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Expression of Four Genes between Chromosome 15 Breakpoints (BP1 and BP2) and Behavioral Outcomes in Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from lack of expression of paternal genes from the chromosome 15q11-q13 region. A *de novo* paternally derived deletion is seen in about 70% of PWS subjects and classified as having either a large Type I (TI) deletion involving chromosome 15q breakpoints BP1 and BP3 or a smaller Type II (TII) deletion involving breakpoints BP2 and BP3. Clinical differences have been reported between those with the typical 15q11-q13 deletion (unclassified) and maternal disomy 15 in verbal IQ, visual memory and maladaptive behavior. In addition, PWS individuals with the TI deletion reportedly have more behavioral and psychological problems than PWS individuals with the TII deletion. Hence, we examined the relationship between expression patterns of four genes (*NIPA1*, *NIPA2*, *CYFIP1*, *GCP5*) located between the two proximal 15q breakpoints (BP1 and BP2) and behavioral, psychological and cognitive assessments previously reported to be different in individuals with PWS having TI or TII deletions. The four genes are deleted in individuals with Prader-Willi syndrome having a TI deletion but not in those with a TII deletion. Thus, they become candidate genes for contributing to reported phenotypic differences between the two typical deletion subtypes.

Our study subjects included eight TI PWS subjects (4 male, 4 female, mean age 25.2 +/- 8.9y) and nine TII PWS subjects (3 male, 6 female, mean age 19.5 +/- 5.8y). We determined gene expression by quantitative RT-PCR using RNA isolated from actively growing lymphoblastoid cell lines from each of our study subjects. We compared the gene expression (C_T) values to behavioral, cognitive and visual assessments previously identified as differing by deletion subtypes in our study subjects. Statistical analysis using the coefficient of determination suggested that expression of the four individual genes accounted for as much as 75% of the variation seen in several behavioral and academic assessments. The joint impact of the four genes explained from 24% to 99% of the assessment scores obtained from our subjects with PWS. We also demonstrated significant correlations between phenotypic outcomes and gene expression of the four genes located between BP1 and BP2. Because behavior and cognition are difficult to quantify, it is obvious that the expression of the four genes between BP1 and BP2 can not explain all of the behavioral and psychological differences observed in our PWS subjects. However, the gene expression values explained more of the variability than the deletion subtype alone. This is in keeping with the fact that haploinsufficiency reduces expression of the four genes in PWS subjects having TI deletions. Our data further suggested that the four genes influence neurodevelopment and function in PWS with the greatest contribution identified for *NIPA2* as well as through direct interaction with other genes yet to be identified.



Dissecting the Phenotype of Prader-Willi Syndrome using Mouse Models of Nervous System Development

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PWS is a multi-gene disorder. It appears likely that different manifestations of PWS are due to the additive loss of function of multiple genes that are essential for normal development. To simplify investigations, we are examining the effects of loss of PWS genes individually. We generated gene-targeted mice for two key PWS genes, encoding *neccdin* and *MAGEL2*, which are both imprinted and inactivated in most individuals with PWS. *Neccdin* is required for migration and neurite outgrowth of a variety of neurons in the developing mouse embryo. Loss of *neccdin* in mice causes a developmental defect 1) in the brain stem and diaphragm causing reduced neonatal respiration, 2) in the sensory nervous system causing reduced pain sensitivity, and 3) in the sympathetic nervous system leading to reduced innervation of target organs, including the salivary glands. These results are consistent with the hypothesis that loss of *neccdin* in PWS contributes to central sleep apnea, altered respiratory responses to hypoxia and hypercapnea, relative pain insensitivity, and reduced salivation. Investigation of the impact of loss of *neccdin* on the enteric nervous system and on sympathetic innervation of the gastrointestinal tract are underway and are highly relevant to altered gastric motility in PWS.

MAGEL2 shares a MAGE protein homology domain with *neccdin* and may have a similar role in promoting the migration and differentiation of neurons in the hypothalamus, where it is most abundantly expressed. Indeed, we present new evidence that *MAGEL2* is important to the normal development of the hypothalamus. Mice with a paternally inherited targeted mutation in *Magel2* have reduced total activity and a less coherent circadian rhythm than control littermates, although they do entrain to a light:dark cycle with a normal period. This suggests a defect in circadian output from the suprachiasmatic nucleus of the hypothalamus, which affects locomotor activity. As sleep, circadian rhythm, appetite, and fertility are coordinately controlled by the hypothalamus, we investigated whether *Magel2*-mutant mice had other phenotypes related to altered hypothalamic function. Compared to control littermates, *Magel2*-mutant mice had slightly reduced birth weights, increased weight gain after weaning onto a moderately high fat diet, and reduced male fertility. These results are consistent with the hypothesis that loss of *MAGEL2* in PWS contributes to hypothalamic deficiency that affects appetite, sleep, and reproduction. We propose that combined loss of *neccdin* and *MAGEL2* act in an additive or cooperative manner to cause delayed or abnormal development of the nervous system, leading to altered neurophysiology in individuals with PWS.



Impaired Pancreatic Islet Organization and Function in a Prader-Willi Syndrome Mouse Model with Neonatal Failure to Thrive

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Prader-Willi syndrome (PWS) is caused by loss of function of multiple imprinted, paternally-expressed loci within chromosome 15q11.2 that encode proteins and snoRNAs, with most predicted to regulate other RNAs. We have shown that many of these loci are controlled by the transcription factor (TF) Nuclear Respiratory Factor (NRF)-1, implicating defective mitochondrial and/or energy metabolism pathways in PWS. The endocrine abnormalities described in children and adults with PWS have been hypothesized to result from a central hypothalamic dysfunction and include deficiencies in growth hormone, insulin-like growth factor (IGF)-1, IGF binding protein-3 and gonadotropins. Increased fasting plasma ghrelin levels and moderate hypoinsulinemia also occur in patients with PWS compared to controls with common obesity. However, the actual basis for the PWS endocrine phenotype is unknown and we have begun to investigate this using a transgenic PWS (TgPWS) deletion mouse model with neonatal failure to thrive and early lethality. In TgPWS mice, we found impaired hepatic glucose homeostasis from postnatal day 2 (P2). Surprisingly, TgPWS compared to wildtype (WT) mice had extremely low plasma insulin and glucagon levels at all postnatal ages and also in fetal life. Moreover, TgPWS mice at P1 showed aberrant pancreatic islet architecture with 50% reduction in β - and α -cell mass; these abnormalities arise in fetal life and hence represent a primary developmental deficit in TgPWS mice. To explore the basis of the TgPWS pancreatic phenotype we probed a cDNA microarray containing \sim 13,000 mouse genes and ESTs expressed in the pancreas with pancreatic cDNA from normoglycemic P1 TgPWS and WT pups. Data integration from two different statistical analyses showed that 69 genes were up-regulated and only 3 genes including *Ndn*, a deleted imprinted gene, were down-regulated in TgPWS pancreas. Approximately 30% of the up-regulated mRNAs were represented by genes encoding pancreatic hormones (e.g., *Ins2*, *Ins1*, *Iapp*, *Sst*, *Ppy*, *Gcg*) and processing/secretory functions (eg., *Sgnt1*, *Tmem27*) suggesting a secretory defect in TgPWS pancreas. Interestingly, several genes with increased transcription in TgPWS pancreas are known to be regulated by the LIM domain homeobox ISL1, which was also 2-fold up-regulated. Moreover, bioinformatic analysis revealed a conserved cluster of core motifs for homeodomain proteins and an E-box site similar to insulin gene enhancer elements adjacent to a regulatory element consisting of four NRF-1 sites within the PWS domain. Chromatin immunoprecipitation studies showed that this regulatory element associates with open chromatin [acetylated and di-methylated (Lys4) histones] in brain and pancreatic β -cell lines and that this CpG-rich region is unmethylated in brain and pancreas. Studies aimed at identifying the TFs that bind to these sites are ongoing. In conclusion, our studies identify a primary pancreatic deficit associated with low levels of circulating islet hormones that may contribute to the TgPWS fetal and neonatal phenotype.



