

USA  
**PRADER-WILLI SYNDROME ASSOCIATION**  
*Still hungry for a cure.*

## Scientific Abstract Submissions

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# The Medical/Nutrition Review

# Perinatal Complications in a U.S. Population with Prader-Willi Syndrome

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**Introduction:** Prader-Willi syndrome (PWS) is characterized by prenatal-onset hypotonia, infantile poor feeding and growth, childhood-onset obesity, short stature, facial dysmorphic features, psychomotor delay, and a distinct behavioral phenotype. Deficiency of imprinted genes in 15q11-q13 cause this disorder.

**Method:** We assessed 64 patients over three days for multiple historical and clinical parameters, including detailed history of the pregnancy, labor and delivery obtained from parental report and available record review. This is a fairly unique cohort of subjects with PWS as the recruitment was aimed at equal numbers with UPD 15 and deletion 15q11-13 in order to study genotype-phenotype correlations and data was gathered with investigators blind to the genotype. There were 34 males and 30 females, ages 3-38 years, 34 with deletion on chromosome 15q (53.1%) and 30 (46.9%) with UPD.

**Results/Discussion:** From the results of the 63 patients with perinatal information, 16 were born prematurely (delivery prior to 37 weeks) (25.6 %). 32 were term (38 to 42 weeks) (47.6%) and 15 were post-dates (>41 weeks) (23.8%). 21 required pictocin induction (36.2%) and 27 had caesarean section (45.6%). Only seven had placental pathology review: Of these, three were normal, one meconium stained, one “abnormal” (no records), one small, and one had calcifications. Fetal movements were reported as subjectively decreased in 52/62 (86.6%). Mean age at diagnosis was significantly delayed in the UPD group (4.7 years) compared with the deletion group (3.2 years) ( $p < 0.017$ ). It is unclear whether this was due to the limitations of the testing that was available at that time or if it is due to a milder phenotype leading to later diagnosis.

**Conclusion:** Although the data collected may have been affected by recall bias, there is clearly a high rate of pregnancy and delivery complications, especially:

1. Very high caesarean section rate
2. Decreased fetal movements
3. High induction of labor rate.

Similar results were previously reported in Europe including France (Dudley et al 2007) and the UK (Whittington et al 2008). This had not been reported previously in a US population. The cause of these perinatal difficulties is not known, but may relate to hypotonia and/or hormonal differences. These perinatal problems might play a role in causing characteristic early postnatal difficulties (lethargy, poor suck) and subsequent developmental and behavioral manifestations.

# Studies of the Frequency of Assisted Reproductive Technology Births and Twinning in Prader-Willi Syndrome

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**Introduction/Background:** Prader-Willi Syndrome (PWS) is an imprinting disorder characterized by affected individuals with typical facial features and major cognitive, behavioral, neurologic, endocrine, and psychiatric issues. There are three main causes of PWS, ultimately resulting in the complete absence of the paternal chromosome 15q11.2-q13, also called the PWS region. These three genetic causes are deletion of the paternal PWS region, maternal uniparental disomy of chromosome 15, and imprinting center defects of the PWS region. Recent studies have suggested an increased risk of imprinting disorders, such as Beckwith-Wiedemann Syndrome and Angelman Syndrome, in children born after assisted reproductive technologies (ART). However, the relationship between PWS and ART has not been extensively researched. The purpose of this study was to determine the association between ART and PWS by evaluating the frequencies of ART-births in three distinct groups of individuals with PWS. The frequency of ART-births in the United States was also determined to make comparisons to the frequencies found in this study.

**Methods:** Data on PWS patients were collected from 1)General health and family surveys administered by the Prader-Willi Syndrome Association of the USA (PWSA(USA)), 2)Rare Diseases Clinical Research Network (RDCRN) Natural history PWS and morbid obesity clinical protocol (IRB protocol 2007-5605), and 3)Review of medical records from patients seen by the Genetics Division at UCI Medical Center.

**Results/Discussion:** The frequency of ART-births in the PWSA(USA), UCIMC, and RDCRN populations was 2.9% (50/1688), 2.0% (1/50), and 0.9% (1/108), respectively. The mean frequency of ART-births of the three distinct PWS populations is 2.8%. There is no increase above the estimated ART-birth frequency of 2% for the U.S (Shiota and Yamada 2005). Interestingly of the total PWS patients in this study, 2.4% (45/1688) were reported to be co-twins. This frequency is higher than the U.S. twin frequency of 1.6% ( $p= 0.006$ ) (Hoekstra, Zhao *et al.* 2008). Ten of these 45 PWS patients reported to be co-twins were born after ART procedures.

