FOUNDATION FOR PRADER-WILLI RESEARCH

Foundation for Prader-Willi Research Scientific Day 2015

PROGRAM

Radisson Hotel, Austin, TX
September 25th, 2015

FOUNDATION FOR PRADER-WILLI RESEARCH

KEYNOTE ADDRESS

Dissecting the neural circuits for maladaptive feeding and reward

Garrett Stuber, PhD, Assistant Professor of Psychiatry, Cell Biology and Physiology, UNC Chapel Hill, School of Medicine

Abstract:
In order to survive and effectively navigate an ever-changing and unpredictable environment, organisms must readily adapt their behavior to seek out needed resources, while simultaneously avoiding life-threatening situations. These opposing processes are controlled by neural circuitry that is readily engaged by both environmental and physiological factors to promote behavioral output. In addition,dysfunction of particular neural circuits may trigger deviations from adaptive motivated states. The bed nucleus of the stria terminalis (BNST) and the lateral hypothalamus (LH) are crucial neural substrates for motivated behaviors, but the precise functional neurocircuitry within these structures that control specific aspects of motivated behavior have not been defined. I will discuss our recently published findings that demonstrate that precise circuits in the extended amygdala and hypothalamus regulated specific aspects of motivated behavioral states related to feeding behavior. In addition, I will discuss data that show how LH GABAergic neurons encode behaviors related to reward seeking and consumption. These findings may help explain how dysregulated activity at a number of unique circuit nodes can result in a cascading failure within a defined brain network to produce maladaptive behavioral states related to feeding.

FPWR FUNDED PROJECT:
Garrett Stuber - Inhibitory circuits and transmission in the hypothalamus in a mouse model of PWS

Aberrant brain reward pathways in the *Magel2*–null mouse model of Prader-Willi syndrome

Presenting Author: Rachel Wevrick

Additional Authors: Chloe E. Luck

Institution: Department of Medical Genetics, University of Alberta

Abstract:
*Introduction***:** Although the consequences of obesity cause considerable morbidity in people with PWS, disordered eating including preoccupation with food, excessive hunger, lack of satiety and binge-eating behavior, often occur even in the absence of obesity. Disordered food-related behaviors severely impact on the ability of people with PWS to live independent lives. *MAGEL2* is one of a set of genes inactivated in PWS, and lack of *MAGEL2* may contribute to disordered eating in people with PWS.

*Aim***:** We investigated the pathways governing food-related behaviors in mice lacking *Magel2*. Our long-term goal is to develop sensitive and consistent outcome measures useful for preclinical testing in anticipation of therapeutic interventions for disordered eating in PWS.

Methods and Results: The *Magel2* gene is expressed in regions of the brain important for reward-based behavior. Mice lacking *Magel2* exhibit abnormal behaviors in responses to stimuli such as cocaine and time-limited exposure to highly palatable food. In standard feeding conditions, total amounts of neurotransmitters in both the serotonergic and dopaminergic pathways were abnormal in many regions of the *Magel2* mouse brain as measured by high-performance liquid chromatography. We measured the abundance of dopamine and serotonin-related metabolites in specific brain regions in mice fed a standard diet, a high fat diet, or withdrawn from a high fat diet. We also measured levels of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis, in brain samples from mice under the three dietary conditions. Further, we detected phosphorylated CREB and phosphorylated AKT, robust markers of neuronal activation, in these samples. Data were analyzed to detect changes associated with diet, genotype, and diet x genotype interactions in wild-type versus *Magel2*-null mice.

*Conclusions***:** The loss of *Magel2* in the mouse causes abnormal responses to stimuli that impact the feeding circuits in the brain, such as withdrawal from a high-fat diet, time-limited exposure to highly palatable food, and cocaine. Our results provide a framework for the development of appropriate endpoints for effective and standardized design of preclinical trials for treatment of disordered eating in PWS.

Funded by the Women and Children's Health Research Institute, University of Alberta.