Psychopathology and 5HT Levels in Prader-Willi Syndrome

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Introduction: Prader-Willi syndrome (PWS) has a behavioral phenotype that includes behaviors such as severe tantrums, outbursts and stubbornness, as well as many obsessive-compulsive features like hoarding and needing to ask and tell. We and other groups have hypothesized that many of the behaviors associated with PWS may be due to aberrant or low levels of serotonin. SSRIs, which increase serotonin at the synaptic level, are often used in PWS populations (with variable success) to help mediate these behavior problems. The current study looks at the plasma levels of 5HT, a precursor to serotonin, in individuals with PWS and whether 5HT levels are related to several measures of adaptive and maladaptive behavior.

Methods: Plasma 5HT levels were collected from 37 individuals (20 females and 17 males) with PWS (mean age=19.3 years +/- 10.4 years) at Vanderbilt Kennedy Center as part of an ongoing longitudinal study looking at development of psychopathology and compulsive behaviors in PWS. Caregivers also completed a number of maladaptive and adaptive behavior questionnaires including the CBCL, Vineland, and the Yale Brown Obsessive Compulsive Scale (YBOCS).

Results: Participants with PWS had plasma 5HT levels that were similar to that of the general population, and levels of 5HT decreased with age. Higher levels of 5HT were seen in younger participants; older persons the lowest levels. As expected, those participants with PWS who were currently using SSRI medications (n=13) had lower levels of 5HT than those with PWS who were not taking SSRIs. 5HT levels did not significantly vary across gender, genetic subtypes (paternal deletion vs. maternal uniparental disomy) nor was 5HT consistently associated with compulsive or other behavior problems. Relation with 5HT to other factors such as prosocial behaviors and weight will be further discussed.

Discussion: Behavior problems, such as compulsive features and tantrums, are hallmark features of PWS, and are often the logical symptom targets of SSRI trials. Differences in 5HT levels in individuals with PWS may explain some of the variable response to SSRI medication, although maladaptive behaviors and treatment responses are likely determined by multiple risk and protective factors. Discussion will also include possible associations between 5HT and more subtle features such as arousal or alertness, as well as issues related to 5HT supplementation in those with PWS.

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Gastrointestinal Complications Associated with Death in Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is the most common known syndromic cause of life threatening obesity, yet few studies have examined the causes of death in PWS. Early mortality and unexpected sudden deaths have been documented in PWS, but choking has not been previously reported as a cause of death. Objective: The objective of this study was to examine the contribution of choking and gastrointestinal complications leading to mortality in PWS.

Methods: In 1999, a brief survey was made available from the Prader-Willi Syndrome Association (USA) bereavement program, which documented demographic data and causes of death. Families were subsequently offered the opportunity to fill out a detailed questionnaire and release medical records. Results: Demographic information was available on 178 individuals with PWS who were deceased, and cause of death was available on 152 individuals. Updated questionnaires were completed by 50 families. Of the deceased individuals with completed questionnaires, 39% reported a history of choking. Choking was listed as the cause of death in 12/152 (7.9%). Of those who died of choking the average age at death was 24 years (range 3-52y; median 22.5y), and only 2 individuals were less than 8 years. Clinical information was available for study including food records and feeding activities around the time of death. Stomach rupture/necrosis was reported as cause of death in 4/152. Two additional patients reportedly died after episodes of suspected gastric bleeding. Of those who died due to choking 11/12 were of male gender, and all individuals with stomach rupture or gastric bleeding were of male gender.

Conclusions: Choking episodes are most common in the general population between the ages 1 and 4 years, but in our cohort the average age of death from choking was 24 years suggesting that risks associated with choking are different in the PWS population compared with the general. Potential causes of increased choking in PWS include poor oral/motor coordination, poor gag reflex, hypotonia, hyperphagia, decreased mastication and voracious feeding habits. Implementation of preventive measures including the Heimlich maneuver training for care providers, supervised meals, better food preparation and diet modification to avoid high risk choking items may decrease mortality.



Post-operative Pulmonary Edema in Two Children with Prader-Willi Syndrome

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Introduction

Prader-Willi syndrome (PWS) is a genetic disorder characterized by obesity, developmental delay, progressive behavioral problems, and hypotonia. This hypotonia, along with a fundamentally abnormal response to hypercapnea and hypoxia, predisposes these children to alveolar hypoventilation during sleep secondary to both obstructive and central apnea. Delayed recovery and other complications post-anesthesia have been reported in individuals with PWS. We report two children with PWS with pulmonary edema occurring during the first 24 hours post anesthesia.

Case 1

A 2 year old, non-obese (BMI=19, +1.5 SD) male, oxygen-dependent since birth due to respiratory failure associated with congenital tracheomalacia and on growth hormone (GH) since 9 months previously, underwent elective tonsillectomy and adenoidectomy. Four hours post-operation (post-op) he became irritable and began crying inconsolably. By eight hours post-op he was wheezing with significant intercostal retractions and increased secretions and was moved into the pediatric intensive care unit (PICU) for closer monitoring. He rapidly progressed into severe respiratory distress unresponsive to oxygen, racemic epinephrine, albuterol and intravenous corticosteroids. Arterial blood gas showed a pH of 7.16, pCO₂ of 89, and he was subsequently intubated. Chest X-ray (CXR) at that time showed signs consistent with pulmonary vascular congestion. Significant improvement in blood gases and CXR were noted after administration of dexamethasone and furosemide, with complete recovery 48 hours post-op.

Case 2

A 3 year old, non-obese (BMI=24, +1.7 SD), non-GH treated male underwent elective bilateral orchiopexy for undescended testicles. He was given general anesthesia and oxygen via face mask and was reportedly breathing spontaneously throughout the procedure. Within 30 minutes after this uncomplicated surgery he was found to have facial cyanosis, tachypnea, and stridor with progressive respiratory distress. He was quickly moved into the PICU. Blood gas at this time was within normal limits (pH of 7.4, PCO₂ of 42) and CXR demonstrated mild cardiomegaly and signs consistent with pulmonary vascular congestion. The patient responded rapidly to furosemide and complete recovery was noted within 12 hours post-op.

Discussion

Infants and children with PWS are at increased risk for the development of respiratory complications following general anesthesia and sedation from a variety of reasons including abnormal respiratory control response to hypercarbia, craniofacial configuration, obesity and hypotonia. These two cases presented illustrate that this risk is not limited to airway procedures or to children on growth hormone. Awareness and anticipation of respiratory complications in this patient population suggest that preoperative screening for sleep related breathing disorders, postoperative observation in a monitored inpatient unit and availability of respiratory support are important factors in maximizing the perioperative care to patients with PWS.



Neural Mechanisms Underlying Hyperphagia in Prader-Willi Syndrome: Genetic Subtype Differences?