**Conclusion:** Multiple studies have concluded that the effects of ART procedures may be restricted to imprinting disorders in which the maternal allele is hypomethylated or in which an imprinting defect accounts for a significant proportion of affected cases. Two other studies did not suggest an association between PWS and ART, also an imprinting center defect in an ART-born individual with PWS has not been previously reported. The results from this study are consistent with previous results, and no increased frequency of ART-births in a PWS study population above the general population was found. Also, an increased frequency of twinning in this PWS study population was reported which was not due to ART procedures. At this time, the mechanisms causing this association have not been fully established but could be related to maternal age (Aston 2008). The possible relationship between ART, twinning, and PWS is an area of future research.

# **Food Preferences in Prader-Willi Syndrome: Feeding Practices, Family Food Environment and Implications for Weight Management**

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**Introduction/Background:** Research into the food preferences of children with Prader-Willi Syndrome [PWS] has focused on behavioral and homeostatic investigations within the child. The purpose of this study was to assess the food preferences of children with PWS in relation to the family's feeding practices and food environment control.

**Methods:** Information regarding the study was posted on the PWS Association USA website. Interested families were asked to contact the investigator and were mailed a questionnaire. 51 families participated in the survey, which asked about the PWS child's likes and dislikes of foods offered from different food groups, home food environment control, and eating habits of the family and the individual with PWS. There was an even age distribution among children with PWS ranging from three to seventeen-years-old, with the mean age of eight.

**Results/Discussion:** Individuals with PWS in this study were given foods from all the food groups with no elimination of any particular food group. They were offered a variety of foods within each food group as well as were allowed to eat snack type and sweet foods. Although a good majority (86%) of the children with PWS were on a weight management diet, only 34% of the families were consulting a nutritionist. Some families (39%) attempted to individualize the diet of the PWS child for weight management by including more or less of certain foods. Second helpings of foods were allowed to 63% of the individuals with PWS. Vegetables and fruits were the most likely foods to be offered as second helpings followed by starches, meats, dairy, sweets and snacks. While it was reported that individuals with PWS generally accepted foods from all food groups, they clearly preferred some foods over others. Families reported that 73-82% of the foods offered from the snack type, starchy, and sweet foods were "liked" by individuals with PWS, followed by fruits (70%), meats (66%), dairy foods (65%) and vegetables (60%). No specific dislikes were reported for any of the food categories except for vegetables. The child's food preferences for each category were reported at above 90% for ages 3-5 with a steady decrease in preference across categories with an increase in age. A 26% decrease in liking for vegetables was reported between ages 3-5 years and 11-17 years. 33% of the families locked the food cabinets to prevent food stealing. Sweets and snack type foods were reported "most likely" to be stolen; and vegetables and meats reported "less likely" to be stolen by individuals with PWS. This behavior further offers evidence of the food preferences of individuals with PWS.

**Conclusion:** Children with PWS are offered a variety of foods from all food groups. Although they accept most foods well at a younger age, they develop food preferences as they get older. Notable among these, is a reduced liking for vegetables which can affect compliance and success with the weight loss diet. An awareness of this behavior by families of young children with PWS may help in developing feeding practices and home food environment that is conducive to weight management.

## Central Adrenal Insufficiency in Individuals with Prader-Willi Syndrome

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**Introduction:** A high prevalence of central adrenal insufficiency (CAI) in individuals with Prader-Willi Syndrome (PWS) has recently been suggested (de Lind van Wijngaarden et al, JCEM 2008; 93:1649) using the metyrapone stimulation test, which is not available in the USA, and is no longer a commonly used test for CAI. Therefore, we investigated the prevalence of CAI in individuals with PWS using the glucagon stimulation test (GST) – a more widely available, established dynamic test to assess the hypothalamic-pituitary-adrenal axis.

**Methods:** We performed a GST for CAI in 49 individuals with genetically confirmed PWS (ages 18 months – 35 years; 31 Deletion, 17 UPD, 1 Imprinting Defect). Glucagon was given as an intramuscular injection (0.03 mg/kg body weight to a maximum dose of 1 mg). Serum cortisol levels were measured at 8 AM (before glucagon injection), and 120 minutes and 150 minutes after the injection to identify the peak cortisol level. CAI was defined as a peak serum cortisol level < 18.1 mcg/dL (500 nmol/L). None of those tested were taking estrogen replacement or exogenous steroids (oral, spray, inhalers, or creams). Forty-six subjects were on GH replacement.