FPWR FUNDED PROJECT:
Rachel Wevrick - Development of leptin dysregulation in a mouse model of obesity in PWS

FOUNDATION FOR PRADER-WILLI RESEARCH

Metabolic and muscular dysfunction in the *Magel2* mouse model of Prader-Willi syndrome

Presenting Author: Rachel Wevrick[1]

Additional Authors: Ain A. Kamaludin[1], Jocelyn M. Bischof[1], Christa Smolarchuk[1], Fred B. Berry[1,2], Rachel Wevrick[1]

Institution: [1]Department of Medical Genetics, [2]Department of Surgery, University of Alberta, Edmonton, AB, Canada

Abstract:
*Introduction***:** Hypotonia of prenatal origin, motor delays and reduced muscle mass are nearly universal in PWS. Muscle dysfunction impairs quality of life, contributing to scoliosis, exercise intolerance and impaired respiratory function in children with PWS. Reduced lean mass also decreases energy expenditure and caloric need. Targeted interventions that build muscle mass and improve exercise tolerance are key to increasing activity and combatting obesity in PWS. Recently, children were identified who carry inactivating mutations of only one of the PWS genes, namely *MAGEL2*. These children with Schaaf-Yang syndrome have a PWS-like phenotype including hypotonia with congenital orthopedic manifestations (contractures, hip dysplasia, club feet). This suggests that loss of *MAGEL2* causes neonatal hypotonia and contributes to hypotonia-related complications in children with PWS.

*Aim***:** We investigated metabolism and muscle structure and function in mice lacking *Magel2*, to develop sensitive and consistent outcome measures useful for preclinical testing in anticipation of therapeutic interventions for hypotonia in PWS.

Methods and Results: Magel2–null mice run a quarter of the distance that control mice do when a running wheel is placed in the cage. Each running bout is four times shorter in duration, demonstrating a decreased capacity or motivation for voluntary exercise. A treadmill test of endurance revealed that *Magel2*-null mice are unable to remain on the treadmill at speeds that wildtype mice can easily maintain. Mutant mice have reduced grip strength that decreases further as they age. *Magel2*-null mice have twice the body fat mass and 10% lighter leg muscles compared with wildtype. The capacity to burn fuel is a key determinant of muscle fitness. We measured the respiratory exchange ratio (RER) using metabolic cages and found an increased ratio of fatty acid/carbohydrate oxidation in *Magel2*-null mice. RER typically switches from 0.9 (primarily using stored carbohydrates as fuel) during the active phase to 0.7 (stored fat used as fuel) during sleep. *Magel2*-null mice have lower RER in both phases compared to wildtype, indicating a reduced ability to use carbohydrates as fuel. Last, we found increased expression of a key gene that is up-regulated during processes that induce atrophy, such as denervation and starvation.

*Conclusions***:** The loss of *Magel2* in the mouse provides a genetic and physiological model for metabolic and muscular dysfunction in Prader-Willi syndrome and Schaaf-Yang syndrome. Our results provide a framework for the development of appropriate endpoints for effective and standardized design of preclinical trials for treatment of muscle dysfunction in PWS.

FPWR FUNDED PROJECT:
Rachel Wevrick - Development of leptin dysregulation in a mouse model of obesity in PWS

FOUNDATION FOR PRADER-WILLI RESEARCH

Sleep phenotype of MAGEL2 null mice

Presenting Author: Carrie E. Mahoney

Additional Authors: Thomas E. Scammell

Institution: Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

Abstract:
Daytime sleepiness, disrupted sleep, and cataplexy-like falling episodes are common in PWS, but the cause of these symptoms is unknown. Using EEG, EMG, and video recordings, we have examined sleep/wake behavior in MAGEL2 null mice, a model of PWS. These mice have normal total amounts of wake (W), Non-REM sleep (NR), and REM sleep (R) when housed on a 12 hour light:dark cycle. However, their wake and NR bouts are shorter than normal during the dark period, and they have more frequent W and NR bouts. These short bouts and frequent state transitions indicate that MAGEL2 null mice lack the ability to maintain wake or sleep for long periods, similar to orexin null mice, a mouse model of narcolepsy. In addition, we observed behavioral arrests in MAGEL2 null mice that appear distinct from the cataplexy seen in orexin null mice. These findings in MAGEL2 null mice are similar to the sleepiness, disrupted sleep, and falling episodes described in PWS, and further research with these mice should provide helpful insights into these troubling symptoms.

FPWR FUNDED PROJECT:
Thomas Scammell - Mechanisms of sleepiness and other sleep abnormalities in a mouse model of Prader-Willi Syndrome

The role of MAGEL2 in neurobehavioral phenotypes

Presenting Author: Michael D. Fountain, Jr.

