

9th IPOSO CONFERENCE

hosted by FPWR Canada



SCIENTIFIC ABSTRACT BOOKLET

Toronto, Ontario, Canada
JULY 20th – 24th, 2016

SCIENTIFIC CONFERENCE SPEAKER ABSTRACTS

THURSDAY, JULY 21

11:00 AM	SESSION 1: PWS through the Lifespan Dr. Jennifer Miller	Dominion Ballroom
1:40 PM	SESSION 2: Mechanisms for Molecular and Cellular Dysfunction in PWS Session Chair: Rachel Wevrick, Ph.D	Dominion Ballroom
1:40 PM	SESSION 3: Mental Health and Challenging Behaviour Session Chair: Professor Tony Holland	Churchill Room

FRIDAY, JULY 22

8:00 AM	SESSION 4: Advances and Bottlenecks in Novel Therapies and Clinical Trials Session Chair: Theresa V. Strong Ph.D.	Dominion Ballroom
1:00 PM	SESSION 5: Preclinical Models of PWS Session Chair: Sebastien G. Bouret, Ph.D.	Dominion Ballroom
1:00 PM	SESSION 6: Lifelong Monitoring and Multidisciplinary Clinics Session Chair: Jessica Bohonowych, Ph.D.	Churchill Room

SPEAKER ABSTRACTS

SPEAKER ABSTRACTS

1.1 - The role of *MAGEL2* and interacting partners in neurobehavioral phenotypes

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Background: The maternally imprinted, paternally expressed *MAGEL2* gene (affected only when inherited from the father, unaffected when inherited from the mother) located in the Prader-Willi critical region 15q11-13 was recently reported as the first single gene responsible for Prader-Willi syndrome (PWS) and Prader-Willi-like phenotypes. Preliminary studies identified four individuals with truncating mutations in *MAGEL2*, expressing hypotonia, feeding difficulties, and intellectual disability. A diagnosis of autism spectrum disorder (ASD) was also described. While these patients resemble PWS, they are also phenotypically distinct from classic PWS. As such, the syndrome caused by *MAGEL2* point mutations has been renamed from Prader-Willi-like syndrome to Schaaf-Yang syndrome (SHFYNG) (OMIM #615547).

Methods and Results, I: Since the time of this first publication, we have identified 40 new individuals with Schaaf-Yang syndrome from 14 families, including one family with a total of 15 affected individuals across two generations. All cases harbor truncating mutations of *MAGEL2*, and nucleotides c.1990-1996 arise as a mutational hotspot, with 10 individuals and one fetus harboring a c.1996dupC (p.Q666fs) mutation and two fetuses harboring a c.1996delC (p.Q666fs). The phenotypic spectrum of Schaaf-Yang syndrome ranges from fetal akinesia to individuals with hypotonia, feeding difficulties requiring assisted feeding, hypogonadism, contractures, DD/ID, ASD, and a characteristic behavior profile, among others.

Conclusions, I: These findings provide strong evidence for the pathogenicity of truncating mutations of the paternal allele of *MAGEL2*.

Methods and Results, II: To elucidate the role of *MAGEL2* interactors, we investigated the functional network of *MAGEL2*, and identified the *USP7* protein to be a direct interactor of *MAGEL2*, critically important for endosomal protein recycling. Database queries identified seven patients with *de novo* heterozygous loss-of-function variants, including six contiguous, non-synonymous deletions including *USP7*, and one likely pathogenic nonsense variant of *USP7*. The patients' clinical phenotypes involved developmental delay/intellectual disability, ASD, seizures, hypogonadism, hypotonia, and aggressive behavior.

Conclusions, II: These findings suggest a molecular and clinical spectrum of disorders, which highlights the important role of *MAGEL2* and *USP7* in human neurodevelopment.

Summary: Prader-Willi syndrome, Schaaf-Yang syndrome, and *USP7*-associated disorder represent a molecular and clinical spectrum of human neurodevelopmental diseases. Our findings lead us to hypothesize that other genes encoding for proteins involved in WASH ubiquitination and endosomal actin assembly are candidate genes for related neurodevelopmental disorders. As technology progresses and more affected individuals are being identified, the clinical and molecular signatures of the various disorders will become increasingly clear.

SPEAKER ABSTRACTS

1.2 - A novel cost-effective screening test for abnormal *FMRI/SNRPN* methylation in symptomatic children and newborns.

David E Godler,^{1#} Xin Li,¹ Yoshimi Inaba,¹ Quang M Bui,² David Francis, MS,¹ David J Amor,^{1,3} Justine Elliot,³ Tiffany Wotton,⁴ Jonathan Cohen,⁵ Carolyn Rogers,⁶ Mike Field,⁶ Howard R Slater.^{1,3}

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Background: This study describes a novel low cost quantitative methylation test named Methylation Specific Quantitative Melt Analysis (MS-QMA), and its use for combined screening of epi-mutations associated with fragile X syndrome and chromosome 15 imprinting disorders in symptomatic children and newborns. The test targets *FMRI* and *SNRPN* promoters, with the lower limit of detection for abnormal methylation of 1%. **Methods and Results:** MS-QMA *FMRI* assay was performed on lysate (without DNA extraction) from a single 3 mm punch per newborn screening dried blood spot. Sensitivity and specificity were >99% for fragile X syndrome full mutation (FM) alleles (30 FM males; 25 FM females; 89 male controls; 95 female controls); for Prader-Willi syndrome (PWS) and Angelman syndrome (AS) these were 100% (17 PWS, 12 AS, 42 controls, all confirmed by micro-array and MS-PCR) using venous blood DNA. As expected, there was no significant difference between *SNRPN* methylation in the PWS and AS groups for those diagnosed at birth (n=15) and those diagnosed at >1 year of age (n=14). Furthermore, the *SNRPN* assay performed equally well with newborn blood spot lysates (n=11) and venous blood DNA collected at birth. In a separate cohort of DNA samples retrospectively collected from 57 patients referred for chromosome 15 imprinting disorder testing, MS-QMA identified 14 abnormal cases (8 PWS - 100% methylated; 3 AS - 0% methylated; 3 Chromosome 15 duplication syndrome - 72% (+/-2%) methylated; with control range 44-57%). These were all consistent with the MS-MLPA and micro-array results. MS-QMA also identified 4 patients (normal by MS-MLPA and micro-array), referred with obesity and developmental delay, with abnormal 63-66% *SNRPN* methylation and 2 patients with 38% methylation, suggestive of mosaicism for PWS and AS, respectively.

Conclusions: *FMRI/SNRPN* MS-QMA may be a cost-effective approach with enhanced analytical and diagnostic sensitivity that is suitable for testing and screening for four early onset epigenetic disorders with overlapping cognitive and behavioural phenotypes.

Summary: We have developed a highly sensitive, cost-effective test for symptomatic children and asymptomatic newborns that can be effectively used to simultaneously screen for DNA changes associated with Prader-Willi syndrome, and three other common childhood disorders.

SPEAKER ABSTRACTS

1.3 - Energy expenditure, physical activity and maximal oxygen uptake in adults with Prader-Willi syndrome

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*These authors contributed equally to this work.

Background: Prader-Willi Syndrome (PWS) is the most common syndromal cause of life threatening obesity. Strict adherence to a low-calorie diet and regular physical activity prevent extreme obesity. Unexpectedly, direct measurement of maximal oxygen uptake (VO₂ max), the “gold standard” for assessing aerobic exercise capacity, has not been described in PWS. The objective of this study was to assess aerobic capacity by direct measurement of VO₂ max in adults with PWS, and in age and BMI-matched controls (OC). Compare the results with values obtained by indirect prediction methods.

Methods and results: 17 individuals (12 males) ages: 19-35 (28.6±4.9), BMI: 19.4-38.1 (27.8±5) kg/m² with genetically confirmed PWS who exercise daily and 32 matched OC (22 males) ages: 19-36 (29.3 ±5.2), BMI: 21.1-48.1 (26.3±4.9) kg/m². All filled out a medical questionnaire and performed strength and flexibility tests. During a graded exercise test on a treadmill VO₂ max was determined by measuring oxygen consumption. VO₂ max (24.6±3.4 vs 46.5±12.2 ml/kg/min, p<0.001) and anaerobic threshold (20±2 and 36.2±10.5 ml/kg/min, p<0.001), maximal strength of both hands (36±4 vs. 91.4±21.2 kg, p<0.001) and flexibility (15.2±9.5 vs. 26±11.1 cm, p=0.001) were all significantly lower for PWS compared to OC.

Conclusions: Aerobic capacity, assessed by direct measurement of VO₂ max, is significantly lower in PWS adults, even in those who exercise daily, compared to OCs. Indirect estimates of VO₂ max are accurate for OC, but unreliable in PWS. Direct measurement of VO₂ during a graded exercise test is preferred, when tailoring a personal training plan.

Summary: Effective exercise is an essential part of treatment for individuals with PWS. We documented that aerobic capacity is much lower in PWS adults when compared to OCs and that direct calculations of aerobic capacity are needed when planning clinical studies or individual plan of exercise in PWS.

SPEAKER ABSTRACTS

1.4 - Causes of deaths in French patients with Prader-Willi syndrome

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Background: Death reports are a critical issue in Prader-Willi syndrome (PWS). We published a study analyzing the causes of death in children with PWS reported in the literature in 2008. Our objective is now to identify the number and to describe the causes of death in children and adults with PWS.

Methods and Results: We conducted a retrospective study from 2004 to 2012 collecting death certificate with a notification of CIM-10 « Q87.1 » from the national death register: Centre of Epidemiology on the Causes of Death in France (CépiDC). In addition we contacted the 22 competence centres for PWS in France. We identified 85 patients, including 12 children, with a mean age 29 years [0-58] and a sex ratio (M/F) of 46/39. We then classified the deaths depending on causes: we found 41 (48.2%) respiratory causes (infection, respiratory insufficiency or cardiorespiratory), 8 (9.4%) non-respiratory infections (gastroenteritis, other infection), 10 (11.7%) cardiac events (pulmonary embolism, cardiac insufficiency, other cardiac event), 15 (17.7%) sudden deaths, 1 (1.2%) food choking during feeding, 1 (1.2%) accident and 4 (4.7%) other causes. There was no notification for 5 patients (5.9%). Of note when cardiorespiratory arrest was notified without other precision we classified the death as sudden death.

Conclusions: Our data documented the younger age at death in PWS 31 years vs. 73 years in the general population. The first cause of death is respiratory failures representing 48.2% in PWS vs. 6% in the general population. Thirty three % of children died from respiratory failure vs. 50.6% in adults. The second cause is sudden death 17.7% (25% in children and 16.4% in adults) and the third cause is cardiac 11.7% (8.3% in children and 12.3% in adults). Prospective studies are required in different countries in order to describe more precisely the causes of deaths and implement specific prevention strategies.

Summary: we investigated the number of death reports 2004 to 2012 in the French PWS population and classified the different causes of death in children and adults with PWS.

SPEAKER ABSTRACTS

2.1 - Characterizing the processing, localization, and function of *Snord116* noncoding RNAs at the Prader-Willi locus

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Background

The noncoding RNA cluster *SNORD116*, located within the Prader-Willi critical locus at 15q11-13 is GC-skewed, resulting in R-loop formation, histone displacement, and chromatin decondensation specifically of the paternal allele upon neuronal transcription. *SNORD116* processing produces two noncoding RNAs: *SNORD116* snoRNAs, which localize to the nucleolus of maturing neurons, and *116* host gene (*116HG*), which is retained within the nucleus, localizing to its paternally decondensed site of transcription and forming an RNA cloud.

Methods and Results

To understand the processing, localization, and functional relevance of each component of *SNORD116*, we engineered novel transgenic mice to test complementation of the PWS mouse model, *Snord116del*. Complete transgene wild-type (*Ctg/WT*) mice carrying all of the elements of the *Snord116* repeat were generated and bred to *Snord116del* males to produce mice deficient for endogenous paternal *Snord116* but expressing the transgene (*Ctg/Snord116del*). RNA fluorescence *in situ* hybridization (FISH) analysis of brain showed that spliced *116HG* RNA localized in a distinct cloud at its decondensed transcription site and mature snoRNAs localized to the nucleolus in wild-type (WT) but not *Snord116del* neurons. In non-neuronal *Ctg/WT* tissues and all *Ctg/Snord116del* tissues, no *116HG* RNA cloud or snoRNAs were detected, but in *Ctg/WT* neurons, nucleolar snoRNAs were increased and a significantly larger single *116HG* RNA cloud was detected. RT-PCR analysis demonstrated that splicing of the *Snord116* transgene was largely restricted to neuronal tissues of both *Ctg/WT* and *Ctg/Snord116del* mice, despite widespread expression from the CMV promoter in many tissues. Rbfox3 (NeuN), a neuronal specific splicing factor was tested for its role in *Snord116* splicing by siRNA knockdown in neurons derived from *Ctg/Snord116del* NPCs. Rbfox3 knockdown reduced spliced *Ctg* transcript levels without affecting the abundance of the primary transcript.

Conclusions

These results suggest that processing of the *Snord116* transcript is dependent on neuronal-specific splicing factors including Rbfox3 and/or chromatin states, particularly the decondensed *Snord116* paternal allele. Analyses of a spliced *116HG/Snord116del* transgenic mouse is in progress to determine whether the process of splicing facilitates localization or whether providing a pre-spliced transcript circumvents the need for an endogenous active *Snord116* locus.

Summary: The *Snord116* gene was inserted into the genome of a mouse model for Prader-Willi syndrome, in which mice lack a paternal copy of this gene. In wildtype mice, this extra copy contributes to the RNA products typically produced, however in the Prader-Willi mice, no mature products of the *Snord116* gene are detected. Based on our results, this appears to be due to the brain specific protein Rbfox3, which is required for processing of *Snord116* products, as well as the structure of the DNA at the original paternal location.

SPEAKER ABSTRACTS

2.2 - Ghrelin acylation by ghrelin *O*-acyltransferase: A potential target to treat hyperphagia in Prader-Willi syndrome

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Background: Obesity and hyperphagia present two major health threats to patients with Prader-Willi syndrome (PWS). The discovery of the hormone ghrelin and its role in controlling appetite suggests a potential role for ghrelin in PWS. Elevated levels of ghrelin in children and adults with PWS, coupled with ghrelin's role in stimulating appetite, highlights inhibition of ghrelin signaling as a potential treatment avenue for PWS-associated hyperphagia. Recent studies have demonstrated high ghrelin levels in the early postnatal period can lead to life-long metabolic disturbances, providing further support for ghrelin as a therapeutic target in PWS. Ghrelin requires a unique chemical modification (octanoylation) catalyzed by the enzyme ghrelin *O*-acyltransferase (GOAT) to bind and activate its receptor. Ghrelin is the only predicted substrate for GOAT within the human proteome, supporting inhibition of GOAT-catalyzed ghrelin acylation as a specific and targeted avenue to treat health conditions potentially impacted by ghrelin signaling such as hyperphagia in PWS patients.

Methods and Results: Our research focuses on understanding the substrate specificity and catalytic mechanism of GOAT, working towards development of GOAT inhibitors. Through structure-activity analysis of both GOAT substrates and inhibitors, we have identified multiple sites within ghrelin involved in substrate recognition by GOAT. Targeted mutagenesis studies coupled with functional studies offer insight into the location of the active site and substrate binding sites within GOAT and suggest potential catalytic mechanisms. Using rational approaches and small-molecule library screening, we have identified several new classes of GOAT inhibitors for optimization to realize increased potency and bioavailability. Several of these molecules may serve as mechanism-based inhibitors, the first such examples identified for GOAT.

