heart rate variability (HRV) has been associated in non-PWS studies with appetite/food exposure, adolescent food cravings and food signals, diseases (e.g., diabetes), psychiatric disorders (e.g., binge eating disorder), narcolepsy, and autism. Alterations in HRV have been reported in small PWS studies that did not use consumer wearable devices (e.g., smartwatches) to collect data. Technological improvements in wearables may make them suitable for collecting HRV and other data in PWS studies. *Objective:* To assess feasibility of using wearable devices in PWS studies and formats for collecting daily contextual data from caregivers. *Methods:* We conducted two focus groups (FGs) of English-speaking participants: one with parents of a child with PWS aged 7-17 years (*n*=9), and one with adults living with PWS (*n*=7). FGs were moderated using a semi-structured guide tailored to each group (e.g., reading level). A quasi-deductive analysis was conducted on transcripts to elicit themes. *Results:* Parents mostly identified as mothers (78%), were comfortable with technology (100%), and had participated in a PWS clinical trial (78%). Adults with PWS had a median age of 21 years (IQR= 18-28), were mostly female (57%), white (71%), were comfortable with technology (86%), and had participated in a PWS clinical trial (57%). Both FGs endorsed the feasibility and acceptability of a smartwatch versus a stick-on patch for collecting HRV and other data. Over half of the adults with PWS volunteered that they wear a smartwatch. Those who do not wear it at night agreed that they could. Similarly, most parents reported that a smartwatch would be unintrusive and even desirable for their child with PWS, whereas there were concerns from some about sensory issues, compulsive removal, and lack of engagement (i.e., no screen) with the patch. For daily collection of contextual data, both FGs preferred flexibility (e.g., email or text). Parents suggested that use of wearables may be less successful in younger children with PWS. *Conclusion:* A smartwatch was endorsed as acceptable and feasible for collecting HRV data. However, age and functioning level of individuals with PWS should be considered, and contextual data collection options need to be developed and piloted. *Next steps:* A FG of Spanish-speaking parents is planned, followed by wearables pilot testing in a group home setting.

Acknowledgements/Funding: This work was funded by a grant from Foundation for Prader-Willi Research and by the RTI International Fellow Program.

Eye Tracking Technology as a Tool for Understanding Social-Cognitive Pathways in Individuals with Prader-Willi Syndrome

Presenting Author: Sarah-Marie Feighan[1]
Assistant Professor of Surgery, Drexel University College of Medicine
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Additional Authors: Gunnar Wolfe, Allen Browne, Renee Riddle, Erin Hall, John Fam, David Tichansky

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 $\geq$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 0f 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

Result from a Quantitative Survey of Current Care Paradigms for People with PWS –GLP-1 Agonist Prescribing Patterns

Presenting Author: Kimya Bhargava

Additional Authors: Meredith Manning, Mayank Misra, Anish Bhatnagar MD, Neil Cowen PhD

Institution(s): Soleno Therapeutics

Abstract:

Soleno Therapeutics commissioned a study with a third party, life sciences strategy, insights, and analytics provider. A quantitative healthcare professional (HCP) research survey was conducted consisting of primary interviews with HCPs to understand current care paradigms for patients with PWS. In the study, 53 HCPs were interviewed. The participants included pediatric and adult endocrinologists (n=32, 60%), psychiatrists (n=14, 27%), and PCPs (n=7, 13%). 15% were KOLs with experience in publications, guidelines, and/or clinical trials for PWS and 85% were community based. 23 doctors manage adult PWS patients (ages 18+) and 30 manage pediatric PWS patients (ages 0-17). Approximately 92% of their time was spent in direct patient care. Approximately 66% of HCPs recruited for this study were practicing in community settings (hospital, office or clinical based) while the remainder were in specialized centers or universities or teaching hospitals. The median number of PWS patients in an HCP's practice was 10 and the average years in practice was 19 years.

We found that with no current approved therapies for PWS, prevailing approaches focus on managing comorbidities. Across age groups, GLP-1 agents are more commonly prescribed by community treaters. Patients receiving care in community centers are 25% more likely to receive a GLP-1 vs academic treaters. Additionally, analysis of claims data suggests that 25% of PWS patients are using GLP-1s today, of which 75% are 26+ years old. 75% of HCPs believe obesity complications are a key driver of mortality in PWS patients, and 75% of HCPs indicate managing hyperphagia is key to managing obesity in PWS.

While substantial anecdotal data exists regarding GLP-1 use in PWS, there is limited data on the efficacy and safety of GLP-1s from placebo-controlled studies in people with PWS. The primary MOA of GLP-1s is to decrease gastric emptying, which can increase the risk of gastric rupture for PWS patients. Another potential complication is that GLP-1 decreases lean mass at the same time as fat mass. In a large, published weight loss study with semaglutide (NEJM 2021), GLP-1 treatment over 68 weeks resulted in a loss of 6.9 kg of lean body mass (-13.2%), and lean body mass loss accounted for 40% of weight loss. Given the potential to markedly reduce lean body mass, and the results from clinical studies in people with PWS, the use of GLP-1 agonist in people with PWS warrants more study to better characterize risks and benefits of their use.

Acknowledgements/Funding: N/A

Healthcare Provider Survey and Analysis of Healthcare Claims – Care Paradigm for Pediatric and Adult Patients with Prader-Willi Syndrome

Presenting Author: Annabelle Tiller
Additional Authors: Mayank Misra MS, Anish Bhatnagar MD, Neil Cowen PhD, Meredith Manning MBA
Institution(s): Soleno Therapeutics

Abstract: Soleno Therapeutics commissioned a study with a third party, life sciences strategy, insights and analytics provider. A quantitative healthcare professional (HCP) research survey was conducted through primary interviews with HCPs to understand current care paradigms for patients with PWS. In the study, 53 HCPs were interviewed. The participants consisted of pediatric and adult endocrinologists (n=32, 60%), psychiatrists (n=14, 27%), and PCPs (n=7, 13%). Overall, 15% were thought leaders with experience in publications, guidelines, and/or clinical trials for PWS and 85% were community-based. 23 doctors manage adult PWS patients (ages 18+) and 30 manage pediatric PWS patients (ages 0-17). Approximately 92% of their time was spent in direct patient care. Approximately 66% of HCPs recruited for this study were practicing in community settings (hospital, office or clinical based) while the remainder were in specialized centers or university or teaching hospital. The median number of PWS patients they managed was 10 and the average years in practice was 19 years. Additionally, a retrospective analysis of a large healthcare claims database was conducted to understand patient demographics, symptoms, manifestations, care settings, multidisciplinary healthcare teams, and medical insurance coverage associated with PWS patients.

HCPs report that about half of all patients were diagnosed before reaching age 4, 28% between 4 and 11, while about 10% were diagnosed as adults. Across both pediatric and adult PWS patients there is a need for an ongoing and complex care team with many co-managing physicians. Psychiatric treatment increases as patients age and develop more severe psychiatric symptoms. The HCPs suggest that about 20% of all ages may not have been seen by an endocrinologist in the last 2 years. As patients age, they typically move away from living with a full-time caregiver/parent and move into various full time supervised care facilities; a significant proportion of older patients (aged 26+) are living in a PWS specific supervised full time care facility.

Based on claims analysis, PWS patients could be divided into two populations, pediatric (ages 1-25) and adult (age ≥25). The typical pediatric patient visits a HCP 6 times per year, while the typical adult patient visits a HCP 3 times per year. Pediatric patients are most frequently seen by a pediatric or adult endocrinologist, while adult patients are most frequently seen by adult endocrinologists and psychiatrists. Pediatric patients primarily live with a caregiver at the caregiver's home while adult patients more frequently live in Residential Homes, or with/near a caregiver. Further results from the claims analysis will be presented.

In summary, care levels (frequency of healthcare provider visits) decrease as individuals age, the care setting changes, and the composition of the HCP team shifts to include more psychiatric support. Adult patients with PWS receive reduced care, and up to 20% have not seen an endocrinologist in the past 2 years, potentially leaving their endocrinological needs unaddressed. Further details regarding the care paradigms will be shared in the poster/presentation.

Acknowledgements/Funding: N/A

Outcomes of Participation in Leisure on the Mental Well-being of Pediatric Individuals with Trauma or Prader-Willi Syndrome

Presenting Author: Dr. MaryClaire Attisano, B.S, OTD

Additional Author: Dr. Shannon Strate, OTD, OTR/L

Institutions: Johnson and Wales University

Abstract

Background: Children and adolescents with trauma or Prader-Willi Syndrome lack leisure-based activities within programming supporting development. Sustaining engagement in leisure-based activities enhances mental health. Implementation of leisure activities will consistently enhance the personal growth and psychological well-being of both populations. Methods: Latham's facility will implement leisure activities with self-report measures. Scales include the (WHO-5) Well-being Scale, The Zones of Regulation Scale, and four qualitative follow-up questions.

Aim: The intention of the implementation of leisure-based activities within Latham Centers is to provide improvements in mental well-being. The need for leisure activities is increasingly evident for children and adolescents with trauma or Prader-Willi Syndrome (PWS) especially following the mental health implications of the COVID-19 pandemic. The implementation of leisure activities will foster engagement, facilitate learning experiences, promote personal growth, and work to enhance psychological well-being. Design: The facilitator will use the mixed methods approach to incorporate both qualitative and quantitative data. Method: The facilitator will collect all data outcomes and evaluate them through the following scales including the World Health Organization (WHO-5) Well-being Scale and The Zones of Regulation scale. The quantitative data will be collected by a series of follow-up questions following the leisure activity sessions. Limitations: The potential barriers to the implementation of leisure activities within Latham's facility includes programming within a set timeframe and limited activity selection due to the safety protocols of the COVID-19 pandemic. Impact: Increased mental well-being of both populations suggests promising future results and can have implications for other residential or non-residential facilities housing similar populations. This project can assist future occupational therapy practitioners in maximizing health, well-being, and quality of life for children and adolescents with PWS, trauma, or similar diagnoses to support their overall human development and function.