Additional Authors: Jiani Yin, Chun-an Chen, Huifang Tao, Christian P. Schaaf

Institution: Baylor College of Medicine; Texas Children's Hospital Neurological Research Institute

Abstract:
The maternally imprinted, paternally expressed *MAGEL2* gene located in the Prader-Willi critical region 15q11-13 has recently been reported as the first single gene, point mutations of which can cause Prader-Willi (PWS) and Prader-Willi-like (PW-like) phenotypes. In the initial report, four individuals with truncating mutations in *MAGEL2* had been reported. Since then, our group has identified 14 additional individuals. Eight of these additional individuals harbor the same, frame-shifting mutation in *MAGEL2*, c.1996dupC (p.Q666fs), suggesting a mutation hotspot within *MAGEL2*. Two children with this mutation are cousins, and represent the first familial case, with genotypes and phenotypes following an inheritance pattern that is consistent with a maternally imprinted, paternally expressed gene (affected only when inherited from the father, unaffected when inherited from the mother). The emerging phenotypic spectrum caused by truncating *MAGEL2* mutations involves neonatal hypotonia, feeding difficulties, intellectual disability, autism spectrum disorder, etc. While some of the patients may resemble PWS, they are also phenotypically distinct from classic PWS, so that the syndrome caused by *MAGEL2* point mutations was renamed from Prader-Willi-like syndrome to Schaaf-Yang syndrome (SYS) (OMIM #615547). The identification of additional patients with truncating *MAGEL2* mutations has allowed for the refinement of clinical phenotypes associated with SYS and highlights the implications for genetic counseling of families with imprinted genetic disorders.

In a translational bench-to-bedside approach, *Magel2* null mice were utilized to assess the presence of autism-like behaviors. *Magel2* null male and female mice, and age- and sex-matched wild-type littermates, were subjected to a battery of established neurobehavioral assays for social recognition and interaction, and learning and memory. *Magel2* null mice were shown to have an altered social phenotype, in particular a deficit in appreciation of social novelty. Patients diagnosed with PWS have been described as being able to engage in social interactions and making friends, however, there are noted deficits in the amount of time spent with friends and interpersonal communication, which may have some similarity to what we observe in this mouse model.

FPWR FUNDED PROJECT:
Christian Schaaf - Evaluation of autism-like behaviors in mice deficient for Magel2

Regulation of *Mkrn3* expression by leptin

Presenting Author: Stephanie A. Roberts

Additional Authors: Ana Paula Abreu, M.D., Ph.D., Victor M. Navarro, Ph.D., Caroline Maguire, Joy Liang, Rona S. Carroll, Ph.D., Ursula B. Kaiser, M.D.

Institution: Brigham and Women's Hospital, Boston, MA

Abstract:
MKRN3, located within the Prader-Willi syndrome region on chromosome 15q11.2, has recently been described as the first imprinted cause of central precocious puberty. Patients with Prader-Willi syndrome exhibit a range of pubertal abnormalities, most commonly delayed and incomplete puberty due to primary and/or secondary hypogonadism. A small percentage of patients with Prader-Willi syndrome have central precocious puberty. A paternal copy of *MKRN3* is lost in most patients with Prader-Willi syndrome, and may be contributing to the pubertal abnormalities seen is these patients. In mice, hypothalamic expression of *Mkrn3* in the arcuate nucleus, where the control of pubertal onset is believed to be located, declines immediately before the onset of puberty and remains low into adulthood. This expression pattern in mice, together with the identification of loss-of-function mutations in *MKRN3* in children with central precocious puberty, support a role for *MKRN3* as a 'brake' or inhibitor of GnRH secretion during childhood.

The function of *MKRN3* is not known, but based on protein homology it is believed to be associated with protein ubiquitination. The mechanisms regulating *MKRN3* expression are not known. Leptin's action is permissive for pubertal onset and a certain level is required. Therefore, we hypothesized that leptin might negatively regulate *Mkrn3* expression to contribute to its decline prior to the onset of puberty in mice. Thus, in leptin-deficient (*ob/ob*) mice, we hypothesized they will not exhibit the prepubertal decline in *Mkrn3* and therefore mRNA expression levels will be elevated in adult *ob/ob* mice compared to adult wild type mice.

We extracted the RNA from the mediobasal hypothalamus (which includes the arcuate and ventromedial hypothalamic nuclei) of four male and four female wild type and four male and four female *ob/ob* mice at postnatal days 12 (prepubertal) and 30 (pubertal). RNA was reverse-transcribed and quantitative real-time PCR analysis was performed to measure *Mkrn3* mRNA levels, normalized to ribosomal protein L19. Statistical analysis was performed using GraphPad Prism 6.