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Introduction: The behavioral phenotype of Prader-Willi syndrome (PWS) includes hyperphagia; however, few studies have systematically investigated brain structure and function in PWS. The current study extends current research to investigate the neural basis of abnormal food motivation in PWS using fMRI to measure brain responses to visual food stimuli before and after eating a meal.

Methods: Based on previously developed research paradigms, fMRI was used to investigate the neural mechanisms associated with response to visual stimuli of food. Results from Holsen et al. (in press) will be summarized in which nine individuals (8 males, 1 female; 7 15q deletion, 2 maternal disomy 15) with PWS (mean age 14.7y) and nine age-matched typically developing, healthy weight control (HWC) individuals (mean age 14.4; HWC group) were scanned once before (pre-meal) and once after (post-meal) eating a standardized meal. Visual stimuli of food, animals, and blurred control images were presented for passive viewing in a counterbalanced block design during acquisition of whole-brain functional MRI data. Data from 56 studies were analyzed and reported regions of interest (ROIs) were significant at $p < 0.01$. Data were subsequently analyzed for three individuals with maternal disomy 15 and three individuals with the Type II typical 15q deletion (TII) using the same paradigm. For this set of preliminary analyses, the threshold was relaxed to $p < 0.10$.

Results: Statistical contrasts in the HWC group showed greater activation to food pictures in the *pre-meal* condition in the amygdala, OFC, medial PFC, and frontal operculum. By comparison, the PWS group exhibited greater activation to food pictures in the *post-meal* condition in the OFC, medial PFC, insula, and hippocampus. Between-group contrasts confirmed pre- and post-meal group differences in food motivation networks. When the two PWS subtype groups were analyzed, the TII group showed greater activation than the maternal disomy group in the dorsolateral prefrontal cortex and insula pre-meal, while post-meal, the maternal disomy group exhibited greater activation in the amygdala, insula, and medial PFC during the post-meal scan.

Discussion: Holsen et al. (in press) provides initial evidence for a distinct neural mechanism associated with hyperphagia in PWS and suggest that neural systems involved in food motivation are disrupted to the extent that satiation mechanisms may fail to operate normally. In fact, results in the PWS group were most atypical *after* eating rather than when they are most likely to be “hungry”. In addition, primary group effects post-meal may be attributed to the maternal disomy status, although behavioral data supports more overeating and food-related problem behavior in the deletion subtype.



Nutritional Intake by Young Children with Prader-Willi Syndrome

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Objective: There is limited knowledge of nutritional intake among children, age 0-4, with Prader Willi syndrome (PWS). The aim of this study is to describe and evaluate the intake of calories and essential micronutrients.

Material and method: We have followed a group of children with PWS for a period of two years. The first year there were 5 children in the group, age 2-3 years. The second year the group was expanded to 8 children, age 0-4 years. They constitute the total known population of children with PWS in Norway born between 2000-2003. All children in this study were diagnosed with PWS in their first months of life. Assessment of their food intake was made twice a year during the study period by structured food interview with parents, carried out by a nutritionist. All interviews are performed by the same person. Interviews have taken place during a one week family course held at Frambu (spring 2003, fall 2004) and by visits to the families homes (spring 2004). One assessment was completed using the 24-h recall method via telephone (fall 2003), with one of the parents. All interviews, except the 24-h recall, were translated in to a typical three day food consumption for each child. For the 24-h recall calculations are made only using information from the food consumption for the 24-hour period. The recorded data was encoded and analyzed using a food database and a software systems developed at the Institute of Nutrition Research, University of Oslo.

Results: We have total n=25 assessments on nutritional intake for these 8 children. We found a range of calorie intake per kg in the age group 0-1years (n=2) representing 2 children from 65 kcal/kg - 99 Kcal/kg. In the age group 1-2 years (n=3) representing 3 different children from 69 Kcal/kg - 99Kcal/kg, mean 83 Kcal/kg, from 2-3 years (n=11) representing nutritional values for 6 children 54 kcal/kg - 99 Kcal/kg, mean 72 Kcal/kg, median 70 kcal/kg. And in the age group 3-4 years (n=9) representing 5 children 48 Kcal/kg - 76, Kcal/kg, mean 65 Kcal/kg, median 68 Kcal/kg.

The mean percentage of energy from fat in group age 0-1: 40 E% (range 35-45 E%), age 1-2: 27 E% (range 23-36E%), age 2-3: 25 E% (range 15-39 E%) and age 3-4: 24 E% (range 19-32 E%). Levels of intake of essential fatty acids below the recommended 3 E%, were observed in 20 % of the assessments.

All individuals had enough protein in their diet to meet the recommended level of intake on all nutrition assessments. However when we calculated the intake of micronutrients without dietary supplementation we found the percentage of assessments where the individual did not reach recommended level of intake for micronutrients: Retinol 12 %, vitamin D 96 %, tocopherol 85 %, thiamine 40 %, riboflavin 16 %, niacin 92 %, ascorbic acid 24 %, calcium 48 %, iron 68 % and magnesium 28 %. For niacin the percentage was 8 %, when recommended level of intake was individually calculated using their caloric intake.

Conclusion: Children with PWS consume fewer calories than reference value for age and gender, even though a variation in calorie consumption is seen within the PWS group. We found that special attention needs to be drawn towards the fat-soluble vitamins; vitamin D and tocopherol and the minerals; calcium and iron and in some cases essential fatty acids. More research is required to be able to give parents and caregivers for children with PWS better nutritional counselling.



Effectiveness of the RYG Dietary System in the Nutritional Management of Young Children with Prader-Willi Syndrome

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OBJECTIVES: To determine the effectiveness of the Red, Yellow, Green (RYG) dietary education program used at North York General Hospital (NYGH) to prevent and/or control obesity in children with Prader-Willi syndrome (PWS).

PATIENTS and METHODS: A convenience sample of clinic patients with Prader-Willi syndrome ($n=30$, mean age, 5.04 ± 4.5 at first clinic visit (FCV)) were studied by an observational, retrospective chart review. Entry criteria included a confirmed genetic diagnosis of PWS and a minimum of 3 clinic visits. Subjects were divided into two groups: a younger group (YG) who were less than six years of age at FCV ($n=18$, mean age, 1.9 ± 2.0), and an older group (OG) who were six years of age or older at FCV ($n=12$, mean age, 9.7 ± 2.6). Patient charts were reviewed for age, height, and weight, and percent ideal body weight (% IBW) was calculated using the Tanner and Whitehouse (1975) growth charts for males and females.

RESULTS: No significant difference was found in mean % IBW from FCV (139.7 % IBW) to most recent visit (MRV) (136.3% IBW) for the sample as a whole ($n=30$; $p < .5$). As well, no significant difference was found in the mean % IBW in the YG from FCV (110.9 % IBW) to MRV (115.7 % IBW; $p < .5$). There was, however a significant difference in the mean %IBW of the OG at FCV (183.1% IBW) and MRV (167.2% IBW) with a mean decrease of 15.9%; $p < .05$. When the YG and OG were compared, it was noted that the mean age of the YG at MRV (3.96 ± 2.9) was statistically different from the mean age of the OG at FCV (9.7 ± 2.6 ; $p < .001$). However the two groups were compared as the YG mean age was approaching the FCV mean age of the OG. Notably, there was also a significant difference between the %IBW of the YG at MRV (115.7% IBW) and the OG at FCV (183.1% IBW; $p < .001$).