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Prader-Willi syndrome: Impact on caregiver and family quality of life

Presenting Author: Ann Johansson, PhD, DNP, CRNP, FNP-BC [1]

Additional Authors: Cynthia Danford, PhD, CRNP, PPCNP-BC, CPNP-PC, FAAN;[2] Suneeta Madan-Khetarpal, MD [1]

Institution(s): 1 UPMC Children's Hospital of Pittsburgh; 2 Cleveland Clinic

Abstract:

Background/Purpose: Quality of life (QOL) may be defined as subjective satisfaction with physical, psychosocial, and material wellbeing. Prader-Willi syndrome (PWS) involves physical and psychosocial morbidity for not only diagnosed children, but their families. This study aimed to evaluate caregiver-reported QOL upon initiating patient care at an interdisciplinary PWS clinic.
Methods: This descriptive study included caregivers of children with PWS attending a children's hospital in the northeastern United States. Caregivers completed a demographic questionnaire and the PedsQL2.0 Family Impact Module, a 36-item questionnaire using 5-point Likert scales, describing caregiver QOL and family functioning in the past month. Once transformed to a 0-100 scale, higher scores reflect better QOL. Scale scores are the mean of items in the scale. Mean scores were grouped into poor (≤37.5), moderate (>37.5-<62.5), and high (≥62.5) QOL. Analysis included descriptive statistics.
Results: Five caregivers of children with PWS (N=6, 1 caregiver with 2 adopted children) consented to participate. All were mothers, most (n=3) 20- and 40-years old, and almost all (n=4) Caucasian, with mostly female children (n=4) aged 4-months to 13-years (median=3.6[IQR=1.7-8.9] years). The Total Scale, Caregiver Summary, and Family Summary Scores reflect moderate QOL (mean=60-62). Caregiver QOL was reported as poor (mean=25.0-37.5) for tiredness, frustration, and worrying about their child's future; moderate (mean=41.7-54.2) for worry about treatments and how the child's illness is affecting other family members, feeling others do not understand their situation, and feeling angry, sad, and anxious; and high (mean=62.5-87.5) for cognitive function, getting support from others, physical symptoms, and communicating with others. Family QOL was moderate (mean=41.7-50.0) for finding time and energy for activities; and high (mean=62.5-79.2) for communicating and making decisions as a family, and few conflicts between family members.
Conclusions/Implications: PWS impacts caregiver's and family's QOL physically, emotionally, cognitively, and socially. Healthcare providers should give special attention to assessing QOL of family members and connecting them with appropriate services to effectively support families affected by PWS.

Acknowledgements/Funding: None

Understanding the Utility of Coping Strategies in Daily Stress Levels for Parents of Children with Prader-Willi Syndrome

Presenting Author: Lyndsey N. Graham, PhD, CCLS

Additional Authors: Bridgette Kelleher, PhD

Institution(s): Purdue University

Abstract:

Parents of children with Prader-Willi Syndrome are faced with varying amounts of stress on a day to day basis. Many parents are able to handle their stress effectively through the use of coping strategies. Coping can take on many forms, though some strategies tend to be more useful than others. While many coping strategies would be expected to reduce stress, other strategies may be related to increases daily stress levels, or may not impact stress at all. To that end, we examined the usefulness of a set of daily coping strategies for rare disorder caregivers. Fifty rare disorder caregivers participated in biweekly 7-day ecological momentary assessment (EMA) surveys across 8 weeks, with over 950 reports from 9 PWS families. All parent caregivers completed short “snapshot” surveys four times per day throughout the study, reporting on daily stress levels, mood, social support, and health behaviors. Preliminary analyses indicated that 67% of the variability in daily stress was at the within-person level, suggesting that caregivers experience major fluctuations in their stress levels from moment to moment. We will describe the utility of various coping strategies in addressing daily stress levels in parent caregivers; we will specifically look at how the experiences of PWS families differ compared to other rare disorder caregivers across indices of stress and coping strategies. Ongoing studies suggest that the needs of PWS families may differ compared to other rare disorder caregivers; implications for researchers, practitioners, and caregivers will be discussed.

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Neuroanatomical differences in Prader-Willi Syndrome subtypes: Insights from MRI analysis

Presenting Author: Chun-Xia Yi[1,2,4]

Additional Authors: Esther M. Speksnijder[1,2], Marten J. Peters[1,3], Felipe Correa da Silva[1,2,4], Ingrid Caroline van Nieuwpoort[1], Leopold M. G. Curfs[5], Dirk Jan Stenvers[1,2], Madeleine L. Drent[1].

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Abstract:
Prader-Willi Syndrome (PWS) is a complex neurodevelopmental genetic disorder. Approximately 70% of individuals with PWS have a de novo deletion of the paternally derived 15q11-q13 region, resulting in two subtypes, namely Type I (PWS T1) and Type II (PWS T2). The larger T1 deletions involve four additional haploinsufficient genes. Patients with T1 deletions have a higher risk of developing obsessive-compulsive behaviors, self-injury, defective visual processing, and lower academic achievement than those with the T2 deletion. However, the brain mechanisms responsible for these differences remain unclear. Our current study employed neuroimaging analysis to explore whether there are differences between PWS T1 and PWS T2. T1-weighted MRI images from 6 PWS T1 patients, 6 PWS T2 patients, and 13 non-PWS controls were used to explore volumetric brain structure using a sophisticated segmentation method. In addition, a specified hypothalamic segmentation tool was used to obtain the volume of the hypothalamus and its sub-regions. Interestingly, in our cohort, the only brain region with volume differences between PWS T1 and PWS T2 was the cerebellum, which was significantly larger in PWS T1 patients. While most of the brain regions did not show clear differences in volume between PWS T1 and T2, there were significant differences between the PWS group and controls. These include the anterior-superior and tubular inferior hypothalamic subunits, which were smaller in the PWS patients. The cerebellum white matter and brainstem were marginally smaller in the PWS patients, whereas the lateral ventricle, third ventricle, left choroid plexus, cerebrospinal fluid, and total ventricle & choroid plexus volume were larger in the PWS patients compared to the controls. Our results suggest that the anatomical differences in the cerebellum might underlie the different cognitive dysfunction observed in PWS patients with T1 or T2 deletions.

Acknowledgments/Funding: N/A

The role of Lateral Septal Neurons in Prader-Willi Syndrome

Presenting Author: Alyssa Koehler

Additional Authors: Whitnei Smith, Estefania Azevedo

Institution(s): Medical University of South Carolina

Abstract:

Prader–Willi syndrome (PWS) is a neurodevelopmental disorder characterized by hyperphagia, obesity and intellectual disability. PWS results from deletion of a part of chromosome 15 which affects several genes including MAGE family member L2(MAGEL2). In situ RNA hybridization shows abundant expression of MAGEL2 in the lateral septum, a region that directly connects to several nuclei in the hypothalamus. The lateral septum (LS) is a key node that processes and integrates salient information and relays those downstream to the hypothalamus, a master regulator of feeding behavior. Our group has showed that the LS contains a specific population of neurons that are important to the development of obesity. Silencing of neurotensin-expressing neurons in the LS (LS^{NT}) induce hyperphagia, high-fat diet consumption leading to obesity. On the other hand, activation of LS^{NT} neurons induce an anorexigenic phenotype. Several LS populations, including LS^{NT} neurons, project to several hypothalamic nuclei to control feeding behavior and obesity (i.e. lateral hypothalamus, tuberal nucleus, suprammamillary nucleus). These data suggest that LS is a key node controlling feeding and obesity. In MAGEL2-null mice, data showed that dysfunctional signaling for vasopressin and oxytocin were observed in the LS. However, the identity of lateral septal neurons and the molecular mechanisms by which these neurons become dysfunctional and lead to the hyperphagic and obese phenotype observed in PWS is unknown. We hypothesize that MAGEL2 deletion leads to dysfunctional signaling in specific molecular pathways in specific LS populations and contributes to the hyperphagic and obese phenotype observed in PWS. We propose to use innovative state-of-the-art tools in neuroscience, including single nuclei RNA sequencing in the lateral septum of MAGEL2-null mice, a widely-used mouse line that mimics several phenotypes observed in PWS. Using this powerful technique, we will obtain an unbiased, comprehensive transcriptome of genes, including druggable receptors, that mark dysfunctional lateral septum populations in MAGEL2-null mice compared to controls. Top gene candidates will be orthogonally validated using optogenetics and pharmacology with the ultimate goal of finding potential druggable targets for treating hyperphagia and obesity in PWS patients. Considering that drug development can take decades, we will also focus in finding genes and pathways that can be corrected with drugs that are already FDA approved and commercially available, but not yet used to specifically treat PWS due to lack of research. Our data will shed light on the neuronal and molecular mechanisms by which MAGEL2, a gene which mutation mimics many of the same features of PWS patients, alter a key feeding node in the brain and contributes to the hyperphagic and obese phenotype observed in PWS. The data derived from these studies will identify novel neural pathways and druggable molecular targets that potentially can rescue the feeding alterations observed in PWS. Our ultimate goal is to find new targets to treat PWS in humans.

Acknowledgements/Funding: PWS Foundation, MUSC, Whitehall Foundation

Comprehensive Therapeutic Potential of EPM301 for Hyperphagia, Anxiety, and Sleep Disturbances in Prader-Willi Syndrome

Presenting Author: Phillip J. Rose[1]

Additional Authors: Joseph Tam[2], Peter J Welburn[1]

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[2] Obesity and Metabolism Laboratory, Faculty of Medicine, School of Pharmacy, The Institute for Drug Research, The Hebrew University of Jerusalem, Jerusalem, Israel

Abstract:
The primary unmet need for individuals with Prader-Willi syndrome (PWS) is the management of hyperphagia, which, if uncontrolled, can lead to obesity. In addition to obesity, PWS patients often experience high levels of anxiety, resulting in obsessive-compulsive behaviors, rigidity, stubbornness, argumentativeness, and temper outbursts. Sleep disturbances, leading to excessive daytime sleepiness, are also common among PWS patients. Current Phase III drugs for PWS target specific symptoms such as hyperphagia (Carbetocin, DCCR) or sleep disturbances (Pitolisant). EPM301, a novel CBD analogue in pre-clinical evaluation by EPM Therapeutics, shows potential as a comprehensive treatment for PWS. Early studies suggest EPM301 may address hyperphagia, anxiety, and daytime sleepiness. In the *Magel2* null mouse model of PWS, EPM301 administration (20 and 40 mg/kg/day, intraperitoneally) significantly reduced body weight and food intake in high-fat diet-fed mice[1]. Additionally, EPM301's effect on anxiety-like responses was evaluated in rats using the light-dark box emergence test. At a dose of 0.01 µg/kg (intraperitoneally), EPM301 significantly reduced stress-induced anxiety[2]. Further studies investigated EPM301's impact on the sleep-wake cycle in male Wistar rats. EPM301 demonstrated a dose-dependent (0.1, 1.0 or 100 µg/kg, intraperitoneally) increase in wakefulness and a decrease in slow-wave sleep duration, with no significant change in rapid eye movement sleep[3]. These promising animal model results suggest that EPM301 could offer multiple therapeutic benefits for individuals with PWS.

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Enhanced Bioavailability and Therapeutic Potential of EPM301, a Novel Cannabidiol Analogue, in the Treatment of Prader-Willi Syndrome

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Abstract: Cannabidiol (CBD), the principal non-psychotropic compound derived from *Cannabis sativa*, has shown therapeutic efficacy in treating various health conditions, including epilepsy, pain, nausea, and anxiety[1], attributed to its anti-inflammatory, immunomodulatory, antioxidant, and neuroprotective properties. However, CBD's high lipophilicity and low oral bioavailability in humans, due to incomplete absorption and significant first-pass metabolism (with 70–75% of an oral dose metabolized hepatically before systemic circulation), present challenges for its clinical use.