There was a statistically significant difference between groups for males as determined by one-way ANOVA ($F(3,12) = 33.48$, $p < 0.0001$). Post-hoc comparisons using the Tukey HSD test revealed *Mkrn3* mRNA expression levels were not significantly different for male *ob/ob* mice (2.165 ± 0.110) compared to male wild type mice (2.48 ± 0.158) on postnatal day 12. Similarly, there was no significant difference in *Mkrn3* mRNA expression levels in male *ob/ob* mice (5.959 ± 0.260) compared to male wild type mice (5.195 ± 0.575) at postnatal day 30. The difference between groups was also statistically significant for females as determined by one-way ANOVA ($F(3,12) = 55.73$, $p<0.0001$). The Tukey HSD post-hoc test revealed *Mkrn3* mRNA expression levels were not significantly different for female *ob/ob* mice (1.897 ± 0.386) compared to female wild type mice (1.738 ± 0.132) at postnatal day 12. There was also no significant difference in *Mkrn3* mRNA expression levels in female *ob/ob* mice (4.781 ± 0.342) compared to female wild type mice (5.805 ± 0.189) at postnatal day 30. *Mkrn3* mRNA expression levels significantly decreased in wild type and ob*/ob* males and females at postnatal day 30 compared to postnatal day 12. There was no significant difference in expression levels between wild type males and females at postnatal days 12 and 30.
In summary, leptin-deficient (*ob/ob*) mice showed a decline in *Mkrn3* mRNA expression levels similar to wild type mice from postnatal day 12 to 30. *Mkrn3* mRNA expression levels were not different in adult *ob/ob* mice compared to adult wild type mice. Therefore, leptin does not appear to be involved in the regulation of expression of *Mkrn3*. Further studies are needed to better understand the mechanism of action and regulation of *MKRN3*, including the potential for epistatic control by other genes in the Prader-Willi region.

FOUNDATION FOR PRADER-WILLI RESEARCH

ZNF274 KO in PWS-specific neurons derived from iPSCs leads to 15q11.2-q13 maternal genes re-expression

Presenting Author: Maeva Langouet

Additional Authors: Heather Glatt-Deeley, Erin Banda, Chris Stoddard, Elodie Mathieux, Leann Crandall, Marc Lalande

Institution: Department of Genetics and Genome Sciences, School of Medicine, University of Connecticut

Abstract:
Prader-Willi syndrome (PWS) is a multisystemic complex genetic disorder caused by the lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region. This genomic imprinting disorder is characterized by neonatal hypotonia and failure to thrive during infancy. Clinical manifestations change as the individual ages and other features as short stature, hyperphagia, obesity, developmental delay, cognitive disability and behavioral problems become evident.

We previously used a knockdown approach via RNA interference in iPSCs to investigate the role of a complex composed of the zinc-finger protein ZNF274 and the histone H3 lysine 9 (H3K9) methyltransferase SETDB1 in silencing the maternal copy of the PWS critical region (CR) encompassing the SNORD116 cluster.

Here, we have knocked out ZNF274 expression in PWS-specific iPSCs by the CRISPR-mediated strategy and show that the extent of transcriptional re-activation of PWS-CR genes varies during *in vitro* neurogenesis. While PWS-CR gene expression in ZNF274 KO iPSCs is ~5 % of normal levels, wild-type mRNA levels are completely restored in neurons derived from PWS ZNF274 KO iPSCs. Surprisingly, the DNA methylation at the PWS-IC remains unchanged upon ZNF274 KO, suggesting the existence of a second region regulating the PWS-CR gene re-expression. We are now investigating the upstream region of the PWS-IC to test the hypothesis that this region could act as a second IC later during neurogenesis. For our future directions, we plan to generate additional ZNF274 KO lines from multiple PWS patients with diverse genetic abnormalities (large deletion, small deletion and UPD). Our goal is to demonstrate that ZNF274 is a potential target for future therapeutic applications to rescue the PWS phenotype.

FPWR FUNDED PROJECT:
Marc LaLande - Reactivation of the PWS locus via disruption of the ZNF274 silencing complex

FOUNDATION FOR PRADER-WILLI RESEARCH

Injectable protein based gene activation therapy for PWS

Presenting Author: David J. Segal

Additional Authors: Ben Pyles, Michelle McAlister, Henriette O'Geen

Institution: University of California, Davis

Abstract:
With support from FPWR, we have been developing artificial transcription factors to reactivate maternally-silenced genes in a mouse model of Prader-Willi Syndrome (PWS). My lab has created zinc finger-based artificial transcription factors targeting the *Snrpn* promoter with the intention to repress the long transcript that silences the *Ube3a* gene in a mouse model of Angelman Syndrome (AS). We can now show that the AS factor can be injected IP into a mouse, cross the blood brain barrier, enter the neurons in the brain, and activate expression of *Ube3a*. A simple reconfiguration of the same factor should be sufficient to enable it to activate the long transcript on the maternal allele that encodes a *Snord116* cluster. Similarly, we can design factors that could activate the silenced maternal copy *Magel2* gene to compensate for inactivating mutations in the paternal copy. These genome-engineering tools, and our progress in using them, will be described, as well as our efforts to create a large-deletion rat model of AS/PWS.