**Results:** Twenty-three subjects (47%) had a maximum cortisol <18.1 mcg/dL (500 nmol/L), consistent with CAI. The four subjects with the lowest maximum cortisol (6.4-10.0 mcg/dL) all described symptoms of adrenal insufficiency with daytime fatigue, decreased energy level, and frequent pre-meal hypoglycemia which normalized with physiologic doses of oral hydrocortisone. Four out of 22 individuals who had serum glucose measurements available had hypoglycemia during the GST with blood glucose levels <50 mg/dL (2.8 mmol/L), requiring intravenous glucose rescue. One of these four individuals had a peak cortisol of 10 mcg/dL, while the rest were all >18 mcg/dL.

**Conclusion:** Forty-seven percent of the individuals we tested had evidence of CAI demonstrated by a different stimulation test than de Lind van Wijngaarden et al (2008) used. Unlike other children with CAI, individuals with PWS had the possibility of severe hypoglycemia during the GST, which did not seem related to severe CAI. The gold-standard test for CAI, the insulin tolerance test (ITT), may be difficult to perform in children and can cause dangerously low blood glucose levels, requiring an inpatient setting, whereas the GST can be done in a clinic setting with supervision of the patient. However the glucagon stimulation test is well recognized to be a less reliable test of ACTH reserve than the gold-standard ITT.

Further studies are warranted before a definitive estimate of the prevalence of CAI in PWS can be determined. Nonetheless, our data confirm the findings of the Dutch group that a substantial number of individuals with PWS have CAI. Therefore, we recommend that every patient with PWS undergo a dynamic stimulation test to evaluate for the presence of CAI, as some patients may benefit from physiologic doses of hydrocortisone, while others may only require stress-doses of hydrocortisone during times of illness.

# The Genetics Review

# Profound Pancreatic $\alpha$ - and $\beta$ -cell Defects Arise in Transgenic Prader-Willi Syndrome (TgPWS) Mice from Loss of the PWS-Imprinted Gene Cluster

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**Introduction/Background:** Pancreatic islet hormones are essential for metabolic homeostasis and their deregulation contributes to illnesses such as diabetes. A transgenic-deletion mouse model of Prader-Willi syndrome (TgPWS) recapitulates the most common genetic basis (an ~ 6 Mb deletion) and similar to the human condition, results in severe neonatal failure to thrive. We previously found that TgPWS animals have markedly low levels of plasma insulin and glucagon in fetal and neonatal life prior to and following onset of progressive hypoglycemia. This study aimed to identify deficits in pancreatic islet development and function underlying this unusual phenotype.

**Methods:** Hormonal (plasma and pancreatic), morphological (islet architecture, replication and apoptosis), gene expression (QRT-PCR and microarray), and functional (insulin secretion) studies of TgPWS and control littermates were performed in fetal and early neonatal life.

**Results/Discussion:** Immunohistochemical analysis of the endocrine pancreas demonstrated disrupted morphology of TgPWS islets with reduced  $\alpha$ - and  $\beta$ -cell mass, arising from an increase in apoptosis. In accordance with islet cell mass, major hormone contents are moderately decreased in TgPWS pancreas. However, mRNA levels for genes encoding key islet hormones (eg., Ins2, Ins1, Gcg, Sst, Ppy) and many secretory/exocytosis gene products (eg., Sgna1, Iapp, Tmem27, and others) are up-regulated in TgPWS pancreas either due to a compensatory response to deficient plasma hormone levels or a primary effect of a deleted gene(s). Further, insulin secretion is markedly impaired in TgPWS  $\beta$ -cells both in vitro (by decreased basal and glucose-stimulated insulin release from isolated islets) and in vivo (by analyzing the time-course of pancreatic expression of an Insulin-C-Timer fluorescent reporter). As the insulin/C-peptide ratio in TgPWS pancreatic extracts was unchanged this indicates normal processing of proinsulin and suggests that the secretory block is distal to hormone processing in the secretory granule.