Current research includes two development programs investigating cannabinoids in patients with Prader-Willi syndrome (PWS). Radius Health initially developed RAD011, a synthetic oral CBD, to treat hyperphagia in PWS, but the program was discontinued in September 2022. A small clinical trial is now evaluating the effects of orally administered cannabidivarin (CBDV) on hunger and behavior in PWS patients.

EPM301, a novel CBD synthetic analogue developed by EPM Therapeutics, is a promising candidate for PWS treatment. A recent study demonstrated that EPM301 (20 and 40 mg/kg/day, intraperitoneally) effectively reduced body weight and hyperphagia in a high-fat diet-fed *Magel2* null mouse model of PWS[2].

An *in vitro* biotransformation of EPM301 using rat, dog, minipig, and human hepatocytes characterized its metabolites. Unlike CBD, EPM301 exhibited limited metabolism across all species after incubation with hepatocytes for up to 4 hours. The parent compound, EPM301, constituted 82.7% of the total peak area response in rat, 93.6% in dog, 81.2% in minipig, and 85.8% in human hepatocytes. The most abundant metabolite, M13, accounted for 4.0%, 0.2%, 5.0%, and 4.0% of the total peak area response in rat, dog, minipig, and human hepatocytes, respectively. The second most abundant metabolite, M9, accounted for 0.2%, 4.0%, 3.3%, and 2.6% of the total peak area response in the respective species. All human metabolites were also detected in at least one animal species.

An ongoing study compares the oral bioavailability of EPM301 and CBD in rats, with results to be presented at the upcoming FPWR conference. Based on the *in vitro* findings and the anticipated outcomes from the rat bioavailability study, EPM301 is expected to exhibit superior oral bioavailability compared to CBD, offering significant therapeutic advantages for PWS patients.

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Acknowledgements/Funding: This work was supported by EPM Therapeutics

Loss of Magel2 Alters Protein Translatome in the Mouse Hypothalamus and Pituitary

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Abstract: **Introduction:** Translational regulation of gene expression occurs on polysomes and is in particularly important in secretory cells, including neuroendocrine cells of the hypothalamus, to achieve high protein synthesis levels without triggering the protein stress response. Dysregulation of mRNA translation contributes to several neurodevelopmental diseases. However, its contribution to the pathogenesis of Prader-Willi syndrome (PWS) is not known. PWS is a neurodevelopmental disorder, whose hallmark is improper hypothalamic neuroendocrine function. PWS is caused by the loss of several genes, including melanoma antigen L2 (*MAGEL2*), which was recently shown to be critical for the regulated secretion of the hypothalamus. We hypothesize that MAGEL2 contributes to proper hypothalamic neuroendocrine function also on the translational level. **Methods:** To investigate the potential role of MAGEL2 in translational regulation, we performed polysome profiling of the hypothalamus and pituitary of 16 wild-type and *Magel2*$^{p\Delta/m+}$ mice. Hypothalamic and pituitary lysates were loaded on the top of a sucrose gradient, and ultracentrifugation was performed to separate mRNAs associated with different numbers of ribosomes, including monosomes, light polysomes, and heavy polysomes which correspond to the translation efficiency from low to high, respectively. To evaluate the role of Magel2 on translatome, we extracted RNA from each group and performed RNA sequencing. **Results:** RNA sequencing data were analyzed to determine differentially translated genes in KO vs. normal tissues. We found that several mRNAs were differentially present in the *Magel2*$^{p\Delta/m+}$ hypothalamus and pituitary monosomes, light polysomes, and heavy polysomes fractions compared to the wild-type animals; specifically, in the pituitary, we found 402 differentially present genes in monosomes, 404 genes in light polysomes, and 198 genes in heavy polysomes. This suggests that Magel2 contributes to the translation efficiency of several mRNAs in the pituitary and hypothalamus. **Conclusion**: Our data provide novel insight into the potential role of MAGEL2 in protein synthesis and hormone secretion with implications for symptom development in patients with PWS. Future analyses are warranted to determine the underlying molecular mechanism and potential therapeutic potential.

Acknowledgements/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.).

Single-Nucleus RNA Sequencing Offers Novel Insights into the Magel2 Function in Fed and Fasted Conditions

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Abstract:
The MAGE family protein MAGEL2 is coded within the critical region of chromosome 15 (15q11-q13) and is key in the occurrence of Prader-Willi syndrome (PWS), a rare, genetic, multisystemic, neurodevelopmental disorder. While the deletion of the whole chromosomal region causes PWS, the mutations in the gene *MAGEL2* represent the underlying cause of a similar disorder called Schaaf-Yang syndrome (SYS). On a cellular level, deletion, and mutations of MAGEL2 were recently associated with the reduction in proteins associated with secretory granules, due to MAGEL2 regulating retromer-dependent endosomal trafficking, suggesting MAGEL2's critical role in hypothalamic neuroendocrine function. Recent data indicate that the MAGE gene family has evolved to protect cells from environmental stress, such as nutritional deprivation, facilitating stress adaptation, but the role of MAGEL2 during stress is not known.
To address this question, we exposed wild-type and *Magel2*$^{p\Delta/m+}$ male mice to 24h-fasting-induced stress and measured serum corticosterone levels. Then we performed single nucleus RNA sequencing (snRNAseq) on hypothalami from 16 mice from 4 different groups, 4 mice per group: 1 - wild-type/control, 2 - wild-type/24h fasting, 3 - *Magel2*$^{p\Delta/m+}$/control and 4 - *Magel2*$^{p\Delta/m+}$/24h fasting group. For snRNAseq library generation, 2 animals per group were pooled and 2 libraries per group were analyzed. Initial quality control (QC), alignment, and quantification were done with CellRanger (v7.2.0), and data was filtered by the percentage of mitochondrial content, number of counts, and number of detected genes. Normalization, dimension reduction, clustering, and differential expression were done with Seurat (v5.0.3). After quality control, we obtained 130,000 nuclei per library, which were subsequently classified into 23 cell types based on established cell type markers, including nonneuronal (astrocytes, oligodendrocytes, microglia, tanycytes, ependymal and endothelial cells) and neuronal cells (several excitatory and inhibitory neurons). Then, we analyzed cellular composition among 4 major treatment groups and found that the ratio of cell types was not significantly changed between different groups, except oligodendrocytes. Then we performed differential gene expression (DEG) analysis in each cell type to determine the effects of fasting and the Magel2 knockout. The cell types that express the highest number of DEGs upon starvation are endothelial cells, microglia, and excitatory neurons. The cell types with the highest DEG upon Magel2 depletion are microglia, endothelial cells, and oligodendrocyte precursor cells. These findings suggested that Magel2 affects several cell types in the hypothalamus in a fed and

fasted state. With ongoing gene ontology analysis, we aim to get the first insights into the molecular mechanisms regulated by Magel2 in specific cell types, which may lead to a new understanding of Magel2's role in hypothalamic function under nutritional stress and in the pathogenesis of PWS and SYS.

Acknowledgements/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.).

Metabolic Inputs to the Prefrontal Cortex Mediating Cognitive Processes in Magel2-null Mice

Presenting Author: Antonin Cornillon

Additional Authors: E. Saraceno, M. Cooke, K. Callahan, R. Calitri, R. Shansky, R. Ross

Institution(s): Albert Einstein College of Medicine, Northeastern University

Abstract:
Prader-Willi Syndrome (PWS) is a complex neuroendocrine disorder that is marked by an inability to detect metabolic states such as hunger and satiation. Accompanying these metabolic dysregulations, PWS also presents with symptoms of cognitive dysfunction including cognitive rigidity and difficulty adapting to change, which may play a role in the hyperphagia seen in people with PWS. To understand whether the metabolism and cognitive functions are related in the brain, we used a mouse model for PWS, the magel2-null mouse, in a combination of molecular and behavioral studies. We evaluated cognitive deficits and neuropeptide receptor expression in different hunger states focusing on the prefrontal cortex region, a region involved in cognitive function. Magel2-null mice demonstrate key trends in their metabolic function when compared to controls, notably marked by progressive weight gain and increased fat pad weight, mirroring characteristics of PWS. Additionally, they display a delay in associative learning during fasted states, decreased impulsivity and decreased motivation during a chronic fast. This motivation difference was absent after an acute overnight fast. When comparing neuropeptide receptor expression in Magel2-null mice to controls, oxytocin receptors do not change between ad libitum and acute fasted states, yet demonstrate decreased expression after a chronic fast. In addition to this, orexin receptors are chronically upregulated in Magel2-null mice compared to control mice, regardless of metabolic state. Together this shows that hunger state induces tangible molecular changes in metabolic peptide signals in the prefrontal cortex, and we hypothesize that this may underlie the cognitive deficits seen in our behavior tasks of learning, impulsivity, and motivation. Better understanding of these changes in the Magel2-null mice may help provide new insight and potential treatment targets to help mitigate the symptoms of PWS.

Acknowledgements/Funding: Funding for this project was generously provided by the Foundations for Prader-Willi Research to Dr. Rachel A. Ross and by the Northeastern URF PEAK Awards to Antonin B. Cornillon.

September 26th, 2024
SPEAKER ABSTRACTS MORNING SESSION 1A

The Epitranscriptome of Hypothalamic Neurons During Fasting is Altered in a T1 PWS Human Model

Presenting Author: [1]Donna M Lehman
Additional Authors: [1]Li Yao, MD, PhD, [1]Erika Espinosa, [2]Juan Peralta
Institution(s): [1]Texas A&M University San Antonio and Texas A&M University School of Medicine, [2]South TX Diabetes and Obesity Institute, UT Rio Grande Valley School of Medicine

Abstract:
We are investigating whether the paternal PWS deletion globally alters the epitranscriptomic fine-tuning of hypothalamic neurons to lead to aberrant signaling which may underlie the hyperphagia clinical manifestation. The epitranscriptome comprises many classes of biochemical modifications to the RNA, each causing functional changes to the molecule to control or adapt its task, allowing for rapid adjustments in activity in response to developmental or environmental cues. Using iPSC-derived hypothalamic neuronal cultures, we previously mapped N6-methyladenosine (m6A) modifications, which is the most abundant post-transcriptional modification among mRNA, and identified m6A signatures of human hypothalamic neuronal activation and subsequent inactivation via nutrient/hormonal signaling. We now have mapped m6A modifications genome-wide of mRNA, lncRNA, and small RNAs under experimentally “fed” and “fasted” conditions for hypothalamic neuronal cultures derived from iPSC that were CRISPR edited to mimic the paternal Type 1 PWS deletion and the isogenic non-deleted controls. The m6A modifications were identified using the Arraystar human m6A-mRNA&lncRNA epitranscriptomic microarray. Differentially m6A-modified mRNAs, lncRNAs and other ncRNAs based on "m6A quantity" passing fold change (FC) and statistical significance cutoffs were identified and compiled. Hierarchical clustering heatmap analysis was performed for differentially m6A-modified RNAs. Differentially m6A-modified mRNAs were analyzed for GO and pathway enrichment. Applying a FC cutoff of >2 and FDR of >0.05, we observed the set of mRNA differentially modified between PWS deletion-bearing vs control hypothalamic neurons under fasted conditions to be enriched for the KEGG pathway identifiers “insulin secretion”, “linoleic acid metabolism”, “neuroactive ligan-receptor interaction” ($p<.01$ for all), and the disease term “waist-hip ratio” ($p=5E-3$). These results support the hypothesis that the epitranscriptome is altered in hypothalamic neurons due to the PWS Type 1 deletion and these changes may underpin the metabolic phenotypes.