Conclusions: Defining interactions used by hGOAT to bind, recognize, and modify ghrelin offers new insight into the structure of the hGOAT active site and has guided the design, identification, and optimization of hGOAT inhibitors. Development of these hGOAT inhibitors is an essential step towards evaluation of ghrelin signaling as a route for treating hyperphagia in PWS.

Summary: The protein hormone ghrelin plays a key role in controlling appetite. Patients with PWS have high levels of ghrelin in their bloodstreams, suggesting the voracious appetite that accompanies PWS may arise from excessive ghrelin signaling. Ghrelin *O*-acyltransferase (GOAT), an enzyme that activates ghrelin, offers a target for specifically blocking ghrelin activity. By using biochemistry and synthetic chemistry to understand how GOAT binds and activates ghrelin, we are learning how to create molecules that block ghrelin activation by GOAT. These new GOAT inhibitors are essential for evaluating ghrelin's potential as a therapeutic avenue for treating hyperphagia in patients with PWS.

SPEAKER ABSTRACTS

2.3 - Knockout of the zinc finger protein ZNF274 in PWS-specific stem cell neurons activates expression of repressed maternal 15q11.2-q13 transcripts

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Background

Prader-Willi syndrome (PWS, MIM 176270) is characterized by neonatal hypotonia and failure to thrive during infancy, and, subsequently, by obesity, hyperphagia, cognitive disability and behavioral abnormalities. Hypothalamic dysfunction is hypothesized to be responsible for many of the PWS features. This neurobehavioral disorder of genomic imprinting is caused by the lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region. The detailed genetic basis of PWS is complex involving the loss of active copies of a cluster of non-coding box C/D class small nucleolar RNAs, the SNORD116 snoRNAs.

Methods and Results

We have generated stem cell models of PWS via induced pluripotent stem cell technology and used these to identify a new complex that is involved in silencing the maternal copy of the PWS critical region (PWS-CR) encompassing the SNORD116 cluster. This epigenetic complex is composed of the zinc-finger protein ZNF274 and the SET domain, bifurcated 1 (SETDB1) histone H3 lysine 9 (H3K9) methyltransferase. Here, using genome editing approaches, we have knocked out ZNF274 expression in PWS-specific iPSC lines from multiple PWS patients with diverse genetic abnormalities (large deletion, small deletion and UPD) and showed that the extent of transcriptional re-activation of PWS-CR genes varies during *in vitro* neurogenesis. While PWS-CR gene expression in ZNF274 knockout (KO) iPSCs is ~5 % of normal levels, wild-type mRNA levels are completely restored in neurons derived from PWS ZNF274 KO iPSCs. Surprisingly, the DNA methylation at the PWS-IC remains unchanged upon ZNF274 KO, suggesting that the ZNF274 complex acts as a second imprinting locus regulating the PWS-CR gene expression during the course of neurogenesis.

Conclusions

Our data further support the finding that ZNF274 may represent a potential target for future therapeutic applications to rescue the PWS phenotype.

Summary

Our team has discovered a component of the switch off mechanism leading to the parent-of-origin specific gene expression in the 15q11.2-q13 region: a protein called ZNF274 that specifically binds the maternal SNORD116 cluster. By targeting ZNF274 for destruction (genetic knock out or KO) in stem cells created from PWS skin cells, we have reversed the off-switch and turned on the maternal chromosome 15 set of genes. Importantly, we demonstrated that the rescue is maintained during the course of neuronal differentiation, opening novel opportunities for potential therapeutic interventions in PWS.

SPEAKER ABSTRACTS

2.4 - Mechanisms of hormone secretion deficits in Prader-Willi syndrome (PWS)

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Background: PWS is a multisystem disorder caused by loss of function of a cluster of ~12 paternally-expressed, imprinted genes in human chromosome 15q11.2. Neonatal failure to thrive (FTT) is followed by childhood-onset hyperphagia, obesity, neurocognitive and behavioral issues, and deficits in growth hormone (GH), gonadotropin-releasing hormone (GnRH), and other hormones. These clinical features are assumed to arise from hypothalamic deficits. In contrast, using a mouse model with a deletion of the orthologous cluster of PWS-imprinted genes, we have shown that FTT is associated with fetal pancreatic endocrine abnormalities and a postnatal onset of severe hypoglycemia. Mechanisms contributing to this phenotype included a developmental defect with increased islet-cell apoptosis and reduced β - and α -cell mass, deficient basal and glucose-stimulated insulin secretion leading to hypoinsulinemia, and concurrent hypoglucagonemia with lack of a counter-regulatory response to hypoglycemia. These mice had upregulated expression of genes encoding pancreatic hormones and proteins involved in hormone secretion; however, this was insufficient to overcome the hormonal deficits. Indeed, a similar metabolic profile with hypoglycemia is seen in PWS. These findings indicate that PWS-imprinted genes are required for the prevention of hypoglycemia as well as the development, survival, and secretory function of pancreatic endocrine cells. Based on the mouse data implicating defective maturation or exocytosis of insulin secretory granules (SGs), we hypothesize that PWS genes are required for secretory function of pancreatic and neuroendocrine cells including for insulin, glucagon, GnRH, GHRH or GH, and other hormones (e.g., oxytocin).

Methods/Results: To establish hormone-secreting cell models, we utilized CRISPR/Cas9 genome editing within rodent cell lines that specifically secrete high levels of GnRH or insulin, the latter also expressing an mCherry fluorescent protein-propeptide hormone biosensor to monitor trafficking within SGs. Initial transfections with pairs of gRNAs and Cas9 vectors led to ~10% of cells with a deletion of the ~3.16 Mb PWS-orthologous imprinted domain. Subsequently, we generated isogenic, clonal PWS-deletion and control cell lines based on deletion-PCR of genomic DNA, and are currently completing molecular cytogenetics, gene expression and hormone secretion assays, and examining SG maturation and exocytosis.

Conclusions: We have developed pancreatic and neuroendocrine cells with deletions of PWS-imprinted genes, as a model system to identify which PWS-imprinted genes control secretion of multiple hormones and the molecular, biochemical, and cellular mechanisms involved.

Summary: This study will identify which PWS genes regulate secretion of which hormones and how, and could lead to new hormone or small molecule treatment approaches to supplement the benefits provided by GH for individuals with PWS (or similar endocrine and metabolic defects).

SPEAKER ABSTRACTS

2.5 - Identification and mechanistic studies of a novel PWS-associated gene

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Background: The MAGE-L2 gene is in the Prader-Willi Syndrome (PWS) susceptibility locus and is deleted or mutated in individuals affected with PWS or Schaaf-Yang syndrome. Furthermore, MAGE-L2 knockout mice phenocopy several aspects of PWS. Recently, my group has uncovered the cellular function of MAGE-L2. We showed that MAGE-L2 functions with the TRIM27 E3 ubiquitin ligase to promote recycling of membrane proteins from endosomes back to the plasma membrane or the trans-Golgi network, thus sparing them from lysosomal degradation. It does so through ubiquitination and activation of the actin nucleating protein WASH on endosomes. However, it has remained unclear how MAGE-L2 is regulated.

Methods and Results: Utilizing a number of different biochemical and cell biological assays, we have identified a novel interacting partner of MAGE-L2, the USP7 deubiquitinating enzyme. We show that MAGE-L2-TRIM27 stably associates with USP7 where USP7 is essential for two critical aspects of MAGE-L2 function. First USP7 protects TRIM27 from auto-ubiquitination and proteasomal degradation. Second, USP7 regulates ubiquitination of the MAGE-L2-TRIM27 substrate, WASH, to properly tune the amount of endosomal actin and control the endosomal protein recycling pathway. Depletion of USP7 impairs the MAGE-L2-regulated endosomal protein recycling pathway in cells. Importantly, we identify de novo heterozygous loss-of-function mutations of USP7 in individuals with a neurodevelopmental disorder whose symptoms overlap with children with MAGE-L2 mutation, including intellectual disability, autism spectrum disorder, and hypotonia.

Conclusion: USP7 is a novel component of the MAGE-L2-TRIM27 ubiquitin ligase complex and is necessary to allow proper regulation of WASH ubiquitination and endosomal actin accumulation and protein recycling. Furthermore, we identify individuals with mutation in USP7 that have overlapping symptoms to those with mutation of MAGE-L2. These results provide unanticipated insights into endosomal trafficking, illuminate the cooperativity between an ubiquitin ligase and a deubiquitinating enzyme, and establish a role for USP7 in human neurodevelopmental disease.

Summary: We have uncovered the cellular function of the PWS-associated gene, MAGE-L2. MAGE-L2 functions to facilitate proper cellular protein homeostasis by controlling protein trafficking and degradation. Furthermore, we identify a MAGE-L2 interacting protein, USP7, which collaborates with MAGE-L2 in this cellular process and is also mutated in children with symptoms similar to MAGE-L2 mutation.

SPEAKER ABSTRACTS

2.6 - Function and therapeutic substitution of snoRNAs in Prader-Willi syndrome

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Background

The Prader-Willi critical region contains five C/D box snoRNAs (SNORDs) (107, 109, 64, 115 and 116) that are not expressed in PWS patients. The 47 copies of SNORD115 are almost identical and regulate alternative splicing of the serotonin receptor 2C pre-mRNA, where they promote the formation of the full-length, active receptor. In contrast, no functions and possible targets of the other SNORDs are known. The 29 SNORD116 copies are more diverse than SNORD115 and fall into five classes of higher sequence identity. Loss of SNORD116 results in a Prader-Willi like phenotype, underlining the importance this SNORD.

Method and Results

We devised a method to knock-down SNORDs using antisense oligos composed of nucleotides with different chemistry. Using five different oligos, we knocked-down all SNORD116 copies in the neuroblastoma cell line SH-SY5Y. RNAseq of these samples revealed that SNORD116 promotes the inclusion of 51 exons, about 19 of which are microexons. The microexons are less than 50 nt in length and keep the reading frame. Most of them are located near or in known protein domains. The effect of SNORD116 on most individual exons is small (<50%), but most exons are located in proteins that affect vesicle transport. Bioinformatic predictions show short sequence complementarities between SNORD116 and target exons. When nuclear extract is fractionated under native conditions and separated using glycerol gradients, about 30% of SNORD116 purifies in fractions devoid of fibrillarin and affinity purification of SNORD116 reveals its association with hnRNPs. This suggests that SNORD116 forms atypical SNORD-protein complexes and regulates proteins involved in vesicular transport via direct RNA:RNA interaction.

We previously developed an oligonucleotide that promotes alternative exon inclusion of the serotonin receptor 2C and could thus substitute the loss of SNORD115. Systemic injection of the oligo results in marked decrease of food uptake in mice. To explain this effect we re-analyzed serotonin receptor 2C expression in mouse tissues and found expression in the pituitary where the oligo rapidly promotes exon inclusion. Both SNORD115 and SNORD116 are expressed in pituitary, suggesting that they regulate vesicular trafficking and receptor localization in this tissue.

Conclusions

The SNORDs with the strongest expression missing in PWS, SNORD116 and SNORD115 all act in promoting alternative exons that work in vesicular transport and serotonin receptor 2C localization/function. Their expression in the pituitary suggests a role of this gland in PWS.

Summary

SNORD116 and SNORD115 possibly regulate vesicle transport and serotonin receptor 2C activity in the pituitary, which could explain some of the hormonal deregulations in PWS. The SNORDs can be substituted with oligonucleotides, suggesting that oligo delivery through injections could be a therapeutic avenue to treat PWS.

SPEAKER ABSTRACTS

3.1 - Aberrant, autistic, and food-related behaviors in adults with Prader-Willi syndrome: The effects of age and genotype

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Background: This study aims to explore the difference of age as well as genotype in regards to the severity of behavioral symptoms in Prader-Willi syndrome (PWS), with special emphasis on the comparison between young adults and adults.

Methods and Results: The Food Related Problem Questionnaire (FRPQ), the Aberrant Behavior Checklist Japanese Version (ABC-J), and the Pervasive Developmental Disorders Autism Society Japan Rating Scale (PARS) were administered to 45 PWS patients. The participants consisted of 33 young adults (ages 18 to 28) and 12 adults (ages 30 to 45), including 23 young adults and 11 adults confirmed as having a paternal chromosome 15q deletion (DEL), and 10 young adults and one adult confirmed as having maternal uniparental disomy (mUPD) of chromosome 15. To examine that the difference between young adults and adults have on the symptom's severity, Mann-Whitney U tests were conducted. Statistically significant differences were found in ABC-J ($p=.035$) and PARS ($p=.049$), with higher scores in young adults than adults. Such differences between the two age groups were still true for DEL subgroups (ABC-J: $p=.022$; PARS; $p=.038$). By contrast, there was no significant difference between young adults and adults regarding FRPQ ($p=.79$).

Conclusions: In the total PWS patients, aberrant behaviors and autistic behaviors in young adults were more severe than those in adults. Even in DEL subgroups aberrant behaviors were more severe in young adults. In terms of food-related behaviors, no significant differences were found between young adults and adults. These results suggest that aberrant behaviors and autistic behaviors decline from around the ages of thirty, while food-related behaviors give no indication of diminishing in spite of developmental growth. Hyperphagia should be considered to follow a developmental trajectory independent from that of other behavioral symptoms in PWS such as aberrant and autistic symptomatology.

Summary: In PWS patients aberrant and autistic behaviors decline around the ages of thirty.

SPEAKER ABSTRACTS

3.2 - The PRASOC project: Assessment of the impact of cognitive, executive and emotional abilities on the difficulties of adaptation and socialization of patients with Prader-Willi Syndrome, preliminary results

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Background: While behavioural descriptions and recommendations in terms of diagnosis and management are relatively documented (cf. National protocol of diagnosis and care for rare diseases, HAS, 2012), the specific cognitive and emotional disorders and their incidence on the daily lives of individuals with PWS are still poorly understood. Intellectual deficit cannot completely explain most of the daily difficulties observed in people with PWS. Our recent studies showed that patients with PWS exhibit an executive deficit, in part independent of intellectual level. (Chevalere et al, 2013; 2015). In addition, the emotional component seems to be an important factor involved in cognitive treatments and relationships with others. Few studies so far have reported the main influence of emotions in performing executive tasks in PWS. Finally, as disturbed eating behaviour is a central feature of PWS, it appears essential to study the physiological and behavioural reactions of patients when confronted with food.

Methods and Results: The project PRASOC (Prader-Willi Socialization) is a French multicentric research program which implies a laboratory of Psychology, two reference centers of PWS (adults and children) and the French Association of Prader-Willi. The objective is to provide a precise description of the executive and emotional capacities linked with food at various ages. We presented the preliminary results about the executive functioning in association with emotional components. We interested about inhibition, working memory updating and flexibility capacities assessed by laboratory cognitive tasks applied to children (N=32, m= 11.14) and adults (N=37, m=30.0) compared with two control groups, one matched to chronological age and another to biological age. The results show that in spite of a global impairment of executive functions, some of them seems not be impaired or at least not specific to the PWS but linked with the intellectual level. Moreover, it is quite surprising to note that the processing of emotion is not systemically impaired in the PWS and that the relationship with food seems not to modify the processing of information.

Conclusion: The study sheds light on the executive capacity of people with SPW and their relationship with emotional component from childhood to adulthood. Some deficits seem linked with intellectual level but some difference indicated that some capacities are similar to the control population (especially for the adults) and others tend to be more similar with impaired intellectual people.