CONCLUSION: This study indicates that the RYG program utilized at NYGH is successful in not only reducing the degree of obesity in older children with PWS, but may also be successful in delaying the onset of obesity among younger children with PWS. This is evident by the fact that while the OG entered the clinic with a mean %IBW of 183.1%, (the typical progression of obesity seen in PWS) the younger children at their MRV (mean age approaching OG at FCV) were effectively kept under 120%IBW and therefore not clinically obese. Hence, the progression to obesity that is expected with PWS has not happened to our younger group of children. The results of this study are promising, but further data over an extended period of time would be necessary to determine whether the RYG program is effective in preventing the onset of obesity in children/people with PWS.



Final Adult Height in Children with Prader-Willi Syndrome after Completing Growth Hormone Treatment

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Background: Short stature is characteristic of children with Prader-Willi syndrome (PWS). The mean final adult height (AH) for an individual with PWS is approximately 2 standard deviation (SD) below the relevant population mean. While previous studies have demonstrated acceleration of linear height velocity with GH treatment, the long-term benefit on AH has not been reported.

Objectives and Methods: We reviewed the records of 21 children (aged 8.3 ± 2.7 years, 13 boys and 8 girls) with PWS and confirmed GH deficiency that attained AH after receiving human GH treatment (0.25 ± 0.06 mg/kg/week) for a period of 7.9 ± 1.7 years. A group of 39 non-GH treated adults (13 males and 26 females) with matched initial height SD score (SDS) at age 6.8 ± 1.3 years was used as control.

Results: For the GH-treated patients their mean initial and AH-SDS was -1.9 ± 1.7 and -0.3 ± 1.2 respectively ($p < 0.0001$), whereas the mean initial and AH-SDS for the non-GH treated was -1.9 ± 1.3 and -3.1 ± 1 respectively ($p < 0.0001$). Scoliosis was seen in 43% and 39% in the GH and non-GH treated adult individuals respectively. None of GH treated individuals developed diabetes mellitus type 2.

Conclusions: GH treatment improves AH to within normal limits without significant adverse events. Non-GH treated children with PWS attain AH below their growth potential. Further long-term studies are necessary to correlate AH with anabolic effects as well as related morbidity and mortality of GH in children with PWS.



Polisomnography Study in Prepubertal Children with Prader-Willi Syndrome: Prior and During Growth Hormone Treatment

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Sleep-disordered breathing (SDB) is a common problem in PWS patients and hypotonia and obesity are considered important risk factors. Adenotonsillar hypertrophy is the leading cause of obstructive sleep apneas (OSA) in childhood.

Many studies have documented several benefits of growth hormone therapy (GHT) in children with Prader-Willi syndrome. Nevertheless, some children with PWS are at risk of sudden death during the first months of GHT, probably due to oropharyngeal soft tissue growth that increases the incidence of OSA. In our study we evaluated the possible role of GHT on OSA and sleep-disordered breathing in prepubertal children with Prader-Willi syndrome.

We studied 14 children (10 male and 4 female, aged 5.3 ± 3.4 years, BMI: 22.3 ± 6.9) with genetically confirmed PWS, before and after (25.9 ± 16.1 months) the onset of GHT (dosage: 0.23 ± 0.04 mg/kg/w).

All patients underwent an overnight cardio-respiratory sleep study (polysomnography) using a SomnoStar PT2 (Sensor Medics) (7 channel recorder) both in basal condition and during GH therapy. We measured the following respiratory parameters: MOAHI (number of mixed/obstructive sleep apnea-hypopnea/hr); CAI (number of central apneas/hr); mean SaO₂% (oxygen saturation during sleep); SaO₂<90 (% TST: total sleep time with SaO₂ lower than 90%); ODI (number of desaturations $\geq 4\%$ of baseline value per hour of sleep). OSA was defined as an MOAHI ≥ 1 event/hr.

There weren't any significant differences between respiratory parameters performed before and during growth hormone therapy: MOAHI (1.84 ± 2.03 vs 1.59 ± 1.99 , $p=0.71$); CAI (0.65 ± 0.48 vs 0.66 ± 0.53 , $p=0.95$); mean SaO₂% (96.57 ± 0.65 vs 96.64 ± 1.22 , $p=0.65$); SaO₂<90 (%TST) (0.33 ± 0.49 vs 0.89 ± 2.09 , $p=0.37$); ODI (1.75 ± 1.79 vs 2.31 ± 3.92 , $p=0.55$).

These results are confirmed also when we consider PWS subjects with (n.10) and without (n.4) obesity. Six children (43 %) at baseline and 8 during GHT (57%) showed OSA ($p=0.4386$).

In conclusion, GHT does not seem to promote the development of OSA or a significant worsening of respiratory parameters in children with PWS. These results are confirmed also when we consider PWS subjects with and without obesity. Nevertheless, our findings refer to a relatively small population and further studies are needed. We suggest individually undertaking a polysomnography study and an otorhinolaryngological examination during GHT in all PWS patients.



Phenotyping of Adult Mice with a Deletion of the Prader-Willi Syndrome Imprinting Center

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Introduction: Prader-Willi syndrome (PWS) is caused by the loss of imprinted gene expression on chr 15q11-q13. The PWS-imprinting center (PWS-IC) is a positive regulatory element required for bidirectional activation of a number of paternally expressed genes in this region. Until recently, all mouse models of PWS were post-natally lethal, precluding them from studies of late onset phenotypes, such as hyperphagia and obesity. We have established a strain-specific survival model of PWS through paternally inherited deletion of the PWS-IC on an FVB-B6 F1 background.

Methods: PWS-IC^{+/-del} mice were compared to wild-type littermates. Mice were separated and housed in groups by sex and genotype. Body weight and food intake were measured twice weekly from weaning at 3 weeks until 22 weeks, and then weekly until 48 weeks. At around 50 weeks, after a 6 hour fast, organs were removed for histology, and blood taken for measurement of plasma insulin, leptin, ghrelin and IGF-1. Adult mice had *in vivo* brain (11 Tesla) and *ex vivo* whole body (4.7 Tesla) magnetic resonance imaging (MRI) to examine brain structure and body composition.

Results and Discussion: Body weights for both male and female PWS-IC^{+/-del} mice remained reduced compared to wild-type mice throughout the post-weaning period into adulthood (n = 10-16 per group), without evidence for any reversal of pre-weaning failure-to-thrive. Compared to wild-type littermates, the body weight of male PWS-IC^{+/-del} mice was 54% at 3 weeks and 52% at 48 weeks (mean ± SEM: 29.5 ± 0.8 vs. 55.8 ± 1.8g, P<0.001), and of female PWS-IC^{+/-del} mice was 55% at 3 weeks and 63% at 48 weeks (21.5 ± 0.6 vs. 39.2 ± 3.1g, P<0.001). Female PWS-IC^{+/-del} mice had delayed vaginal opening compared to wild-type females (39 vs. 27 days, n = 7, P<0.001).

Both sexes of adult PWS-IC^{+/-del} mice had a large reduction in inguinal adipose tissue weight (by 45-83% at 50 weeks of age), and fat content was visibly reduced on MRI. PWS-IC^{+/-del} mice had significantly reduced plasma insulin, leptin and IGF-1 levels compared to wild-type littermates, but there were no significant differences in plasma ghrelin (n = 6-12 per group). Post-weaning food intake (corrected for body weight) was in fact reduced in male PWS-IC^{+/-del} mice up to 14 weeks of age, and in female PWS-IC^{+/-del} mice up to the 48 week point, compared to wild-type littermates.