**Conclusions:** This study indicates that the imprinted, paternally expressed gene cluster in mouse chromosome 7C, which is orthologous to the PWS domain in human chromosome 15q11.2, regulates islet endocrine cell development, survival, and function. Additionally, this mouse model reveals coordinate gene regulation for all hormones and many secretory/exocytosis polypeptides produced by the endocrine pancreas. In PWS, pancreatic insufficiency is seen with reduced insulin levels relative to the degree of obesity, reduced pancreatic polypeptide levels in response to nutrients, and with either insulin-dependent or -independent diabetes described in ~ 25% of individuals. Given our observations and the well known growth hormone and gonadotropin deficiencies in PWS thought to be due to deficient pituitary secretion and hypothalamic GnRH release (in which Necdin has been implicated), respectively, we hypothesize that similar neuroendocrine mechanisms underlie abnormal pancreatic, pituitary and hypothalamic hormone and peptide secretion in PWS. Understanding the molecular and genetic pathways for hormone release in PWS is essential to develop successful strategies for the treatment of PWS and other metabolic disorders such as diabetes and obesity.

## **Role of snoRNA MBII-85 in a mouse model of Prader-Willi syndrome**

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**Introduction/Background:** Prader-Willi syndrome (PWS) is a complex neurodevelopmental disorder caused by paternal deficiency of 15q11-q13. Recently, several pieces of evidence from human and PWS mouse models suggested that the C/D box snoRNAs HBII-85/MBII-85 may be responsible for most of the phenotype of PWS in humans and neonatal lethality in mice. This group of snoRNAs is unique and of great interest since they exist as multiple copies and their expression is brain specific and imprinted. Furthermore, they are known as “orphan snoRNAs” as they have no predicted targets in any rRNA, tRNA or snRNA species. Therefore, their biological functions and the molecular mechanisms contributing to Prader-Willi syndrome remain unknown.

**Methods:** To study genes targeted by MBII-85 and the downstream pathways, we utilized a mouse model for PWS that has the paternal inheritance of a large deletion from Snrpn to Ube3a. Transcriptome from the hypothalamus of PWS and wild-type (WT) mice were compared using Affymetrix exon array.

**Results/Discussion:** Among 18,713 transcript clusters analyzed, 89 genes were found to be significantly different between PWS and WT mice (false detection rate < 0.01, fold change > 1.5). Interestingly, 84 genes were up-regulated in PWS mice vs. WT mice; only 5 genes were down-regulated with 3 of the 5 located within deleted region. Gene ontology analysis showed that the most enriched clusters are extracellular proteins, transport proteins, and proteins involved in hemostasis, retinoic acid and hormone metabolism, structure development, enzyme regulation, and intercellular junctions.

**Conclusion:** The dramatic expression difference between PWS and WT mice indicates a likely repressive role of MBII-85 in regulating downstream targets. To further confirm the results and identify direct targets of MBII-85, expression profiling by mRNA-seq is under progress.

## **Necdin is Required for Normal Cell Migration and Neuronal Activation**

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**Background:** Two PWS candidate genes, namely necdin and MAGEL2, are members of the MAGE protein family implicated in cellular proliferation, differentiation, and apoptosis. Necdin is normally expressed in the developing nervous system, in muscle, and in other cells of mesenchymal origin. Overexpression of necdin promotes neurite outgrowth and differentiation, whereas loss of necdin in mice causes defects in the hindbrain resulting in central respiratory deficiency, and defects in the differentiation of the dorsal root ganglia. Loss of necdin also causes delayed migration of the sympathetic neurons of the superior cervical ganglia, which normally migrate rostrally during development. The observation that loss of necdin affects both migration and axonal outgrowth suggests defects in the intracellular cytoskeletal processes common to cellular migration and neurite outgrowth.

**Methods:** Cellular reorientation and directed migration in mouse embryonic fibroblasts and human fibroblasts were examined in tissue culture. These experiments were performed in control cells and cells with constitutive absence of necdin, either derived from necdin knockout mice, or from individuals with PWS. Activation of neurons by neurotrophic factors and migration of muscle cell precursors were also examined.

**Results:** Cells deficient in necdin are delayed in the initiation of cell migration and response to pro-migratory stimuli compared to control cells. Changes in cell shape during migration require coordinated movement of the actin cytoskeleton, and are regulated by cytoplasmic signaling pathways that include Rho GTPases. We identified altered regulation of these pro-migratory signaling pathways in necdin-deficient cells.

**Conclusion:** Neuronal migration is critical to normal brain development, and defects in migration are implicated in many genetic forms of developmental delay. The regulated migration of other cell types is also essential to prenatal development. Our results demonstrate dysregulation of cytoskeletal rearrangement consequent to loss of necdin, and provide the first evidence for an intracellular signaling defect in murine and human cellular models of Prader-Willi Syndrome.