Summary: The first results of a research program in cognitive psychology are presented. By comparing two groups of participants with PWS (children and adults) with a group matched by age without deficit and another group matched by age with the same intellectual deficit than the PWS group, we began to have a more precise description of the cognitive capacities of the PWS and especially those links with emotional component.

SPEAKER ABSTRACTS

3.3 - The human centred development and initial evaluation of a task switching training video game for children with PWS

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Background

A deficit in task switching – one's ability to respond flexibly to stimuli or events, depending on external demands – has been associated with change-triggered temper outbursts in children with PWS. Task switching can be improved via computerised practise but these improvements do not necessarily extend to gains in daily life functioning (gains don't always transfer). However, certain kinds of commercial video game include features that promote learning transfer and can mediate serendipitous improvements in task switching.

We developed a prototype video game for training task switching in children with PWS. We evaluated changes in task switching ability mediated by engagement with the game in a placebo controlled cross-over design.

Methods and results

Seven children with PWS collaborated in the design of TASTER, a switching training game. This collaboration ensured usability. Additionally, a systematic analysis of commercial video games that have positive effects on task switching identified features which train switching and promote learning transfer; these features were included in TASTER.

Four children with PWS have completed the first stage of a placebo controlled cross-over evaluation, involving engagement with either an experimental or placebo (no demands on switching) version of TASTER. Children completed four neurocognitive tests of switching twice before training and again after 1-3 hours of engagement with the game, spread over four weeks. Results to date indicate gains in task switching limited to the individual who has already engaged with the experimental version of the game. Caregivers completed an informant report measure of task switching before and after training but these data show small gains in switching across all children. The results of the cross-over phase and those from further participants will be discussed.

Conclusions

Human-centred design with children with PWS allowed a prototype switching training game to be developed that establishes several conditions that are known to facilitate high learning transfer. Initial results suggest that even short term engagement with the training can have a positive effect on task switching ability.

Summary

Temper outbursts due to changes to plans or routines have been linked with a reduced ability to switch quickly between tasks in children with PWS. We have collaborated with a group of children with PWS to design and develop a video game to train this ability, with the aim of reducing such outbursts. Initial evidence suggests that playing the game can improve task switching.

SPEAKER ABSTRACTS

3.4 - Aberrant Behavior Checklist Scores in Children with Prader-Willi Syndrome compared to those with Autism

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Background: Children with Prader-Willi Syndrome (PWS) have behavioral difficulties that can be impactful on families and caregivers. Various instruments have been utilized to evaluate behavior, including the Aberrant Behavior Checklist (ABC). This questionnaire is also used in children with autism. Individuals with PWS can have behaviors similar to those with autism. In this study, we compare ABC results in youth with PWS and those with autism.

Methods and Results: ABC scores from de-identified populations of youth with PWS from Seattle Children's Hospital (SCH) and University of Florida (UF) [n = 66 (44 females, 22 males), mean age 10.5 ± 6.6 years, age range 3-37 years] and children with autism (without PWS) from UF [n = 102 (21 females, 81 males), mean age 10.4 ± 3.5 years, age range 5-18 years]. The ABC was filled out by parents or caregivers. Scores were calculated using standard methods and compared using two-tailed t-tests. ABC scores were evaluated as total score and sub-scores (hyperactivity, irritability, lethargy, speech, and stereotype). Scores are presented as mean±SD. The relationship between BMI z-score and ABC scores in the SCH PWS population was evaluated using linear regression. IRB approval for retrospective data review was obtained from SCH.

There was a significant difference in mean ABC total and sub-scores between the PWS (total 28.8±22.5, hyperactivity 8.1±7.6, irritability 10.4±8.6, lethargy 5.2±5.2, speech 2.7±2.5, stereotype 2.2±2.9,) and autism groups (total 61±29.5, hyperactivity 20.5±11.3, irritability 16±10.3, lethargy 13.5±9.2, speech 4.2±3.0, stereotype 6.8±5.0,) with p-values all <0.001.

There were no significant differences in ABC scores in the following PWS subgroups: deletion vs. non-deletion genetic subtype, male vs. female, age <13 vs. ≥13. In the PWS group from SCH (n = 24), there was no significant relationship between BMI z-score and ABC scores.

Conclusion: ABC scores in youth with PWS are lower than those in autism despite overlap of behavioral abnormalities and need of similar behavior, psychiatry, and education services. The results may indicate fewer behavioral abnormalities in youth with PWS, or it may be an indication of the inadequacy of the ABC as a behavioral assessment in PWS. Also to be considered is the potential influence of under reporting of abnormal behavior on score outcomes.

Summary: The ABC has been used to gauge abnormal behavior in different populations including PWS and autism. Perhaps surprisingly, the results of our study show that children with PWS have lower ABC scores correlating with better reported behaviors than those with autism. There was no difference in ABC scores among PWS children of different genetic subtypes, genders, or ages.

SPEAKER ABSTRACTS

3.5 - Oxytocin vs. Placebo for the Treatment of Repetitive Behaviors, Disruptive Behaviors, and Social Communication in Children and Adolescents with Prader-Willi Syndrome: Preliminary Results

Bonnie P. Taylor, Ellen Doernberg, Anahid Kabasakalian, Casara Ferretti & Eric Hollander

Albert Einstein College of Medicine/Montefiore Medical Center (all authors)

Background. Oxytocin (OXT) has been implicated in the pathophysiology of Prader-Willi Syndrome (PWS). Studies have shown that the number and size of OXT neurons is significantly reduced in individuals with PWS. Further, animal studies have demonstrated that a reduction in OXT neurons leads to overeating, which reverses after OXT administration. OXT administration in individuals with ASD impacts social communication skills, trust in others, and repetitive behaviors- all significant issues for children with PWS. The few clinical trials of OXT in PWS have yielded some promising results, including improvements in eye gaze and trust in others, and a reduction in anxiety. OXTs effects on tantrums and appetite have been equivocal. To date, there have been no double-blind placebo-controlled trails in children with PWS that examined the effects of OXT over the course of more than 5 days.

Methods. We conducted an 8-week double-blind placebo-controlled treatment study of intranasal OXT (IN-OXT) in 24 children with PWS. Patients received an OXT dose of 16 IU per day (2 puffs per nostril/4 IU each). The primary outcome measure was Eating Behaviors, including the Revised-Dykens Hyperphagia Questionnaire, gut hormone measurements, dietary diary and BMI. Secondary and exploratory measures assessed repetitive behaviors (RBS-R and Y-BOCS), disruptive behaviors (ABC-I), social communication (ABC-SW and SRS), quality of life (WHOQOL), hypotonia and oxytocin levels (oxytocin receptor genotype and salivary oxytocin).

Results. An analysis of the sample that has completed the study by May 1st, 2016 will be presented. The demographics of the sample will be described, including age, gender, and subtype (i.e., PWS by deletion, UPD and imprinting). Analysis will be conducted examining end-study OXT-placebo differences (controlled for baseline) for repetitive behaviors (RBS-R and Y-BOCS), disruptive behaviors (ABC-I) and social communication (ABC-SW and SRS).

Conclusions. Based on the results, we will discuss the benefits of 8-weeks of intranasal oxytocin administration versus placebo on repetitive behaviors, disruptive behaviors and social communication in children 5-18 years old with PWS.

Summary. Oxytocin, a naturally occurring hormone in the body, may improve PWS-associated behaviors, including repetitive behaviors, disruptive behaviors and social communication. To examine this, we conducted a pilot 8-week double-blind treatment study of intranasal OXT vs. placebo in 24 children with PWS. Results and conclusions will be briefly described.

SPEAKER ABSTRACTS

3.6 - An overview of cognition in PWS

Joyce Whittington and Tony Holland

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Why study cognition?

- (1) Scientific interest: How does the genetic defect in PWS lead to the cognitive phenotype?
- (2) Education issues: Aspects of cognition that affect learning
- (3) Social/behavioural issues: How cognition affects observed behaviour and social interaction

Examples of contributions in each of these areas.

Drawbacks and limitations to research on cognition

As with many aspects of research in PWS, much of our view of cognition in PWS is based on old reports, which in turn were based on small samples and included ‘clinically’ diagnosed participants. Some of these reports contain findings which have never been replicated and which are perpetuated in the literature by references to the original studies. Because of the difficulties inherent in research on rare conditions, not least because of the difficulties of obtaining reasonable sample sizes and representative populations, replication is essential if we are to get a true picture. This is well illustrated by the literature on cognition in PWS.

Rapid review of research on cognition

Findings are presented on general ability (IQ), attainment, language, comprehension, executive functions, and social cognition. The latter includes investigations of theory of mind, emotion recognition, face processing and knowledge of social norms.

SPEAKER ABSTRACTS

4.1 - Evaluation of the Effects of GH Therapy in Adult Patients with Prader-Willi Syndrome

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Background: The clinical picture of Prader-Willi Syndrome (PWS) strongly supports the presence of GHD. Apart from short stature, both PWS and GHD are characterized by body composition abnormalities, impaired psychological abilities, altered physical strength, and reduced bone mineral density. GH treatment has been shown to normalize skeletal growth in PWS children and to have additional beneficial effects on body composition, muscular performances, and cognitive level. It has hitherto not been settled if treatment should be continued in PWS adults. The objective of this study was to analyze the effects of GH therapy in a group of PWS adults in comparison to what observed in PWS patients without GH treatment.

Methods and results: Forty-one subjects with PWS (21 females, aged 31.3±1.3 yr (mean±SE), del15:UPD15= 31:10) were studied. Twenty-one PWS were undergoing GH therapy from 7.5±0.7 years (Group A), while the other 20 patients were without GH treatment (Group B). The examination of PWS patients included: anthropometric measurements [Body Mass Index (BMI: (kg/m²), waist circumference], body composition and bone mineralization (DXA), resting energy expenditure (indirect calorimetry), glucose metabolism (OGTT-derived indexes), lipid profile, thyroid function, IGF-I level, blood pressure, liver ultrasonography, cardiac assessment (M-mode, two-dimensional, and pulsed Doppler echocardiographic studies), pulmonary function (spirometry). The comparison of the 2 study groups showed the following results: BMI: 42.2±1.8 (A) vs 42.7±1.8 (B) (p=ns); Fat mass (%): 51.0±1.5 (A) vs 54.6±0.9 (B) (p<0.05); Lean body mass (kg): 49.0±2.2 (A) vs 40.8±1.4 (B) (p<0.003); Lean body mass (%): 49.0±1.5 (A) vs 45.4±0.9 (B) (p<0.05); Vertebral T-score: -1.46±0.30 (A) vs -1.47±0.20 (B) (p=ns); Femoral T-score: -1.31±0.24 (A) vs -0.59±0.20 (B) (p<0.03); Glycaemia mg/dl (basal): 89.2±5.4 (A) vs 111.8±10.6 (B) (p=0.06); Glycaemia mg/dl (120' OGTT): 120.3±6.2 (A) vs 147.3±9.6 (B) (p<0.03); Insulin (basal) (μU/ml): 13.0±1.8 (A) vs 12.3±1.4 (B) (p= ns); HbA1c (%): 5.5±0.09 (A) vs 6.2±0.29 (B) (p< 0.03); Total cholesterol (mg/dl): 181.9±9.4 (A) vs 208.2±9.1 (B) (p<0.05); IGF-I (μg/L): 179.1±10.1 (A) vs 102.7±9.6 (B) (p<0.0001); Moderate-severe NAFLD (%): 38.1% (A) vs 68.4% (B) (p<0.05); Vital capacity (L): 3.76±0.14 (A) vs 3.35±0.13 (B) (p<0.05); Forced vital capacity (L) (%ref): 71.8±2.2 vs 59.6±4.2 (B) (p<0.02); Forced expiratory volume at 1.0 second (FEV1) (L) (%ref): 87.1±4.2 vs 68.5±4.3 (B) (p<0.005); Forced expiratory flow 25-75 (L/min) (%ref): 80.4±3.4 vs 72.6±4.3 (B) (p<0.001).

Conclusions: Our results suggest that GH therapy may have a role in the strategic perspective of the more appropriate care to adult patients with PWS.

Summary: Long-term GH therapy in adults with PWS is able to improve body composition, total cholesterol levels, IGF-I levels, liver morphology, and pulmonary function, in the absence of negative effects on carbohydrate metabolism and cardiac function.

SPEAKER ABSTRACTS

4.2 - Hyperphagia: Cause or Consequence of Obesity?

Mark I. Friedman, Ph.D.

Nutrition Science Initiative

Early nutritional stages defined in Prader-Willi Syndrome (PWS) by Miller et al (2011) include increased fat accumulation and body weight prior to the onset of hyperphagia. This observation suggests that PWS is primarily a fuel partitioning disorder, in which hyperphagia is not a primary cause of the obesity.

Historically, both common obesity and that in PWS have been thought to be caused by a defect in energy intake (hyperphagia) that results in increased fat accumulation and, eventually, obesity. In contrast to this prevailing view, obesity has also been seen as a primary metabolic disorder of excessive fat deposition, which triggers hyperphagia as a compensatory response to the “loss” of energy into adipose tissue. This overeating in turn exacerbates fat gain. In addition to the example of PWS, other findings are consistent with this alternative perspective. For example, studies using animal models of obesity also indicate that excessive lipogenesis and body fat accumulation occur before the onset of hyperphagia and, in addition, that restricting energy intake to normal levels does not prevent the increase in body fat mass.

The mechanism by which shifts in fuel partitioning towards fat storage stimulates eating behavior is not yet established. In general terms, an increased flux of metabolic fuels into adipose tissue would reduce the availability of energy-producing substrates much like limiting the supply of food through fasting does. More specifically, animal studies suggest that a reduced capacity for hepatic fatty acid oxidation and its resultant effect on the generation of a hepatic metabolic “hunger” signal may be involved.

The propensity for fat accumulation is in part genetically determined and is usually expressed through a susceptibility to diet-induced obesity. This disposition to obesity is seen mostly clearly when the diet contains a combination of large amounts of carbohydrates – particularly, refined grains and sugars – and fat. Such a diet would promote obesity by facilitating lipid synthesis and storage, while inhibiting fatty acid mobilization and oxidation.

In keeping with the assumption that overeating causes obesity, treatment typically emphasizes caloric restriction. This is true for all forms of obesity including that in PWS. But if hyperphagia is an attempt to compensate for a loss of calories into body fat, then the persistent metabolic hunger driving overeating can only be exacerbated by caloric restriction. From this perspective, treatment of obesity should focus instead on modifying metabolism to shift fuel partitioning away from fat storage toward pathways of oxidation. Drugs like Belanorib may work in this way. Alternatively, a low-carbohydrate or ketogenic diet could be effective in this regard because they would minimize fat synthesis and storage and maximize the mobilization of stored fat and its oxidation. Thus, treating the obesity should reduce or minimize hyperphagia and obviate the need to restrict calories.

SPEAKER ABSTRACTS

4.3 - Efficacy and Safety of Diazoxide Choline Controlled-Release Tablet in Prader-Willi syndrome: results of the Double-Blind Arm of the Study

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Background: Prader-Willi syndrome (PWS) is a complex, multisystem genetic disorder characterized by multiple endocrine, neurological and behavioral abnormalities. To date, no medication has proven effective in regulating appetite in PWS. It is conceivable that Diazoxide Choline Controlled-Release (DCCR) tablet can be used to control appetite and possibly improve on behavioral symptoms of PWS patients, through the activation of a diverse set of downstream mediators. DCCR works by mimicking sensitivity to leptin and insulin in AgRP and POMC neurons, reducing de-novo fatty acid biosynthesis, stimulating β -oxidation of fat and normalizing GABA response, reducing hyperphagia, fat mass and aggressive behavior in PWS patients.