FPWR FUNDED PROJECT:
David Segal - Injectable protein gene activation therapy for PWS

FOUNDATION FOR PRADER-WILLI RESEARCH

Changing alternative splicing patterns of the serotonin receptor 2C inhibits food intake

Presenting Author: Zhaiyi Zhang[1]
Additional Authors: Paul Gresch[2], Ronald Emerson[2], Stefan Stamm[1]
Institution: [1] Department of Biochemistry and Molecular Biology, College of Medicine, University of Kentucky, Lexington, KY, [2] Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN

Abstract:
Introduction/Background: The loss of SNORD115 expression contributes to Prader-Willi syndrome. Recently, we found that SNORD115 promotes inclusion of the alternative exon Vb of the serotonin receptor 2C. This receptor forms at least 25 protein isoforms due to a combination of RNA editing and alternative splicing. Pre-mRNA splicing generates two isoforms, RNA1 and RNA2. RNA1 encodes a truncated receptor that is located intracellularly. Heterodimerization of the truncated receptor with the full-length receptor, encoded by RNA2, leads to a sequestration of the full-length receptor inside the cell, which switches off serotonin signaling. In the hypothalamic arcuate nucleus, the serotonin receptor 2C regulates food intake. PWS patients have more RNA1 than RNA2 in the hypothalamus, indicating a decrease of serotonin signaling.
Methods: To substitute the loss of SNORD115, we identified an oligonucleotide (oligo#5) that promotes inclusion of exon Vb, i.e. formation of RNA2. Further analysis showed that oligo#5 promotes only inclusion of the non-edited form of exon Vb. To test the effect of oligo#5 on food uptake, we delivered it to the third ventricle through intracranial injection and through injection into the blood and analyzed food intake.
Results/Discussion: Oligo#5 delivery through injection in to the third ventricle caused a significant reduction in food intake that lasted for about 12 hours post injection. We observed the effect on food intake after repeated injections and behavioral analysis in metabolic cages showed no adverse effects. Injection of oligo#5 into serotonin receptor 2C knock out mice had no significant effect on food intake. As expected, we saw a change in RNA1 to RNA2 ratios and an induction of POMC after injection. Surprisingly, injection into the blood via a catheter leads to oligo#5 accumulation in the brain and reduction of food uptake, even if mice are fed ad libitum all the time. To gain insight into the mechanism, we constructed an EGFP tagged genomic construct that expresses the serotonin receptor 2C protein, after exon Vb inclusion. Using Flp-in stable cells with this construct, we investigated the effect of oligo#5 on receptor localization. We found that oligo#5 increases the expression of the receptor protein on the cell surface, which correlates with the loss of the truncated isoform. To our surprise, we found expression of the serotonin receptor 2C in the pituitary, which has not been reported. Strikingly, PWS subjects express only RNA1 in the pituitary, consistent with the absence of SNORD115, whereas control subjects express RNA1 and RNA2-encoded isoforms. The ratio of these isoforms is variable and depends on feeding status and sex of mice. Importantly, in the pituitary the full-length receptor binds to the ghrelin receptor, which regulates growth hormone secretion, linking growth hormone levels to SNORD115 absence.
Conclusion: The ratio of serotonin receptor 2C isoforms is regulated by SNORD115 and deregulated in PWS, as SNORD115 is missing. The deregulation of the serotonin receptor 2C isoforms impacts on food intake and likely contributes to hyperphagia. The strongest deregulation is seen in the pituitary, where the full-length form is absent, which could contribute to the low growth hormone levels, and possibly the elevated ghrelin levels. The serotonin receptor isoforms in the pituitary and the levels of SNORD115 in the pituitary are influenced by the feeding of animals, suggesting that the loss of SNORD115 exaggerates a natural 'sensor-function' of SNORD115. The loss of SNORD115 can be substituted with an oligonucleotide through injection, offering a treatment option for PWS.