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## **Molecular Function of snoRNAs Missing in Prader-Willi Syndrome**

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**Introduction:** The loss of expression of small nuclear RNAs (snoRNAs) encoded in the Prader-Willi critical region on chromosome 15q11-q13 is the major molecular cause for the Prader-Willi syndrome (PWS). We recently showed that one of the missing snoRNAs, HBII-52, changes splice site selection. HBII-52 is a C/D box small nucleolar RNA (snoRNA). SnoRNAs have been mainly implicated in 2'O-methylation of non-messenger RNAs.

**Results/Discussion:** Using a bioinformatic approach, we identified more than 200 exons that could interact with the antisense box of its mouse orthologue MBII-52. Experimentally, we identified five pre-mRNAs ((DPM2, TAF1, RALGPS, PB1, and CRHR1) containing alternative exons that are regulated by MBII-52, but not the related MBII-85 snoRNA. The dependency of alternative exon in these pre-mRNAs on MBII-52 was observed in three experimental systems: (i) the splicing of the endogenous genes changes after ectopically expressing HBII-52; (ii) reporter gene constructs alter their splicing pattern after snoRNA expression and (iii) differences in splicing are seen when brain RNA from wild-type and MBII-52 knock out animals is compared. Including G-U base pairing, always 15 of the 18 possible nucleotides exhibit base complementarity between the antisense box and the RNA targets, and six positions of the antisense box showed always complementarity to their targets.

We investigated the expression of a single MBII-52 copy by RNase protection. We detected four additional shorter RNAs. The formation of these shorter RNAs depends on the presence of either the C or D box, which suggests that they originate from the full-length MBII-52 snoRNA through an additional processing step. To identify associated proteins, we employed an oligonucleotide bearing sequence complementarity to the antisense box of MBII-52. Using this affinity approach, we identified several hnRNPs associated with the isolated RNAs, but could not detect any proteins associated with canonical C/D box snoRNAs.

**Conclusion:** In summary, our data indicate that MBII-52 is processed into smaller RNAs that associate with hnRNPs and regulates alternative splicing of numerous pre-mRNAs. The processing of snoRNAs into smaller RNAs represents a novel mechanism to generate regulatory RNAs. It also indicates that the loss of miRNA-like snoRNA derivatives could be the cause for PWS, which offers therapeutic approaches for the disease.

# Differential Gene Expression in an Imprinting Center Deletion Mouse Model of Prader-Willi Syndrome by Whole Genome Microarray Analysis

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**Background:** Prader-Willi syndrome (PWS) is a genetic disorder caused by deficiency of imprinted gene expression from the paternal chromosome 15q11-15q13 and clinically characterized by neonatal hypotonia, short stature, mental retardation, hypogonadism and hyperphagia leading to morbid obesity and diabetes. Unlike maternal-specific expression of *UBE3A* which is spatially restricted to brain in the human and mouse, paternally expressed genes maintain the imprint in most or all tissues. Several additional paternally expressed imprinted genes that encode proteins have been identified as a cluster of intronless genes on 15q11-q13 and mouse 7C, including two MAGE protein family members: *NDN* and *MAGEL2*, and *MKRN3* (makorin RING finger protein gene family). Three families (HBII-13, HBII-85/MBII-85 and HBII-52/MBII-52) of C/D box snoRNA genes have been mapped between *SNURF-SNRPN* and *UBE3A*. We have compared the gene expression microarray data between the brain and muscle tissues of the PWS IC del mice versus wild type littermates.

**Methods:** We used microarray analysis to assess gene expression levels in the paternally derived imprinting center (IC) deletion (PWS IC deletion) 6 month old mouse tissue. Quantitative RT-PCR was used to confirm the gene expression levels. Enzyme activities of mitochondrial oxidative phosphorylation (OXPHOS) complexes in the brain, heart, liver and muscle were assessed. Electron microscopy of the PWS IC del mice was employed to observe the ultrastructures in the heart and the muscle when compared to their wild-type littermates.