Methods and Results: Thirteen obese subjects with genetically confirmed diagnosis of PWS, between 10-22yrs were enrolled in the study PC025. Two subjects were withdrawn from the study due to a prior psychiatric illness in one and progressively compromised glycemic control at the highest dose in the other. Eleven subjects completed the open-label treatment phase, were designated as Responders (hyperphagia response rate 92%) and randomized into the double-blind phase of the study. Data was analyzed for both open-label treatment phase and double-blind phase.

We found significant improvements in hyperphagia in DCCR treated subjects at the end of the open-label treatment period (-31.6%, $p=0.003$) and in those who continued on DCCR in the double blind phase (-29.2%, $p=0.006$). There was significant reduction in 'aggressive', 'threatening', 'destructive' behavior in comparison to all other PWS associated behaviors at the end of open label treatment phase (62.5% Vs 29.8% respectively, $p=0.01$). The effect of DCCR on behavior seems to be independent of the effect on hyperphagia. Significant impacts were seen on fat mass (-3.8%, $p=0.011$), lean body mass (+5.4%, $p=0.001$), lean body mass/fat mass ratio (+9.8%, $p=0.002$) during open-label treatment. The impacts on body composition were of similar magnitude in GH treated and GH naïve subjects. DCCR treatment significantly reduced TG, Tot-C, and Non-HDL-C in both the open-label and double blind phase. The most common adverse events included peripheral edema which was responsive to dose reduction and diuretics, hyperglycemia which responded to dose reduction and hirsutism all of which were reversible.

Conclusion: DCCR significantly reduces hyperphagia; reduces fat mass while increasing lean body mass, reduces aggressive, threatening and destructive behavior and significantly improved cardiovascular risk factors.

Summary: Diazoxide is effective in reducing hyperphagia, body fat and behavior problems in Prader-Willi syndrome. Side effects include fluid retention, hyperglycemia and hirsutism.

SPEAKER ABSTRACTS

4.4 - Oxytocin vs. Placebo for Hyperphagia, Eating Behaviors and Quality of Life in Children and Adolescents with Prader-Willi Syndrome

Eric Hollander, Bonnie P. Taylor, Ellen Doernberg, Casara Ferretti & Anahid Kabasakalian

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Background. Oxytocin (OXT) has been implicated in the pathophysiology of Prader-Willi Syndrome (PWS). Studies have shown that the number and size of OXT neurons is significantly reduced in individuals with PWS. Further, animal studies have demonstrated that a reduction in OXT neurons leads to overeating, which reverses after OXT administration. OXT administration in animal models and human studies of obesity and type-2 diabetes have demonstrated an impact on eating behavior, weight, and insulin release and sensitivity. The few clinical trials of OXT in PWS have yielded some promising results in eye gaze, trust in others, anxiety and hyperphagia, although differences in dosing may have a differential effect on temper-tantrums due to cross-binding to vasopressin receptors. To date, there have been no double-blind placebo-controlled trials in children with PWS that examined the effects of OXT over the course of more than 5 days.

Methods. We conducted an 8-week double-blind placebo-controlled treatment study of intranasal OXT (IN-OXT) in 24 children with PWS. Patients received an OXT dose of 16 IU per day (2 puffs per nostril/4 IU each). The primary outcome measure was Eating Behaviors, including the Revised-Dykens Hyperphagia Questionnaire, gut hormone measurements, dietary diary and BMI. Secondary and exploratory measures included quality of life (WHOQOL), hypotonia and salivary oxytocin levels.

Results. An analysis of the sample that has completed the study by May 1st, 2016 will be presented. The demographics of the sample will be described, including age, gender, and subtype (i.e., PWS by deletion, UPD and imprinting). Analysis will be conducted examining end-study OXT-placebo differences (controlled for baseline) for Eating Behaviors (Revised-Dykens Hyperphagia Questionnaire, gut hormone measurements, dietary diary and BMI) and quality of life (WHOQOL). Adverse event data will be presented.

Conclusions. Based on the results, we will discuss the benefits of 8-weeks of intranasal oxytocin administration versus placebo on eating/food-seeking behaviors and quality of life in children 5-18 years old with PWS.

Summary. Oxytocin, a naturally occurring hormone in the body, may improve PWS-associated behaviors, including hyperphagia, and eating behaviors, body composition, BMI, weight, gut hormone measures and quality of life. To examine this, we conducted a pilot 8-week double-blind treatment study of intranasal OXT vs. placebo in 24 children with PWS. Results and conclusions will be briefly described.

SPEAKER ABSTRACTS

4.5 - Laparoscopic Sleeve Gastrectomy in Children and Adolescents with Prader-Willi Syndrome: A Matched Control Study

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Background: Obesity is a leading mortality and morbidity cause in Prader-Willi syndrome (PWS). Our center, which developed a standardized care pathway for pediatric bariatric surgery, runs a prospective clinical outcome study on children and adolescents undergoing weight loss surgery. This study aimed to assess weight loss and growth after sleeve gastrectomy (LSG) in pediatric patients with PWS compared to those without the syndrome.

Method: Clinical data of all PWS patients who underwent LSG were abstracted from our prospective database that included all pediatric patients who underwent bariatric surgery. These data were then compared to a 1:3 matched non-PWS for age, gender and BMI. Data for up to 5 years follow-up were analyzed.

Results: The twenty-four PWS patients (mean age=10.7; 6 < 8 years old, range 4.9 to 18) had a preoperative body mass index (BMI) of 46.2 ± 12.2 kg/m². All PWS patients had obstructive sleep apnea (OSA), 62% had dyslipidemia, 43% had hypertension, and 29% had diabetes mellitus. BMI change at the first, second, third, fourth and fifth annual visits was -14.7 (n=22 patients), -15.0 (n=18), -12.2 (n=13), -12.7 (n=11), and -10.7 (n=7), respectively in the PWS group, while the non-PWS group had a BMI change of -15.9 (n=23), -19.1 (n=17), -17.4 (n=15), -18.9 (n=9), and -17.9 (n=8), respectively. No significant difference was observed in postoperative BMI change (p-value 0.2 to 0.7) or growth (postoperative height z-score p-value at each annual visit: 0.07-0.9). 95% of comorbidities in both groups were in remission or improved, with no significant difference in the rate of comorbidity resolution after surgery (p-value 0.72). One patient was readmitted 5 years after surgery with recurrence of OSA and heart failure. No other readmissions occurred, and there were no reoperations, postoperative leak, or other complications. No mortality or major morbidity was observed during the five years of follow-up. Among the PWS patients who reached their follow-up visit timepoints, the total follow-up rate was 94.1%, while in the non-PWS group it was 96%. All patients who missed a follow-up visit were subsequently seen in future follow-ups, and no patient was lost to follow-up in either group.

Conclusions: PWS children and adolescents underwent effective weight loss and resolution of comorbidities after LSG, without mortality, significant morbidity, or slowing of growth. LSG should be offered to obese PWS patients with heightened mortality particularly as no other effective alternative therapy is available.

Summary

Prader-Willi syndrome leads to severe obesity and significant comorbidities. To date, there is no effective treatment for the severe obesity associated with this syndrome. In this study, Laparoscopic sleeve gastrectomy is a solution that safely provides significant resolution of obesity and associated comorbidities, with no major complications.

SPEAKER ABSTRACTS

4.6 - Long-Term Outcome After Biliopancreatic Diversion in Prader-Willi Syndrome

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Background: Improvement in weight control remains the most important goal of any treatment program in Prader-Willi syndrome (PWS). Dietary restriction, physical activity and anorexic drugs, together with behaviour interventions, are generally ineffective to induce a long-term weight loss. Thanks to advances of our understanding of safety and efficacy of bariatric procedures in adults with simple obesity, interest in bariatric surgery has increased also for PWS patients. To date, bariatric surgery experience in PWS is limited, and different procedures have been used with varying success. Malabsorptive procedures such as biliopancreatic diversion (BPD) are not always recommended for PWS due to lack of safety data and concerns about long-term nutritional complications. Moreover, data regarding the long-term outcome of this procedure in PWS are still lacking.

Patients and methods: We report 7 patients (4 males) with genetically confirmed PWS (*5 del15, 2 UPD*), who underwent Scopinaro biliopancreatic diversion after an inability to control food intake with the classical approaches and with a progressive and excessive weight gain. The age of surgery was $17,6 \pm 1,6$ yrs (range: 15,5-20) and the BMI (kg/m^2) was >40 in all cases except one ($47,5 \pm 8,5$). At baseline, severe co-morbidities were present, such as obstructive sleep apnea (OSAS), type 2 diabetes mellitus (T2DM), metabolic syndrome and/or steatohepatitis.

Results: No perioperative complications were observed. After a follow-up period of $15,5 \pm 6,8$ yrs (range 8,4-25,8, mean age at follow-up $33,1 \pm 6,2$ yrs) the maximum weight loss (MWL) was $34,4 \pm 20,3$ kg (7-61). Following BPD, BMI decreased in 3 patients, was stable in 2 subjects and significantly increased in 2 individuals after 8,4 and 25,8 yrs, but in these cases MWL was low (10 and 7 kg, respectively). The mean BMI at follow-up was: $42,4 \pm 8,7$ (29,5-51,6).

After BPD, appetite was reduced in 3 cases; 5 subjects had hypocromic anemia and diarrhea; OSAS were present in 4 pts and osteoporosis/osteopenia in all study group. T2DM improved significantly. One patient suddenly died at the age of 37.3. After surgery all patients received iron, calcium, vitamin D, minerals and multivitamin preparations to prevent nutritional deficiency.

Conclusion: Bariatric surgery should be considered in PWS patients as a realistic control of morbid obesity, when other classical approaches have failed. The outcome of BPD is variable in PWS. It could be a good option in the presence of serious comorbidity and in selected and well-disciplined PWS patients, with co-operating families. However, a careful long-term multidisciplinary follow-up is always necessary.

Summary: Long-term outcome with BPD in PWS are variably satisfactory and are associated with improvement of metabolic abnormalities and important weight loss. However, reduced bone mineral density and diarrhea are frequently reported. In this light, a careful long-term multidisciplinary follow-up is mandatory.

SPEAKER ABSTRACTS

4.7 - Histamine H₃ Receptor Antagonist on Sleep Disturbances

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Histamine is an important neurotransmitter in the peripheral and central nervous system. It is mainly acting via four different membrane bound G-protein coupled receptors. Antagonists for the histamine H1 and H2 receptor subtypes are or have been known blockbuster drugs for the treatment of allergy and ulcer, respectively. The histamine H3 receptor is the main modulator of this transmission. It can act as an auto- as well as heteroreceptor and thereby influencing numerous other neurotransmitter innervation. Among others, this modulation is changing the orexinergic, dopaminergic, serotonergic and acetyl cholinergic pathways. Although many histamine H3 receptor antagonists are in the preclinical phase, some have already reached clinical trials. Due to the numerous interaction and cross-reactions the antagonists have been tested for diverse therapeutic indications from ADHS, schizophrenia, obesity, cognitive impairment, epilepsy and neuropathic pain. The most prominent outcome has been achieved on sleep disorders as a strong wake-enhancing agent. Recently the histamine H3 receptor antagonist pitolisant (Wakix®) has been approved by the European Medicine Agency (EMA) as orphan drug on the treatment of narcolepsy. In addition to this, it is in actual late phase clinical trials on daytime sleepiness with Parkinson patients.

Summary. The sum of the different effects and side effect observations in preclinical and clinical studies may suggest the symptomatic use of histamine H3 receptor antagonists on patients with Prader-Willi syndrome after further clinical trials. At this stage the therapeutic potential is highly speculative, but with the given data on the early clinical data a reduction in disease burden may be achieved.

SPEAKER ABSTRACTS

4.8 - AZP-531, an Unacylated Ghrelin Analog, Improves Food-related Behavior in Patients with Prader-Willi Syndrome: A Multicenter, Randomized, Placebo-controlled Study

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Background: Plasma levels of Acylated Ghrelin (AG), the most potent orexigenic hormone, are elevated in Prader-Willi syndrome (PWS) and are hypothesized to contribute to the pathophysiology of hyperphagia. Administration of unacylated ghrelin and AZP-531, a first-in-class peptide analog, have been shown to prevent AG-induced food consumption in animals, and improves glucose control and decreases weight in animal models and in Phase 1 clinical trials.

Methods and Results: In a multicenter, randomized, placebo-controlled, parallel group study, we investigated the safety, tolerability, and efficacy on food-related behavior of AZP-531 over a 2-week treatment period in 23 male and 24 female patients with genetically confirmed PWS and evidence of hyperphagia (mean age: 26.8 ± 6.7 yrs, range: [13-46 yrs], mean BMI: 38.00 ± 12.01 kg/m², range: [20.6-67.4 kg/m²]). Participants were randomly assigned to receive subcutaneous AZP-531 (3 mg or 4 mg) or placebo once daily. Adverse events were monitored throughout the treatment period and up to Day 28. Study assessments were performed on Day -1 (baseline), Day 1 (after the first injection) and Day 14. AZP-531 was well tolerated with no serious or severe adverse events and no clinically significant changes with respect to safety laboratory tests. A significant improvement in food-related behavior, as assessed by the Hyperphagia questionnaire, was noted in AZP-531-treated patients on Day 14 ($P < 0.05$ vs placebo) with particular improvement in the severity score ($P < 0.05$ vs placebo). Findings were supported by reduction in appetite feelings following breakfast on Day 14, as assessed by a newly developed patient-reported outcome scale. No change was observed in body weight which is not unexpected following a short-term treatment in this study population with highly variable weight and BMI at baseline. However, a significant reduction of waist circumference was noted in the AZP-531 group ($P < 0.05$ vs baseline) and not in placebo (NS vs baseline). In addition, AZP-531 tended to improve glucose control and this improvement was greater in patients with higher fasting or post-prandial glucose levels at baseline.

Conclusions: Daily AZP-531 for 2 weeks was well tolerated in patients with PWS, corroborating findings from previous human studies. Data suggest that AZP-531 may be a beneficial treatment strategy in this patient population with elevated ghrelin levels and support further investigation in larger controlled trials to assess long-term safety and efficacy on food-related behavior and metabolic parameters.

Summary: Blood levels of Acylated Ghrelin (AG), the appetite hormone, are elevated in patients with PWS and are hypothesized to contribute to hyperphagia. Unacylated Ghrelin (UAG) is known to antagonize some effects of AG. In this study performed in 47 patients with PWS, we demonstrated that administration of AZP-531, a fragment of UAG that works similarly as UAG, was well-tolerated and improved food-related behavior.

SPEAKER ABSTRACTS

4.9 - Weight loss and Improvement in Hyperphagia-Related Behavior: Results from best PWS, a Phase 3, Randomized, Placebo-Controlled Clinical Trial of Beloranib in Patients with Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is the leading genetic cause of life-threatening obesity. PWS is associated with reduced life expectancy; insatiable hunger characteristic of PWS can cause gastric necrosis and choking while obesity and other metabolic derangements increase the risk of metabolic disease and cardiorespiratory events. Beloranib inhibits MetAp2, an enzyme that influences metabolism, lipid synthesis, and fat storage, and was recently shown to reduce body weight and hyperphagia in a study of 17 patients with PWS.