FPWR FUNDED PROJECT:
Stefan Stamm - Identification of substances that substitute for the loss of snoRNAs from the Prader-Willi critical region

FOUNDATION FOR PRADER-WILLI RESEARCH

Characterizing the processing, localization, and function of noncoding RNAs at the heart of the Prader-Willi locus

Presenting Author: Janine LaSalle

Additional Authors: Rochelle Coulson, Justin Aflatooni, Dag Yasui

Institution: Medical Microbiology & Immunology, Genome Center, M.I.N.D. Institute, University of California, Davis, CA

Abstract:
Prader-Willi syndrome (PWS) is a neurodevelopmental disorder caused by paternal deficiency of the maternally imprinted 15q11-13 noncoding RNA cluster *SNORD116*. This repeat cluster is GC-skewed, resulting in R-loop formation, histone displacement, and chromatin decondensation specifically of the paternal allele upon neuronal transcription. *SNORD116* processing results in two noncoding RNAs: *SNORD116* snoRNAs, which localize to the nucleolus of maturing neurons, and *116 host gene (116HG)*, which is retained within the nucleus, localizing to its paternally decondensed site of transcription and forming an RNA cloud. To understand the processing, localization, and functional relevance of each component of *SNORD116,* we engineered novel transgenic mice to test complementation of the PWS mouse model, *Snord116del*. Complete transgene wild-type (*Ctg/WT*) mice carrying all of the elements of the *Snord116* repeat were generated and bred to *Snord116del* males to produce mice deficient for endogenous paternal *Snord116* but expressing the transgene (*Ctg/Snord116del*). RNA fluorescence *in situ* hybridization (FISH) analysis of brain showed that spliced *116HG* RNA localized in a distinct cloud at its decondensed transcription site and mature snoRNAs localized to the nucleolus in wild-type (WT) but not *Snord116del* neurons. In non-neuronal *Ctg/WT* tissues and all *Ctg/Snord116del* tissues, no *116HG* RNA cloud or snoRNAs were detected, but in *Ctg/WT* neurons, nucleolar snoRNAs were increased and a significantly larger single *116HG* RNA cloud was detected, suggesting that processing and localization of the transgene requires the presence of an active endogenous *Snord116* locus. qRT-PCR analysis demonstrated that splicing of the *Snord116* transgene was largely restricted to neuronal tissues of both *Ctg/WT* and *Ctg/Snord116del* mice, despite widespread expression from the CMV promoter in many tissues. These combined results suggest that processing of the *Snord116* transcript is dependent on neuronal-specific splicing factors and/or chromatin states, including the decondensed *Snord116* paternal allele. Analyses of a spliced *116HG/Snord116del* transgenic mouse is underway to determine whether the process of splicing facilitates localization or whether providing a pre-spliced transcript circumvents the need for an endogenous active *Snord116* locus. Understanding how DNA-RNA interactions mediate the processing and localization required for *Snord116* function and phenotypic rescue is critical for the development of effective PWS therapies in the future.

FPWR FUNDED PROJECT:
Janine LaSalle - Rapamycin treatment to correct the circadian mTOR imbalance in the Snord116 deletion mouse model of PWS

FOUNDATION FOR PRADER-WILLI RESEARCH

Expression analysis in PWS neurons

Presenting Author: Lawrence T. Reiter

Institution: The University of Tennessee Health Science Center

Abstract:
One of the biggest challenges to understanding Prader-Willi syndrome (PWS) at the mechanistic level has been identifying which non-coding snoRNA is the major contributor to PWS phenotypes and how this regulatory micoRNA is able to regulate gene expression in neurons. Our laboratory has been using deciduous teeth from children with a variety of neurogenetic syndromes to investigate gene expression changes in dental pulp stem cell derived neurons directly from individuals with these disorders. I will present current data on both control DPSC neurons and neurons from individuals with Duplication 15q syndrome as a proof of concept. In the near future we will apply this expertise to the identification of gene expression changes in PWS vs controls as well as differences between PWS +/- autism spectrum disorder. In addition, we will use miRNAseq to pinpoint regulatory microRNAs (snoRNAs and miRNAs) that may be contributing to the gene expression changes we find while at the same time looking for new microRNAs that may be regulating some of the transcriptional differences. The identification of both coding and non-coding RNAs differentially regulated in PWS will open new avenues for therapeutic interventions designed to target these transcripts and return expression levels to neurotypical control.