Acknowledgements/Funding: This work is supported by Foundation for Prader Willi Research (DML), and NIH R01DK114661 (DML). Foundation for Prader Willi Research (to Michael Talkowski, PhD, Massachusetts General Hospital and Harvard Medical School) supported development and sharing of the iPSC lines used for this project.

Demethylation of Chromosome 15q11-13 Region in Induced Pluripotent Stem Cells from Prader-Willi Syndrome Patients by Targeted DNA Demethylation Technology Using dCas9–Peptide Repeat and scFv–TET1 Catalytic Domain Fusion

Presenting Author: Hironobu Okuno

Additional Authors: Akisa Nemoto, Kent Imaizumi, Shinji Saito, Mamiko Yamada, Kenjiro Kosaki, Hideyuki Okano

Institutions: Tokyo Medical University, Keio University School of Medicine

Abstract: Prader-Willi syndrome (PWS) is one of the well-known imprinting disorders. This syndrome is caused by dysfunction of the chromosome 15q11-13 region, which contains several genes that are expressed only on the paternal allele, so called paternally expressed genes (PEGs). It is known that 75% of PWS patients have deletions in this paternally derived region 15q11-13. In these patients, the maternal allele is present and sufficient for the gene. However, due to imprinting mechanisms, these genes are methylated and have no gene expression.

Then, we generated iPS cells from two patients with deletion-type PWS and one patient with UPD-type PWS. We modified CRISPR/Cas9 technology to unmethylate the PWS-IC region on the maternal alle without altering the nucleotide sequence (Morita et al. Nature Biotech. 2016). We observed that this region-specific demethylation caused demethylation of PEG downstream of PWS-IC, and that gene expression of PEG was restored accordingly.

We also found that this demethylation did not occur even after iPS cell passaging and induction of hypothalamic differentiation.

This technology is expected to be applied to the elucidation of disease pathology and cell transplantation therapy using patient-derived cells.

Acknowledgements/Funding: This study was supported by funding from the Japan Agency for Medical Research and Development (AMED) (Grant Number 22bm0804003h0006 to H.Okano and Grant Number 23bm14230009h0001 to H.Okuno); and Japan Society for the Promotion of Science (JSPS) (Grant Number 22J11876) to A.N.

Minipig Genome assembly for comparative analyses of the PWS locus

Presenting Author: Nicole M. Tillquist, Ph.D.

Additional Authors: Savannah J. Hoyt, Gabrielle A. Hartley, Patrick G.S. Grady, Nicole Pauloski, and Rachel J. O'Neill

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Abstract:
To determine whether pigs (*Sus scrofa)* can serve as a large-animal model for the human etiology of Prader- Willi Syndrome (PWS), we derived long read based genome assemblies for two minipig strains– *nanopig* and *Yucatan* (Sinclair Bio Resources). Skin biopsies (<5mm) were obtained and cell lines were established for a female *nanopig* and a female *Yucatan* pig. Fibroblasts were grown in culture and cell pellets (≥ 5.0 x 10^6) were isolated, washed with sterile PBS, and frozen at -80°C. High molecular weight (HMW) gDNA was extracted from cell pellets via DNEasy (Qiagen, long read samples) or Monarch HMW (New England Bio Sciences, ultra-long read samples). Library preparation was performed in-house and long read and ultra-long read sequencing was achieved on the Oxford Nanopore Technologies (ONT) PromethION using duplex Q20 chemistry and Dorado super accurate base-caller. Reads were assessed for quality and length using NanoPlot. Passed reads were mapped to the *Sus scrofa* reference genome (Sscrofa11.1, NCBI) using minimap2. Reads aligning to chromosome 1, which contains synteny to human Chr 15q11.2-q13 (the location on the PWS locus), were *de novo* assembled using *Flye*. Each minipig assembly was evaluated for coverage, accuracy, and contiguity to ensure complete coverage of the PWS locus. Both of our pig assemblies carry the full PWS locus in a single contig, in contrast to the currently available *S. scrofa* genome assembly (Sscrofa11.1, NCBI) which contains a misassembly of the PWS locus between *UBE3A* and *SNRPN,* resulting in the placement of *SNRPN* on an unassembled contig. Further development of our new assemblies will assess the full genomic structure of the PWS locus and assess the potential for minipigs as a model to increase our understanding of PWS.

Acknowledgements/Funding:
Funding for this work was provided by the Foundation for Prader Willi Research.

SPEAKER ABSTRACTS MORNING SESSION 1B

Long-Term Safety of Diazoxide Choline Extended-Release (DCCR) Tablets in Participants with Prader-Willi Syndrome from the Completed C601 Randomized Double-Blind Placebo-Controlled Study (DESTINY PWS) and C602 Open Label Extension (OLE) Study

Presenting Author: Michael Huang, MD[17]

Additional Authors: Evelien F. Gevers, MD, PhD[1], Jennifer Lynne Miller, MD[2], Lynne Bird, MD[3], Moris Angulo, MD[4], Jack Yanovski, MD, PhD[5], Parisa Salehi, MD[6], Ashley Hall Shoemaker, MD, MSCI[7], Merlin G. Butler, MD, PhD, FFACMG[8], Kathryn Obrynba[9], David Stevenson, MD[10], Virginia Kimonis, MD[11], John Wilding, DM, FRCP[12], M Guftar Shaikh, MD,[13] Anthony Goldstone, MD, PhD,[14] Melissa Lah, MD[15], Elizabeth Littlejohn, MD, MS[16], Jing Gong, MS[17], Patricia Hirano, MPH[17], Neil Cowen, PhD[17], Anish Bhatnagar, MD[17]

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Abstract:

Objectives and Methods: Prader-Willi syndrome (PWS) is a rare genetic neurobehavioral-metabolic disorder characterized by hyperphagia, excess fat, hypotonia, and behavioral/psychological complications. No approved medications exist for hyperphagia in patients with PWS. DCCR is currently under development as a treatment for PWS. The objective of this analysis was to evaluate the long-term safety of DCCR from 2 completed PWS studies. 125 participants with genetically-confirmed PWS ≥4 years old received daily oral DCCR in studies conducted at 29 sites in the US and UK: a 13-week, double-blind, placebo-controlled study (Study C601, DESTINY PWS) and the long-term, open-label extension (OLE) period of Study C602. Participants had hyperphagia assessed by the Hyperphagia Questionnaire for Clinical Trials. The target DCCR dose was 3.3-5.8 mg/kg.

Results: Mean duration of DCCR treatment was 2.5 years (maximum: 4.5 years) with 105 participants treated >1 year and 90 participants treated >2 years. DCCR was well-tolerated with treatment-emergent adverse events (TEAEs) in 98.4% of participants with most being grade 1 or 2 (75.2%). Drug-related TEAEs occurred in 88.8% of participants. 20 participants reported serious adverse events (SAEs), with 2 patients having drug-related events (peripheral/pulmonary edema and fluid retention). There were no suspected unexpected serious adverse reactions (SUSARs) or deaths. The most common TEAEs were hypertrichosis (68.8%), peripheral edema (34.4%), hirsutism (27.2%), and hyperglycemia (27.2%). TEAEs infrequently

resulted in discontinuation (7.2%), with peripheral edema (2.4%) and hyperglycemia (1.6%) being the most common reasons.
Consistent with the expected TEAE of hyperglycemia, mean fasting glucose and HbA1c increased slightly over the initial 6-9 months and stabilized or returned to near-baseline levels upon continued DCCR dosing. Most participants with hypertrichosis, peripheral edema, hirsutism, or hyperglycemia resolved (or were resolving) with continued DCCR dosing through the end of the study. Infrequently, DCCR dose adjustments or glucose-lowering agents (for hyperglycemia) and diuretics (for peripheral edema) were prescribed to manage participants experiencing these TEAEs.
Conclusions: DCCR was well-tolerated after long-term DCCR use up to 4.5 years. The common TEAEs were expected based on past studies. These included hypertrichosis, peripheral edema, hirsutism, and hyperglycemia, which were typically mild and often resolved without medical intervention.

Acknowledgements/Funding: This work was supported by Soleno Therapeutics.
This abstract was presented at the Pediatric Endocrine Society (PES) 2024 Annual Meeting and published by S. Karger AG, Basel in *Hormone Research in Paediatrics*. Reused with permission.

Delayed gastric emptying and the safety of long-acting GLP-1 receptor agonists in Prader-Willi Syndrome. Results from the ENGAGE PWS Study.

Presenting Author: A/Prof Alex Viardot

Additional Authors: Dr Amanda Hor, A/Prof Tania Markovic, Georgina Loughlin, Prof Lesley Campbell

Institution(s): Garvan Institute of Medical Research, Sydney, Australia

Abstract:
Background: Management of hyperphagia and behavioral problems in Prader-Willi Syndrome (PWS) remains a major challenge. GLP-1 receptor agonists (GLP-1RA) have gained traction in the treatment of diabetes and obesity in people with PWS and have potential beneficial effects in suppressing appetite. However, little is known yet about the safety of this drug class in people with PWS as they have demonstrated to delay gastric emptying (GE).

Methods: This two-part cross-sectional and prospective cohort study aimed to assess the prevalence of delayed gastric emptying assessed by gastric scintigraphy in patients with PWS compared to healthy controls. Furthermore, a 12-week intervention with exenatide extended-release (Bydureon) in PWS subjects examined the effect on GE, appetite, metabolic parameter and body weight.

Results: Our study showed that adults with PWS have a high prevalence of delayed gastric emptying (3/13, 23%), which is underdiagnosed in the clinical setting. The 10 PWS subjects who entered the intervention with Bydureon showed a delay in gastric emptying after 4 and 12 weeks of treatment. These findings challenge the conventional concept of tachyphylaxis associated with long-acting GLP-1RA. Only about 50% of subjects benefited from some degree of weight loss, which is less than reported in non-PWS subjects. We could not observe any changes in hyperphagia behavior or cognition.

