PRADER-WILLI SYNDROME ASSOCIATION USA

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Scientific Abstract Submissions

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The Medical Review

Obestatin is elevated in Young Children with Prader-Willi Syndrome

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Background: Ghrelin levels are elevated in children and adults with PWS, but are normal in infants prior to the development of hyperphagia. Obestatin is derived from the proteolytic cleavage of preproghrelin. Obestatin initially was described as having anorexigenic effects, but other studies have failed to confirm these findings. Recent studies suggest that obestatin inhibits water intake more profoundly than it affects food intake. Since infants with PWS have been found to have reduced water intake, an alteration in obestatin levels might contribute to their unusual drinking behavior.

Objective: The aim of this study was to measure fasting plasma obestatin in PWS infants (n=21) compared to controls of similar age and weight-for-age z-score (n=17).

Design/Methods: Fasting plasma obestatin was measured by ELISA (BioVendor, Candler, NC). Intra- and inter-assay CVs were 3.5-9.9% and 5.6-9.0%, respectively. Median, quartiles, and Mann-Whitney U Test were performed using Sigmastat. Correlations between obestatin and previously measured fasting hormones (ghrelin, total PYY, leptin, insulin, Total and HMW adiponectin) were performed by Spearman correlation using Sigmastat.

Results: PWS and control groups were similar for age, gender, and weight for age z-score (Table 1). Fasting concentrations of obestatin were elevated in infants with PWS compared to controls (PWS: 2.7 ng/mL NC: 2.1 ng/mL; p=0.04). However, Ghrelin: Obestatin ratio was not different between the groups. Fasting obestatin did not correlate with age, weight for age z-score, weight for length z-score, ghrelin, total PYY, leptin, insulin, or total and HMW adiponectin in either the PWS or control groups.

Conclusions: In this study, obestatin levels were higher in infants with PWS than in controls. This finding might indicate increased processing of ghrelin preprohormone by prohormone convertase 2 in PWS. The possibility that obestatin might contribute to the reduced water intake or failure to thrive commonly seen in infants with PWS should be further explored.

Table 1.

	PWS (n=21)	**Control (n=17)**	**p-value**
Age (months)	15.5 (11.0, 30.9)	27.3 (11.4, 44.9)	0.55
Gender (M/F)	12M; 9F	8M;9F	NS
Weight for age z-score	-0.99 (-1.69, 0.89)	0.16 (-0.40, 0.90)	0.09
Weight for length z-score	-0.42 (-1.39, 1.51)	0.53 (-0.38, 0.94)	0.16
Obestatin (ng/mL)	2.7 (2.1, 4.3)	2.1 (1.9, 2.4)	0.04
Ghrelin (pg/mL)	2190.0 (1602.8, 3278.8)	1980.0 (1686.9,2239.3)	0.24
Ghrelin: Obestatin ratio	906.7 (390.9,1218.2)	907.2 (633.5, 1082.4)	0.95

Data reported as medians (interquartile range)

Is the GLP-1 Analogue Liraglutide Safe and Effective for Body Weight and Glycaemic Control in Prader-Willi Syndrome?

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Background: Prader-Willi Syndrome (PWS) leads to childhood-onset hyperphagia and morbid obesity, with a prevalence of type 2 diabetes mellitus ~ 25%. Best practice for T2DM treatment is unclear, with use of Metformin, thiazolidinediones and insulin. The role for insulinotropic GLP-1 agonists, such as Liraglutide, is unclear, but they may also have potential benefits for weight loss. However, there are possible concerns about their safety in PWS because of adverse effects on gastric motility.

Method: We report two 20yo females with PWS and childhood-onset T2DM treated with Liraglutide for 6 months (up-titrated over 2 months from 0.6mg to 1.8mg od) who had assessment of glycaemic control, body weight and gastric emptying using Tc-99 semi-solid meal scintiscan.

Case report 1: Initially controlled with (HbA1c <7.0%, <53mmol/mol), but by 19y glycaemic control was suboptimal Metformin and a thiazolidinedione (HbA1c 7.5%, 58mmol/mol). Despite long-acting insulin therapy (Detemir titrated up to 24 units) glycaemic control worsened over the next 18 months (HbA1c 9.6%, 81mmol/mol), with increase in BMI (36.1 to 39.5kg/m^2). After 6 months of Liraglutide, together with further increase in insulin dose (Novomix30 40units daily), HbA1c had decreased by 1.9% (7.7%, 61mmol/mol), and BMI decreased to 38.3kg/m^2 (2.5% weight loss). $t_{1/2}$ gastric emptying time increased from 48 to 83min (normal <60min) after 3 months of Liraglutide.

Case report 2: Initially controlled at 19y with Metformin (HbA1c 5.6%, 38mmol/mol), but by 20y HbA1c had increased (6.8%, 51mmol/mol) despite BMI decreasing (32.3 to 30.7 kg/m^2). After 6 months of Liraglutide, HbA1c had decreased by 0.8% (6.0%, 42mmol/mol) and BMI remained stable at 30.5kg/m^2 (0.5% weight loss). $t_{1/2}$ gastric emptying time doubled from 25min to 49min after 3 months.

Side-effects: Neither subject reported side-effects. Although subject 2 reported an increase in post-prandial satiety, hyperphagia questionnaire scores did not improve in either case.

Conclusion: GLP-1 analogues may improve glycaemic control in PWS patients with T2DM but may not have a major impact on hyperphagia or weight control other than avoiding or attenuating insulin-associated weight gain. However Liraglutide markedly delayed gastric emptying in these patients (without symptoms). There have been several reports of idiopathic and binge-eating related gastric necrosis and fatal rupture in PWS perhaps related to delayed gastric emptying (unconnected to GLP-1 analogues). Some caution in the use of GLP-1 agonists and monitoring of gastric emptying may therefore be appropriate in patients with PWS.

Peripheral Cannabinoid Type-1 Receptor Inverse Agonism Inhibits Food Intake and Reverses Diet-induced Obesity

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Introduction/Background: Activation of the cannabinoid type-1 (CB_1) receptor promotes food intake and increases lipogenesis in peripheral tissues such as adipose tissue and liver. Indeed, diet-induced obesity (DIO) is associated with increased activity of the endocannabinoid system and CB_1 inverse agonists reduce body-weight and the associated metabolic complications, although adverse neuropsychiatric side effects halted their therapeutic development.

Methods: We treated DIO mice with a potent, orally bioavailable, peripherally restricted CB_1 inverse agonist (JD-5037) and analyzed its pharmacological, behavioral and metabolic profile.

Results/Discussion: Our results indicate that in DIO mice, the peripherally restricted CB_1 inverse agonist JD-5037 is equieffective with its brain-penetrant parent compound, SLV-319, in reducing cumulative food intake and body weight, even though it does not occupy central CB_1 receptors or induce related behaviors. The chronic hypophagic and weight-reducing effects of JD-5037 are mediated by resensitizing DIO mice to endogenous leptin through rapidly reversing their hyperleptinemia. In contrast to their similar effects on consummatory food intake in the fed state, only SLV-319 and not JD-5037 is able to inhibit the initiation of food intake in a fasting/refeeding paradigm both in lean and in leptin-deficient *ob/ob* mice, indicating that this latter effect is centrally mediated and leptin independent.

Conclusion: The anorexic and weight reducing effects of inverse agonism at peripheral CB_1 receptors highlight the therapeutic potential of such compounds in obesity and related metabolic disorders. Promising findings with the globally acting CB_1 inverse agonist, rimonabant, in adults with Prader-Willi syndrome (PWS), may provide the rationale for future clinical testing of peripherally acting CB_1 antagonists for the treatment of obesity and hyperphagia in PWS without eliciting side effects associated with blockade of CB_1 in the CNS.

Hypoglycemia in Prader-Willi Syndrome

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Introduction/Background: Hypotonia, feeding difficulties and poor growth are common characteristics of infants with Prader-Willi Syndrome, but may also be findings associated with hypoglycemia. Hypoglycemia in PWS infants may go unrecognized due to the overlap of signs associated with these conditions. As frequent hypoglycemia in infancy has been associated with developmental delay, determining the presence and characteristics of hypoglycemia in infants with PWS is crucial to improving patient care and outcomes. For this reason, we performed a chart review to assess the frequency of documented hypoglycemia in infants with PWS.