Methods and Results: This 26-week clinical trial investigated biweekly beloranib injection in patients with PWS aged 12-65 years. Co-primary efficacy endpoints were change in body weight and hyperphagia-related behavior (measured by Hyperphagia Questionnaire for Clinical Trials [HQ-CT]). The randomized portion of the study was concluded early due to venous thromboembolic events in beloranib clinical trials. The intent-to-treat population included 107 patients: 74 completed the study, 27 stopped early due to halting of the study (these completed >75% of the study), 6 withdrew due to adverse events (AEs). Baseline characteristics: 52% Male, mean±SD age 20±6y, 101.4±26.3kg, BMI 40.0±10.1kg/m², HQ-CT total score 16.9±6.6 (range 0-36). After 26 weeks, the LS mean±SE weight change from baseline for placebo was 4.2±0.9% (N=34) vs -4.1±0.9% with 1.8 mg beloranib (LS mean difference -8.2%, 95% CI, -10.8 to -5.6, p<0.0001, N=36) and -5.3±0.9% with 2.4 mg beloranib (-9.5%, -12.1 to -6.8, p<0.0001, N=37). The LS mean±SE change in HQ-CT total score (reduction indicates improvement) with placebo was -0.4±1.2 vs -6.7±1.1 with 1.8 mg beloranib (LS mean difference -6.3, 95% CI, -9.6 to -3.0, p=0.0003) and -7.4±1.3 with 2.4 mg beloranib (-7.0, -10.5 to -3.6, p=0.0001). Most common AEs were injection site bruising, aggression, and hyperphagia; only injection site bruising was more frequent with beloranib vs placebo. Psychiatric disorder AEs occurred at a higher rate with beloranib, were primarily mild to moderate in severity and generally resolved (baseline incidence of psychiatric disorders: 66%, consistent with the patient population). The incidence of serious AEs was low and similar across treatment groups (2.7-5.9%). Six beloranib-treated patients withdrew due to AEs during the randomized treatment period (4 psychiatric AEs, 1 injection site pain, 1 pulmonary embolism resulting in death). Investigation of the pulmonary embolism leading to death is ongoing.

Conclusions: This is the first Phase 3 clinical trial to show statistically and clinically significant weight loss and improvement in hyperphagia-related behavior in patients with PWS.

Summary: This study investigated the effect of beloranib on body weight and hyperphagia in patients with PWS. Beloranib produced weight loss and improvements in hyperphagia-related behavior compared to placebo. AEs were generally consistent with the patient population and prior clinical trials.

SPEAKER ABSTRACTS

5.1 - Loss of *Magel2* Impairs the Development of Hypothalamic Anorexigenic Circuits

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Background. Prader-Willi syndrome (PWS) is a genetic disorder characterized by a variety of physiological and behavioral dysregulations, including hyperphagia, a condition that can lead to life-threatening obesity. Feeding behavior and body weight regulation are highly complex and dynamic processes with multiple feedback loops that involve both peripheral and central systems. The arcuate nucleus of the hypothalamus (ARH) is a nucleus of the brain that is critical for the regulation of homeostatic processes such as feeding, and this nucleus develops during neonatal life under the influence of both environmental and genetic factors. Although much attention has focused on the metabolic and behavioral outcomes of PWS, an understanding of its effects on the development of hypothalamic appetite-related neural circuits remains elusive.

Methods and Results. We found that mice lacking *Magel2*, one of the genes responsible for the etiology of PWS, display an abnormal development of axonal projections from the ARH. Notably, the density of anorexigenic α -melanocyte-stimulating hormone immunoreactive axons was reduced in adult *Magel2*-null mice, while the density of orexigenic agouti-related peptide fibers in the mutant mice appeared identical to that in control mice. Based on previous findings showing the pivotal role of neonatal leptin and ghrelin in hypothalamic development, we also measured leptin and ghrelin levels in *Magel2*-null and control neonate mice and found that mutant mice have normal leptin and ghrelin levels.

Conclusion. Together, these findings suggest that a loss of *Magel2* leads to the disruption of hypothalamic feeding circuits, an effect that appears to be independent of the neurodevelopmental effects of leptin and ghrelin.

Summary. This work indicates that the development of neurons that cause satiety is attenuated in a mouse model for PWS. These findings might help understanding the insatiable appetite associated with PWS.

SPEAKER ABSTRACTS

5.2 - Appetite suppression by allosterically enhancing dopamine-2 receptor (D2R) signaling in Snord116 deletion mouse model for Prader-Willi syndrome.

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Background. Prader-Willi Syndrome (PWS) is a complex genetic disorder in which cognitive disabilities, hyperphagia and early childhood obesity are major hallmarks. PWS patients have a delayed activation of brain areas involved in satiety, and hyperactivation of neural circuitry involving motivation, reward, taste and food-seeking behavior. Dopamine is a neurotransmitter that regulates feeding behavior, cognitive function, motivation and reward. We had previously demonstrated formation of heteromers between the ghrelin receptor (GHSR1a) and the dopamine-2 receptor (D2R) on hypothalamic neurons is necessary for dopamine suppression of food intake. PWS is associated with hyperghrelinemia that effectively reduces the concentration of GHSR1a on these neurons. We hypothesize that allosterically enhancing interactions between GHSR1a and D2R in GHSR1a:D2R heteromers will reduce appetite in patients with PWS.

Methods and Results. To test our hypothesis two different classes of GHSR1a antagonists (JMV2959 and YIL781) were injected alone or in combination with a D2R selective agonist (cabergoline), into the Snord116^{+/-} mouse model for PWS. Snord116^{+/-} male mice (Het) and littermate controls (WT) were single housed, fasted for 16 hours and injected with either vehicle, GHSR antagonist (JMV or YIL), cabergoline or the combination of the two and then provided food. Food intake was monitored for 24 hours. Cabergoline significantly suppressed food intake, but was blocked by JMV. By contrast, YIL inhibited food intake and enhanced the anorexigenic effect of the D2R agonist; however the effects of YIL were short-lived. We therefore synthesized a library of new GHSR1a antagonists and screened them in HEK293 cell line, co-expressing GHSR1a and D2R for their effects on Ca²⁺ mobilization. All the compounds inhibited ghrelin-induced Ca⁺⁺ release, and subsets either inhibited (Type A) or enhanced (Type B) dopamine-induced Ca⁺⁺ mobilization. The most potent Type A and B antagonists were tested for inhibition of food intake in Snord116^{+/-} mice in the presence or absence of cabergoline. Type B antagonists enhanced the anorexigenic effect of cabergoline.

Conclusions. We have identified a new class of GHSR1a antagonists that block ghrelin signaling, but enhance D2R agonist-induced reduction in food consumption in the Snord116^{+/-} mouse model of PWS.

Summary. Allosteric modulation of GHSR1a:D2R signaling has the potential to inhibit hyperphagia associated with PWS, providing new opportunities to design pharmacologic agents for therapeutic intervention.

SPEAKER ABSTRACTS

5.3 - Sleep phenotype of Prader-Willi Syndrome is partially recapitulated in MAGEL2 null mice

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Background. Daytime sleepiness, disrupted sleep, and cataplexy-like falling episodes are common in Prader-Willi Syndrome (PWS), but the cause of these symptoms is unknown.

Methods and Results. Using EEG, EMG, and video recordings, we have examined sleep/wake behavior in MAGEL2 null mice, a model of PWS. These mice have normal total amounts of wake (W), Non-REM sleep (NR), and REM sleep (R) when housed on a 12:12 light:dark cycle. However, compared to their wild type littermates (WT), MAGEL2 null mice have shorter NR bouts during the dark and light phases. Additionally, MAGEL2 null mice have more bouts of W and NR during the dark and light phase. MAGEL2 null mice did not have any cataplexy, even with stimuli that should elicit positive emotions.

Conclusion. These short bouts and frequent state transitions indicate that MAGEL2 null mice lack the ability to maintain sleep for long periods.

Summary. These findings in MAGEL2 null mice are similar to the sleepiness and disrupted sleep common in PWS. Future research targeting orexin and oxytocin signaling, which are known to be altered with MAGEL2 deletion, should provide helpful insights into the nighttime awakenings and daytime sleepiness experienced by many people with PWS.

Support: Foundation for Prader-Willi Research

SPEAKER ABSTRACTS

5.4 - Fluoxetine is an effective treatment to reduce respiratory distress in *Necdin* deficient mice

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Background

Prader-Willi syndrome (PW) is a rare neurodevelopmental disorder caused by the genetic loss of paternal expression of several maternally imprinted genes on chromosome 15q11–13, among which *NECDIN*. The symptoms include a various range of sensori-motor, endocrine, cognitive and behavioural disturbances, including breathing deficits starting at birth such as apnea and blunted respiratory responses to hypercapnia. In mice, loss of *Necdin* recapitulates these breathing deficits found in PW patients and it is associated with brain serotonin homeostasis abnormality suggesting the involvement of a serotonopathy in such respiratory symptoms. However, the mechanism at the basis of this pathophysiology is unknown.

Methods and Results

We have investigated the expression of *Necdin* in the 5HT neurons and observed that *Necdin* is expressed at the beginning of the serotonergic lineage. During embryonic development, lack of *Necdin* leads to defects in migration of 5-HT neurons, to an altered 5-HT neuroarchitecture, and to a loss of 5-HT neurons. *Necdin* KO mice present serotonergic dystrophic neurons with enlarged varicosities associated and with increase in spontaneous firing. At the molecular level, we found an increase of 5-HT reuptake activity and an increase expression of the 5-HT transporter. Considering these last results, we assessed in vivo the efficiency of fluoxetine, an inhibitor of 5-HT reuptake, to rescue the respiratory deficits. We demonstrated that during the neonatal period, fluoxetine administration can restore the respiratory response to hypercapnia and reduce the number of apnea.

Conclusions

On the whole, we show that lack of *Necdin* has pleotropic effects on the serotonergic system. However a fluoxetine treatment, allowing an increase of extracellular 5HT, appears sufficient to restore normal breathing in *Necdin* deficient mice.

Summary

The *Necdin* mutant mouse recapitulates the respiratory deficits found in PW patient and thus permitted us to investigate the pathophysiology of this symptom. We found that the homeostasis of brain serotonin, a modulator of breathing activity, is dysregulated and that treatment of mutant newborn mice with fluoxetine can reduce respiratory apnea.

SPEAKER ABSTRACTS

5.5 - Fatty Acyl Amides as Novel Therapeutics for the Treatment of Osteoporosis in PWS

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Background: Decreased bone mineral density (BMD), increased fracture risk secondary to osteoporosis, and short stature are common features of individuals with Prader-Willi syndrome (PWS), affecting 60% to 90% of the patients. Recently, we described the presence of an endogenous long-chain fatty acyl amide, *N*-oleoyl serine (OS), in bone, and demonstrated its critical involvement in the regulation of bone remodeling and osteoporosis in mice.

Methods and Results: Here, we characterized the skeletal phenotype of an established mouse model for PWS, *Magel2*-null mice, and tested the skeletal effects of a newly synthesized OS derivative, named oleoyl α -methyl serine (KAL671), in this model.

In comparison with wild-type control animals, female *Magel2*-null mice showed a low bone mass phenotype, resulting from a combined reductions in trabecular number, connectivity density and cortical thickness. In vitro, KAL671 enhanced the number of osteoblasts, the bone forming cells, and inhibited bone resorption and osteoclastogenesis. In vivo, chronic treatment with KAL671 (0.5 mg/kg for 6 weeks), completely prevented the trabecular bone loss observed in vehicle-treated *Magel2*-null mice. The KAL671-stimulated increase in trabecular bone formation occurred mainly due to increased trabecular number and thickness. Moreover, KAL671 treatment completely rescued the reduction in the femoral cortical thickness.

Conclusions: Our data identify the importance of *Magel2* gene in the regulation of bone remodeling and mass in PWS, and describe the skeletal efficacy of a novel fatty acyl amide for the treatment of osteoporosis-like features in *Magel2*-null mice. Moreover, the present study provides a preclinical proof for further clinical development of KAL671, as a bone anabolic and anti-resorptive agent, for the treatment of osteoporosis in PWS.

Summary: Using an established mouse model for PWS, cell culture experiments and a novel pharmacological tool, this project significantly contributes to the understanding of one of the major pathological processes associated with PWS, osteoporosis, and may enhance the translation of our basic scientific findings to the development of therapeutic interventions for PWS.

SPEAKER ABSTRACTS

5.6 - Genomic imprinting: a new epigenetic regulation of sleep

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Background. Sleep-wake and food-intake disturbances are often reported in Prader-Willi Syndrome (PWS). PWS is a rare neurodevelopmental disorder that is caused by genomic imprinting defects within the small nuclear ribonucleoprotein N (*SNPRN*) cluster of the human chromosome region 15q11-13. The small nucleolar RNA 116 (*SNORD116*, also called HBII-85) gene, a non-coding molecule that participates in the modifications of other small nuclear RNAs has been identified as one of the key players for this syndrome.

Methods. To investigate the link between imprinting defects, sleep and food-intake behaviors in PWS, we studied the mouse mutant model *PWScr^{m+/p-}*, which lacks *Snord116* in the orthologous locus on chromosome 7C. The chromosomal region that is involved in the pathogenesis of PWS comprises a number of imprinted genes that present complex parent-of-origin expression. Some genes are preferentially expressed from the paternal allele while others from the maternal allele. The monoallelically expression of many imprinted genes is tissue-dependent and has been confirmed only in development, neglecting the imprinting status in adulthood. The deleted allele carried by our mutants is placed within the critical region of PWS in which paternally expressed small nucleolar RNAs (snoRNAs) resides. We focused our attention on mice carrying the paternally inherited deletion, since maternally inheritance is phenotypically similar to wild-types.

Results. We observed specific electrophysiological deficits during sleep, which suggest that paternally expressed *Snord116* is involved in the 24-h regulation of sleep-wake cycles. Moreover, we reported important changes in timing behaviors across timescales. In particular, *PWScr^{m+/p-}* mutants show delays in seconds-to-minutes (interval timing) cognitive processes and in food anticipatory activity (FAA) compared to wild-type littermate controls.

Conclusions. In this study we discovered a novel role of the *Snord116* imprinted gene in sleep and working-for-food timing behaviors. We report, for the first time, that imprinting of *Snord116* occurs in mouse brain structures that are important for sleep and timing behaviors.

Summary. We annotate specific sleep and behavioural/cognitive abnormalities as direct and/or indirect endophenotypes of *Snord116* and *Ipw A-C* deletion within the Prader-Willi genomic region. This report adds translational validity of the *PWScr^{m+/p-}* murine model to the investigation of PWS.

SPEAKER ABSTRACTS

6.1 - The Global Prader-Willi Syndrome Registry

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Background: The Global Prader-Willi Syndrome Registry was developed to support and accelerate PWS research and clinical care. The Registry has an Institutional Review Board (IRB)-approved protocol and consent/assent process, and aims to: document the full range of the PWS phenotype to better understand the natural history of the disorder; enable data trend analysis to generate new insights into PWS and identify areas for additional study; facilitate partnerships with academic researchers and pharmaceutical companies; guide the development of standards of care; expedite the completion of PWS clinical trials; and allow participants to store their PWS medical data in one place. The Registry is managed and funded by the Foundation for Prader-Willi Research (FPWR), is hosted by the National Organization of Rare Disorders (NORD), and is a participant in the NIH-supported Global Rare Disease Registry.