Conclusion: This study advances our understanding of abnormal gastric motility in PWS and the effects of long-acting GLP-1RA on GE, emphasizing the need for a personalized approach and careful monitoring due to the potential risks. Screening for delayed GE before initiating and monitoring during GLP-1RA therapy should be considered to prevent disastrous complications such as gastric necrosis. Future research should investigate the newer, more potent GLP-1RA and dual and triple peptide receptor agonists whilst investigating gastric and intestinal motility using gold-standard techniques.

Acknowledgements/Funding: This study was supported by families affected by PWS, and the Curran Foundation, St Vincent's Hospital Sydney.

Craving Friendship: Investigating Friendship Behaviors in Young Adults with Prader-Willi Syndrome

Presenting Author: Carly Knauss

Additional Authors: Robert Hodapp, Elizabeth Roof, Elisabeth Dykens

Institution(s): Vanderbilt University

Abstract:

Like typically developing individuals, those with intellectual and developmental disabilities (IDD) desire social connectivity in the form of friendships. Existent literature suggests differences in friendships among those with versus without IDD in both who they form friendships with, and the reasons that underlie such friendships (Callus, 2017; Gillooly et al., 2022). Although friendships are important to those with PWS, few studies have examined the challenges and benefits that people with PWS face in making and sustaining friendships. A recent, FPWR-sponsored intervention was successful in improving social cognition and interpersonal skills that young adults with PWS need to make and sustain their friendships with others (Dykens et al., 2022). Without such interventions, however, several syndrome-related characteristics (e.g., hyperphagia, rigidity, difficulties with perspective-taking) conspire to make it especially difficult for people with PWS to initiate and sustain friendships. The current study uses in-depth, online Zoom interviews with approximately 50 young men and women with PWS, aged 18-35, to learn more about how they perceive and self-report on their behaviors as a friend. To compare self- versus parental reports of such friendships, mothers of their adult children are being asked similar questions via an online questionnaire. We are examining results using both qualitative thematic analyses and quantitative statistics. Although data collection is ongoing, preliminary findings suggest that many young adults with PWS have a difficult time reporting the challenges they have in being a good friend, and that friendship formation is difficult, especially beyond the school-aged years. Research and practical implications will be discussed.

Acknowledgements/Funding: Special thanks to the individuals with PWS and their mothers participating in this study.

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SPEAKER ABSTRACTS MORNING SESSION 2A

NPAP1: novel insight into its role in Prader-Willi Syndrome

Presenting Author: Angelo Serani1

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Abstract:
Our study unveils a novel evolutionary mechanism involving the human-specific nuclear pore complex-associated protein 1 (NPAP1) in lipid accumulation and circadian rhythm alteration in Prader-Willi Syndrome (PWS). Investigating the modern and ancestral variants of NPAP1 in mouse cells lacking paternal Snord116, we found that the two haplotypes influence adipogenesis and lipid uptake pathways differently. 3D protein modeling indicates conformational changes between the two variants may affect its interaction with other molecules, impacting lipid regulation. This prediction was confirmed by analyzing the impact of NPAP1 variants on cell RNA expression and investigating the molecular partners involved in the protein complexes with high throughput techniques.
Co-immunoprecipitation and mass spectrometry reveal that NPAP1 is mainly involved in transcription regulatory complexes. Among the NPAP1 molecular partners, we found that a group of transcription factors presents at least one binding site on most of the deregulated genes we identified by RNAseq. In particular, we found that NPAP1 is potentially involved in the adipogenesis and lipid accumulation axes mediated by FOXO1 and HGMA1 transcription factors.
Finally, we generated a novel mouse model presenting NPAP1 in the same orthologous region of the human genome to recapitulate the physiological role of this protein in vivo. This model will be the platform for testing a novel epigenetic writer recently developed in our lab. This tool can potentially revert the phenotypes induced by the NPAP1/Snord116 imbalance by modifying the target-specific gene expression in our animal models and potentially represent a novel therapeutic strategy for PWS patients.

Acknowledgements/Funding: None

Snord116 non-coding RNA sequesters *Nhlh2* target mRNA in the nucleus of hypothalamic neurons, suggesting a possible mechanism that accounts for phenotypes found in individuals with Prader-Willi syndrome

Presenting Author: Deborah J. Good[1]

Additional Authors: Shadi Ariyanfar[1], Stuart Tobet[2], Christopher K. Thompson[3]

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Abstract: Prader-Willi Syndrome (PWS) 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@* snoRNAs are cleaved from a larger long non-coding gene, *SNHG14*. In previous work, we showed that levels of *Nhlh2* mRNA were increased in cells that contained a *Snord116* expression construct and had significantly reduced mRNA stability in cells that did not express *Snord116*, mimicking the cellular environment in PWS. Whole-body deletion of *Nhlh2* in mice results in PWS-like phenotypes, such as later onset weight gain, and delayed puberty. In trying to understand "where" and "when" *Nhlh2* and *Snord116* are co-expressed in the brain, we have made significant progress, specifically identifying brain regions such as the hippocampus, habenula, cerebellum, hindbrain, spinal cord, and hypothalamus as key areas for co-expression. In addition, we find co-localization outside of the brain, including the testes, ovaries, and skeletal muscle. Interestingly, in hypothalamic neurons from normal (WT) mice co-expressing *Nhlh2* and *Snord116 (*or the host gene *Snhg14*), *Nhlh2* mRNA levels are high, and co-localized with *Snord116* in the nucleus in 80% of neurons that express both RNAs. However, *Nhlh2* message is evenly distributed between the cytoplasm and nucleus of neurons that do not co-express *Snord116*. Consistent with these findings, *Nhlh2* is almost exclusively distributed to both the cytoplasm and nucleus of hypothalamic neurons from mice that lack *Snord116* (Snord116^{del}-a mouse model of PWS). Additionally, hypothalamic cell lines that co-express *Nhlh2* and *Snord116* expression plasmids recapitulate the nuclear sequestering of *Nhlh2* mRNA, while cell lines that only express *Nhlh2* expression plasmids have the mRNA distributed throughout both the cytoplasm and nucleus. We are continuing our analysis of this mRNA regulatory phenomenon by *SNORD116*, specifically now using human brain tissue from patients with PWS and neurotypical controls to determine if this same regulation pattern occurs. We hypothesize that *NHLH2* mRNA levels will be lower in PWS patients and found evenly distributed between the cytoplasm and nuclear compartments of neurons. Our ongoing studies are the first to demonstrate that *Snord116* non-coding RNAs may regulate mRNA expression of their targets using cellular partitioning between the cytoplasm and nucleus. This work lays the groundwork for fundamentally changing our understanding of gene expression networks in the mammalian brain, providing hope to identify Snord116-targeted therapies for treatment of Prader-Willi syndrome.

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Abnormal sleep-wake dynamics in Snord116^{del} mice

Presenting Author: Ramalingam Vetrivelan

Additional Authors: Whidul Hasan, Shaili Nandigam, Sathyajit Sai Bandaru

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Abstract: In addition to growth and metabolic issues, individuals with Prader-Willi Syndrome (PWS) suffer from significant sleep-wake abnormalities. Specifically, excessive daytime sleepiness and rapid eye movement (REM) sleep dysregulation manifesting as a higher percentage of REM sleep, sleep-onset REM, and cataplexy (sudden loss of bilateral skeletal muscle tone triggered by positive emotional stimuli) are the most debilitating sleep problems in PWS. In contrast to other symptoms, sleep dysregulation in PWS and its neural basis remain poorly understood. One of the issues stalling progress in this direction is that sleep-wake behavior in murine models of PWS has not been well characterized. To address this issue, we examined sleep-wake behavior in transgenic mice sustaining *paternal* deletion of Small nucleolar RNA, SNORD116 (Snord116^{del} mice), an animal model of PWS.
Under anesthesia (100 mg/kg ketamine+10 mg/kg xylazine), adult male Snord116^{del} mice and their wildtype (WT) littermates (n=10 each) were instrumented for electroencephalogram (EEG) and electromyogram (EMG). After 4 weeks of recovery and habituation, EEG/EMG recordings were performed for 24 hours in each mouse to assess their sleep-wake amounts and architecture at baseline conditions.
We found that Snord116^{del} mice displayed a significant increase in REM sleep amounts during both the light and dark periods but the amounts of wake or NREM sleep did not differ from controls. The increase in REM sleep was primarily due to an increase in the number of REM bouts, suggesting a higher rate of NREM-REM sleep transitions. We also observed abnormal REM sleep intrusions in the Snord116^{del} mice. These data confirm that the Snord116^{del} mice exhibit similar REM sleep abnormalities to those observed in individuals with PWS, validating them as appropriate animal models to study the neural mechanisms underlying REM sleep dysregulation in PWS.

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Analysis of hypothalamic fat-sensing pathways throughout postnatal development in *Magel2*-null mice

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Institution(s): Department of Cell Biology, Physiology and Immunology, University of Cordoba (Spain), Maimonides Research Institute of Cordoba (IMIBIC, Spain), Hospital Universitario Reina Sofía (Cordoba, Spain) & Parc Taulí Hospital Universitari-Institut d'Investigació I Innovació Parc Taulí (I3PT-CERCA, Sabadell, Spain).

Abstract: Prader-Willi syndrome (PWS) patients experience a significant metabolic shift throughout development. Newborns display decreased feeding behavior, while children and adults exhibit extreme hyperphagia, leading to obesity. Similarly, *Magel2*-null mice, an animal model of PWS, show decreased body weight before weaning and increased adiposity and weight gain in adulthood. Hypothalamic dysfunction has been suggested as the primary cause of these metabolic changes, but the specific targets and mechanisms remain unknown. Mounting evidence suggests that fat-sensing pathways in the hypothalamus play a role in controlling energy balance. However, their influence on the metabolic changes in PWS during postnatal development has not been investigated. Here, we examined the hypothalamic expression of relevant fatty acid transporters (CD36, FATP1, and FATP4), free fatty acid receptors (FFAR4), and lipid transducers (PPAR-γ), as well as the hypothalamic content of key lipid-signaling molecules (ceramides), in the hypothalamus of *Magel2*-null mice at different postnatal (P) development stages (P7, 14, 23, 35, and 42). Additionally, we analyzed relevant neuropeptides in the control of energy balance, such as *Pomc* and *Npy*, and the metabolic phenotype of the animals in such context. In male *Magel2*-null mice, we observed reduced mRNA levels of *Ppar-γ* at P23, a stage when their body weight was decreased, and a decline of *Pomc* mRNA expression at P35, a period when the animals began to increase food intake and experience more significant weight gain compared to their respective controls. In female *Magel2*-null mice, *Ppar-γ* mRNA levels decreased at P14, while *Fatp1* and *Fatp4* mRNA expression increased at P23. These changes were associated with reduced body weight. At P35, when female *Magel2*-null mice began to experience increased food intake and weight gain, *Pomc* mRNA levels declined, a phenomenon that was associated with higher *Ppar-γ* mRNA levels. In contrast to male Magel2-null mice, female Magel2-null mice exhibited significant changes in certain ceramide species. Specifically, four ceramide species were found to be increased at P35, while three ceramide species were significantly decreased at P42. Our findings indicate that male and female Magel2-null mice experience a comparable metabolic shift during development, but the mechanisms driving these changes may differ. In particular, females show more significant changes in fat-sensing pathways than males throughout development, especially in Ppar-γ and Ceramide signaling. However, additional experiments are necessary to fully confirm this conclusion.