Methods: We reviewed medical records for patients with a diagnosis of Prader-Willi Syndrome seen at the University of Florida Pediatric Endocrinology clinic between January 1, 2011 and June 24, 2012. All children included in this study were four years old or younger during their most recent visit to the clinic. We evaluated medical records for the presence or absence of neonatal hypoglycemia, timing of hypoglycemia, degree of hypoglycemia, patient's genetic type of PWS, and gestational age at birth.

Preliminary Results: 12% (8 of 66) of patient charts showed evidence of hypoglycemia (defined as blood glucose less than 50 mg/dl) prior to discharge from the newborn nursery. Blood glucoses below 40 mg/dl were documented in five out of eight infants. 6 of 8 infants (75%) had deletion, while 2/8 had uniparental disomy. Of the total sample, 38/66 patients (58%) had deletion, while the remainder had UPD or imprinting defects. 4/8 patients with hypoglycemia were male. Of the total sample, 28/66 patients (42%) were male. Infants with hypoglycemia were all between 36 and 41 weeks gestational age at birth.

Discussion: We found that hypoglycemia is occurring in a significant number of infants with PWS. This has not been reported previously. It is interesting that patients with hypoglycemia less than 40 mg/dl were more likely to have deletion compared to the whole sample group, suggesting that hypoglycemia in infancy may explain differences between subtypes in verbal and cognitive development. Through further studies of hypoglycemia in PWS, we hope to gain insight into the impact of hypoglycemia on neuro-cognitive development and hormone regulation in this patient population.

Vagus Nerve Stimulation as a Treatment for Hyperphagia in Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is a genetic disorder involving hyperphagia arising from aberrant satiety signaling, for which there are currently no successful treatments. The central involvement of the vagus nerve in satiety, alongside serendipitous weight loss observed during Vagus Nerve Stimulation Therapy® (VNS; Cyberonics, TX, USA) for other conditions (Pardo et al. 2007), suggests that enhancing vagus signaling may be beneficial in PWS. The safety, acceptability and efficacy of VNS as a novel therapeutic intervention for hyperphagia in PWS are assessed.

Methods: Three individuals with PWS (2 female, 1 male) were recruited and screened for suitability before surgical implantation of VNS. VNS was switched on three months post-implantation, initially set at 0.25mA output current and incrementally increased to 1.5mA as appropriate to each individual. Participants attend monthly visits, with several outcome measures taken to assess safety, acceptability and efficacy. **Safety:** sleep apnea incidents, ECG, side-effect consultations. **Acceptability:** self-report and side-effect diaries. **Efficacy:** weight, body composition, hormone levels, body image, carers' reports, fMRI responses to food images and measures of eating rate and quantity.

Results: No effects of VNS on sleep apnea or heart rate have been observed. Participant reports suggest few side-effects, with voice changes at higher output currents found acceptable. Data concerning weight and food intake is currently equivocal, with participant and carer reports suggesting beneficial effects on everyday eating behaviour but little weight loss or effect on experimental measures of eating. Participants further report more general and marked behavioural benefits, with improvements in temperament, flexibility and social functioning, which have greatly enhanced daily life.

Conclusions: Preliminary findings indicate that VNS is safe and acceptable in PWS. To date, effects on the characteristic overeating in PWS are unclear. More strikingly, positive effects on mood and behaviour have been reported which demand further investigation.

Magel2 is required for Leptin-Mediated Depolarization of POMC Neurons in the Arcuate Nucleus of the Hypothalamus in Mice

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Introduction: The hypothalamus senses circulating factors and controls short- and long-term energy balance. Leptin, a hormone produced by fat tissue, acts on leptin receptors on hypothalamic neurons. The leptin signaling pathway has garnered increased attention because leptin insensitivity is found in both genetic and diet-induced forms of obesity. We investigated leptin responses in mice lacking *Magel2*, the ortholog of *MAGEL2,* one of the genes inactivated in PWS.

Methods: Mice lacking *Magel2* have excess fat mass and reduced activity, suggesting leptin insensitivity. We used pharmacological studies and electrophysiological measurements in hypothalamic neurons to test leptin responses in *Magel2* mice.

Results: Injection of leptin reduced 24h food intake in control but not *Magel2* mice. Food intake is primarily regulated by two types of leptin-sensing neurons in the hypothalamus, which are either inhibited by leptin (neuropeptide Y (NPY) neurons), or excited by leptin (pro-opiomelanocortin (POMC) neurons). Inhibition of NPY neurons reduces the release of orexigenic peptides, reducing the drive to eat. Activation of POMC neurons facilitates the release of anorexigenic peptides, promoting satiety and increasing energy expenditure. Using whole cell patch-clamp recordings in brain slice preparations, we found normal leptin responses in NPY neurons in *Magel2* mice, but POMC neurons failed to be activated by leptin in mice lacking *Magel2*.

Conclusions: *Magel2* is essential for the centrally mediated anorexigenic effect of leptin *in vivo* and for the activation of anorexigenic POMC neurons in the hypothalamus. Leptin insensitivity likely accounts for increased fat mass and reduced activity in mice lacking Magel2. Likewise, loss of *MAGEL2* may contribute to increased fat mass, reduced satiety, and reduced voluntary activity in PWS. Defective leptin signaling in hypothalamic neurons could be the mechanistic link between PWS and other hyperphagia/obesity disorders caused by genetic mutations in the leptin signaling and downstream pathways.

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Study of Body Composition Variables and Bone Mineral Density in Patients with Prader-Willi Syndrome

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Introduction: Prader-Willi syndrome (PWS) is a genetic imprinting disorder resulting from loss of the paternally expressed genes on chromosome 15q11-q13. About 70% of subjects with PWS have paternal deletion (Del) of this region, and 30% have maternal uniparental disomy (UPD) of chromosome 15 or an imprinting center defect. Some individuals with PWS show evidence of low bone mineral density (BMD) and changes in body composition variables. Previous studies have reported improved body composition variables and BMD in children with PWS who are treated with growth hormone. There is limited consensus regarding comparison between the molecular subclasses and the management of bone mineralization problems in individuals with PWS.

Methods: Using dual-energy X-ray absorptiometry (DEXA), we evaluated bone mineralization in 74 individuals with PWS (44 from previously published study at UCI, SC (Galasetti et al), 30 from the UC Irvine RDCRN cohort, males=35, females=39, 43 had a deletion, 25 had UPD, 6 were unclassified, and 47 were on growth hormone.

Results: Individuals with UPD had a lower weight, BMI, whole body fat %, and higher whole body BMD (g/cm2) than those with deletion ($p \leq 0.05$). Individuals on growth hormone treatment were taller, had a lower BMI and whole body fat mass compared to untreated individuals. 24/74 (32%) of the total patients had a hip or spine Z-score of -1 to-2 SD (Del: UPD=8:15), 8 /74 (11%) had a z-score < -2, Del: UPD=6:1).

Conclusion: This is the largest study that compares BMD and body composition variables in deletion and UPD subclasses of PWS. In UPD there was a lower BMI, however BMD was higher compared to individuals with deletion. Individuals treated with Growth hormone had favorable body composition variables. Osteopenia/osteoporosis was seen in 45% of individuals with PWS. This points to the importance of evaluating bone mineralization status regularly. Supplementation with calcium, vitamin D and/or bisphosphonates to prevent fractures needs to be considered. Larger longitudinal studies are required to evaluate the natural history, effects of growth hormone and genetic subtype on bone mineralization in individuals with PWS.

Valproic Acid Related Hyperammonemia among Individuals with Prader-Willi Syndrome: a Case Series

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Background: Valproic acid related hyperammonemia (VRH) occurs in 20-50% of children and adults with seizure disorders and is more likely to occur with antiepileptic polytherapy and longer duration of valproic acid (VPA) therapy. There is a linear relationship between ammonia level, VPA level, and VPA dose; hepatic enzymes may not be elevated. Symptomatic VRH is termed valproic acid related encephalopathy (VRE) and clinical symptoms can include lethargy, ataxia, hypotonia, slowed cognition, confusion, agitation and encephalopathy (confirmed by EEG.) In the majority of cases, elevated levels of ammonia and clinical symptoms of VRE are reversible when the dose of VPA is reduced or discontinued.