FPWR FUNDED PROJECT:
Larry Reiter - Gene Expression Analysis in PWS Subject Derived Dental Pulp Stem Cell Neurons

FOUNDATION FOR PRADER-WILLI RESEARCH

State of the Science - Report from the Prader-Willi Syndrome Mental Health Research Strategy Workshop

Presenting Author: Lauren Schwartz[1,4]

Additional Authors: Anthony Holland[2], Elizabeth Roof[3], Jessica Bohonowych[4], Theresa Strong[4,5], and Elisabeth Dykens[3]

Institutions: [1]Department of Rehabilitation Medicine, University of Washington, Seattle, WA; [2]Department of Psychiatry, Cambridge Intellectual and Developmental Disabilities Research Group, University of Cambridge, UK; [3]Vanderbilt Kennedy Center, Vanderbilt University, [4]Foundation for Prader-Willli Research, Los Angeles, CA, [5]Department of Medicine, University of Alabama at Birmingham, Birmingham, AL,

Abstract:
Background: This presentation will report on the 'Prader-Willi Syndrome (PWS) Mental Health Research Strategy Workshop' (March, 2015). The goal of the meeting was to highlight the state-of-the science regarding the mental health of people with PWS. Mental ill-health and maladaptive behaviors significantly impact quality of life and effective treatments for people with PWS are crucial to improve function and independence.

Methods: a multidisciplinary group of scientists and health care professionals were brought together to discuss the mental health and behavioral needs of people with PWS. The workshop focused on synthesizing established work on PWS with other relevant areas of study including 22q deletion syndrome as an example of another neurodevelopmental syndrome with not dissimilar mental health problems and also a focus on two particularly neurobiological systems that research had suggested were relevant to understanding the broader behavioral/mental health aspects of PWS: the autonomic nervous system and oxytocin/vasopressin pathways. Other relevant topics were considered and recommendations made.

Results: in the posters and presentations it was apparent that no-one approach was sufficient but that theoretically informed studies were needed which included, detailed clinical characterization and measurement, further targeted behavioral studies, investigation into specific neurobiological systems through pharmaceutical or other interventions in the form of proof of concept studies, the use of advanced clinically informed neuroimaging protocols, and molecular biological studies using iPS cells.

Discussion: within this framework participants identified and prioritized key research questions, as well as highlighted current opportunities. Recommendations were made with respect to resource development, collaborative opportunities, and targeted research initiatives.

WORKSHOP REPORT
The Prader-Willi Syndrome Mental Health Strategy Workshop 2015

FOUNDATION FOR PRADER-WILLI RESEARCH

Examining Genetic Subtype Differences in Pretend Play Among Children with Prader-Willi Syndrome

Presenting Author: Anastasia Dimitropoulos[1]
Additional Authors: Zyga, O.[1], Russ, S.[1], Danker, N.[2], & Dykens, E.[2]
Institutions: [1]Department of Psychological Sciences, Case Western Reserve University, Cleveland, OH, [2]The Kennedy Center, Vanderbilt University, Nashville, TN

Abstract:
*Introduction***:** The processes involved in pretend play are associated with the positive development of cognitive, emotional, and social skills in children (Russ, 2004). Deficits in play have been identified in children with various developmental disorders, including autism spectrum disorder (ASD). Play deficits in ASD have been shown to be related to delayed social, language, affective, and creativity development. Specifically, children with ASD can express very high rates of repetitive behaviors, which cause their play to be rigid, stereotyped and lack divergent thinking. Although research suggests individuals with Prader-Willi syndrome (PWS) have social deficits and repetitive behaviors similar to that of ASD with a greater risk associated with the maternal uniparental disomy (mUPD) subtype of PWS, play patterns have not been well studied. While hallmark characteristics of PWS include hyperphagia, obsessive-compulsive symptoms, and cognitive delays, understanding social and emotional risk factors for individuals with the disorder is important for planning intervention and increasing quality of life. Recently, we found pretend play ability in children with PWS to be similar to children with ASD with reduced social cognitive processing in areas such as imagination and organization of play (Zyga et al., 2014). In addition, children with PWS did not differ on measures from those with ASD in individual and joint play and the addition of a play partner increased social cognitive scores for children with PWS. However, we were unable to examine effects of genetic subtype in that small sample. The purpose of the current research is to extend this examination of pretend play in a larger sample of children to identify if early social cognitive processes in play differ by genetic subtype in children with PWS.
*Methods***:** 60 Children with PWS (DEL = 30; mUPD = 30) mean age = 9.56) underwent the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2006) as part of larger studies examining the social phenotype of PWS at either CWRU (n = 14) or Vanderbilt University (n = 46). The ADOS sessions were video recorded and secondary analyses of play abilities were assessed by scoring the “Make-Believe Play” activity from ADOS modules 2 and 3 using a modified Affect in Play Scale (APS; Russ, 2004). The modified APS scored participants on scaled measures of comfort, imagination in play, organization of storyline, affective expression in play, frequency of symbolic versus functional play versus no play acts, and number of repetitive actions. In addition, the “Make-Believe Play” activity included both individual and joint play periods, where the child would play with a trained psychologist.
Results & Discussion: Preliminary analyses indicate that during both the individual and joint play periods, genetic subtype groups did not differ in play ability and showed a similar pattern of deficits across all original measures within the APS (Comfort: t = -.663, p = .513; Imagination: t = .583, p = .565; Organization: t = -.099, p = .922; Affective Frequency: t = -.023, p = .982; Affective Categories: t = .000, p = 1.000). These findings add to previous findings (Zyga et al., 2014) and suggest that children with PWS do indeed have deficits within their play abilities that are not mediated by genetic subtype. Preliminary results also show that both groups had showed gains in most measures with the addition of a play partner. Overall, these results suggest that play facilitation allowed for similar increases in social cognitive processes in play in both of the subtype groups. Intervention tailored to target deficits such as imagination or emotional expression in both groups could be beneficial in increasing play skills and perhaps other, more global processes related to pretend play development.