Acknowledgements/Funding: This work was conducted thanks to the funding support provided by Foundation for Prader-Willi Research.

SPEAKER ABSTRACTS MORNING SESSION 2B

High Rates of Dysphagia and Silent Aspiration in Children with Prader-Willi Syndrome

Presenting Author: Sani Roy, MD[1]
Additional Authors: Amy Trejo, BS[1]; Jennifer McReynolds[1]; Ana Neblett, RD[1]; Keisha Shaheed, DO[1]; Yvette Johnson, MD, MPH[1]; and Kristen Taylor, DO[1]
Institution(s): Cook Children's Medical Center (CCMC)

Abstract:
Background: A few studies to date have noted dysphagia and/or silent aspiration (SA) in children with PWS[1,2]. As symptoms, such as gagging, choking, and coughing with feeds are often the impetus for obtaining a swallow study (SS), it is important to further understand the prevalence of dysphagia and SA in PWS and to investigate associated clinical characteristics and long-term clinical implications.
Methods: We assessed prevalence of dysphagia on bedside swallow study (BSS) and videofluoroscopic swallow study (VFSS) and additionally silent aspiration (SA) by VFSS in children with PWS at CCMC. We investigated 1) associated clinical characteristics, 2) acute visits for pulmonary complications, and 3) ongoing growth hormone therapy at the time of the first VFSS. A two-tailed Fisher's exact test was used to assess if there was a difference in number of acute pulmonary visits in those who had vs. those who did not have SA on their first VFSS.
Results: Fifty total swallow studies (SS) were performed for 24 patients with PWS: 8 BSS and 42 VFSS. The sample was 58.3% male and 62.5% White (25% Black), with mean gestational age 37.7 weeks and mean age at PWS diagnosis 28.5 days (range 0-101); 58.3% had a PWS deletion subtype. The mean age at BSS was 3.0 months. Mean age at initial VFSS was 2.8 months in the majority (*n*=18), and four children had VFSS at older ages of 18.5 months, 21.8 months, 5.2 years, and 13.9 years. Dysphagia was noted in 87.5% on first BSS and in 100% on first VFSS. The most common dysphagia was oropharyngeal both on BSS (62.5%, *n*=5) and on first VFSS (50%, *n*=11). On first VFSS, SA was noted in 36.4% (*n*=8), and the main preceding symptoms included hypotonia (72.7%), poor feeding (72.7%), lethargy/fatigue with feeds (54.5%) and incoordination of suck/swallow (54.5%). Aspiration on VFSS was always silent. Patients with SA on their first VFSS were 7 times more likely to have an acute care visit for pulmonary issues compared to those who did not have SA on their first VFSS (two-tailed p=0.039). Ongoing growth hormone therapy was noted at the time of first VFSS in 25% of those who had SA on their first VFSS, and in 35.7% of those who did not have SA on first VFSS. On the 20 follow-up VFSS, dysphagia persisted in 80% (*n*=16) but SA had resolved in 70% (*n*=14).
Conclusion: Dysphagia was noted in all patients by VFSS and in nearly all by BSS. Aspiration was present on first VFSS in more than a third of patients and was always silent. Patients who had SA on their first VFSS were more likely to have an acute care visit for pulmonary issues than those who did not have SA on first VFSS. Further studies are needed to understand long-term implications of silent aspiration and whether growth hormone therapy plays a role in dysphagia and silent aspiration in children with PWS.
Acknowledgements/Funding: The authors acknowledge Svetlana Camacho for assistance in data extraction. SR's work was supported by the Cook Children's Endowed Chair Program.
References:
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Clinician Survey of Management of Growth Hormone Therapy and Sleep Evaluation in Infants with Prader-Willi Syndrome

Presenting Author: Emily Paprocki DO[1]
Additional Authors: David Ingram MD[1], Janelle Noel-MacDonnell PhD[1], Sani Roy MD[2], Keisha Shaheed DO[2], David Viskochil MD[3], Parisa Salehi MD[4], Maida Chen MD[4], Kelsee Halpin MD[1]
Institution(s): Children's Mercy Kansas City[1], Cook Children's Medical Center[2], University of Utah Health[3], Seattle Children's[4]

Abstract:

Background: Current consensus guidelines encourage polysomnogram (PSG) prior to starting growth hormone (GH) in individuals with Prader-Willi Syndrome (PWS) to avoid adverse effects[1]. However, there are challenges with obtaining and interpreting PSG in infancy; therefore, alternative sleep assessments may be necessary. We conducted a survey collecting clinician perspectives regarding initiation of GH therapy and sleep evaluation in infants younger than 1 year of age with PWS.

Methods: A REDCap survey was distributed to 10 medical societies involved in PWS care. Survey respondents' data were descriptively summarized. Each respondent fell into 1 of 3 categories defined by the number of patients with PWS seen per year: < 5 (low volume, LV), 5-20 (medium volume, MV), and >/= 21 (high volume, HV). Bivariate analysis was conducted between all variables and 3 categorical variables based on volume of patients with PWS seen.

Results: We analyzed 113 surveys from pediatric PWS clinicians across the U.S. Most respondents (80%) specialized in endocrinology followed by sleep medicine/pulmonology (13%). Most were from urban, academic centers (86%) and had been caring for patients with PWS >10 years (56%). Respondents were evenly distributed geographically throughout the United States. Among respondents, *n*=48 (43%), 32 (28%), and 33(29%), fell into LV, MV, HV categories, respectively. HV respondents were more likely to answer "not always" when asked whether they perform baseline PSG prior to GH initiation (LV: 22%, MV: 31%, HV: 67%, *p*=<.001). Top reasons for deferring PSG among HV respondents were 1) avoiding delay in GH (*n*=15/22, 68%) and 2) lower concern with side effects in infants (*n*=13/22, 59%). The median (IQR) ideal age of GH start was 3 months (2-6 months) in MV and HV groups compared to 6 months (4-12 months) in LV group (*p*=.004). All HV respondents said GH should be started by 6 months of age. In contrast, 32% of LV and 19% of MV respondents said GH start between 12-36 months of age was ideal. A subset of HV respondents (*n*=7/33, 21%) routinely start GH < 2 months of age; often in the NICU prior to discharge. Most respondents (*n*=81/108, 75%) supported updating the PWS consensus guidelines. Only 3 respondents report they follow current guidelines mostly as written.

Conclusion: High volume PWS clinicians are more likely to forego baseline PSG to prevent delay of starting GH and have less concern about side effects in infants. All HV respondents aim for GH to be started by 6 months of age. The majority of clinicians support updating the PWS consensus guidelines. These survey findings substantiate the need for more data to evaluate the safety of GH initiation prior to PSG in infants.

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Defecation disorders in children with Prader Willi Syndrome

Presenting Author: Parisa Salehi MD[1,2]

Additional Authors: Hannah Bahram Pour[2], Lusine Ambartsumyan MD[1,2]

Institution(s): [1]Seattle Children's Hospital, [2]University of Washington

Abstract:

Background: Functional defecation disorders, such as Functional constipation (FC) and fecal incontinence (FI) affect children of all ages with a world-wide prevalence of 0.7-29% and 0.8-4.1% respectively. Children with Prader Willi Syndrome (PWS) commonly present with hyperphagia, endocrinopathies, and gastrointestinal abnormalities including FC and FI. **Aim:** To describe the prevalence and characteristics of functional defecation disorders in children with PWS who attend a Multidisciplinary PWS Program. **Methods:** We retrospectively reviewed the medical records of 87 children with PWS who completed a bowel questionnaire as part of their routine multidisciplinary clinic visit. Demographic data was extracted from the medical records. A bowel questionnaire was used to determine if patient met Rome IV Criteria for FC and to characterize defecation and voiding characteristics. **Results**: 81 children with PWS (74% female, mean age 8.5 ± 6.9 years) were included. Thirty percent met Rome IV criteria for FC, 23% reported FI, and 20% reported urinary incontinence. Thirty-five percent were using osmotic and/or stimulant laxatives and 21% were followed by the gastroenterology (GI) clinic. Of children who met Rome IV criteria for FC, 46% were using osmotic and/or stimulant laxative and only 42% were followed in the GI clinic. Notably, 46% had FI and 21% of parents reported that soiling affects their child's quality of life. Interestingly, 46% of parents whose children met Rome IV criteria for FC did not think their child was constipated. **Conclusion**: Defecation disorders are common in children with PWS. Thirty percent of children with PWS met Rome IV criteria for FC of which nearly half reported FI with impact on quality of life. However, most children who met Rome IV criteria were not followed in GI clinic and were perceived by their parents to not be constipated. This study highlights the importance of parent and provider education and recognition of functional defecation disorders in children with PWS.

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EHR-based strategy to explore clinical profiles and comorbidities in Prader-Willi syndrome

Presenting Author: Waheeda A. Hossain, MD, MPH[1]

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Abstract:

Electronic Health Records (EHR) offer a unique opportunity to establish robust clinical profiles for rare genetic syndromes. For individuals with Prader-Willi syndrome (PWS), it is especially important to develop and validate EHR-derived computable phenotypes to curate clinical data for exploration of comorbidities and health outcomes (*e*.g., early morbidity and mortality). An algorithm to accurately identify individuals with PWS using evidence from structured data elements was designed using data from the University of Kansas Medical Center (KUMC) Health system. Cases were defined as individuals with: (1) ≥2 PWS diagnosis codes [ICD9:759.81 and/or ICD10:Q87.11] on different dates, or (2) ≥1 PWS diagnosis code [ICD9:759.81, ICD10:Q87.11, ICD10:Q87.1] and ≥1 prescription for RxNorm strings reflecting growth hormone use (e.g., somatropin [RxCUI = 61148]). Controls were matched on BMI and age with ≥2 visits and no ICD codes for PWS or other congenital malformation syndromes. The algorithm was validated via manual medical record review, including confirmation based on genetic testing followed by inter-rater reliability. Eighty-seven cases met criteria for the computable phenotype including 51% males and 74% reported white race. Controls (n=58) were 45% male and 52% white. Manual chart reviews confirmed 60 (69%) patients with PWS, 21 (24%) confirmed not PWS, and 6 (7%) could not be confirmed. Genetic tests were found for 49 (82%) individuals, others were confirmed by doctor's note. Genetic classification for PWS included Type I deletion 4%, Type II deletion 22%, UPD 47%, atypical deletion 4%—others were deletion or methylation positive without specific subtype. The 21 false positive PWS cases reflected other genetic syndromes, indicating low specificity for the PWS ICD codes. By developing an algorithm using structured EHR data, we successfully identified individuals with PWS within the KUMC Health system. Our findings underscore the challenges in accurately diagnosing PWS, often relying on clinical history rather than genetic testing. Incomplete documentation of PWS genetic test highlights the need for improved data capture and provider education regarding the significance of these assessments. Leveraging EHR-derived data offers promising avenues for advancing our understanding of clinical profiles in PWS, emphasizing the importance of ongoing medical surveillance and tailored care for individuals with this syndrome. Future endeavors will concentrate on utilizing data extracted from other institutes to deepen our comprehension of the correlation between sleep disruptions and PWS, highlighting the necessity for ongoing medical monitoring and intervention.