VPA is used to treat mood instability and aggressive/impulsive behavior occurring in individuals with Prader-Willi Syndrome (PWS). The incidence of VRH in PWS has not been reported. This study explores the incidence of VRH among individuals with PWS residing in a series of group homes in Oconomowoc, WI.

Methods: Chart review of 60 individuals determined the number of individuals receiving VPA; the incidence of VRH and correlation with VPA dose, VPA level, ammonia level, hepatic enzymes; and genetic subtype of PWS. Literature review informed the background and discussion of results.

Results: Of the 60 charts reviewed, 12 individuals are or were receiving VPA; 11 had documented VRH. Hepatic enzymes were normal. More individuals with VRH were receiving the DR form of VPA than other preparations. There was no gender or age specificity. Maternal uniparental disomy subtype was identified in 3 individuals and undetermined in the remainder.

Discussion/Conclusions: Given that 11 out of 12 individuals with PWS in this case series (91%) had documented VRH, we recommend monitoring ammonia levels during VPA treatment. Because individuals with PWS have preexisting cognitive deficits, excessive daytime sleepiness, motor coordination problems, and mood and behavioral lability, it can be difficult to decide if VRH is resulting in neurobehavioral toxicity. Therefore, if ammonia levels are elevated, it is strongly advised to consider reducing VPA dose or discontinuing VPA therapy. The cause of VRH is not known. Potential etiologies include mitochondrial dysfunction, abnormalities of urea metabolism and high protein diet. Individuals with PWS are typically on calorie restricted diets, many of which limit protein content, and this may serve as a protective factor. Serum carnitine levels have been found to be inversely proportional to serum ammonia levels; therefore carnitine supplementation has been recommended. More research is needed.

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The Behavior/Neuropsychiatry Review

Hyperphagia in Patients with Bardet Biedl Syndrome

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Introduction/Background: Early-onset obesity is found with high prevalence among patients with Bardet-Biedl syndrome (BBS). However, phenotyping studies among patients with BBS are limited, and the importance of hyperphagia as a cause for energy imbalance for such patients has not been documented. We therefore compared symptoms of hyperphagia among patients with BBS and controls.

Methods: We studied 18 patients with a clinical diagnosis of BBS (1 BBS6, 1 BBS7, 10 BBS10, 6 unknown genotype) and 21 nonsyndromic obese controls who were selected to have similar mean age, sex, and body mass index z-score (BMI-Z). A 13-item-questionnaire previously validated among patients with Prader-Willi syndrome (Dykens et al., Obesity, 2007;**15**:1816-26) was used to assess hyperphagic behavior, drive, and severity. The questionnaire was completed through phone interviews with the patients' parents/guardians. ANCOVAs were used to compare hyperphagia components among BBS and control groups (adjusted for age, sex, and BMI-Z).

Results/Discussion: Groups did not differ by sex (53 vs. 48% female, p=0.74), age (13.3±6.6 vs. 12.7±6.2y, p=0.80), BMI (34.2±11.3 vs. 31.3±8.1 kg/m^2, p=0.55), or BMI-Z (2.39±0.52 vs. 2.29±0.67, p=0.57). Total hyperphagia questionnaire score was significantly higher in subjects with BBS than nonsyndromic obese controls (27.4±7.7 vs. 21.3±8.1, p=0.032), even after adjustment for age, sex, and BMI-Z. The hyperphagic behavior sub-score was higher for subjects with BBS versus nonsyndromic obese controls (12.3±3.5 vs. 8.6±3.7, p=0.003), but neither hyperphagic drive (11.2±3.6 vs. 9.2±3.5, p=0.132) nor hyperphagic severity (3.9±1.5 vs. 3.5±1.6, p=0.490) was significantly different between groups, indicating that food-seeking activity, rather than preoccupation with food or time spent thinking about food, is the distinguishing feature of hyperphagia in subjects with BBS.

Conclusion: Patients with BBS have greater hyperphagic behavior than expected for their body size. These results support the hypothesis that appetite dysregulation is an important contributor towards obesity among patients with BBS.

The Effect of Atypical Antipsychotic Medications on Metabolic Parameters in Patients with Prader-Willi Syndrome

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Introduction: Atypical antipsychotics (AAP) are often used to treat behavioral and psychiatric disorders in patients with Prader-Willi syndrome (PWS). AAP are associated with metabolic risks such as increased body mass index (BMI), total cholesterol (TC), triglycerides (TG) and hemoglobin A1C, and thus the study objective was to evaluate the effect of AAP on metabolic parameters in patients with PWS.

Methods: A retrospective cohort study was conducted at The Children's Institute of Pittsburgh using the electronic medical records of all patients (n=540) admitted to the inpatient Prader-Willi Syndrome Program from March 1, 2002 through May 14, 2012 exploring effects of AAP on metabolic parameters at time of admission. Pediatric and adult subjects exposed to AAP prior to admission were matched based on age, gender, and race to those not exposed prior to admission.

Results: Pediatric subjects (n=100) exposed to AAP had statistically ($p<0.001$) lower BMI (37.1) at admission compared to those not exposed (47.2). TC, TG, and A1C were similar at admission for pediatric subjects exposed to AAP (166, 124, 5.8) compared to those not exposed (170, 125, 5.9). There were no statistically significant differences observed between BMI, A1C, TC and TG in adult subjects (n=186) exposed to AAP (49.9, 6.1, 186, 159) compared to those not exposed (51.2, 7.5, 178, 148).

Conclusion: There were no differences in metabolic parameters at time of admission between adult patients with and without prior AAP exposure. The only statistically significant difference in metabolic parameters observed in pediatric patients was with respect to BMI, with those exposed to AAP having lower BMI at time of admission. Further research is warranted on the effect of AAP on metabolic parameters.

Risks and Benefits of SSRI Medication in Children, Adolescents and Young Adults with PWS

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Background: Mood and behavioral activation is a known risk factor associated with the use of antidepressant medications of the selective serotonin reuptake inhibitor (SSRI) type. Among persons of all ages with PWS, the first indication of mood and behavioral activation is increased intensity of typical excessive/repetitive behaviors, including food acquisition, perseveration, tantrums and skin picking. As a result, prescribers not experienced with PWS often increase medication dose, mistaking early signs of mood activation as recurrence of target symptoms, which in turn exacerbates symptoms. More severe symptoms of mood and behavioral activation include impulsive suicidal, homicidal, self-injurious, or aggressive behaviors. In the extreme, mood and behavioral activation can be associated with psychosis. The treatment of choice for mood and behavioral activation is the discontinuation of the iatrogenic agent. Here, the serum half-life of these agents determines how fast the medication can be tapered safely, because abrupt discontinuation of SSRI's can precipitate a withdrawal syndrome that is characterized by mood instability. This presentation summarizes the authors' longitudinal clinical experience with treating symptoms of mood and behavioral activation on an outpatient and residential treatment setting, and elucidates a role for low dose SSRI treatment in some individuals with PWS.

Methods: This presentation is informed by literature review and the author's longitudinal clinical experience in outpatient and residential treatment settings. Chart review informs the clinical data.

Results: Among the 25 individuals with PWS, ages 11-27 years, who displayed symptoms of mood activation associated with SSRI treatment, decreasing the medication resulted in some improvement. However, very few individuals remained medication free. The majority of individuals (23/25) went onto require mood stabilizers and/or atypical antipsychotic medication to manage mood and behavior. In several cases, withdrawal emergent effects necessitated treatment with low doses of SSRI medication (equivalent of fluoxetine 5 mg). As described in this clinical sample, neither age, gender, genetic subtype, not family history predicted mood activation, withdrawal emergent effects, or ongoing mood instability.