**Results:** We found 95 and 66 mitochondrial genes differentially expressed in PWS muscle and brain, respectively at  $p < 0.05$  and of the differentially gene expressions at  $p < 0.01$ , 18 genes overlapped in skeletal muscle and brain. All the known genes from the PWS critical region were downregulated and *Ube3a* was significantly upregulated. We further investigated 5 significant genes (3 upregulated and 2 downregulated) in PWS muscle and 2 genes in PWS brain, specifically we found that actin  $\alpha$  cardiac muscle1 (*Actc1*), and  $Ca^{2+}$  sensing receptor (*Casr*) were significantly up-regulated and genes of TBC domain family, member1 (*Tbc1d1*) and metallophosphoesterase 1 (*Mmp1*) were down-regulated in the PWS IC del mice muscle and mitochondrial ribosomal protein L 15 (*Mrp15*) was up-regulated in the brain when compared to their control littermates, several of these genes being potential candidates for mitochondria related energy metabolism. Quantitative RT-PCR for *Actc1*, *Casr*, *Tbc1d1*, *Mmp1* in the PWS muscles and for *Mrp15* in the PWS brain when comparing with their littermates validated our microarray findings. We were not able to replicate the previous microarray findings (Bittel et al. 2007) in one day old mice of elevated *Mcsr*, a melanocortin receptor known to be involved in thermoregulation or of *Pomc* with which it interacts. Mitochondrial enzyme activities of the cardiac complexes II+III were significantly upregulated in the PWS IC del mice when compared to the wild-type mice whereas the enzyme levels in muscle and brain were unchanged. Also noted was increased mitochondrial density by light and electron microscopy in the heart and myopathic changes in skeletal muscle when compared to their wild-type littermates. These studies indicate that genes involved in mitochondria and energy metabolism in muscle, are upregulated in PWS.

**Conclusions:** We found differential gene expressions in an imprinting center deletion mouse model of Prader Willi syndrome by whole genome microarray analysis. These studies suggest that genes involved in mitochondrial energy metabolism in muscle and brain are upregulated, consistent with our mitochondrial enzyme and histology studies and may contribute to the pathophysiology of PWS mice. Future research in this area may lead to improved future therapeutic interventions for PWS patients.

# The Behavior/Neuropsychiatry Review

## Neural Response to Food Cues during Hunger and Satiation

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**Introduction:** In typically developing individuals, fMRI studies on food-related neural circuitry suggest that the hypothalamus, insula, limbic areas, and several regions of the frontal cortex are involved in the control of appetite. Food stimuli have been found to elicit greater response in the amygdala, hippocampal formation, and orbitofrontal cortex during hunger than after satiation. In addition, the motivational salience of food has also been shown to elicit differential activation of neural systems with high-calorie foods activating regions known to be involved in reward-related behavior. How all of this food-related neural circuitry is involved in the eating disorder in people with PWS has not been well established. However, studies to date show evidence of an abnormally delayed satiety response indicated by increased activation in the prefrontal, limbic and paralimbic regions to visual presentations of food after ingestion of a meal or oral glucose load. And, recent research by our group indicates neural activation in PWS is modulated by the motivational salience of food during a fasting state (Dimitropoulos & Schultz, 2008). Whether the saliency of food type continues to be of importance after attempted satiation is not known. The present project examines the brain's response to food during hunger and after attempted satiation for people with PWS and healthy and overweight comparison groups. The goal is to discover if overeating in PWS is mediated not only by abnormal satiety response but also by abnormally heightened responses to rewarding food during hunger and, more importantly, after eating.

**Method:** To date, 16 lean adults and 6 individuals with PWS completed an fMRI block-design task with 3 conditions: high-calorie food (HI), low-calorie food (LO), and nonfood objects during fasting and fed states. In addition to T1-weighted and high-resolution 3D images, functional images were acquired on a 4.0T MR scanner (TR=1950, TE=22ms, flip angle=90). Group data sets were examined using GLM analysis contrasting the experimental conditions.

**Results/Discussion:** Findings with lean adults indicated greater activation to HI vs. LO during fasting in the orbitofrontal cortex (OFC), superior frontal gyrus, and amygdala ( $p < .01$  corrected). Direct comparison of motivational salience (HI vs. LO) by hunger state (fasting vs. satiation) indicates greater activation in the OFC and amygdala to high-calorie foods during hunger. Preliminary results for individuals with PWS indicate greater activation in the ventral striatum, superior frontal gyrus (SFG), OFC, fusiform gyrus, and dorsolateral prefrontal cortex (DLPFC;  $p < .001$  uncorrected) in response to food vs. nonfood objects during fasting state. In addition, high-calorie foods yield greater activation in the insula, hippocampus, inferior frontal gyrus, and the DLPFC ( $p < .001$  uncorrected). After ingestion, individuals with PWS continue to activate the DLPFC and fusiform gyrus to foods and show deactivation in the parahippocampal gyrus ( $p < .001$  uncorrected). The small sample sizes prevent direct group comparisons at this time. Nonetheless, these preliminary results indicate increased activation in areas involved in food-regulation, taste information processing and food reward among individuals with PWS. Based on these findings, greater response in reward circuitry and areas involved in food regulation is expected among individuals with PWS in comparison to HWC during fasting state. Moreover, continued activation of these circuits after meal ingestion is anticipated for those with PWS. These preliminary results give insight into the effect of rewarding foods on the underlying neural mechanisms of food regulation during different hunger states.