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SPEAKER ABSTRACTS AFTERNOON SESSION 1

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

Presenting Author: Lawrence T. Reiter[1,2,3]

Additional Authors: Chidambaram Ramanathan[4], Andrew C. Liu[5], Yang Shen[5], A. Kaitlyn Victor[1]

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Abstract:

Prader-Willi syndrome (PWS) patients are known to suffer from sleep problems, including sleep-disordered breathing and central hypersomnolence. Mouse models of PWS reflect these altered circadian rhythms, including REM sleep alterations and defects in rhythmic DNA methylation. In cultured cells, *Magel2* has been shown to regulate *Bmal1* and *Per2*, two core clock 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 the *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 observed circadian bioluminescence rhythms in these patient-derived neurons across several days. Here we show, for the first time, that circadian period length differs from controls 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 negative feedback loop regulating *Bmal1* and *Per2* in the circadian pathway oscillates as expected. Recent work has focused on cyclic gene expression changes in DPSC neurons using QuantSeq to interrogate four different circadian time points in PWS and control neurons. These studies revealed new candidate genes for PWS pathology with expression that 1) does not cycle properly; 2) cycles opposite to controls or 3) does not cycle at all. 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.

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Pilot Research Awards from the Foundation for Prader-Willi Research

The role of SNORD115 and SNORD116 in neuronal development

Presenting Author: David Tollervey

Additional Authors: Aleksandra Helwak

Institution: Wellcome Centre for Cell Biology

Abstract: Small nucleolar RNAs (snoRNAs) are a family of short non-protein coding RNAs (ncRNAs) whose best studied functions are the chemical modification of pre-ribosomal RNA. Human cells express hundreds of snoRNAs; some are tissue specific and many lack known functions or targets. Two types of “orphan snoRNA”, SNORD116 and SNORD115, are encoded within introns of a very long ncRNA, snoRNA host gene 14 (SNHG14). This gene lies within the maternally imprinted PWS locus, and its expression is defective in Prader-Willi Syndrome. Loss of SNORD116 appears causal in development of PWS, whereas micro-deletions of only the neighboring SNORD115 do not have clear phenotypes.

SNORD116 and SNORD115 show brain-enriched expression and PWS is a neurodevelopmental disorder. To identify roles for SNORD115 and SNORD116 in neuronal development, we generated disease models. Using CRISPR-Cas9 we precisely deleted either SNORD115 or SNORD116 from the expressed, paternal chromosome in an immortalized neuronal progenitor cell line; Lund human mesencephalic (LUHMES) cells. Wild type LUHMES cells differentiate into dopaminergic neurons, and naturally accumulate SNORD115 and SNORD116 during neurodevelopment. We characterized transcriptomic and proteomic signatures of WT and mutant cells across 15 days of differentiation.

Visually, wild type and mutant cells differentiate with similar dynamics, creating extensive neuronal networks. RNAseq analysis revealed that undifferentiated wild type and mutant cells are initially very similar. However, they diverge during the differentiation process. By day 15, a few hundred genes were differentially expressed in mutant cells relative to the WT, with a substantial proportion shared between mutant cell lines. Aiming to find a link between SNORD116 expression and PWS phenotypes we directly compared the two deletion mutants. This analysis produced a short list of 24 significantly altered genes. Based on published literature, these have strong links to PWS phenotype.

To uncover the molecular mechanisms that lead to PWS development, we are currently focusing on defining direct molecular interactions of SNORD115 and SNORD116 with target RNA and proteins. Better understanding of those processes should support future therapeutic interventions.

Acknowledgements/Funding: FPWR, Wellcome Trust

Impact of MAGEL2 on the development of human induced pluripotent stem cell (hiPSC)-derived neurons

Presenting Author: Jannis Bücking[1]
Additional Authors: Tobias Walczuch[1], Michael Eibl[1], Tobias Beschauner[1], Freya Hermann-Sim[1], Susanne Theiss[1], Melanie Spanjaard[1], Katrin Hinderhofer[1], Derek Tai[2], Celine De Esch[2], Michaela Bartusel[3], Byron Lee[3], Nima Jaberi-Lashkari[3], Eliezer Calo[3], Syed Azmal Ali[4], Michael Talkowski[2], Jeroen Krijgsveld[4], Christian P. Schaaf[1], Magdalena Laugsch[1]

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Abstract: *MAGEL2* point mutations observed in Schaaf-Yang Syndrome (SYS) result in a more severe phenotype compared to the large genomic deletions encompassing the whole *MAGEL2* gene as seen in Prader-Willi Syndrome (PWS). However, the specific contribution of *MAGEL2* to SYS and PWS remains to be elucidated. Hence, we aimed to determine cellular pathways affected by aberrant MAGEL2 in neurons. To this end, we generated and validated CRISPR/Cas9-engineered heterozygous and homozygous *MAGEL2* deletion human induced pluripotent stem cell lines (hiPSC) in two different genetic backgrounds. We then differentiated these hiPSC alongside isogenic PWS type I/II deletion and two typical SYS-associated *MAGEL2* mutations lines into cortical neurons and conducted RNA-sequencing and whole proteome analyses. Our preliminary multi-omics data indicate differentially affected pathways associated with changes in the (post)synaptic membrane, the extracellular matrix (ECM), and nuclear division. Due to lack of an N-terminal MAGEL2 antibody, it is difficult to elucidate the specific pathomechanism of how the mutated MAGEL2, potentially resulting in a truncated protein at the C-terminus, leads to a more severe phenotype in SYS compared to the deleted *MAGEL2* in PWS. To address this, we CRISPR/Cas9-engineered an additional panel of hiPSC lines with endogenous N-terminally V5-Spot-tagged wildtype and SYS-mutated *MAGEL2* in a third genetic background with hetero- and homozygous deletions of *MAGEL2* as controls, enabling co-immunoprecipitation and immunofluorescence applications. This will allow us to determine the neuron-specific localization and interaction partners of mutated MAGEL2. Overall, our unique and powerful cellular system will provide data that will deepen the knowledge about the role of MAGEL2 in neurodevelopment and its contribution to PWS and SYS.

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Transcriptional Defects in Prader-Willi Syndrome Cortical-Like Cultures is Driven by Transcription Factors that Regulate the Extracellular Matrix.

Presenting Author: Tayler Hedgecock[1]

Additional Authors: A. Kaitlyn Victor[1], Daniel Johnson[1], David Garmire[2], Lawrence T. Reiter[1]

Institution(s): [1]The University of Tennessee Health Science Center, [2]The University of Michigan

Abstract: Prader-Willi Syndrome (PWS) is a multigenic neurodevelopmental disorder that is characterized by the loss of expression of maternally imprinted genes in the 15q11.2-q13.1 region. Individuals with PWS present with hypotonia, developmental delay, and childhood obesity. Little known about the molecular mechanism during neural development of PWS which leads to intellectual disability and autism. Since current mouse models do not accurately recapitulate PWS phenotypes, cellular models derived from PWS patient samples are crucial to understanding the molecular etiology of disease during early neural development. Our laboratory uses dental pulp stem cells (DPSC)-derived neuronal cultures to investigate a variety of neurodevelopmental disorders including PWS. These neuronal cultures contain a collection of cell types including glia, excitatory and inhibitory neurons. Because of the mixed cellular nature of these cultures, we decided to use single cell RNA sequencing (scRNA-seq) to investigate differences in both cell type and gene expression during early neural development between neurotypical controls and PWS Deletion DPSC derived cultures. We found significant differences between PWS and control lines for all identifiable cell types in our cultures, with excitatory neurons possessing the most differentially expressed genes. Notably, there was downregulation of extracellular matrix receptors and modulators in PWS vs controls, likely driven by the transcription factors; TWIST2, ZNF469, and PRRX1. Additionally, we found decreased expression of ribosomal-related transcripts in excitatory neurons. Identification of dysfunctional molecular pathways in a cell-specific manner will help resolve the neurodevelopmental trajectory of PWS.

Acknowledgements/Funding:

Pilot Research Awards from the Foundation for Prader-Willi Research

Myelinating cortical organoids with paternal PWS deletion show gene profile of autistic and obsessive compulsive behaviors

Presenting Author: Erika Espinosa[1]
Additional Authors: Li Yao[1], Elizabeth Roeder[2], Michael Talkowski[3], Juan Peralta[4], Donna M. Lehman[1]
Institution(s): [1]Texas A&M University San Antonio, [2]Baylor College of Medicine, [3]Massachusetts General Hospital and Harvard University, [4]South TX Diabetes and Obesity Institute, UT Rio Grande Valley School of Medicine
Abstract: Despite the progress in elucidating the genetic map of the paternal deletion on chromosome 15 in TI/TII Prader Willi Syndrome, the molecular and cellular mechanisms for many of the clinical manifestations remain unknown. We are investigating this neurodevelopmental genetic disorder using cortical organoid models generated from patient derived induced pluripotent stem cells (iPSC), which were CRISPR edited to carry a paternal PWS Type 1 deletion, and their isogenic non-deleted control. To further assess the effect of myelination process on autistic-related behaviors and other cognitive-behavioral phenotypes, we utilized a protocol that enhances derivation of oligodendrocytes and myelinating structures. This protocol generates mature myelinated axons in 3D cortical organoids within 30 weeks in culture. We have collected and processed multiple isogenic sets of cortical organoids for RNA-Seq as well as imaging using electron and confocal microscopy at 4 timepoints: 14, 20, 26, and 30 weeks. RNA-Seq of triplicate RNA isolates (each comprised of 4 independently generated organoids) in early development at 14 weeks was recently completed and confirmed the paternal Type 1 deletion with paternally imprinted genes lacking detectable expression in the Type 1 deletion carriers only and non-imprinted genes in the region showing approximately 50% expression level of the isogenic controls. A total of 18,827 transcripts were detected by RNA-Seq, and principal component analyses showed significant clustering of the samples by deletion vs. control. DESeq2 detected 4,780 transcripts to be differentially expressed at an adjusted p-value of <1E-7. No chromosome except chr15 was overrepresented in the DEG set. We conducted pathway and gene-set enrichment analyses of the DEG using Toppgene and further confirmed our results with David and g:Profiler. We observed striking enrichment for Human Phenotype Ontology terms related to obsessive compulsive behavior and autism including “social disinhibition,” “abnormal repetitive mannerisms,” “autistic behavior,” and “aggressive behavior” (qvalFDR B&Y = 2E-6, 2E-6, 3E-6, 6E-3, respectively). We also observed significant enrichment for HPO terms “abnormal cerebral white matter morphology” and “slanting of the palpebral fissure” (qvalFDR B&Y =3E-2 and 6E-2). The top GO terms for “cellular component” category were synapse, neuron projection, and glutamatergic synapse (padj=3E-87, 2E-72, and 3E-44); and for “biological process” category was “neuron projection development” (q=2E-83). Reactome pathway analyses of the DEG genes belonging to the HPO set of social disinhibitions primarily pointed to glutamate binding and GABA activity and roles in GPCR signaling. Collectively, these results suggest that neurobehavioral manifestations of PWS begin very early in brain development, can be modeled, and warrant further investigation of the human cerebral organoid systems at this early timepoint and longitudinally as they mature, and are ongoing in our lab.
Acknowledgements/Funding: This work is supported by Foundation for Prader Willi Research (DML), NIH R01DK114661 (DML), NIH R03HD108551-01A1 (DML); Foundation for Prader Willi Research (MT) supported development of the iPSC lines used for this project.