Discussion: Despite the growing awareness of mood activation as a problem associated with SSRI use in PWS, the authors continue to see this phenomenon as an underlying reason for behavioral crises, hospitalization, and psychiatric morbidity. The neurochemical mechanism for mood activation is not known. Serotonin is a neuromodulator of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS; low dose SSRI's function as GABA agonists, increasing *cortical inhibition*. The authors hypothesize that as the SSRI dose increases in PWS, GABA dysfunction results in cortical disinhibition, resulting in the increased intensity of phenotypic behaviors, mood instability, and psychosis. Decreased cortical inhibition caused by GABA dysfunction is noted in major depressive disorder, schizophrenia and obsessive compulsive disorder. Mood activation in PWS appears to be a strong predictor for ongoing mood instability that requires the use of mood stabilizers (potent GABA agonists) and/or atypical antipsychotic medications.

Participant and Carer Experiences of Participation in Invasive Biomedical Research: Perspectives of Individuals with Intellectual Disability

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Background: Prader-Willi Syndrome (PWS) is a neurodevelopmental disorder of genomic imprinting associated with mild to moderate intellectual disability (ID). Most prominent, however, is the characteristic hyperphagia, for which there are currently no successful treatments. Given the complex brain-gut interplay in appetite regulation, successful interventions are likely to target the central nervous system. Developing such treatments may require invasive biomedical research. There is some anxiety about conducting research of this nature with individuals with ID, based on the essential need to protect vulnerable individuals from exploitation. However, in practice, this reluctance may result in the disempowerment of these individuals and hinder the development of beneficial treatments. We consider the experiences of a group of individuals with PWS participating in a study to investigate the use of vagus nerve stimulation to treat their overeating, as well as those of the people supporting their involvement. In doing so, we consider ethical implications of intrusive research in adults with ID.

Methods: Semi-structured qualitative interviews were conducted with 3 adults with PWS and their primary carers regarding their experiences of having PWS and taking part in intrusive research. This data was transcribed and analysed using thematic analysis.

Results: The interview data demonstrated that men and women with PWS and their family carers can directly benefit from participating in intrusive research, value the opportunity to be involved, and can overcome potential barriers through extensive planning and information exchange with the research team.

Conclusions: Invasive research is likely to be crucial in developing interventions for the hyperphagia in PWS, as well as in other issues relevant to vulnerable populations. Participation of people with ID in research projects of this nature can be a rewarding and enriching experience. These positive experiences depend on reflexive ethical and practical consideration by researchers, working in collaboration with participants and carers.

The Genetics Review

A Chromosome 15q11.2 Microdeletion involving SNORD116 with Hyperphagia, Childhood-Onset Morbid Obesity and Hypogonadotrophic Hypogonadism but without Short Stature, GH Deficiency, Mental Retardation or Developmental Delay

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Background: Three previous cases of childhood-onset obesity, hyperphagia, male hypogonadism/hypergenitalism and mild-moderate mental retardation with microdeletions of the paternally-inherited chromosome 15q11.2 involving a limited number of Prader-Willi syndrome (PWS) region genes have implicated the snoRNAs, particularly SNORD116 cluster, in these phenotypes.

History: A 24 year old man with morbid obesity was referred to the PWS clinic at Hammersmith Hospital. He had neonatal weak cry and sleepiness but no history of infantile hypotonia, feeding difficulties or delay in developmental milestones. He developed active food seeking after 2y, obesity from age 4y and stealing food with binge eating episodes between 7-14y, but hyperphagia attenuated from his late teens. Height was 1.82m (mid-parental height 1.86m), weight 188.4kg, BMI 57.2 kg/m^2. He had obstructive sleep apnoea treated with CPAP. Neither his parents nor sister were obese. There was no history of mental retardation. He had a previous history of childhood depression and verbal outbursts but no other problem behaviours or high pain threshold. He had undescended testes and a unilateral orchidectomy aged 14y. He had no facial features of PWS or dysmorphism other than a slightly downturned mouth, and did not have dry saliva or small hands/feet. He had reduced body hair, micropenis, and scrotal hypoplasia with no palpable testes.

Investigations: He had hypogonadotrophic hypogonadism but otherwise normal pituitary function tests including cortisol and GH responses to insulin-induced hypoglycaemia. He had normal blood pressure, lipids and glucose tolerance but fasting hyperinsulinaemia. MRI brain/pituitary was normal. X-ray revealed thoracolumbar scoliosis. SNRPN methylation-specific PCR was normal but array CGH demonstrated a 218kb chromosome 15q11.2 deletion. The microdeletion was not present in parental DNA samples. This deletion is predicted to include SNRPN exons 6-7, SNORD116 and copies 1-22 of SNORD115 clusters.

Conclusion: Although fine mapping and expression studies are awaited, this case further supports a role for these snoRNAs, particularly SNORD116, in the regulation of eating behaviour and hypothalamic-pituitary-testicular development. Such microdeletions should be sought in childhood-onset obesity with hypogonadism even in the absence of short stature, mental retardation and other PWS phenotypes.

Growth Hormone Receptor (*GHR*) Gene Polymorphism and Impact on Growth in Prader-Willi Syndrome

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Introduction/Background: Prader-Willi syndrome (PWS) is a genomic imprinting disorder characterized by infantile hypotonia; failure to thrive; a poor suck and feeding difficulties; hypogonadism with genital hypoplasia; growth hormone deficiency with short stature and reportedly small hands and feet; hyperphagia leading to early childhood obesity; mental deficiency and behavioral problems. This syndrome is due to loss of paternally expressed genes from the 15q11-q13 region usually from a paternally derived deletion followed by maternal disomy 15 or an imprinting defect. When treated with growth hormone (GH), PWS individuals respond favorably in stature and body composition with decreased fat and increased muscle mass. The exon-3 deletion polymorphism (*d3*) in the growth hormone receptor (*GHR*) gene is reported to occur in about 50% of Caucasians in the general population. This polymorphism is reportedly associated with an increased growth response to GH therapy in non-PWS patients. The aim of our study was to assess whether *GHR* alleles impact height, weight, head circumference and body mass index (BMI) in PWS at baseline prior to GH treatment and the rate of growth during treatment.

Methods: We examined and recorded growth parameters on 69 genetically confirmed individuals with PWS (30 males, 39 females; average age ± SD = 20.1 ± 12.8y). PCR amplification was performed using primers to generate *GHR* gene fragments representing the normal full length (*fl*) allele or the *d3* allele. Thirty-nine individuals had the 15q11-q13 deletion (57%) and the remaining subjects had maternal disomy 15 or an imprinting defect.

Results/Discussion: The vast majority (i.e., 95%) of our PWS subjects were Caucasians with the distribution of alleles (*fl/fl*, n=36 or 52%; *fl/d3*, n=25 or 36%; *d3/d3*, n=8 or 12%) similar to reported data in Caucasian control subjects. There were no gender or PWS genetic subtype differences identified in the distribution of *GHR* alleles. We found a negative correlation with age for standard deviational height scores ($r=-0.49$; $p<0.0001$) and a positive correlation with age for standard deviational weight scores ($r=0.26$; $p<0.02$) and for BMI ($r=0.46$; $p<0.0001$). Adjusting for effects of age and gender, we found that individuals with PWS carrying the *d3* allele before GH treatment showed a significant increase in BMI compared with those having the full length (*fl*) allele ($F=3.9$, $p<0.02$). This analysis explains about 35% of the shared variance in BMI with 5% of the unique variance attributed to *GHR* alleles. No differences in height, weight or head circumference standard deviational scores were found. We then examined for differences in rate of growth during GH treatment while in a therapeutic range by measuring plasma IGF-I levels in 12 children with PWS (age range of 3 months to 9 years) with a minimum of 3 length measurements over an average treatment duration of 17 months (range of 2 to 54 months). We found that children with PWS having the *d3/d3* or *f1/d3* genotypes had significantly higher rates of growth at 1.50 cm/month compared with 0.87 cm/month in those with the *f1/f1* genotype (Wilcoxon-Mann-Whitney test; $p<0.04$).

Conclusions: The *d3* allele was associated with significantly increased BMIs in our cohort of PWS subjects prior to GH treatment but not for height or weight alone. The growth rate was positively impacted by the *GHR* genotype with *fl/d3* or *d3/d3* subgroups having nearly twice the rate of growth compared with the *fl/fl* subgroup. The presence of the *d3* allele and its impact on BMI before GH treatment and growth rate during treatment in PWS may influence the care and surveillance and should be addressed in expanded studies.