At 50 weeks of age, PWS-IC^{+/-del} mice had reduced body length (by 9-10%, P<0.001), reduced brain, liver, stomach, kidney and testes weights (by 10-54%, P<0.02) compared to wild-type mice. No histological abnormalities in the liver, stomach, duodenum, pancreas, kidney, salivary gland, ovary or testis were seen in PWS-IC^{+/-del} mice on hematoxylin and eosin staining. Spermatogenesis and folliculogenesis appeared normal. No gross brain defects were seen in adult PWS-IC^{+/-del} mice using MRI except for mild ventriculomegaly (n = 4 of each sex, aged 54 to 77 weeks), nor with thionine or myelin staining (n = 7 of each sex, aged 48 to 109 weeks).

Conclusion: This is the first detailed description of adult PWS-IC^{+/-del} mice. As in humans with PWS, these mice have loss of expression of similarly imprinted genes, and display pre-weaning failure-to-thrive and growth retardation which persist into adulthood. However, by contrast to the human condition, these mice do not develop hyperphagia, obesity or infertility. In fact, adult PWS-IC^{+/-del} mice are resistant to the development of age-related adiposity. Current studies are addressing abnormalities in detailed brain morphology or behavior, as they are observed in humans with PWS.



Long-acting Octreotide (Sandostatin LAR[®]) Decreases Ghrelin Levels in Subjects with Prader-Willi Syndrome

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There is presently no effective treatment for the excessive weight gain in Prader-Willi syndrome (PWS). Concentrations of ghrelin (an orexigenic hormone mainly produced by the stomach) are markedly elevated in children and adults with PWS, suggesting that ghrelin may be partly responsible for the increased appetite seen in PWS. Previous studies have confirmed that treatment with short-acting octreotide (a somatostatin analogue) suppresses ghrelin levels in patients with PWS.

Hypothesis: Long-acting octreotide (Sandostatin LAR[®], L-Oct) treatment decreases circulating ghrelin levels and the compulsive behavior towards food in subjects with PWS.

Objective: To investigate the effect of L-Oct on plasma concentrations of acylated (“active”) and total (“active” + “inactive”) ghrelin concentrations during an oral glucose tolerance test (OGTT) and on parental/subject attitude towards food in subjects with PWS.

Methods: Nine subjects, with a confirmed genetic diagnosis of PWS, participate in this randomized, double blind, cross-over trial. Subjects receive either L-Oct (30 mg) or saline (placebo) intramuscularly every 4 weeks for 16 weeks (phase 1). After a washout period of 24 weeks, subjects are switched over to the other treatment modality (phase 2). An OGTT (with measurement of active and total ghrelin, glucose and insulin levels) is performed at the beginning and at the end of each phase, as well as measurement of glycosylated hemoglobin (A1C) and ultrasound of the gallbladder (for safety reasons).

Results: In the 4 subjects (ages 12-17 years), who have completed the study thus far, L-Oct causes a 45-65 % decrease in active ghrelin and a 33-50 % decrease in total ghrelin concentrations on fasting and during the OGTT test. These preliminary results also show that the ghrelin decrease was associated with a decrease in compulsive behavior but was not associated with a significant change in body mass index (BMI) Z-score before and after treatment.

L-Oct treatment was associated with a decrease in insulin levels without significant change in glycosylated hemoglobin (A1C). Two of the 4 patients developed asymptomatic gallstones during the course of the study which improved when therapy was discontinued.

Conclusion: Long term L-Oct treatment causes a marked and sustained decrease in ghrelin concentrations in subjects with PWS. The effect of these changes on behavior, appetite and weight remain to be evaluated once all subjects have completed the study.



Food Security: A Tool for Teaching and for Behavioral Analysis in Prader-Willi Syndrome

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Day to day management of PWS is a challenge for parents who often express a need for a succinct means of expressing to schools and other caretakers what the child with PWS needs.

Food Security is a term the authors have given to a patient-centered paradigm for simultaneous weight and behavior management. The central feature of this paradigm is: how does the patient *perceive* his environment with respect to food; it does not focus on weight control alone. As a result, behavior problems are analyzed not only from a functional view point (i.e., what response does the behavior achieve) but from a perceptual view point (i.e., what was the person expecting). This analysis informs clinical decision making and results in a clinically successful means of managing behavior. The concept of Food Security is presented using drawings and a repeated slogan to train persons managing children and adults with PWS. Food Security consists of “No Doubt, No Hope, No Disappointment”. Food security is a means of “managing expectations”.

“No doubt” addresses the person’s need always to know what and when he will be eating. The framework for “no doubt” is the menu and the schedule. Adjunctive measures included reminders and reassurances, especially when the menu or schedule must be modified. “No doubt” improves behavior by reducing anxiety.

“No hope” addresses the element of “chance” related to food acquisition which is highly rewarding. It refers to the measures needed to prevent the child or adult from acquiring extra food with an emphasis on the person’s *perception* of whether or not he should “get his hopes up”.

“No disappointment” is related to unfulfilled expectations based upon chance occurrences; unfulfilled expectations are a major source of unexplained behavior problems in PWS.

Food Security does not come automatically with controlled access to food (locks etc.). Behavior problems can often be traced to lack of food security in persons with PWS with or without well-controlled weight. Lack of food security is a major unrecognized source of stress for persons with PWS.

Analysis of the child’s entire environment (all locations, at all times) using the food security paradigm is the *first step* in assessing difficulties with behavior. Obvious or subtle, any lapses in food security should be addressed before considering medications for managing behavior problems.



Evaluation of a Computer-Assisted Technique for Measuring Injury Severity

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We evaluated the utility of an inexpensive, efficient, and noninvasive technique for measuring the severity of tissue damage produced by self-injurious behavior (SIB). The technique involved computerized measurement of wound surface area (WSA) based on digital photographs.

In Study 1, we compared photographic measurement to a more commonly used procedure, transparency measurement, in estimating the WSA of 20 wound models that varied in shape and size. Results showed that both methods were reliable and that there was a high degree of correspondence between the two sets of measures. In Study 2, we compared photographic WSA measures to direct-observation measures of behavior in documenting changes over time in the SIB exhibited by a woman diagnosed with Prader-Willi syndrome.

Results showed that increases and decreases in observed SIB during baseline and treatment conditions corresponded with changes in WSA measures, indicating that the computer-assisted photographic technique may be useful as a corroborative measure or as a primary dependent variable when direct observation of SIB is not feasible.



Prevalence and Functions of Self-injurious Behavior in Prader-Willi Syndrome

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University of Florida

It has been noted that individuals with Prader-Willi syndrome (PWS) often engage in self-injurious behavior. The most commonly reported form of SIB is skin picking (Dykens & Shah, 2003). In the current study, we established the prevalence, frequency, and severity of SIB in individuals with PWS by way of a structured questionnaire sent to all providers registered with the National Prader-Willi Syndrome Association of the USA. Second, we conducted experimental analyses to identify the functional characteristics of SIB in a sample of PWS individuals. Results are discussed in terms of form and function of SIB in individuals with PWS, as well as the implications these findings have for treatment development.