SPEAKER ABSTRACTS AFTERNOON SESSION 2

The VNS4PWS Study: a Patient Centric Device Trial to Reduce Temper Outbursts in PWS

Presenting Author: Lisa Burnett, PhD

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Abstract: Prader-Willi Syndrome (PWS) is a uniquely complex and challenging neurodevelopmental disorder. Among the most difficult features of PWS is a behavioral phenotype that includes a marked propensity for extreme temper outbursts. Temper outbursts affect between 60% to 80% of people with PWS and are a major cause of family stress and placement breakdown, with a pronounced detrimental effect on quality of life (Mazaheri *et al.*, 2013). Treatment options for temper outbursts are very limited; psychiatric medications are often used in crises without evidence of benefit. Vagus nerve stimulation (VNS) therapy from implanted devices is FDA approved for treatment-resistant epilepsy, treatment-resistant depression, and cluster headaches. Two small pilot studies of VNS therapy in people with PWS suggest that this may be a potential noninvasive, nonmedication treatment option for developmentally inappropriate temper outbursts in people with PWS (Manning, *et al.,* 2016; Manning *et al.,* 2019). The present study, *A Phase 3, Randomized, Double-Blind, Dose-Ranging Evaluation of Transcutaneous Vagus Nerve Stimulation (tVNS) to Reduce Temper Outbursts in People with Prader-Willi Syndrome (PWS)*, or *VNS4PWS*, seeks to understand if tVNS treatment is safe, acceptable, and effective in the reduction of developmentally inappropriate temper outbursts in people with PWS aged 10-40. This is a multicenter trial sponsored by the Foundation for Prader-Willi Research. The protocol was developed with input from patient advocates, and is designed to minimize burden on participants, caregivers and clinical trial sites.

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Oral ARD-101, a Bitter Taste Receptors (TAS2R) Pan-Agonist: Novel Mechanism of Action and Early Clinical Evidence of Hyperphagia Control

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Abstract:

Hyperphagia of Prader-Willi Syndrome (PWS), an insatiable and intense hunger, is associated with severe anxiety and aggressive food-seeking behavior. Over three decades ago, it was discovered that individuals with PWS have impaired food-stimulated secretion of intestinal cholecystokinin (CCK), the satiety hormone known to play a key role in the gut-brain signaling of hunger control. Since then, CCK has represented an important therapeutic target for the treatment of hyperphagia. However, several programs utilizing systemic administration of long-acting CCK receptor agonists were discontinued due to severe "on-target, off-tissue" toxicities, including pancreatitis and neurotoxicity. By contrast, oral ARD-101, a bitter taste receptor (TAS2R) pan-agonist, stimulates the localized secretion of CCK by enteroendocrine I-cells, which acts in an autocrine/paracrine manner to activate the gut-brain axis. This activation may result in reduced hunger, normalization of metabolic markers, and reduced inflammatory cytokines implicated in obesity and the psychopathological symptoms in PWS. Importantly, orally administered ARD-101 is gut-restricted (systemic exposure $\leq$ 1%), which contributes to the excellent safety profile documented in animal models and humans.

Early clinical findings from an ongoing Phase 2a trial of oral ARD-101 in young adults with PWS confirmed safety and provided early evidence of reduced hyperphagia with marked improvement in anxiety and food-seeking behavior in several treated individuals. It is postulated that binding of ARD-101 to TAS2Rs in the intestines stimulates the secretion of CCK, thereby restoring the otherwise impaired CCK-mediated control of hyperphagia. The key findings and plans for a large confirmatory clinical trial will be discussed.

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PATH for PWS: A Non-Interventional, Observational, Natural History Study in Prader-Willi Syndrome (PWS)

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Abstract:

Introduction: The *PATH (Paving the way for Advances in Treatments and Health) for PWS* study (NCT03718416) is a 4-year, prospective, non-interventional PWS natural history study, sponsored by the Foundation for Prader-Willi Research (FPWR). This caregiver-reported study captured serious medical events, PWS behaviors, and medication use over a four-year period, ending in January 2024, to help contextualize observations in clinical trials and aid in development of new therapies for the treatment of PWS. The PATH study was entirely remote and was a sub-study of the Global PWS Registry, hosted on the National Organization of Rare Disorders (NORD) IAMRARE Registry Platform.

Methods: Participants, age 5 and up, were enrolled in PATH from October 2018 through June 2019. Enrolment numbers exceeded recruitment goals with a total of 647 participants consenting and completing the initial assessments. Respondents in the study complete surveys every 6 months, providing data on serious medical events that required hospitalization, emergency department or acute care visits. Interviews were completed to document each serious medical event, with a detailed de-identified narrative of the event completed. Events are being coded using MedDRA version 27.0. Additional data was collected on height and weight, concomitant prescription medications, PWS behaviors, and food security.

Results/Discussion: From October 2018 through January 2024, participation in the PATH study showed a high retention rate with more than 83% of study participants active through the entire study period. More than 850 serious medical events were documented. Most commonly, these events involved mental health/extreme behaviors, gastrointestinal events, orthopedic problems, infections, and seizures. The mortality rate was ~0.5% per year, with most of the deceased individuals in the Class 3 obesity category (BMI>40). Documentation of medication use demonstrates that behavior/psychiatric medications are frequently prescribed, and the use of these drugs increases with age such that more than 60% of individuals over the age of 18 are taking medication for behaviors and 47% are taking multiple medications. A longitudinal analysis of hyperphagic behaviors as measured by the Hyperphagia Questionnaire for Clinical Trials (HQ-CT) showed a broad range of scores across all ages and an increasing linear trend in mean HQ-CT score with increasing age through childhood and adolescence, with stable or slightly decreasing scores in later adulthood. HQ-CT scores were higher in individuals who were overweight or obese compared to those who were normal weight. Measures to limit access to

food, as assessed by the Food Safe Zone questionnaire, increased with the age of the participant and were positively associated with higher HQ-CT scores.

Conclusions: Mental health/extreme behaviors are the most commonly reported serious medical event in the PATH for PWS study, highlighting the need for effective treatments of this aspect of PWS. Polypharmacy is common and increases with age. Hyperphagic behaviors increase throughout childhood and adolescence, and limitations on food access increases with age.

Acknowledgements: This study was funded by the Foundation for Prader-Willi Research. We are extremely grateful to all of the families who participated in the PATH for PWS study.

Validation of the Food Safe Zone Questionnaire for Families of Individuals with Prader-Willi syndrome

Presenting Author Elisabeth M Dykens, PhD.

Additional Authors: Elizabeth Roof, MA, HSP, Hailee Hunt-Hawkins, P-MHNP, FNP, Theresa Strong, Ph.D.

Institution(s): Vanderbilt University, FPWR

Abstract:

Background. Hyperphagic individuals with PWS are chronically hungry yet rarely feel sated, and often engage in food-seeking behaviors. To avoid obesity in their children, families implement food security strategies (e.g., locking food sources, supervision around food). Yet such accommodations may undermine parental appraisals of hyperphgia (e.g., it's not a problem as we lock food). Indeed, the FDA and clinical trial sponsors have questioned if such parental accommodations could undermine their accurate appraisals of hyperphagia in clinical trials aimed at attenuating hyperphagia. We developed the Food Safe Zone (FSZ) questionnaire to minimize parental accommodation by reminding parents of their food safety practices prior to them evaluating their child's hyperphagia.

Methods. Our team developed 20 FSZ items that were revised for clarity and completeness in an iterative feedback process with stakeholders, including parents of individuals with PWS, researchers and specialists in PWS, and individuals with PWS. The FSZ was pilot tested (n=133), descriptive findings were reviewed by additional stakeholders, and then administered to 624 parents in a large-scale study. Based on an open-ended question, "Is there anything else you do to ensure food safety?" two additional items were added and evaluated in a follow-up study (n=119).

Results. Principal component analyses revealed that 21 FSZ items loaded onto 5 factors that were readily interpretable, accounting for 67% of test variance: Alerting Others and Food Supervision in the Community; Locking or Restricting Food Sources; Checking for Food; At Home Supervision and Meals; and Avoiding Food Settings. Internal consistency and test-rest reliability were robust. Construct and content validity were well-established via the iterative revision process with stakeholders. Convergent validity analyses revealed that regardless of age, parents implemented FSZ strategies in response to the severity of their child's hyperphagia, especially their food-seeking behaviors.

Conclusions. The psychometrically sound 21-item FSZ stands to enhance accurate parental assessments of hyperphagia in future studies or clinical trials. Analyses of the open-ended question also shine a light on the extraordinary measures that parents use to ensure the health of their loved one with PWS.

Acknowledgements/Funding: We are grateful to the over 700 families who participated in this research, the partnership with FPWR in accessing the Global PWS Registry, and the FPWR Clinical Trials Consortium and other stakeholders. RedCap support for data collection was provided by Vanderbilt University Medical Center's NCATS/NIH grant UL1 TR000445.

PWS Research Resources

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

GLOBAL PRADER-WILLI SYNDROME REGISTRY

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.

PWS CLINICAL TRIAL CONSORTIUM

www.pwsctc.org

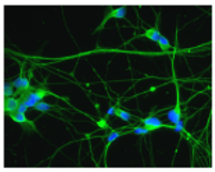
The ***International PWS Clinical Trial Consortium*** leverages the expertise from industry, academia, and patient organizations to address the unmet scientific, technical, clinical, and regulatory needs of clinical trials for PWS

AutismBrainNet

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 (lreiter@tulane.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 CLIC

CLINICAL INVESTIGATION COLLABORATIVE

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.