Methods and Results: The Global PWS Registry launched in May 2015 and has ~800 participants as of early 2016. It is comprised of a series of 37 web-based surveys covering medical history, developmental history, behavior, mental health, medications and quality of life. The platform is flexible and allows the capture of longitudinal data and addition of new surveys as needed. Data is self-reported by a parent or guardian of individuals with PWS, with future plans for a clinical portal that will link physician entered to participant reported data through a global unique identifier (GUI). The initial marketing and enrollment push has been through social media, newsletters, and e-mails from the Foundation for Prader-Willi Research (FPWR) and the Prader-Willi Syndrome Association (PWSA(USA)). A number of help resources have been created to guide families through the registration and informed consent process. These include a “getting started video”, a “getting started PDF”, a private Facebook group, webinars, brochures, FAQ documents, and presentations at family conferences. The second phase includes working with group homes, PWS clinics, physicians, and PWS groups globally. There are plans for the registry to be available in multiple languages.

Conclusions:

The Global PWS Registry successfully launched in 2015. With growing participation, the registry is poised to begin leveraging de-identified data through collaborations with researchers, industry, and other partners to advance the understanding and treatment of PWS.

Summary:

The Global PWS Registry is a web-based natural history study. The Registry launched in 2015 and the coming year includes plans to continue enrollment, promote survey completion, and begin leveraging de-identified data through collaborations with researchers, companies, and other parties involved in advancing solutions for PWS.

SPEAKER ABSTRACTS

6.2 - Multidisciplinary outpatients clinic for adults with Prader-Willi syndrome: the Rotterdam experience

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Introduction Health issues in people with Prader-Willi Syndrome (PWS) show a wide variability, ranging from mild, to complex and severe. In the Netherlands, multidisciplinary health care for PWS children is well organized. However, for adults, multidisciplinary care is not available. Since part of the health problems in PWS can be prevented by good medical support, we have launched a multidisciplinary outpatients clinic (OPC) for adults with PWS. This PWS-OPC is part of the Dutch National Expert Center for PWS, a collaboration between the university hospitals of Nijmegen, Maastricht and Rotterdam which is currently being launched. The aim of the OPC is to improve quality of life of adults with PWS by 1. treating underdiagnosed health issues 2. supporting patients and caregivers in dealing with behavioral problems 3. to treat obesity using a specialized diet.

Methods The multidisciplinary PWS-OPC took place in the Erasmus MC of Rotterdam. All patients visited the endocrinologist, the physician for people with intellectual disabilities, the pedagogist-psychologist and the dietitian. Before the first visit, we asked all the patients for their expectations regarding the multidisciplinary visit. During the visit, we collected data regarding age, sex, genetic subtype, living situation and medication, including growth hormone (GH) treatment, past and current health problems, as well as behavioral problems. Data of complete physical examination including blood pressure, BMI, PWS-related physical characteristics, edema and skin picking scars were also collected. After the visit, we collected laboratory data considering hematology, kidney function, liver function, electrolytes and pituitary hormone levels and we handed out patients satisfaction questionnaires.

Results We will present data of the first 40 patients visiting the multidisciplinary PWS-OPC. Patients were either young adults from the children's cohort of the Dutch Growth Research Foundation, or new patients referred by the General Practitioner. We will show an overview of demographic data, frequencies and types of past and current medical treatment, behavioral problems as well as physical parameters. Furthermore, we will report on pharmacological treatments (including psychiatric medication), behavioral, physical and biochemical data. We will present all relevant parameters according to genetic subtype and the presence or absence of GH treatment. Finally, we will show the results of the patients' satisfaction questionnaires as a tool for colleagues who want to launch a similar multidisciplinary OPC.

Conclusion Within the Erasmus MC, we have launched a new multidisciplinary outpatients clinic for adults with Prader-Willi syndrome. As expected, patients on GH treatment had less physical problems and less behavioral problems than GH-naïve individuals. Hypogonadism was the main underdiagnosed health issue. Satisfaction questionnaires show that patients appreciate both the medical, behavioral and nutritional support.

Summary: We have launched a new outpatients clinic (OPC) in Rotterdam, the Netherlands, where adults with PWS are supported by a team of PWS experts. We will show the (anonymized) data of the first 40 patients and show their opinion regarding the OPC.

SPEAKER ABSTRACTS

6.3 - Venous Thromboembolism in Prader-Willi Syndrome: A Questionnaire Survey

Ann M. Manzardo¹, Merlin G. Butler¹, Janalee Heinemann², Barbara McManus², Carolyn Loker², James Loker³

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Introduction: Prader-Willi Syndrome Association (USA) monitors the ongoing health and welfare of individuals with PWS through active communication with members, membership surveys and data registries (e.g., Causes of Death Registry established in 1973). Pulmonary embolism has recently emerged in clinical studies and data reviews as a significant risk factor for injury and death in Prader-Willi syndrome (PWS). Prader-Willi Syndrome Association (USA) conducted a survey of its membership to advance understanding and further characterize the frequency, risk and protective factors of thromboembolism in PWS.

Methods: A 66-item questionnaire probing the frequency of venous thromboembolism, pulmonary embolism and related characteristics including obesity, health problems, medications and family history of clots was developed by a panel of PWS medical and scientific experts with input from Prader-Willi Syndrome Association (USA) leadership. The survey was administered electronically to the Prader-Willi Syndrome Association (USA) membership with confirmed and active e-mail addresses. An interim analysis of descriptive data including means, standard deviations and frequencies of blood clots was carried out on data from the first round of respondents. The characteristics of those with and without reported history of blood clots were tabulated and compared.

Results: Interim results from 539 current responders (277 females, 262 males) identified 22 (4%; 14 females, 8 males) individuals with a history of blood clots. There were no differences in gender, but individuals with clots were significantly older 27.6 ± 16 yrs v 18.2 ± 12 yrs ($F=12$, $p<0.001$) and more likely to have a reported history of obesity ($N=16$, 76%), edema ($N=13$, 59%), hypertension ($N=5$, 24%), vasculitis ($N=6$, 33%) and family history of blood clots ($N=7$, 33% with clots; $N=73$, 15% without clots, $\chi^2=4.9$, $p<0.03$) than individuals without clots. Individuals with clots were less likely to have received growth hormone ($N=10$, 45% with clots vs $N=408$; 81% without clots, $\chi^2=16$, $p<0.0001$) than individuals without clots.

Summary: The preliminary results suggest an increased risk of venous thromboembolism in PWS with increased age, obesity history, lower extremity edema, vasculitis, and family history of clots. Further study is needed to evaluate the effectiveness of interventions such as weight loss, anticoagulation, risks associated with genetic predisposition and PWS subtype and potential benefits of growth hormone therapy on the causation and development of thromboembolism in PWS.

SPEAKER ABSTRACTS

6.4 - A PWS clinic – is it worth the effort?

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Boden Institute, University of Sydney, Sydney Australia

Prader-Willi Syndrome (PWS) is a genetic syndrome associated with severe obesity and a historic life expectancy of late 20s. PWS obesity results from an energy imbalance influenced by hypothalamic dysfunction. People with PWS have cognitive limitations, emotional immaturity and often demonstrate behavioural outbursts. Maintaining health in this population is difficult and commonly requires unpopular strategies. Medical presentations can differ markedly from the general population, thus complicating or delaying management. Given the complexities of this syndrome, we sought to identify a successful model of care for people with PWS. Evaluation of the PWS clinic in a large Australian public teaching hospital was undertaken to determine if a specific clinic achieves meaningful outcomes for clients with PWS and their families/carers and to determine what methods were most effective in the treatment of their obesity. Data collected over 24 years was used to determine weight and health outcomes.

Eighty six patients diagnosed with PWS have attended the clinic, 46 of whom have attended regularly. The mean age was 30 yrs (range 11 to 59) with 5 patients >50yrs (to December 2015). 20% of patients are currently in the healthy or overweight range. The mean maximum BMI of the patients was 47kg/m² and the mean BMI of the clinic population at present is 40 kg/m² (range 22 – 76). Of the cohort 17 have died, five of whom resided in appropriate residential care. 81% of deaths were from obesity related complications.

Residential care was the most important marker for successful treatment. Restrictive practices, consistent management and home-based exercise equipment contributed to superior results. Preliminary carer feedback indicated specialised healthcare, training of caregivers and advocacy were the most beneficial components of the clinic.

Summary: A specialised PWS clinic effectively addresses the complex needs of this population. It also provides support and education for their families, caregivers and health professionals contributing to greater longevity and improved health for the client.

SPEAKER ABSTRACTS

6.5 - Multicenter study of early screening and prevention of Prader-Willi Syndrome

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Background: The current diagnostic criteria for Prader-Willi syndrome (PWS) is proposed by Holm *et al.* in 1993. Although the criteria are widely accepted, it was challenging to be implemented in Chinese population. The present study collected PWS cases from 12 centers across China. By analyzing the clinical manifestation during early infancy, we aimed to provide data for clinical characteristics, screening strategy and effect of growth hormone (GH) treatment in Chinese PWS patients.

Methods and Results: We screened 63 suspected PWS cases in 11 centers from May-2012 to Aug-2013 using MS-PCR. Patients diagnosed by MS-PCR further underwent analysis by MS-MPLA and STR to identify PWS genetic markers. Data on patients' history, clinical manifestation, anthropometrics and clinical biochemistry test before/after GH treatment were collected for analysis. Among our enrolled subjects, 16 were confirmed by MS-PCR. Further analysis using MS-MLPA and STR analysis showed that 13 were associated with paternal deletion while the rest 3 were maternal uniparental disomy (mUPD). Among the 16 diagnosed PWS, 13 were delivered at full term, 1 were preterm birth, 2 postterms, 4 delivered vaginally, 12 delivered by cesarean section. Fetal distress was diagnosed in 10 cases while abnormal fetal position found in 5 cases. All patients had reduced fetal movement, hypotonia and infant feeding difficulties. Characteristic facial appearance was found in 6 cases when 13 showed hypogonadism, 8 had hypopigmentation. There were 4 patients received rhGH treatment. When we found patients treated with GH had improved physical development, no difference was found in thyroid function, plasma IGF-1 levels, fasting blood glucose, fasting insulin levels and blood lipid levels.

Conclusions: PWS might account for 25% of infants with idiopathic hypotonia and infant feeding difficulties. Screening using MS-PCR in suspected cases is critical to identify PWS patients. Hypogonadism and hypopigmentation are important clues for diagnosis. GH treatment during infancy can improve physical development in PWS patients, however how to improve cognitive development and function of endocrine system in PWS patients requires future studies.

Summary: We screened 63 neonates suspicious of PWS with idiopathic hypotonia and feeding difficulties among 11 Chinese centers during the period from May-2012 to Aug-2013. 16 were confirmed by genetic analysis, 13 with paternal deletion and 3 with maternal uniparental disomy. 4 patients treated with GH had improved physical development, no difference was found in thyroid function, plasma IGF-1 levels, fasting blood glucose, fasting insulin levels and blood lipid levels.

**SCIENTIFIC
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P1. Ayabe, Tadayuki. Reduced cerebrospinal fluid orexin level in Prader-Willi syndrome patients with excessive daytime sleepiness. Tadayuki Ayabe, MD, Mayu Omokawa, MD, Yuji Oto, MD, Maki Fukami, MD, PhD, Nobuyuki Murakami, MD, Takashi Kanbayashi, MD, Toshiro Nagai, MD. National Research Institute for Child Health and Development, Tokyo, Japan

P2. Galesanu, Corina. Hypogonadism in a Female with Prader Willi Syndrome. Case Report. Corina Galesanu. University of Medicine and Pharmacy “Gr.T.Popa”, Iasi- Romania

P3. Oto, Yuji. Latent Central Adrenal Insufficiency may exist in some patients with Prader-Willi syndrome. Yuji Oto, Keiko Matsubara, Tadayuki Ayabe, Nobuyuki Murakami, Tomoyo Matsubara, Hiroyuki Ogata, Masao Gito, Hiroshi Ihara, Toshiro Nagai. Dokkyo Medical University Koshigaya Hospital, Saitama, Japan

P4. Murakami, Nobuyuki. Association of atrophy and asymmetry of the paravertebral muscles with the development of scoliosis in Prader-Willi syndrome. Nobuyuki Murakami, MD, Yuji Oto, MD, Tadayuki Ayabe, MD, Tomoyo Matsubara, MD, Toshiro Nagai, MD. Dokkyo Medical University Koshigaya Hospital, Saitama, Japan

P5. Vijaykumar, Padmavathi. Analysis Of Chromosomal Abnormalities And Genomic Imprinting Gene At SNRPN- SNURF In Prader Willi Syndrome –Tamilnadu Population. Padmavathi Vijaykumar, Keshavarao Sasikala and Vellingiri Balachandar. Department of Human Genetics and Molecular Biology, School of Life Sciences, Bharathiar University, Coimbatore, India.

P6. Passone, Caroline. PWS in Brazil: 6 months follow-up in a reference center. Caroline de Gouveia Buff Passone MD, Simone Sakura Ito MD, Ruth Rocha MD, Durval Damiani MD PhD. Pediatric Endocrinologist Instituto da Criança- São Paulo University- Brazil

P7. Ito, Simone. Obese Prader-Willi versus Obese controls: Metabolic Profile in Brazilian Patients. Simone Sakura Ito MD, Caroline de Gouveia Buff Passone MD, Ruth Franco Rocha MD, Durval Damiani MD PhD. Pediatric Endocrinologist Instituto da Criança- São Paulo University- Brazil

P8. Salles, Juliette. Deficits in voice and multisensory processing in patients with Prader-Willi syndrome. J. Salles, K. Strelnikov, C Mantoulan, D. Thuilleaux, V. Laurier, C. Molinas, M. Tauber and P. Barone. Centre de Référence du Syndrome de Prader-Willi, Hôpital des Enfants, Toulouse, INSERM, France

P9. Hasegawa, Tomoko. A Young Adult with PWS Encourages a Mother of a Baby with PWS by Emails. Tomoko Hasegawa, Gaku Harada, Fumiko Harada, Tomoko Iwasaki. PWSA Japan

P10. Matsubara, Keiko. Risk assessment of medically assisted reproduction and advanced maternal ages in the development of Prader-Willi syndrome due to UPD(15)mat. Keiko Matsubara*, Nobuyuki Murakami, Maki Fukami, Masayo Kagami, Toshiro Nagai, and Tsutomu Ogata*. National Research Institute for Child Health and Development, Japan

P11. Stegmann, Jorgelina. Are frequent the Cholethiasis in subjects with Prader Willi Syndrome? Stegmann, Jorgelina; Quaglia, Federico C.A.; Biasatti, Cintia; Ceccomancini, Romina; Paradiso, Alex D. SPINE Foundation, Buenos Aires, Argentina

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P12. Camblats, Anna-Malika. PRASOC project: Study of the cognitive capacities to process emotional and social informations in the Prader-Willi syndrome. A.-M. Camblats, J. Maire, J. Chevalère, S. Roux, S. Mathey, C. Bellamy, M. Glattard, M. Tauber, D. Thuilleaux, V. Laurier, F. Besnier, & V. Postal. Université de Bordeaux, Bordeaux, France

P13. Diene, Gwenaëlle. French experience of assessment of hyperphagia in PWS patients. G. Diene, C. Molinas, S. Faye, I. Kieffer, M. Valette, M. Glattard, B. Jouret, S. Cabal-Berthoumieu, C. Noual, M. Tauber. Centre de Référence pour le Syndrome de Prader-Willi; Hôpital des Enfants, Toulouse, France