Determinants of Food Preference in Individuals with Prader-Willi Syndrome

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University of Florida

Previous research has shown that several characteristics of reinforcers and their delivery, including quality, magnitude, delay, etc., may affect preference. We examined the influence of those characteristics on food preferences in individuals with Prader-Willi syndrome (PWS), a genetic disorder in which excessive food consumption is a major problem behavior. Preference assessments were conducted initially to identify foods that were of “high quality” (highly preferred). Next, baseline sessions were conducted to examine behavioral sensitivity to reinforcer quality, magnitude, and delay. Two response options were available; one response was associated with the optimum value of a characteristic; the second response was associated with a lower value of a characteristic (e.g., one response resulted in immediate reinforcer delivery; the other response resulted in delayed reinforcer delivery).

The relative influence of each characteristic on responding was evaluated during a final phase, in which the values of two characteristics were simultaneously manipulated, and response allocation was measured. Results are discussed in terms of implications for the assessment and treatment of dietary management and food-related problem behaviors.



Descriptive and Experimental Research on Exercise in Prader-Willi Syndrome

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Abstract: Physical exercise is an important therapeutic intervention in the management of life-threatening obesity, a prominent clinical feature of Prader-Willi syndrome (PWS). However, very few studies have been conducted on exercise behavior in individuals with PWS.

We conducted a descriptive study initially to identify types of physical activity (e.g., sitting, laying down, walking, running, cleaning) exhibited by individuals with and without PWS throughout their daily routines. We subsequently evaluated the effects of a reinforcement contingency to increase the frequency of exercise by individuals with PWS. Of particular interest was an assessment of the utility of conjugate reinforcement schedules as maintenance procedures. Access to preferred activities (music, television) was available under conjugate or more traditional ratio schedules, and both performance and preference were examined. Results of this comparison are discussed with respect to the use of non-food interventions to increase the occurrence of exercise for individuals diagnosed with PWS.



Cognitive and Achievement Abilities in Individuals with Prader-Willi Syndrome and Early-Onset Morbid Obesity

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Introduction: The literature on cognitive functioning and achievement in Prader-Willi syndrome (PWS) is somewhat diverse (Whittington et al., 2004). Individuals with PWS have been described as showing mild to moderate mental retardation and multiple severe learning disabilities with relative weaknesses in short-term memory and mathematical skills and relative strengths in reading skills and on tasks that assess attention to visual detail, visual-motor coordination, perceptual planning, and spatial organization. Dykens and colleagues (1992) found that the mean level of achievement for individuals with PWS was approximately 2 years above their mean mental processing composite age score. However, Whittington et al. (2004) reported that levels of achievement were lower than what was predicted based on IQ among individuals with PWS.

The purpose of the present study was to determine the extent to which individuals with PWS and early-onset morbid obesity (EMO) of unknown etiology reached the attainments predicted by their IQ and to investigate factors that might be associated with any discrepancy between their level of achievement and IQ. Both the PWS and EMO groups were compared to their normal control siblings.

Methods: Cognitive and achievement testing was done with PWS, EMO, and normal control sibling participants using the Woodcock Johnson Tests of Cognitive Abilities, Third Edition (WJIII-Cog) and the Woodcock Johnson Tests of Achievement, Third Edition (WJIII-TA; Woodcock et al., 2001). Extensive genetic testing was conducted on both the PWS and EMO subjects.

Results and Discussion: The overall achievement score (TIA) for the PWS and EMO groups is slightly higher than their IQ, while the TIA for the normal control siblings is slightly lower than their IQ. The average TIA (67.26) for the PWS group is 3.29 points higher than their average IQ (63.98), the average TIA (78.06) for the EMO group is 0.06 points higher than their average IQ (77.99), and the average TIA (107.03) for the Control group is 2.71 points lower than their average IQ (109.74). While all three groups scored higher on the Incomplete Words subtest of the WJIII-Cog than their IQ, the PWS and EMO groups scored significantly higher on that subtest compared to their IQ score, while the control group did not. There is a 26.1 point difference between the average Incomplete Words subtest score (90.09) for the PWS group and their average IQ (63.98), a 23.7 point difference between the average Incomplete Words subtest score (101.65) for the EMO group and their average IQ (77.99), and a 2.86 point difference between the average Incomplete Words subtest score (112.60) for the Control group and their average IQ (109.74). Both the PWS and EMO groups scored the highest on the Incomplete Words subtest compared to all of the other subtest and cluster scores.



Eco-Environmental Interventions in Prader-Willi Syndrome

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For most parents and providers, locking the refrigerator and securing food access are *the* major environmental interventions for the management of PWS. But food is not the only determinant of stress in the life of an individual with PWS. Their unique dependency on the environment makes them super sensitive to environmental cues. When an individual's capacity to predict what will happen next in the environment is challenged, a stress response occurs. For individuals with PWS, this inevitably results in behavior problems. The stress sensitivity and poor adaptability associated with the syndrome mandate that an *ecological approach* is necessary. Ecology pertains to the study of the relationship between an organism in its environment. Analogously, the goodness of fit between a person with PWS and their environment will predict their level of functioning. Therefore, as the predictability of the daily plan increases, the anxiety related to uncertainty diminishes, and a higher level of functioning is realized.

The principles of food security (no doubt, no chance, no disappointment) can be applied to every aspect of a person's life in order to decrease stress. When one knows what is expected to happen next, even if it is an unpleasant event, anxiety actually decreases. The easiest way to achieve this predictability is through a linear schedule.

The TRAIN program (**T**ool to **R**educe **A**nxiety, **I**nsecurity & **N**oncompliance) is an eco-environmental management system for handling the cognitive, behavioral and social communication deficits associated with PWS. It runs on a linear schedule of activities with predictable stops for fuel, rest and recreation/leisure. The orderly progression through the activities of the day is called *flow*; transitions are effectively managed through the generous use of non-contingent reinforcement by the caregiver. Added motivation is achieved with scheduled *contingent* reinforcement.

The sequence of the day is pre-set by the caregiver; it is posted and reviewed with the person(s) with PWS. If noncompliant behavior or a "shut down" interferes with the flow of the day, the schedule stops until the individual's behavior is extinguished and the flow of the schedule is restored. Depending on the amount of time expended during the interruption, the outcome (goals) of the scheduled activities may be subject to change, but the expectation of compliance with the activity(s) does not diminish. If the behavioral disturbance is externalized (acting out), then predetermined consequences to that behavior are applied. If the incident requires a "time out," adaptations to the times allotted by the schedule will follow, but the *sequence* of activities to be completed does not change.

Thus, the person with PWS knows with certainty what will happen next; they know that their needs will be met; and their challenges to the set plan will be addressed allowing the consistent implementation of the daily schedule. This approach to reducing stress minimizes many of the classic PWS traits and behaviors such as dawdling, tantrums, argumentativeness, shut downs, perseveration, daytime sleepiness, and excessive/ repetitive behaviors.



Impact of the Red-Yellow-Green Diet on Weight of Subjects with Prader-Willi Syndrome

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The purpose of this study is to describe the impact of the Red-Yellow-Green System (RYG) for Weight Control (The Children's Institute, Pittsburgh, PA) on the weight and behavior of subjects with Prader Willi syndrome (PWS). We studied a convenient sample of 9 PWS patients aged 2.7-38 y. Anthropometric measures were taken before and after treatment using the RYG system for at least 1 year, and medical complications and their progress were reported. A Likert scale questionnaire and individual interviews were administered to the main caregivers, to determine changes in behavior and food-related problems, since the implementation of the RYG dietary system.