Brain-Derived Neurotrophic Factor in Prader-Willi Syndrome

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Background: Brain-derived neurotrophic factor is believed to play an important role in energy homeostasis. In a small pilot study, obese patients with PWS were observed to have lower serum BDNF concentrations compared with obese and lean control subjects, suggesting that BDNF insufficiency may contribute to the hyperphagia of PWS. Our objective was to replicate these findings in a separate, larger cohort of patients with PWS at different stages of body composition and eating behavior.

Methods: Our recruitment goal was to enroll 75 subjects with PWS (25 infants, 25 non-obese children, and 25 obese children). Thus far, we have enrolled 18 subjects with PWS and 18 healthy controls (HC). Fasting serum BDNF concentrations were measured by commercial ELISA (R&D Systems). Because BDNF is stored in and released from platelets in peripheral circulation, platelet count was included as a covariate in analyses.

Results: PWS and HC were not significantly different for age (mean±SD, PWS vs. HC: 6.1±4.6 vs. 8.6±4.1y, p=0.10), sex (56 vs. 72% female, p=0.49), BMI (18.1±4.3 vs. 20.2±5.7 kg/m^2, p=0.22), BMI-Z (0.19±1.31 vs. 0.92±1.27, p=0.16), and platelet count (285±72 vs. 290±63 K/μL, p=0.81). PWS had significantly lower serum BDNF concentrations than HC (17.1±6.6 vs. 21.9±6.6 ng/mL, p=0.036). After adjusting for platelet count and BMI-Z, the difference remained significant (p=0.043).

Conclusions: Our preliminary findings support previous findings of lower BDNF in PWS, even after adjustment for body size and platelet count. We will continue to enroll subjects to meet our recruitment goal and plan to compare patients with PWS at different phases of development.

Alternative Splicing Regulation of Serotonin Receptor 2C (5HT2C) as a Therapeutic Strategy for Hyperphagia in PWS

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Introduction/Background: Hyperphagia has been identified by physicians and patients as the primary unmet need presenting in PWS patients. While the biological mechanism underlying the satiety dysfunction still remains unknown, the management of the insatiable hunger mostly relies on food access restriction. PWS is caused by the loss of a region of Chromosome 15, which includes small nucleolar RNAs (snoRNAs) that are processed into shorter psnoRNAs with different biological functions. One of the snoRNAs targets is the serotonin receptor 2C (5HT2C) and psnoRNAs promote the generation of the most active form of the protein through a change in alternative splicing. Previous pharmacological and genetic studies established 5HT2C as the major receptor modulating appetite control. We tested several antisense oligonucleotides targeted against the serotonin receptor to develop a therapeutic strategy for hyperphagia in PWS.

Methods: Nine 18-mer RNA Antisense Oligonucleotides (ASO) covering exon5/intron5 junction region were tested in cell-based splicing assays. The RNA ASO showing strongest splicing effect was further characterized for an *in vivo* effect in mice. ASO was injected into mouse brain and food consumption was recorded in treated and control mice. To understand the mechanism of action of 5HT2C splicing, the same RNA fragment was used in RNA pull down experiments with Hela nuclear extract, to determine binding proteins with regulatory functions.

Results/Discussion: An18-mer RNA ASO against 5HT2C intron 5 significantly increases the expression of RNA isoform encoding the most active receptor in cell culture. The injection of the most effective RNA ASO into the 3rd ventricle of mouse brain inhibits animal food consumption for eight hours post the injection. *In vitro* protein pull down assays have identified potential associated protein factors, including an RNA helicase and other double-stranded RNA binding proteins. RNA probing assays were preformed to characterize the mechanism of action of oligonucleotide activity on 5HT2C. The results suggest the RNA structure formed in exon5/intron junction region is involved in the splice site selection and this structure is modified by the ASO.

Conclusion: Antisense RNA oligonucleotides can manipulate the splicing of 5HT2C gene and provide a novel therapeutic strategy in hyperphagia management for PWS patients.

Genomic and Cytogenetic Characterization of the Porcine Imprinted and Three Non-Imprinted Domains Orthologous to the Human Prader-Willi Syndrome Chromosome Region

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Introduction/Background: Prader-Willi syndrome (PWS) is a multisystem disorder caused by loss of function of a ~2 Mb cluster of ~12 paternally-expressed, imprinted genes in human chromosome 15q11.2. Cardinal features include neonatal failure to thrive, abnormal body composition, short stature with GH deficiency, and childhood-onset hyperphagia and obesity, among other endocrine and behavioral abnormalities. Although mouse models of PWS recapitulate some of the clinical components of the disorder, none develop early onset hyperphagia or the severe obesity of the human disease. Therefore, alternative animal models are needed to study the biomedical basis and therapeutic approaches for the eating disorder and obesity. Miniature pigs may provide an ideal model for PWS and other body fat disorders, since they have a more similar body size, physiology, anatomy, and genome to human than does the mouse, and hence may be more susceptible to development of obesity. Furthermore, technologies exist in the pig to produce genetic models of disease.

Results/Discussion: Prior to this study, the pig PWS-orthologous region was poorly represented in the pig genome sequence. Using sequence databases to screen for phylogenetically conserved sequences from the PWS domain, we generated *in silico* BAC and fosmid contigs spanning most of the pig PWS-homologous imprinted domain, including the ~150-kb *cis*-acting imprinting center (IC). Seven of these genomic clones were then sequenced by the Wellcome Trust Sanger Institute. Eleven orthologs of PWS imprinted genes have been identified. These new sequences have allowed us to identify regulatory elements controlling genomic imprinting in this domain and that control neuronal gene expression of a polycistronic *Snurf-Snrpn* mRNA-snoRNA lncRNA, including four classes of small nucleolar RNAs. Surprisingly, by FISH mapping of BAC clones for imprinted and non-imprinted PWS-gene regions, we found that the porcine PWS-orthologous domain has split into three segments, with the IC-*Snurf-Snrpn*-snoRNA locus mapped to *Sus scrofa* chromosome (SSC) 1q18 and the non-imprinted PWS-orthologous loci mapped to SSC1q18, SSC15q14, and SSC15q21.

Conclusions: This work has identified the genetic structure of imprinted genes, transcriptional and imprinting *cis*-regulatory elements, and chromosome evolutionary breakpoints in the PWS-orthologous domain in pig. As a consequence, it is now possible to consider the development of pig models of PWS.

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Brain-Derived Neurotrophic Factor (BDNF) in Human Subjects with Function-Altering Melanocortin-4 Receptor (MC4R) Mutations

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Background: In rodents, BDNF appears to act downstream of the leptin-proopiomelanocortin pathway. No prior studies have examined BDNF in humans with loss-of-function (LOF) or gain-of-function (GOF) *MC4R* mutations.

Methods: Fasting serum BDNF and leptin were measured by ELISA in 148 Pima Indian subjects [age (mean±SD) 15.7±6.5y, 50% female, BMI-Z 1.63±1.03] and plasma BDNF and leptin were measured in 60 Hispanic subjects (age 10.7±3.7y, 47% female, BMI-Z 1.55±1.03). All subjects underwent sequencing of *MC4R*. In the Pima cohort, heterozygous (n=40) or homozygous (n=3) LOF and heterozygous GOF (n=20) subjects were separately matched by age, sex, and BMI to homozygous wild-type (WT) control subjects [LOF-C (n=65) and GOF-C (n=20), respectively]. In the Hispanic cohort, heterozygous LOF (n=15), heterozygous GOF (n=20), and homozygous WT (n=25) subjects were compared.