P14. Kalarchian, Melissa. Weight Management for Youth with Prader-Willi Syndrome: Delivery to Parents via Telemedicine. Melissa A. Kalarchian, PhD, Jennifer P. Elliott, PharmD, Khalid M. Kamal, M. Pharm., PhD, Roxann Diez Gross, PhD, Heather Roach, MS, Gina Salvatori, RD, LDN, Gregory Cherpes, MD. Duquesne University and the Children's Institute of Pittsburgh, USA

P15. Achutegui, Irune. PWS & Self-Determination. Helping Parents & Teachers To Enhance Child Resources To Grow In Autonomy. Irune Achutegui. San Raffaele Hospital, Milan, Italy

P16. Bellamy, Caroline. First results of the larger study on the evaluation of the impact of cognitive and executive abilities on socialization difficulties in childhood with PWS. Bellamy C., Glattard M., Camblats A., Chevalère J., Diene G., Cabal-berthoumieu S., Tricot J., Thuilleaux D, Besnier F., Chirossel C, Tauber M., Postal.V. Centre de Référence pour le Syndrome de Prader-Willi; Hôpital des Enfants, Toulouse, France

P17. Kato, Yoshiro. Manifestation of behavioral problems and BMI scores in person with PWS by age groups, and living condition of adults with PWS in Japan. Yoshiro KATO a, Koji MINOSAKI. Kansai University of Welfare Sciences, Japan

P18. Tauber, Maïthé. Cerebellar hyperperfusion in PWS patients: the link for satiety? C. Mantoulan, PhD, P. Payoux, MD, K. Boulanouar, G. Diene, MD, M. Glattard, PhD, B. Rogé, PhD, C. Molinas, A. Sevely, MD, P. Celsis, PhD, M. Tauber, MD. Centre de Référence pour le Syndrome de Prader-Willi; Hôpital des Enfants, Toulouse, France

P19. Aman, Lucie. Psychosis in Prader-Willi syndrome. Lucie Aman, Katherine Manning, Anthony Holland. University of Cambridge, UK

P20. Chevalère, Johann. Relationship between electrodermal activity and behavioural responses to inhibition and working memory updating tasks in Prader-Willi syndrome: A sub-section of the PRASOC project. J. Chevalère, J. Jauregui, P. Copet, V. Laurier, D. Thuilleaux, A.M. Camblats, C. Bellamy, M. Tauber, & V. Postal. Université de Bordeaux, Bordeaux, France

P21. Eggert, Rachelle. Muscle development and function in a Magel2 mouse model of Prader-Willi syndrome. Rachelle Eggert, Ain A. Kamaludin and Rachel Wevrick. University of Alberta, Edmonton, AB, Canada

P22. Debladis, Jimmy. Altered gaze behavior during face processing in patients with Prader-Willi syndrome. J. Debladis, K. Strelnikov, C Mantoulan, J Salles, D. Thuilleaux, V. Laurier, C. Molinas, M. Tauber and P. Barone. Centre de Référence du Syndrome de Prader-Willi, Hôpital des Enfants, Toulouse, INSERM, France

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P23. Wijesuriya, Methsala. Role of the Prader-Willi Syndrome protein MAGEL2 in intracellular pathways regulating the leptin receptor. T.Methsala Wijesuriya, K. Vanessa Carias, Leentje De Ceuninck, Jan Tavernier and Rachel Wevrick. University of Alberta, Edmonton, AB, Canada

P24. Fountain, Michael. Elucidating the neurobehavioral phenotypes of Magel2-null mice. Michael D. Fountain, Huifang Tao, Chun-An Chen, Jiani Yin, Christian P. Schaaf. Baylor College of Medicine, USA

P25. Sanderson, Matthea. Interactions between the Prader-Willi syndrome candidate protein MAGEL2 and neuronal proteins. Matthea Sanderson, Herman Cortes, and Rachel Wevrick. University of Alberta, Edmonton, AB, Canada

P26. Wishnefsky, Elana. The Impact of Hyperphagia and Food Restriction on Siblings of Individuals with Prader-Willi Syndrome. Elana Wishnefsky, Anne Matthews, Shawn McCandless, Michelle Merrill, Aaron Goldenberg. Case Western Reserve University, USA

P27. Carias, Vanessa. The role of MAGEL2, a protein implicated in Prader-Willi syndrome, in the regulation of RING-zinc finger-type E3 ubiquitin ligases. Vanessa Carias and Rachel Wevrick. University of Alberta, Edmonton, AB, Canada

P28. Woodcock, Kate. Characteristics of change triggered temper outbursts in children with PWS and ASD and how these may impact on the efficacy of behavioural intervention strategies. Kate Anne Woodcock, Clare McGeady, Glenda Preston, Aine Fitzpatrick, Nigel Robb, Katerina Dounavi & Leah Bull. School of Psychology, Queen's University Belfast

P29. Roof, Elizabeth. Beloranib Improves Hyperphagia-Related Behavior: Results from a Phase 3, Randomized, Placebo-Controlled Clinical Trial of Beloranib in Patients with Prader-Willi Syndrome. Elizabeth Roof, Jaret Malloy, Dongliang Zhuang, Nerissa Kreher, Elizabeth M Dykens. Vanderbilt University, Nashville, TN, USA

P30. Even-Zohar, Noa. Parental approaches regarding prenatal diagnosis and termination of pregnancy in Prader Willi syndrome. Noa Even- Zohar, Talia Geva-Eldar, Yehuda Polak, Harry Hirsch, Itai Gross, Varda Gross-Tsur. Multidisciplinary Prader-Willi Syndrome Clinic, Shaare Zedek Medical Center, Jerusalem, Israel

P31. Biliou, Vasiliki. Correlation Of Five Distinct Characteristics Of Prader-Willi Syndrome: A Four Year Case Study On A Daily (School Days) Basis. Biliou Vasiliki¹, Christina Theodoropoulou, Efstratios Alexandrou. National Association of Prader-Willi Syndrome Hellas, Athens, Greece

P32. Chanioti, Eleni. Determining The Mathematical Profile Of A Child With Prader-Willi Syndrome And Applying The Constructivism Model As A Learning Tool. Chanioti, Eleni. Scientific Advisor to the National Association for Prader-Willi Syndrome Hellas, Greece

P33. Haqq, Andrea. Abnormal body composition phenotype in children with Prader-Willi Syndrome. Camila E. Orsso, Michelle Mackenzie, Carla M. M. Prado, Angela Alberga, Andrea M. Haqq. Department of Pediatrics, University of Alberta, Canada.

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P34. Weisensel, Nicolette. Methylene tetrahydrofolate reductase (MTHFR) polymorphisms, genetic subtype, psychiatric diagnosis and symptom severity in PWS adults residing in group homes. Nicolette Weisensel MD, Marge Royle PhD, Linda Gourash MD and Janice Forster MD. Prader-Willi Homes of Oconomowoc and Marion University, Wisconsin, USA

P35. Kimonis, Virginia. Early Diagnosis of Prader-Willi syndrome delays onset of obesity. Virginia Kimonis, MD, Roy Tamura, June-Anne Gold, MD, Abhilasha Surampalli, Javeria Manazir, Jennifer L. Miller, MD, Elizabeth Roof, MA, Elisabeth Dykens, PhD, Merlin G. Butler, MD, PhD, and Daniel J. Driscoll, MD, PhD. University of California, Irvine, California, USA

P36. Stamm, Stefan. An oligonucleotide changes alternative splicing of the serotonin receptor 2C, reduces food intake in mice and could substitute SNORD115. Zhaiyi Zhang, Marina Falaleeva and Stefan Stamm, University of Kentucky, Lexington, KY, USA

P37. Forster, Janice. Von Economo neurons (VENs) in post mortem brains in PWS: a preliminary report. Janice Forster MD, Merina Varghese PhD and Patrick Hof MD. Pittsburgh Partnership, Pittsburgh, PA, USA

P38. Muscatelli, Françoise. Functional and therapeutical investigation of the oxytocin system in the Magel2-deficient mouse model. Françoise Muscatelli, Valery Matarazzo, Fabienne Schaller, Alessandra Bertoni, Marie-Solenne Felix, Laura Caccialupi, Claude Villard, Amélie M Borie, Michel G. Désarmenien. Institut de Neurobiologie de la Méditerranée, Marseille, France.

P39. Mathieux, Elodie. An in vitro model of Prader-Willi Syndrome by generation of hypothalamic neurons and genome editing of induced pluripotent stem cells. Elodie Mathieux, Erin Banda, Chris Stoddard, Heather Glatt-Deeley, Maeva Langouet, Leann Crandall and Marc Lalande. Stem Cell Institute and Institute for Systems Genomics University of Connecticut, Farmington, CT, USA

P40. Melki, Judith. Truncating Mutations of MAGEL2, a Gene within the Prader-Willi Locus, Are Responsible for Fetal Akinesia and Arthrogyrosis. Dan Mejlachowicz, Flora Nolent, Jérôme Maluenda, Hanitra Ranjatoelina-Randrianaivo, Fabienne Giuliano, Ivo Gut, Damien Sternberg, Annie Laquerrière, and Judith Melki. Inserm and University of Paris-Saclay, Le Kremlin Bicêtre, France

P41. Manning, Katherine. Brain and Behaviour in PWS. Katherine Manning, Roger Tait, John Suckling, Howard Ring, Anthony Holland. University of Cambridge, UK

P42. Woodcock, Kate. The development of resistance to change in individuals with PWS. Kate Anne Woodcock & Elaine Laura Haig. School of Psychology, Queen's University Belfast

P43. Mahoney, Carrie E. Oxytocin neurons promote arousal in a mouse model of PWS. Carrie E. Mahoney, PhD, Valery Grinevich, MD, PhD, and Thomas E. Scammell, MD. Beth Israel Deaconess Medical Center, Harvard Medical School, USA

P44. Salehi, Parisa. Abnormal Videofluoroscopic Swallow Studies (VFSS) in Infants with Prader-Willi Syndrome Indicate a High Rate of Silent Aspiration. Parisa Salehi, MD, Maida Chen, MD, Anita E. Beck, MD, PhD, Amber McAfee, ARNP, Soo-Jeong Kim, MD, Lisa Herzig, MD, Anne Leavitt, MD. Seattle Children's Hospital, Division of Endocrine, University of Washington, USA

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P45. Tam, Joseph. Role of the Endocannabinoid System in Prader-Willi Syndrome. Ibrahim Knani, Brian J. Earley, Shahar Azar, Saja Baraghithy, Shiran Udi, Anna Permyakova, Rivka Hadar, Daniela P. Reyes-Capo, Harry J Hirsch, Talia Eldar-Geva, Itai Gross, Andrea M. Haqq, Joan C. Han, Varda Gross-Tsur, Rachel Wevrick, Joseph Tam. The Hebrew University of Jerusalem, Jerusalem Israel

P46. Diez Gross, Roxann. Discovery of Dysphagia in Children and Adults with Prader-Willi Syndrome. Roxann Diez Gross PhD, CCC-SLP, Ronit G. Gisser, MSc, CCC-SLP, Gregory L. Cherpes, MD, Katie Hartman, MA, CCC-SLP, Rishi Maheshwary, MD. The Children's Institute of Pittsburgh, Pittsburgh, Pennsylvania, USA

POSTER ABSTRACTS

POSTER ABSTRACTS

P1 - Reduced cerebrospinal fluid orexin level in Prader-Willi syndrome patients with excessive daytime sleepiness

Tadayuki Ayabe, MD^{1,2}, Mayu Omokawa, MD³, Yuji Oto, MD², Maki Fukami, MD, PhD¹, Nobuyuki Murakami, MD², Hiroshi Ihara, Professor⁴, Takashi Kanbayashi, MD³, Toshiro Nagai, MD^{2,5}

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Background: Patients with Prader-Willi syndrome (PWS) often show excessive daytime sleepiness (EDS). The pathogenic mechanism is assumed that obstructive sleep apnea syndrome and hypothalamic dysfunction are both implicated. Orexin (known as hypocretin) is a hypothalamic neuropeptide known to present in lower levels in the cerebrospinal fluid (CSF) of patients with sleep disorders such as narcolepsy compared with healthy individuals. The objectives of this study are to investigate the reduction in CSF orexin concentration, and the relationship between the severity of EDS and CSF orexin concentration in Japanese PWS patients.

Methods and Results: The CSF orexin levels were evaluated in 12 patients (9 males and 3 females) of the Pediatric Department of Dokkyo Medical University Koshigaya Hospital. The genotypes were all chromosome 15q deletion. The median age at the time of the CSF sample was 21.5 years old with an IQR of 16.2–28.2 years. Their median Epworth sleepiness scale (ESS) score of 12 subjects was 10.0 (IQR 3.3–13.8), and 6 subjects (50%) had an ESS score ≥ 11 (criterion for pathological EDS). The median CSF orexin level of these 12 subjects was 192.0 pg/mL with an IQR of 159.5–235.8 pg/mL. Based on the standard values (normal: ≥ 200 pg/mL, intermediate: 110 to 200 pg/mL, low: ≤ 110 pg/mL), seven of the 12 subjects (58%) exhibited below normal concentrations, including 7 subjects with intermediate levels. A multiple regression model with the ESS score as the outcome variable and the CSF orexin concentration and other variables as explanatory variables was generated. A Wald test revealed that CSF orexin concentration could significantly explain the ESS score (regression coefficient -0.098, $p = 0.003$).

Conclusions: Reduced CSF orexin concentration was noted in about three-fifths of Japanese PWS patients. A significant correlation was observed between ESS score and CSF orexin concentration. The present study suggests that reduced CSF orexin contribute to severity of EDS in Japanese PWS patients.

Summary: At least some of Japanese PWS patients have insufficient orexin function in brain. The lower the CSF orexin concentration is, the worse daytime sleepiness PWS patient has.

POSTER ABSTRACTS

P2 - Hypogonadism in a Female with Prader Willi Syndrome. Case Report

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Background: Prader-Willi syndrome (PWS) is a genetic disorder characterized by mental retardation, dysmorphic features, and behavioral dysfunction, most notably food-related problems such as hyperphagia, food seeking, and a high risk for obesity. Clinical features are attributed mainly to hypothalamic dysfunction. The main phenotypic features include neonatal hypotonia and feeding problems; hypogonadism, hyperphagia, and obesity; short stature; and characteristic facial appearances. Individuals with PWS have mild to moderate intellectual disability.

Case presentation: A girl at 16 years old was presented at the consultation with her mother for the absence of the cycle. At a first inspection a young obese with low stature. Height=147cm (-2.0 SDS), Weight = 74.5kg (+2.5 SDS), BMI= 34.4 Kg/m². Clinical examination of sexual characters: poorly developed for age: Tanner stage I breast development and Tanner stage I/II pubic hair. Menses had not yet started. Laboratory routine investigations: urea and electrolytes, liver function tests, calcium, and phosphate were normal. A fasting and a 2-hour post-prandial blood sugar were normal. Thyroid and adrenal function were normal. Serum levels of LH and FSH was abnormally low <1.0IU/l. The karyotype of the patient's mother and father were normal. The girl, at genetics consult subsequent chromosome studies revealed a deletion in chromosome 15, positive for Prader-Willi syndrome. At psychiatric examination, she was found to be functioning in the borderline area of subnormality IQ76. History: She was born at term, with Apgar of 7. Her birth weight was 2830 g, and birth length was 49 cm. She had poor initial respiration, genital hypoplasia and she was hypotonic and quiet. The latest submitted to the school difficulties with psychiatric records for behavior problems. Sex hormone treatment has been initiated with transdermal estradiol.