FPWR FUNDED PROJECT:
Anastasia Dimitropoulos - Evaluating the Parent-focused Remote Education To Enhance Development (PRETEND) Program in PWS

FOUNDATION FOR PRADER-WILLI RESEARCH

Assessment of Affective Speech Recognition in Children with Prader-Willi Syndrome: An Introduction to the Affective Speech Recognition Task-Social Communication (ASRT-SC)

Presenting Author: Bonnie P. Taylor, Ph.D.

Additional Authors: Casara Ferretti, M.A., Ellen Doernberg, B.A., Eric Hollander, M.D.

Institution: Albert Einstein College of Medicine, Montefiore Medical Center. Bronx, New York.

Abstract: Social functioning in children with Prader-Willi Syndrome (PWS) has recently become a topic of scientific interest and examination. Many of the social deficits found in children with PWS overlap those exhibited by children with autism spectrum disorder (ASD), for example, difficulty relating to peers and making friends, an inability to understand personal space (Dimitropoulos et al., 2013), restricted variety and frequency of affective responding (Zyga et al., 2015) and difficulty responding appropriately to the changes of mood in others (Dimitropoulos et al., 2013). Like children with ASD, children with PWS have demonstrated poor ability in recognizing and discriminating emotions that are conveyed in facial expressions (Key et al., 2013; Whittington & Holland, 2011). Children and adults with ASD also demonstrate a reduced ability in identifying spoken affective prosody. Understanding the emotional states of others via nonverbal communication (i.e., facial expression and affective prosody) is essential for developing and maintaining meaningful relationships. As such, it is important that this skill be evaluated in children with ASD and PWS in order to clarify the nature of the deficits, and to assess potential changes in social perception after behavioral and/or pharmacological based treatments.

The Affective Speech Recognition Task-Social Communication (ASRT-SC) is an objective, reliable measure of an individual's ability to decode and identify emotional prosody that has been validated for adults with high functioning ASD in two treatment studies. It is currently being adapted into a more child-friendly version (ASRT-SC-C) that can be used across all levels of intellectual functioning, thereby providing an accessible and objective behavioral measure/outcome of social communication. The proposed presentation will talk about the development of the ASRT-SC, how it was utilized in clinical treatment trials in ASD, and why it would be useful as a measure in the PWS population. In addition, future directions of the ASRT-SC-C will be discussed.

FPWR FUNDED PROJECT:
Eric Hollander - Oxytocin vs. placebo for the treatment of hyperphagia in Prader-Willi syndrome

The Global PWS Registry

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Institution: Foundation for Prader-Willi Research

Abstract:
The Global PWS Registry launched in May 2015 with the mission to support and accelerate PWS research. The Registry is managed and funded by the Foundation for Prader-Willi Research (FPWR), and is hosted by the National Organization of Rare Disorders (NORD), a nonprofit organization that has served the rare disease community for more than thirty years. The Registry is IRB approved and aims to: document the full range of PWS characteristics; enable data trend analysis to generate new insights into PWS and identify areas for additional study; facilitate partnerships with university researchers and pharmaceutical companies; guide the development of standards of care; expedite the completion of PWS clinical trials; allow participants to store their PWS medical data in one place; and accelerate solutions for PWS.

A web-based platform, the Registry provides informed consent and consists of a collection of electronic surveys covering comprehensive clinical medical history, developmental history, behavior, mental health, and quality of life. Data is self reported by a parent or guardian of individuals with PWS, with future plans for a physician entered clinical portal.

There are currently >600 participants in the Registry. The coming year includes plans to continue enrollment, promote survey completion, and begin leveraging de-identified data through collaborations with researchers, companies, and other parties involved in advancing solutions for PWS.

LINK TO THE GLOBAL PWS REGISTRY