Results: In the Pima cohort, LOF had higher BMI-Z compared to GOF (1.91±0.83 vs. 0.96±1.19, p<0.001). LOF and LOF-C were similar in age (p=0.73), sex (p=0.97), BMI-Z (p=0.69), leptin (median [25-75th percentile]: 22.7 [6.4-39.8] vs. 16.4 [7.7-32.5] ng/mL, p=0.51], BDNF (23.3 [19.1-28.1] vs. 21.9 [17.9-25.0] ng/mL, p=0.12), and BDNF-to-leptin ratio (1.2 [0.5-2.6] vs. 1.2 [0.6-2.4], p=0.80). GOF and GOF-C were similar in age (p=0.92), sex (p=1.00), BMI-Z (p=0.97), and leptin (10.3 [1.2-20.6] vs. 10.8 [3.6-28.6], p=0.48), with a nonsignificant trend toward higher BDNF in GOF (25.0 [19.1-26.0] vs.19.0 [15.3-23.2], p=0.06), but similar BDNF-to-leptin ratio (1.8 [0.9-13.0] vs. 1.6 [0.6-6.7], p=0.28). In the Hispanic cohort, we observed no significant differences between LOF, GOF, and WT for age (p=0.87), sex (p=0.84), BMI-Z (p=0.29), leptin (p=0.33), BDNF (p=0.66), or BDNF-to-leptin ratio (p=0.57).

Conclusions: In subjects with function-altering *MC4R* mutations, serum leptin, serum and plasma BDNF, and BDNF-to-leptin ratio were similar to WT subjects with comparable BMI-Z, suggesting that peripheral BDNF may not reflect hypothalamic BDNF or that MC4R signaling is not a significant regulator of BDNF expression in humans.

A Transgenic Mouse with Hyperactive Brain Limbic Region and Hyperphagia-Mediated Morbid Obesity

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Prader Willi Syndrome (PWS) due to loss of paternally imprinted gene expression at the PWS locus in humans is associated with hyperphagia-mediated morbid obesity with endocrinological and neurobehavioral abnormalities. PWS is also associated with high levels of ghrelin, in contrast to other obesity syndromes where ghrelin levels are inversely correlated with obesity. Ghrelin signaling is mediated centrally by neurons of the arcuate nucleus of the hypothalamus neuropeptide Y (NPY) and agouti-related protein (AgRP), which reduces the activity of proopiomelanocortin (POMC) neurons. Therefore, NPY and AgRP are mediators of the orexigenic effect of circulating ghrelin via inhibition of melanocortin signaling.

We have generated transgenic mice misexpressing the RNA editing enzyme ADAR2 that mimic many aspects of PWS. ADAR2 transgenic mice display affective disorder and hyperpahgiamediated morbid obesity. Significantly altered 5HT2CR editing in the prefrontal cortex brain region coincides with enhanced depression-like behavior prior to obesity in ADAR2 transgenic mice. Plasma levels of leptin, glucose, insulin, triglyceride and cholesterol are normal in preobese ADAR2 transgenic mice but show significantly elevated levels of corticosterone. Recently we have found that obese ADAR2 transgenic mice have significantly elevated basal active ghrelin levels without changes in total ghrelin when compared to controls. Since ghrelin mediates homeostatic and non-homeostatic reward-based overeating, we examined if feeding behavior was reward-mediated and whether related genes associated with feeding and reward were altered in ADAR2 transgenic mice. Positron emission tomography (PET) imaging shows significantly hyperactive hypothalamus and brain limbic regions that are implicated in reward pathways in ADAR2 transgenic mice. Gene expression changes associated with feeding and reward show significantly increased ADAR2, GHS-R, NPY, AGRP, CART, MC4R, D1-R, D2-R, and mu-opioid-R; deceased POMC and MC3R expression; and no significant changes in CRH and 5HT2CR expression in the hypothalamus of ADAR2 transgenic mice. By comparison, in the striatum significantly increased D1-R expression and no changes in ADAR2, D2-R and mu-opioid-R expression are observed. In a competing rewarding environment of running wheel activity and food intake, where both are rewarding to mice, ADAR2 transgenic mice show significantly increased food intake and no significant changes in running wheel activity when compared to controls. Together these results indicate that the ghrelin-mediated reward pathway is involved in overeating behaviors of ADAR2 transgenic mice. Thus, the ADAR2 transgenic mouse mimics both hormonal and overeating behaviors associated with PWS.

Stem Cell-Based Approaches to the Neurobiology of Obesity in Bardet-Biedl Syndrome

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Introduction/Background: Bardet-Biedl Syndrome (BBS) is a rare autosomal recessive disease characterized by multiorgan dysfunction, including polydactyly, obesity, retinal degeneration, renal cysts and mental retardation. BBS is caused by mutations in a group of proteins that are components of the basal body of the primary cilium. However, the underlying mechanism of how BBS proteins influence the appetite and energy expenditure in human is not well understood.

Methods: To investigate the neurobiology of obesity in BBS, we established an *in vitro* model by reprogramming skin fibroblasts from BBS patients segregating for *BBS1*$^{M390R/M390R}$ and *BBS10* $^{C91fsX95/C91fsX95}$ mutations, into induced pluripotent stem cells (iPSCs) and further differentiating these cells into insulin- or leptin-sensing neurons with dual SMADs inhibition. iPSCs derived from unaffected healthy subjects were used as control in this study. Then we explored ciliogenesis, insulin and leptin signaling with these iPSC-derived neurons, in order to identify the underlying mechanism of the hyperphagia in BBS patients.

Results/Discussion: We first demonstrated that the ciliogenesis *per se* was not affected in *BBS1* mutant iPSC-derived neurons while *BBS10* mutant neurons displayed longer cilia. Furthermore, insulin-induced AKT phosphorylation at Thr308 site was greatly reduced in both *BBS1* and *BBS10* mutant neurons compared to controls. Leptin signaling was investigated in *BBS* mutant fibroblasts expressing transfected LEPR. Both BBS mutations impaired leptin-mediated STAT3 activation. Furthermore, lentivirus mediated expression of the wildtype *BBS1* transgene in *BBS1* $^{M390R/M390R}$ fibroblasts rescued leptin signaling measured by STAT3 phosphorylation.

Conclusion: These data demonstrate that BBS proteins play an important role in leptin and insulin signaling, and suggest that ciliary proteins could be potential therapeutic targets for treating obesity and diabetes.

The Poster Review

Analysis of NIPA1 and NIPA2 in Neuronal Development and Neurodegeneration using Zebrafish Motor Neurons as Experimental Model System

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Introduction: The Prader-Willi (PW) and Angelman syndromes include genomic deletions in the proximal long arm of chromosome 15 that are flanked by the proximal breakpoints (BP1 or BP2) and by the distal BP3. Individuals carrying the larger type I deletion flanked by BP1 and BP3 show a more severe phenotype than the ones with type II deletions (BP2 - BP3), indicating that genes located between BP1 and BP2 contribute to the phenotypes of these syndromes. The region between BP1 and BP2 contains four evolutionarily conserved genes (*NIPA1, NIPA2, CYFIP1*, *and GCP5*) that are not imprinted. However, the role of these not imprinted genes in neural development and their contribution to PW syndrome is not well understood. The *NIPA1* and *NIPA2* genes are highly conserved across species, and encode integral membrane proteins of ~330 amino acid residues with nine predicted transmembrane domains and Mg^{2+} transporter activity. Missense mutations in the *NIPA1* gene are associated with autosomal dominant hereditary spastic paraplegia 6 (AD-HSP6). NIPA1 regulates Bone Morphogenetic Factor (BMP) signaling and affects axon growth and synapse formation in *C. elegans* and *Drosophila.* NIPA1-mediated regulation of BMP signaling is disrupted by missense mutations in the *NIPA1* gene that cause HSP6, and expression of NIPA1 mutated proteins causes neurodegeneration, suggesting that NIPA1-mediated regulation of BMP plays an important role in neuronal development and cell survival. However, the mechanisms whereby NIPA1 and NIPA2 regulate neuronal development and BMP signaling in vertebrate neurons have not been investigated. The goal of this project is to elucidate the roles of *NIPA1* and *NIPA2* in axon growth and formation of neuromuscular junctions (NMJ) using the zebrafish spinal cord motor neurons as a research tool. A "research tool" is a biological system that provides insights into the mechanisms underlying a human disease, even though the system may not phenocopy all biological aspects of the disorder.

Methods: The zebrafish (*Danio rerio*) is a widely used experimental model organism for the study of neural development and for the modeling of neurological disorders. Its external and rapid growth, small size, morphological and physiological similarity to mammals, and tissue transparency, allows the study of development and of pathological processes *in vivo* and in fixed specimens. The analysis of neuronal development is facilitated by the use of cell-type specific and inducible promoters for the temporal and spatial control of transgenic expression of wild type and mutated proteins.