## Nicotine Dependence and Cessation among Adults with Prader-Willi Syndrome: a Case Series

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**Background:** PWS is typically known for food-related behaviors, but individuals with the disorder also display a variety of excessive and repetitive behaviors related to non-nutritive substances, e.g., lotions, shampoo, collections, etc. The precise neural mechanism underpinning the regulatory dysfunction in appetitive and satiety circuits has yet to be illuminated. Some researchers have suggested that addiction paradigms can be used to understand the excessive and repetitive behaviors associated with the syndrome. Cigarette smoking is one of those behaviors that can be seen as both an addiction and a compulsion. The incidence of cigarette smoking among individuals with PWS in the USA is not known, but the prevalence is very high in European countries where access to tobacco products is less restricted.

**Methods:** This study reviews a naturalistic case series of adults with PWS who met criteria for nicotine dependence. It reviews the course of their disorder as well as the pathways and obstacles to smoking cessation. Results: Nicotine dependence is diagnosed in individuals who engage in daily cigarette smoking for at least several weeks. The nicotine dependent individuals with PWS in our case series were introduced to smoking in their 20's when they made the transition to adult services and saw other adults smoking. About half of them had a family history of smoking or other addictions. Only one of them articulated cravings, but some individuals displayed cigarette foraging behaviors. The only factor limiting the total amount of smoking per day was the number of cigarettes to which they had access; this number related to both monetary funds and opportunity. No one traded cigarettes for food, but some offered cigarettes to others. The use of conventional smoking cessation methods such as nicotine replacement systems or nicotine receptor agonists to facilitate withdrawal was ineffective. In fact, many individuals continued to smoke against medical advice while receiving the nicotine patch or gum. External regulation of access was essential to treat or manage nicotine dependence. In our collective experience with PWS, only a few of the smokers were able to self regulate to the extent that they saved some part of their cigarette allotment for later use. The clinical manifestations of physiological withdrawal have not been observed in medical settings. Anecdotal reports from caretakers and parents across several nations affirm that external control of cigarette access is essential to manage smoking behavior in persons with PWS.

**Discussion:** Nicotine access and use appears to be one of those public health issues that is viewed both as a nuisance and as a personal right, even in the presence of compromised health. Whereas food-related behaviors in PWS are generally agreed to require environmental control, other excessive and repetitive behaviors are not. Nicotine dependence among individuals with PWS is both a health risk for the person as well as their housemates through second and third hand smoke exposure. The apparent failure of traditional smoking cessation methods, and the relative ease with which nicotine dependent individuals respond to scheduled access to cigarettes as well as environmental controls over where smoking behavior occurs suggest that nicotine dependence in PWS should be managed like a food-related behavior.

**Conclusion:** Traditional smoking cessation methods failed to achieve the desired results in this case series. External controls to manage nicotine use were effective and should become part of the support plan for each person with PWS who is a smoker in the same way that food-related behaviors are a focus of restricted access and limited intake.

# **Stress and Coping in Parents of Children with Prader-Willi Syndrome: Initial Assessment of a Structured Plan of Care within the Utah PWS Clinic and Care Coordination Project**

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**Introduction/Background:** In 2008 the Utah Department of Health launched the Utah PWS Clinic and Care Coordination Project to promote the welfare of individuals, families, and communities affected by PWS. The project provides quarterly multispecialty medical clinics, an interdisciplinary care manager for follow-up care and counseling, as well as various educational interventions for parents and providers. An actionable, structured “Plan of Care” was developed from published guidelines including the PWS Association (PWSA) consensus statements. The Plan of Care covers the areas of health care, education, community integration, family dynamics, legal support, financial security, and support services. The Care Manager introduces the Plan of Care to each family individually. We hypothesized that the introduction of this Plan of Care would decrease stress levels and increase utilization of coping behaviors in parents. The specific objectives of this study are: (1) to evaluate levels of stress in a cohort of parents of children with Prader-Willi syndrome; (2) to describe the usefulness of specific coping behaviors; and (3) to assess the impact of introducing the formally structured, interdisciplinary Plan of Care on familial stress and coping behaviors. An independent study will assess whether long-term benefit accrues from compliance to the Plan of Care.