The results demonstrate a mean decrease in % Ideal Body Weight (%IBW) of 47.1% (range: -14 – 140, $P = 0.027$) over the course of the dietary management application. Behavioral improvement of 77% (SD: 11%) was noted. Reported improvements in medical status included discontinuation of diabetes medications, and cessation of sleep apnea. Caregivers reported the RYG system was “Easy to follow, with clear guidelines.” Caregivers also commented that their children stopped arguing and debating about how much food they were allowed, corroborating the behavioural improvements noted in the questionnaire.

The RYG system for weight management is an effective tool for caregivers and patients with PWS to manage weight and reduce food-related negative behaviors reducing the potentially fatal health risks associated with obesity.



Clinical, Genetic and Molecular Study of 89 Chilean Patients with Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is a neurogenetic disease characterized by neonatal hypotonia, retarded mental and motor development, hypogonadism, hyperphagia, morbid obesity and dysmorphic facial features. It has an incidence of 1:12.000-15.000 newborns and is caused by abnormalities in genes located in 15q11q13. PWS is one of the most frequent genetic disorders and microdeletion syndromes. It is also the most common cause of obesity from genetic origin and it was the first disease in which imprinting and uniparental disomy were recognized as cause of genetic disorders. According to literature, 70-75% of PWS cases are due to 15q11q13 deletions, 20-25% to uniparental disomy and 1% to mutations in the imprinting center.

Aim: To analyze the clinical, genetic and molecular features of patients with PWS diagnosed in Chile.

Patients and methods: Retrospective review of 89 patients (41 males and 48 females) with PWS diagnosed at the Molecular Cytogenetics Laboratory at the Institute of Nutrition and Food Technology (INTA), University of Chile.

Results: Only 60 of the 89 patients had been study with fluorescence in situ hybridization (FISH). Between them, 38 (63%) patients had a deletion and 22 (37%) patients did not have a deletion. In 29 patients, with abnormal methylation test, FISH analysis was not performed, therefore the presence of deletion is unknown. Six of the 23 patients with normal FISH, were studied for UDP, between them two presented heterodisomy, and 4 isodisomy. Forty patients have chromosomal analysis, in 10 of them the deletion was detected cytogenetically and three have other chromosomal abnormality (46,XX, t(13;14), del15q11-13; 46,XX,t(13;15), 46,XY/47,XY+mar). The clinical score performed in 55 patients was 8 points for patients younger than 3 years (n=11) and 11.5 points for patients older than 3 years (n=34); for patients aged 12 months or less, the clinical score was 7 points. Mean clinical score was 11 points for patients with deletion and 10 points for patients without deletion.

Conclusions: Most Chilean patients with PWS have a deletion although the frequency of deletion is lower than the frequency described by literature. The phenotype depends on age and the clinical score demonstrated to be useful for Chilean patients with PWS



Bioenteric Intra-gastric Balloon for Treatment of Obesity in Prader-Willi Syndrome

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Obesity in Prader-Willi syndrome (PWS) is often progressive and severe (BMI > 40) and is almost always resistant to dietary, pharmacological and behavioral treatment. A drastic reduction in body weight is necessary in these subjects in order to reduce the possibility of cardio-respiratory and metabolic complications and to improve the prognosis “quoad vitam”. The insertion of a silicon balloon (Bioenteric Intra-gastric Balloon - BIB) in the gastric cavity represents an effective alternative to the more complex and invasive bariatric surgery (bilio-pancreatic diversion, gastric bypass etc). Adult patients with morbid obesity have been treated with BIB with encouraging results. There is no data in literature about the use of BIB in patients with PWS. In our study we evaluated the use and effectiveness of BIB in patients with PWS.

Eleven patients (8 females, 3 males), with genetically confirmed PWS and morbid obesity (BMI: 49.9 ± 8.1), aged from 8.1 to 30.1 years (18.6 ± 7.4 yrs.) were enrolled. In all patients a BIB was endoscopically positioned and removed after 6 months (0.7 ± 0.09 years). An auxological, clinical and nutritional evaluation was performed every 2 months, for 6 months. Body Composition by DEXA (Dual Energy X-ray Absorbiometry) was evaluated before inserting and immediately after removal of the BIB. In 4 cases the treatment was repeated 5 months after removal.

One female patient (28.5 years and BMI 60.2) died 1 month after positioning the BIB due to gastric perforation. In another female (26.2 years and BMI 57.6) the BIB was removed after 25 days due to abdominal pain and vomiting. The remaining 9 patients showed a significant reduction in body weight and BMI ($48.3 \pm 8.2 - 41.5 \pm 9.8$; $p=0.0002$), without any side effects and with temporary reduction of appetite. In all patients the evaluation of body composition showed a significant reduction of fat tissue (52.9 ± 1.7 vs 49.1 ± 5.3 %; $p=0.017$) (5222 ± 1266 vs 45785 ± 14070 gr; $p=0.003$) after removal of the BIB. After this treatment no significant modification in the lean mass ($p=0.106$), nor in the body mineral density (BMD) was found.

In conclusion, with this treatment (only performed in a few PWS patients, so far) it is possible to obtain a control of body weight in PWS patients with morbid obesity, after failure of other non- invasive pharmacological therapies. However, in all cases a careful clinical follow-up is necessary in order to avoid complications, some of them because of the continuous introduction of food.



The PWS Personality: What is “Within Normal Limits” for PWS?

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Among individuals with PWS, some temperamental and behavioral characteristics are so commonly present that they are considered to be part of the **behavioral phenotype**. These features are the “background noise” when considering psychiatric diagnoses in patients with PWS; changes in the severity of these symptoms are clues to changes in mental status. *All of the characteristics of this phenotype can become exaggerated with **stress**.*

The behavioral phenotype of PWS defines the PWS PERSONALITY; it corresponds to the DSM IV Axis I Diagnosis of *Personality Change Due to a Medical Condition (310.1)*. There are five domains of psychiatric/behavioral symptoms in the PWS PERSONALITY: food related behaviors, oppositional defiant behaviors, cognitive rigidity/inflexibility, anxiety/insecurity, and skin picking.

PWS PERSONALITY: FIVE DOMAINS of PSYCHIATRIC/BEHAVIORAL SYMPTOMS in PWS

<p>I. Food related behaviors (in order of ascending severity):</p> <ul style="list-style-type: none"> • overeating of typical food • eating atypical food (frozen, raw, spoiled food or pet food) • sneaking food in the home • night time foraging in the home • arguing or manipulating to get food • tantrumming to get food • opportunistic food theft (shoplifting from a store or stealing food from school or work) • planned food foraging expeditions in the neighborhood or community • nonconfrontational, invasive food access (breaking locks on cabinets, refrigerator or freezer) • confrontational food access (using verbal or physical threats or actual aggression to access food) 	<p>II. Oppositional defiant behaviors:</p> <ul style="list-style-type: none"> • noncompliance • argumentativeness • tantrums/shut downs • manipulation • lying/confabulation <hr/> <p>III. Anxiety/insecurity:</p> <ul style="list-style-type: none"> • stress sensitivity • somatic complaints • stimulus seeking • constant need for reassurance • collecting and hoarding • affective lability
<p>IV. Cognitive rigidity/inflexibility:</p> <ul style="list-style-type: none"> • perseveration, "sticky thinking" • inability to tolerate uncertainty • difficulty with transitions or changes • selective interests (jig saw puzzles, word searches) • impaired judgment • single mindedness: difficulty taking multiple view points • egocentrism 	<p>V. Skin picking:</p> <ul style="list-style-type: none"> • occurs commonly, but not universally • as a habit behavior, opportunistic typography: <ul style="list-style-type: none"> • arms, face and scalp • nasal septum • pulling out toe nails • peeling skin from the soles of the feet • trichotillomania • as an intense, severe, reactive behavior in the presence of ongoing stress: <ul style="list-style-type: none"> • gouging, self mutilation • targets rectum and/or vagina

An understanding of the psychiatric/behavioral characteristics of these five domains will assist the clinician in the diagnosis of psychiatric disorders co-morbid with the PWS Personality.