Results: To study the role of NIPA1 and NIPA2 in neuronal development, we have developed a motor neuron specific promoter from the *mnx1* transcription factor that becomes active only in post-mitotic motor neurons. Using this promoter, we generated transgenic fish lines expressing membrane-bound enhanced green fluorescent protein (EGFP) and Gal4 specifically in motor neurons for the visualization of neuronal arbors and for transgenic expression using the Gal4/UAS transcriptional activation system. We are currently using these tools to generate transgenic fish lines expressing wild type and mutant NIPA1 in motor neurons. These transgenic lines will be used to examine the role of NIPA1 in motor axon growth and development of the pre- and post-synaptic components of the NMJ, and to analyze the effect of the mutant NIPA1 forms that cause spastic paraplegia on neuronal development and neurodegeneration. Finally, we will examine the role of NIPA1 and NIPA2 in BMP signaling by expressing deletion mutants of the BMP1 and BMP2 receptors in motor neurons (which interfere with canonical and non-canonical BMP signaling), and by treatments with pharmacological agents that inhibit BMP.

Conclusion: The analyses of the role of NIPA1 and NIPA2 in axon growth and synapse formation using the zebrafish motor neurons as experimental model system will contribute to elucidate the cellular and signaling mechanisms whereby NIPA1 and NIPA2 regulate neural development. Our understanding of the biological mechanisms whereby these genes participate in PW and Angelman syndromes will facilitate the identification of potential therapeutic targets.

Probing Genes for Hyperphagia in Rare Obesity-Related Syndromes

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Introduction/Background: Hyperphagia and obesity are key features of several rare genetic obesity-related syndromes associated with epigenetic regulatory mechanisms and genomic imprinting including Prader-Willi syndrome (PWS), Alström syndrome (ALMS) and fragile X syndrome (FXS). An understanding of the regulatory basis of hyperphagia will provide a more comprehensive picture of mechanisms that control food intake, energy balance and obesity. Although monogenic causes of morbid obesity are uncommon in the general population, knowledge gained from genetic obesity-related disorders should impact our understanding of the genetic causation or molecular mechanism(s) for exogenous obesity. Coding and non-coding RNAs, specifically microRNAs and snoRNAs, play important regulatory roles in a variety of biological systems. We propose to use state-of-the-art microarray technology with advanced bioinformatics and integrate clinical, hyperphagia and obesity measures with genetic datasets which should lead to discovery of unique molecular signatures and pathways for each disorder amenable for early detection and pharmaceutical interventions.

Methods: We are performing coding and non-coding expression pattern analysis using both the Affymetrix GeneChip miRNA 2.0 Array including 4,592 human miRNA probe sets and GeneChip Human Exon 1.0 ST Array including 1.4 million probe sets with RNA isolated from lymphoblastoid cells and brain cortex from 10 individuals each with PWS, ALMS and FXS compared with 20 controls (N=10 obese and N=10 non-obese). These probesets are background corrected, normalized and summarized using the Robust Multichip Average (RMA) procedure and a standardized bioinformatics approach with a false discovery rate (FDR) of ≤ 0.3. Exon expression data were analyzed at the gene level.

Results/Discussion: To date, we have analyzed lymphoblastoid cell lines on 7 genetically confirmed adults with PWS, 7 adults with non-syndromic exogeneous obesity and 7 adults with a normal body mass index (BMI). As predicted, lower snoRNA expression for the 15q11-q13 region (e.g., HBII-85) distinguished PWS from lean and obese controls, but no disturbances were seen comparing obese and lean subjects. For miRNA analysis in PWS vs lean, we found overexpression of the mature and immature miR-34a constructs and downregulation of miR-885-3p in PWS. For PWS vs obese, we found overexpression of miR-541-star in PWS. Examination of obese vs lean, we found upregulation of 8 miRNAs in obese subjects with the highest being miR-27a-star and downregulation in 12 miRNAs. Exon array analysis found only downregulation of SNRPN in PWS compared to obese and lean subjects, while obese and lean subjects were distinguished by downregulation in obese subjects of metallothionein 1G (MT1G) and 1X (MT1X), a family of proteins that may provide protection against metal toxicity and oxidative stress. Metallothionein is a target mRNA for miR-27a-star, miR-23a-star, mir-296, and miR-513a-3p which are upregulated in obese subjects compared with lean.

Conclusions: Initial evaluation has identified miRNA disturbances in the regulation of metallothionein expression in obesity. MiRNA expression in PWS does not appear to broadly influence exon expression analyzed at the gene level in lymphoblasts but further investigations will include expression at the level of individual exons. Upregulation of miR-34a in PWS may impact upon the GABA-A receptor family. The discovery of molecular pathways or mechanisms controlling eating behavior common to the obese phenotype and hyperphagia in those with known obesity-related disorders and controls with exogenous obesity should stimulate new directions for research. We continue to establish lymphoblastoid cell lines and obtain brain specimens from individuals with Alström and fragile X syndromes for study.

The Effect of Atypical Antipsychotic Medications on Body Mass Index in Patients with Prader-Willi Syndrome

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Introduction: Atypical antipsychotics (AAP) are often used to treat behavioral and psychiatric disorders that accompany Prader-Willi syndrome (PWS). Treatment with AAP has been associated with weight gain in the general population and thus, the study objective was to evaluate the effect of AAP on body mass index (BMI) in patients with PWS.

Methods: A retrospective cohort study was conducted at The Children's Institute of Pittsburgh using the electronic medical records of all patients (n=540) admitted to the inpatient Prader-Willi Syndrome Program from March 1, 2002 through May 14, 2012 assessing the effect of AAP on patient's BMI. Pediatric and adult patients exposed to AAP at admission and during stay were matched based on age, gender, and race to those not exposed to AAP. A second analysis focused on both adult and pediatric subjects not exposed to AAP at admission but subsequently initiated on AAP during their stay.

Results: Pediatric subjects (n=104) not exposed to AAP experienced a greater decrease in BMI (-5.5127) when compared to those exposed (-0.8800), although not statistically significant (P=0.294). Adult subjects (n=194) exposed to AAP experienced a nearly equal change in BMI (-6.7062) when compared to those not exposed (-6.1307) (P=0.735). The second analysis of adult and pediatric subjects (n=22) not exposed to AAP at admission and during stay experienced a statistically significant greater decrease in BMI (-7.2880) when compared to those not exposed to AAP at admission but subsequently initiated on AAP during their stay (-3.0771) (P=0.027).

Conclusion: All subject groups in this review experienced a decrease in BMI regardless of AAP exposure. The only statistically significant difference in BMI change observed was between AAP naïve subjects who were initiated on an AAP during their stay and matched subjects not exposed to an AAP, with those not exposed experiencing a greater decrease in BMI than those exposed. The effect of AAP on BMI in PWS warrants further research.

A Stem Cell Model of Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is a genetic form of early onset, hyperphagic obesity in the context of neurologic, developmental, endocrine and other characteristic phenotypes. Attempts to understand the molecular bases for the PWS nervous system phenotypes have been limited by shortcomings in PWS mouse models and restricted access to relevant human material. Technologies allowing reprogramming of human fibroblasts to induced pluripotent stem cells open new possibilities for the study of PWS, specifically, the opportunity to identify the genes and molecular mechanisms underlying the CNS phenotypes. PWS fibroblast lines were obtained from 2 patients with Type 1 and Type 2 deletion genotypes. Type 1 and Type 2 deletions are 5-7 Mb deletions on 15q11.2-11.3 and make up ~70% of PWS genotypes. Fibroblasts were reprogrammed to iPS cell (iPSC) lines expressing the expected pluripotency markers. Because reprogramming can erase some epigenetic marks, PWS iPS cells were investigated to determine if maternal methylation on 15q11.2-11.3 remained intact. Genes within the PWS region, including SNURF and NDN, showed persistence of methylation patterns after iPS reprogramming, while SNRPN and SNORD116 remained unexpressed. These data indicate that PWS iPS cell lines retained maternal methylation in the PWS region. Because PWS imprinting and gene expression patterns were preserved after reprogramming, it may be anticipated that PWS iPS-derived neuronal-like cells would display characteristic differences in transcripts related to energy intake. PWS and control iPSC differentiated with equal efficiency into neuron-like cells. PWS neuron-like cells express neuronal markers including MAP2, TUJ1, Nefl, and NES. By qRT-PCR, SNRPN and SNORD116 remained silenced after differentiation into neuron-like cells. Quantitative and qualitative aspects of the transcriptional repertoire of these cells are under investigation.