**Methods:** A total of 57 parents, mothers and/or fathers recruited through the Utah Prader-Willi Syndrome Association answered the 14-item Perceived Stress Scale (PSS-14) and the Coping Health Inventory for Parents (CHIP), as well as demographic surveys. A sub-cohort of these parents (N=38) answered the PSS and CHIP surveys 4-6 month after the introduction of the interdisciplinary plan of care by the care manager.

**Results/Discussion:** Consistent with other studies, parents of children with PWS in this study experienced higher mean levels of stress ([PSS-14]  $28.9 \pm 8.7$ ) compared to the general population (19.6). The level of perceived stress was associated with the child’s age, the child’s residential setting (group home placement vs. family home), the parent’s age, and the number of children living at home. The most useful coping pattern for the parents was found to be “maintaining family integration, cooperation and an optimistic definition of the situation.” Coping behaviors related to “maintaining social support, self-esteem, and psychological stability” and “understanding the medical situation through communication with other parents and consultation with medical staff” were associated with parental gender. Parental stress decreased after the Plan of Care introduction ([PSS-14]  $26.2 \pm 8.1$ ,  $P=0.035$ ). Coping behaviors related to “maintaining family integration” increased after the Plan of Care introduction ( $P=0.042$ ). Differences between women and men persisted after the intervention.

**Conclusion:** The introduction of a structured Plan of Care decreased stress and increased coping behaviors related to family stability for parents of children with PWS. Attention to respite care and communication support for parents of children with PWS may facilitate utilization of coping mechanisms.

## Cortisol and Stress in Prader-Willi Syndrome

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**Introduction:** Cortisol, a physiological marker of stress, is becoming a robust measure of overall health and coping. Single measures of levels of cortisol in PWS are generally in the normal range (Burman, 2001; Butler & Garg, 2009), but cortisol is best measured over time. It is unknown whether persons with PWS show a typical diurnal pattern with sharp increase in the time after waking and a brisk cortisol response to mealtimes (post-prandial). A normal cortisol pattern in typical adults is related to healthy measures of BMI, insulin/glucose ratio, and triglycerides. Given the abnormalities in these levels in PWS, plus a host of behavioral disturbances related to lower BMI in PWS, we hypothesize that these may be related to disruptions in the L-HPA response to stress. In contrast to patterns in the general population, others find increased problems, primarily internalizing symptoms, in adolescents or adults with PWS and lower BMI's. Individuals with PWS and lower BMI's have increased repetitive, compulsive behaviors, and are more nervous, tense, tearful, distressed, upset, agitated, cognitively disorganized, and high strung (Dykens & Cassidy, 1995; Dykens, 2004; Hartley et al., 2005; Whitman & Accardo, 1987).

**Methods:** 47 participants with PWS (males =24) ranged in age from 4-48 years of age ( $M$  age =17.20,  $M$  BMI=31.71). Genetic subtypes of PWS in this sample were: Type I Deletion=11, Type II Deletion=12, Maternal UPD=14, Translocation/Imp Defect=2, Deletion not characterized =6. Participants with PWS and their caregiver completed a number of assessments and questionnaires focusing on adaptive/maladaptive behaviors, cognitive functioning, and hyperphagia. Salivary samples were collected 6 times (Sarstedt kits) during a typical 8 hour day for each participant with PWS and analyzed for cortisol in the VUMC Endocrinology Core lab.

**Results:** In general, those with PWS, who have a normal BMI, have higher morning cortisol levels and a normal decline than those with PWS with obesity appear to have blunted, flat cortisol levels throughout the day. In addition, Hyperphagia severity scale was negatively correlated with cortisol change scores with ( $r$ 's ranging from -.43 to -.62,  $p < .05$  to .008). Cortisol change score was also negatively correlated with lower anxiety summary score on Achenbach Child Behavior Checklist ( $r$ 's ranging from -.42 to -.55,  $p < .02$  to .004).

**Discussion:** Diurnal cortisol levels in PWS may be related to BMI, hyperphagic severity and anxiety symptoms. Those with PWS who show a normal cortisol curve have lower BMI, lower hyperphagia severity and report fewer anxiety symptoms. Salivary cortisol sampling may be a novel way to relate physiological drive for food in PWS with associated stress and increases in BMI. Further analyses and discussion will include gender, PWS genetic subtype and post-prandial cortisol secretion and subtype to determine significant factors in PWS.