An Assessment of Motor Function and Development of Young Children with Prader-Willi Syndrome

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Background

In the last decade, Norwegian paediatricians have acquired more knowledge about PWS; most children born with the syndrome are now diagnosed in the neonatal period. This study was planned after a family-course at Frambu National Centre for Rare Disorders in the spring of 2002. At initiation, nine children born in 2000-2003 with genetically proven PWS were included (age range 1-5 year). Two of the children in this sample died.

Purpose

The aim of this study was to better understand the change in motor development and function over time in 7 children with PWS

Method

The children were assessed 3 to 5 times over a period of 3 years. The assessment tools that were used were the Pediatric Evaluation of Disability Inventory (PEDI) and the Motor part of Bayley Scales of Infant Development. The parents were also asked to report their children's ages at which they achieved selected motor milestones.

Results

The results show that all of the children had significantly delayed performance in all measured areas. The psychomotor development index score of the Motor scale of Bayley were less than 50 at all assessments. The PEDI scores indicate that for most children, the degree of delay in development increases with age as compared to children without disability.

Conclusion

PWS is associated with a significant delay in motor development. The development and evaluation of effective intervention programs should be a research priority. Larger studies of children with PWS are needed to confirm the findings of this pilot study.



Quantitative Microsphere Hybridization (QMH) Array Analysis of the Prader-Willi and Angelman Syndrome Regions

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We previously developed a novel quantitative microsphere suspension hybridization (QMH) assay for the high-throughput determination of genomic copy number by the direct hybridization of single copy (sc) probes to genomic patient DNA followed by flow cytometric analysis (Newkirk et al. 2006). Here we describe the first clinical application of this assay examining the Prader-Willi (PWS) and Angelman syndrome (AS) region on chromosome 15q11-13. We designed 17 sc test probes (80 nucleotides each) spanning ~3.2Mb of the PWS/AS region and a disomic reference probe (HOXB1, chromosome 17q21), which were each conjugated to one of ten spectrally distinct polystyrene microsphere levels. All probes were hybridized to biotin-labeled genomic patient DNA in multiplex QMH reactions, and hybridization was detected using phycoerythrin-labeled streptavidin and analyzed by dual-laser flow cytometry. Copy number differences were distinguished by comparing mean fluorescence intensities (MFI) of the test probes to the reference probe. The MFI ratios for deleted loci were 0.58 ± 0.072 (n=80) as compared to the MFI ratios for normal loci, 0.98 ± 0.075 (n=186). A multiplex QMH assay could readily distinguish class I from class II deletions, as well as small (~4.3kb) imprinting center (IC) deletions, with no overlap in MFI value as compared to normal loci. Using this diagnostic QMH assay, the precise deleted genomic interval could be ascertained in all PWS/AS patients examined in the present study.



Growth Hormone (GH) Improves Lean Body Mass (LBM) without Glucose Impairment in Diverse Growth Hormone Deficient (GHD) Prader-Willi Syndrome (PWS) Adults: Results from the U.S. Multi-Center Trial

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INTRODUCTION Prader-Willi syndrome (PWS) is an important complex genetic disorder of hyperphagia, GHD and hypogonadotropic hypogonadism, with marked phenotypic variation. GH has well documented benefits (1-6) and is FDA approved in PWS children. Its use in PWS adults has been limited by an absence of documented benefits and risks as determined by larger multi-site studies.

METHODS We conducted an open-label, multi-center trial of GH in diverse GHD adults (mean age 30.7 years) with PWS genotype to evaluate efficacy, safety, and dose optimization. The study included lean and obese adults with a wide range of cognitive skills, behavioral abnormalities, GH exposure, sex steroid replacement, and living arrangements. GH was initiated at 0.2mg/day and increased monthly by 0.2mg to a maximum dose of 1.0 mg/day, as tolerated. The primary study endpoints were DXA-determined LBM and %fat. Glucose tolerance tests with insulin levels and echocardiograms were performed; covariates were assessed with non-parametric statistics and paired t-tests (SPSS 13.0). Of 40 patients screened at 4 sites, 30 completed 6-12 months of GH (Figure 1).

RESULTS Significant improvements were observed in both LBM and % fat. LBM increased from 42.84 ((se) 2.28) to 45.45 (2.31) kg ($p \leq .0001$) and %fat decreased from 42.84(1.12) to 39.95(1.34)% ($p = .025$). Changes were independent of age, sex, initial BMI, sex steroid use, and social setting and were noted at 6 months. HbA1C and FBS did not vary significantly during the study: 5.5(.4) to 5.6(.3)% and 79(3) to 82(3)mg/dL. Fasting 4.5(.5) and AUC-insulin, 55.3(6.7) increased to 7.0(1.1) and 81.3(11.3) μ U/ml (p 's .015, .010) but remained in normal range in 22 tested (non-diabetic) participants. Worsened ankle edema (5 patients) was the most serious treatment emergent adverse event; one patient withdrew from the study because of myalgias. IGF-1 SDS improved from -1.9(.2) to -0.2(.3) at a median final daily dose of 0.6mg (range .4-1.0 mg).

CONCLUSIONS GH treatment improved LBM and % fat, without glucose impairment and was well tolerated in a multi-center study of diverse PWS genotype adults.

REFERENCES

1. Lindgren AC, Hagenas L, Muller J *et al.* Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favourably. *Acta Paediatr* 1998; 87(1):28-31.
2. Eiholzer U, Gisin R, Weinmann C *et al.* Treatment with human growth hormone in patients with Prader-Labhart-Willi syndrome reduces body fat and increases muscle mass and physical performance. *Eur J Pediatr* 1998; 157(5):368-377.
3. Davies PS, Evans S, Broomhead S *et al.* Effect of growth hormone on height, weight, and body composition in Prader -Willi syndrome. *Arch Dis Child* 1998; 78(5):474-476.
4. Carrel AL, Myers SE, Whitman BY, Allen DB. Growth hormone improves body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome: A controlled study. *J Pediatr* 1999; 134(2):215-221.
5. Carrel AL, Myers SE, Whitman BY, Allen DB. Benefits of Long-Term GH Therapy in Prader-Willi Syndrome: A 4-Year Study. *J Clin Endocrinol Metab* 2002; 87(4):1581-85.
6. Haqq AM, Stadler DD, Jackson RH, Rosenfeld RG, Purnell JQ, LaFranchi SH. Effects of Growth Hormone on Pulmonary Function, Sleep Quality, Behavior, Cognition, Growth Velocity, Body Composition, and Resting Energy Expenditure in Prader-Willi Syndrome. *J Clin Endocrinol Metab* 2003; 88(5):2206.