Autistic Symptomatology in Prader-Willi Syndrome

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Background/Objectives: Prader-Willi syndrome (PWS) is caused by either the structural loss of material or the absence of gene expression from the paternally inherited copy of chromosome 15 (q11-q13). In addition to a well-described behavioral phenotype that includes hyperphagia, obsessive-compulsive symptoms, disruptive behavior, cognitive delays, research also suggests that some persons with PWS have repetitive behavior and social deficits reminiscent of autism spectrum disorders (ASD). In particular, it appears as though those individuals with the maternal uniparental disomy (m-UPD) subtype of PWS are at greater risk for autistic symptomatology than those with paternal deletions (DEL) of 15q11-q13. These findings are particularly intriguing in light of data implicating maternal duplications of the same chromosomal interval in idiopathic autism, as well as evidence that functional alterations of genes in this region are associated with social deficits found in a variety of neurodevelopmental disorders. The purpose of this research is to examine social functioning in individuals with PWS and to further test the hypothesis that m-UPD is a specific risk factor for autistic symptomatology.

Methods: 42 individuals with PWS (23 DEL, 19 m-UPD) and 19 individuals with an ASD (7-36 years old) and their caregivers comprised the total sample. Participants underwent intelligence testing (WISC-IV, WAIS-III, or WASI), adaptive functioning (Vineland Adaptive Behavior Scales), and the Autism Diagnostic Observation Schedule (ADOS). Parents completed the Autism Diagnostic Inventory-R (ADI-R), as well as the Social Competence Inventory (SCI; Rydell, 1997) and Social Responsiveness Scale (SRS; Constantino & Gruber, 2005).

Results/Discussion: For social responsiveness, SRS-Total score significantly differed between ASD, m-UPD, and DEL groups ($F = 7.275$, $p = .002$; controlling for age and IQ). Post-hoc comparisons indicate significantly more social difficulties were present for participants with ASD and m-UPD than those with DEL. 78.9% of m-UPD and 36.4% of DEL participants scored in the highest clinically significant range indicating severe interference in everyday social interactions. Groups also differed significantly on measures of social competence. Participants with m-UPD and ASD evidenced greater impairment in the SCI Prosocial Orientation Subscale (e.g., empathy, understanding of others, helpfulness) than those with DEL ($F=7.2$, $p=.002$ controlling for age and IQ). No differences were found between groups on the Social Initiative Subscale [DEL = 3.04(.82), m-UPD = 2.64(.72), ASD = 2.51(.72)]. Among those with PWS, the SRS-Total score and SCI Prosocial Orientation was significantly negatively correlated with Vineland Social Subscale. These findings indicate individuals with PWS have difficulty initiating social interaction (e.g., making contact with unfamiliar peers) and may be prone to social hesitancy or withdrawal similar to those with an ASD. Prosocial behaviors such as generosity, empathy, and helpfulness were more evident in those with DEL subtype than in individuals with m-UPD or ASD. Results will be discussed in relation to ADOS and ADI-R diagnostic criteria. These findings give further insight into the social functioning of persons with PWS and indicate need for social-skills intervention in this population.

Weight Differences in Neurofunctional Activity to Visual Food Cues: A Meta-Analysis of Neuroimaging Studies

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Introduction/Background: Obesity is a complex disorder associated with serious health risks. Examining the brain's activity in response to food and how it varies between weight groups may help us understand the source of the behaviors that lead to overeating and obesity and in understanding them, help prevent them. Multiple neuroimaging studies have investigated weight differences in functional activity to food cues but have found inconsistent results due to a lack of reliability in neurofunctional studies. By performing a series of meta-analyses of functional neuroimaging studies of the weight differences in activity to food images, this study aims to isolate consistent differences in processing food stimuli between the healthy weight and the obese

Methods: In this study, eleven papers on functional activity to food images (224 coordinates, 324 subjects), were analyzed using an Activation Likelihood Estimation meta-analytic approach to examine neurofunctional differences between the healthy-weight and obese when viewing food cues when hungry or satiated.

Results/Discussion: Preliminary results show clusters of greater activity found in the obese when hungry in the dorsal striatum, amygdala, and anterior cingulate. When fed, greater activity was found in multiple prefrontal regions, the thalamus, parahippocampal gyrus, and superior temporal. Activity in these areas may indicate greater reward activity and evaluation when hungry and continued attention to food, taste processing and continued inhibition when satiated. Greater activity in the healthy-weight was found when hungry in the prefrontal, insula, hippocampus, and parahippocampal gyrus. These activations may reflect differential taste processing and greater inhibition when hungry.

Conclusion: Identifying the functional differences found in obesity may help in understanding the source of overeating. The results of this study suggest that differences in reward functioning, attention, taste processing, and inhibition may be involved in overeating. Developing interventions focused on bolstering inhibition or reducing reward response may help counter overeating behaviors.

Abnormal Proteins Drive the Hyperphagia in Prader-Willi Syndrome

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Introduction: Prader-Willi syndrome (PWS) manifests as extended infantile failure-to-thrive followed by lifelong, treatment-refractory hyperphagia resulting in morbid obesity, significant morbidity, and early mortality. Among those affected, hyperphagia and the associated food-related behavior constellation is ***the*** primary concern impacting overall health and quality of life. In addition, PWS is considered a model for understanding obesity in general, offering insights not only into malignant hyperphagia, but also into a spectrum of obesity related neuroendocrine disturbances including: 1) metabolic disturbances, 2) abnormal body composition and energy expenditure, 3) hypothalamic/pituitary abnormalities including growth and sex hormone deficiencies with concomitant growth and physiologic retardation, 4) as well as insights into the role of peripheral signals including insulin, plasma leptin, ghrelin, Peptide YY, and other gastrointestinal peptides/hormones as they interact with hypothalamic and other central pathways that regulate feeding and energy expenditure. Fifty years of genetic, endocrine, neurochemical and neuroimaging research has failed to isolate the hyperphagic etiology or provide pharmacotherapy.

Hypothesis: We hypothesize that the hyperphagic etiology resides in abnormal signaling mechanisms via serum proteins or peptides unique in type or magnitude compared to those from obese non-PWS and normal weight controls,

Objective: To identify unique serum proteins and/or peptides involved in the mechanism of hyperphagia and obesity in PWS.

Methods: This project employs a previously unapplied, state-of-the-art approach to this critical issue, utilizing serum proteomics to identify up- or down-regulated protein spots in serum from 30 individuals with PWS across the age span compared with age, gender and BMI matched normal controls. Following a fasting blood sample, a standardized breakfast is provided and a 30 minute post-prandial blood sample is obtained.

In the first approach, we are using an immune-affinity column to remove 14 high abundant proteins from the serum samples: Human Serum Albumin, IgG, Fibrinogen, Transferrin, IgA, IgM, Haptoglobin, alpha2-Macroglobulin, alpha1-Acid Glycoprotein, alpha1-Antitrypsin, HDL (Apo A-I & Apo A- II), Complement C3, and LDL (ApoB). These proteins account for more than 90% of the total protein mass in human serum. The removal of the high abundance species allows us to detect changes in low-abundant proteins. The resulting two groups of proteins are then analyzed by the two dimensional difference gel electrophoresis (DIGE) system. Next, we are investigating the changes in posttranslational modifications of 2-D gel detected serum proteins. Depending on the ultimate findings, we will then reconfirm the identified protein expression by western blot analysis, and finally, will purify candidate proteins for developing a confirmatory mouse model based on the underlying defect.

Results: Although we are just completing sample collection, some initial 2D gel runs have been conducted and some differences identified. We will present the resulting images in our poster.