Discussion and Conclusions: We presented a case of PWS, diagnosed later than having regard as the first distinctive sign hypogonadism. The clinical expression of hypogonadism in females with PWS is variable: genital hypoplasia, delayed and incomplete pubertal development. Although some females with PWS undergo spontaneous menarche, most have primary or secondary amenorrhea or oligomenorrhea. Primary ovarian dysfunction is an important contributor to the hypogonadism in women with PWS. Sexual development is incomplete in most PWS patients, consistent with arrested pubertal development.

Summary: Early diagnosis and treatment in women with PWS and hypogonadism can improve quality of life for these persons.

POSTER ABSTRACTS

P3 - Latent Central Adrenal Insufficiency may exist in some patients with Prader-Willi syndrome

Yuji Oto 1, Keiko Matsubara 2, Tadayuki Ayabe 2, Nobuyuki Murakami 1, Tomoyo Matsubara 1, Hiroyuki Ogata 3, Masao Gito 3, Hiroshi Ihara 3, Toshiro Nagai 4,

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[Background] The annual death rate of Prader-Willi syndrome (PWS) is reported to be as high as around 3%. The causes of deaths in children are often sudden and unexplained. Several reports hypothesized that patients with PWS may have latent central adrenal insufficiency (CAI). However, it remains unclear whether PWS subjects are suffered from an alternation of the HPA (Hypothalamus-Pituitary-Adrenal; HPA) axis or not. The aim of this study was to explore the HPA axis in our PWS patients.

[Methods and Results] We evaluated the HPA axis in our PWS patients (24 males and 12 females, aged 0.7-59 years) using insulin tolerance test. In addition, we presented clinical features of five cases who are suspected to have CAI. All 36 patients showed normal basal ACTH (12.9 ± 8.0 pg/ml), cortisol (17.5 ± 8.5 µg/dl) and serum cortisol response (20.8 ± 8.5 µg/dl) to insulin tolerance test. However, 25 of 36 patients showed delayed response of peak cortisol levels (120 minutes after stimulation). In addition, in the presented five cases, several clinical manifestations such as status epilepticus, lethargy and cardiopulmonary arrest were seen. Four of five patients showed hyponatremia and low serum IGF-1 level (Na 122, 125, 135 and 121 mEq/l, IGF-1 8, 21, 6 and 19 ng/ml, respectively).

[Conclusion] We should consider the existence of CAI and evaluate the adrenal function in cases of hyponatremia with unexplained neurological symptoms.

[Summary] We should treat with steroid if CAI is suspected clinically, even though laboratory data do not indicate CAI. Especially, patients with under nutrition (low level of IGF-1) should be carefully monitored.

POSTER ABSTRACTS

P4 - Association of atrophy and asymmetry of the paravertebral muscles with the development of scoliosis in Prader-Willi syndrome

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Background: Scoliosis is one of the most serious complications of Prader-Willi syndrome (PWS). The incidence of scoliosis is significantly higher than that of idiopathic scoliosis in PWS. Poor strength in the paravertebral muscles is considered to be a factor that exacerbates scoliosis in PWS. However, the mechanism underlying the development of scoliosis in PWS remains unknown. In the present study, we aimed to investigate whether atrophy or asymmetry of the paravertebral muscles is a cause or a result of scoliosis in PWS.

Methods and Results: Thirteen patients (10 males and 3 females; age range, 6–19 years) with PWS (deletion type, n = 7; uniparental disomy type, n = 6) were examined. All patients were receiving growth hormone (GH) therapy, and had developed scoliosis during GH therapy. Cobb angles were measured every 6 months, to evaluate the degree of scoliosis. At each assessment, a single slice CT scan was obtained at the level of the umbilicus, to evaluate paravertebral muscle volume.

The age at which scoliosis developed ranged from 2 to 15 years (median, 5 years). The duration between starting GH treatment and developing scoliosis ranged from 1 month to 6 years and 7 months (mean, 2 years). Paravertebral muscle atrophy with fatty change was observed in 7 patients prior to the development of scoliosis. Asymmetry of the paravertebral muscles (>5% difference between right and left paravertebral muscle volume) was observed in 4 patients prior to the development of scoliosis.

Conclusion: Abnormalities of the paravertebral muscles (atrophy with fatty changes or asymmetry) were observed in a substantial proportion of patients with PWS, before they developed scoliosis. This finding suggests that these abnormalities of the paravertebral muscles are not a result of scoliosis. Instead, paravertebral muscle atrophy with fatty changes or asymmetry could be causally related to scoliosis in PWS.

Summary: Atrophy or asymmetry of the paravertebral muscles could be a cause of scoliosis in PWS.

POSTER ABSTRACTS

P5 - Analysis of Chromosomal Abnormalities and Genomic Imprinting Gene at *SNRPN-SNURF* in Prader Willi Syndrome –Tamilnadu Population.

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Background

Praderwilli syndrome (PWS) is a neuro-genetic disorder characterized by low birth weight, severe hypotonia and feeding difficulties in early infancy, followed by hyperphagia and obesity starting in early childhood. Short stature, small hands and feet, narrow bifrontal diameter, almond-shaped eyes, triangular mouth, a temper tantrums, motor milestones and speech delayed, in both sexes hypogonadism. Many of the characteristic features of PWS result from the loss of function of paternally imprinted genes including *SNRPN-SNURF*, *NDN* and a cluster of *snoRNAs*. Their silencing on the maternal chromosome is mainly attributed to DNA methylation and histone modifications at the PWS- Imprinting center (IC) conferred by the Angelman syndrome (AS)-IC, which together form a bipartite IC regulating the imprinted status of this region. **The aim of the present study was to analyze the chromosome abnormalities and paternally imprinted genes of *SNRPN-SNURF* expression at chromosome region 15q11-q13 of PWS patients in Tamil Nadu population.**

Methods and Results:

Totally 6 samples were selected including 3 PWS patients and their parents and equal number of controls and PBLC culture and genomic DNA extraction from peripheral blood to execute molecular screening of PWS. We reported PWS patients were exposed to have the predictable higher degree of chromosomal aberrations karyotypes of del(15) (pter> q11::q13 >qter). Methylation specific – PCR assay were revealed high quantity loss of paternal methylation at *SNRPN* gene to confirmed diagnosis of PWS.

Conclusion:

We observed that the identification of cytogenetic abnormalities is not only important for providing a cause for the PWS in a single individual but is also critical for accurate counseling regarding recurrence risks to parents and family members. We suggest that *SNRPN* might represent a major susceptibility gene for PWS.

Summary:

In our pilot study, cytogenetic and molecular screening studies are to facilitate investigation of paternally imprinted genes distribution of PWS, which is adjustment of the guidelines for preventive management in adulthood.

POSTER ABSTRACTS

P6 - PWS in Brazil: 6 months follow-up in a reference center

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Background

Prader-Willi Syndrome (PWS) patients have been followed in our country in different ways but we do not have a society or a reference center to spread adequate information about the disease.

In January 2015, we started a PWS reference center in Sao Paulo University to promote a better care for patients and families and to support them with a multidisciplinary team.

Methods

Forty two patients, between 2 and 21 years old, were followed for a 6 month period in our PWS clinic. Body mass index-SDS (BMI-SDS) was evaluated at the beginning and after 6 months and these data were compared. The following items were analyzed: 1) use of growth-hormone 2) metabolic profile: LDL, triglycerides, glycated hemoglobin (HbA1c), fasting glucose and insulin levels. 3) polysomnography. All patients received orientation in diet (900 calories/day independent of weight), physical activity and behavior. Our team is composed by pediatric endocrinologist, dietician, nurses; neurologist specialized in sleep disorders and otorhinolaringologist.

Results

The mean age was 9.8 ± 5.2 DP. BMI-SDS at the first visit was 2.8 ± 1.9 DP and after 6 months 2.41 ± 1.8 DP. Metabolic profile showed that 21.6% patients had high LDL-c level (LDL-c > 130mg/dL), 48.6% had low HDL-c level (<40mg/dL), 18.6% had hypertriglyceridemia (>150mg/dL), 23.5% had high A1c ($\geq 5.8\%$) and 44.8% had insulin resistance. Only 12 patients used rhGH at the first visit and at the latest we had 29 (69%) patients on rhGH use. The reason that thirteen patients were not in use of rhGH is due to polysomnography alterations and patients were waiting for surgery or CPAP. Polysomnography revealed that 47.8% patients had an apnoea-hypopnoea index (AHI) >5 events/hour, 20.8% had O₂ saturations under 92% and 56.5% had reduced sleep efficacy.

Conclusions

Most of our patients could lose weight with the correct approach in diet, behavior and physical activity. The use of rhGH was increased after the beginning of the clinic and the benefit of this therapy is well known in the literature. Alterations in polysomnography were a major problem revealed in the follow up and the correct approach of the multidisciplinary team is essential to support this disorder.

Summary

To start a PWS reference center in Sao Paulo – Brazil was the first step to promote a better care for PWS patients and families. Our team could support them with the correct approach in diet, rhGH use and all others disorders related to the syndrome.

POSTER ABSTRACTS

P7 - Obese Prader-Willi *versus* Obese controls: Metabolic Profile in Brazilian Patients

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Background: Prader Willi syndrome is a rare condition (1:15000) that starts with intense hypotonia in the first years of life to reach a condition of voracious appetite which leads to life threatening obesity. The obese Prader Willi syndrome patient (OPWS) has peculiar characteristics which could confer different metabolic profiles compared with obesity of other causes. The aim of this study is to describe and compare the metabolic profile in obese patients and OPWS patients followed in a Pediatric Endocrinology outpatient unit.

Methods: We evaluated in a cross-sectional study 45 obese patients and 22 OPWS between 8 and 20 years old and compared them according to cholesterol levels, triglycerides, glycated hemoglobin (HbA1c) and fasting glucose.

Results: The mean age of the 67 patients was 14.1 (± 3.2) years old, 45 were male and the mean BMI Z SCORE was +3.1SD (± 0.6 SD). Both groups did not differ in sex, age and BMI Z SCORE. The metabolic profile in OPWS versus obese patients showed: high LDL-c level (LDL-c ≥ 130 mg/dL) in 18.2% X 11.1%, low HDL-c level (< 40 mg/dL) in 36,4% X 46,7%, hypertriglyceridemia (≥ 150 mg/dL) in 13.6% X 24.4%, respectively; Probably due to the low number of patients, there was no significant difference between both groups. However, there was a significant difference ($p < 0.001$) in abnormal Hb1Ac ($\geq 5.8\%$) between OPWS (73.3%) and obese patients (7.1%). Only 1 patient in each group had high fasting BLOOD glucose (> 100 mg/dL).

Conclusion: The comparison between obesity in Prader Willi syndrome and in other patients shows that HbA1c tends to be higher in OPWS. The differences in lipid levels show a tendency of more elevated levels in OPWS but the number of patients is small to reach statistical significance.

Summary:

The comparison between obesity in Prader Willi syndrome and in other patients shows that HbA1c tends to be higher in OPWS and there was no difference in lipid levels.

POSTER ABSTRACTS

P8 - Deficits in voice and multisensory processing in patients with Prader-Willi syndrome

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Background. Neuropsychological studies have reported that patients with Prader-Willi syndrome (PWS) display altered social interactions with a specific weakness in interpreting social information and in responding to them, a feature also observed in autism spectrum disorders (ASD). Based on the hypothesis that atypical multisensory integration such as face and voice interactions would contribute in PWS to social impairment we investigate the abilities of PWS to process communication signals including the human voice.

Methods: 21 Patients with PWS were recruited from the national reference center for PWS. In parallel, 21 typically developing (TD) individuals were also selected and recruited to be age- and gender-matched with the PWS cohort. In addition, a second set of five PWS patients was recruited to complete the genetic subgroup of patients presenting with UPD. They performed two tasks. Firstly a simple reaction time (RT) task of stimuli presented in uni- or bimodal condition and the task was to respond as fast as possible to the stimulus by pressing a response key. Secondly a 2 AFC voice recognition task that consisted of discriminating a human voice (laughing, coughing, vocal sounds) from natural environmental sounds.

Results. Compared to control typically developing individuals, PWS present low d-prime values reflecting a specific deficit in discriminating human voices from environmental sounds. Further, while PWS present some degree of multisensory benefits as expressed as a shortening of bimodal RT, this audiovisual gain was much lower than that of the TD. Moreover PWS display an absence of violation of the race model indicating a lack of convergence and integration of multisensory information prior to the initiation of the behavioral response. All the deficits observed in patients with PWS were stronger for the subgroup of those presenting Uniparental Disomy compared with those with chromosomal deletion, fitting with the fact that they are more sensitive to ASD.

Conclusion. Altogether, our study suggests that the deficits in social behavior observed in PWS derive at least partly from an impairment in deciphering the social information carried by voice signals, face signals, and the combination of both. In addition, our work is in agreement with the brain imaging studies revealing an alteration in PWS of the "social brain network" including the region involved in processing human voices.

Summary. In the present study, we were able to reveal specific deficits in patients with PWS using simple tasks and natural stimuli having high ecological value, such as human voices.

POSTER ABSTRACTS

P9 - A Young Adult with PWS Encourages a Mother of a Baby with PWS by Emails

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Background

Mothers of a baby with PWS in Japan usually receive support from professionals and peer parents. However, an adult person with PWS taking part in supporting a mother of an infant with PWS is not a common practice.

Report

This report introduces email interactions between a young adult male person with PWS and a mother of a baby with PWS. Fumiko Harada is a mother of a young adult with PWS, Gaku (co-author). She was giving peer support to a young mother of a 3-year-old girl Mi (assumed name) with PWS, who asked questions if Gaku ever felt it was hard to have PWS himself, and what kind of times he had felt that way. In response to this, Gaku's mother suggested her son that he should answer the questions by himself. Gaku answered the questions of Mi's mother and gave advice from the perspective of a person with PWS with an insight into himself and the syndrome. Gaku wrote Mi's mother that she needed to discard the anxiety as the baby was able to read the mother's mind. Gaku hoped Mi's mother to treat her daughter as a normal child, and not as a 'PWS child'. He also gave advice on overeating that one should not hold oneself too much from eating and that a mother should give her child a lot of love instead of worrying.

Results

The mother of Mi was thankful for Gaku and was encouraged by him. She wrote Gaku that she was going to try not to build up her stress, and find what her daughter can enjoy more than eating. Reading Gaku's email, his mother (Fumiko Harada) was pleased how her son had been thinking deeply, and that he could think about other person's feelings. Knowing what is in his mind better, they became able to build a better relationship. These changes made her feel much more relaxed than before, and treat and speak differently. When difficult situations arise, she could now overcome them because she was able to wait.

Conclusions

In this report, the person with PWS was given an opportunity to objectively understand himself. Support by people with the same syndrome had been regarded as difficult, as they could be quite egocentric and have weak self-insight. But this case showed it may be possible. Positive advice from persons with PWS will encourage parents who are anxious about their children's future.

Summary

We presented encouragement and advice by an adult with PWS to a mother of a baby with PWS. We recommend advising under supervision of parents and/or caregivers as this could have positive impact on the person with PWS as well as the mother of an infant with PWS.

POSTER ABSTRACTS

P10 - Risk assessment of medically assisted reproduction and advanced maternal ages in the development of Prader-Willi syndrome due to UPD(15)mat

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[Background] Recent studies have suggested that disomic oocyte-mediated UPD(15)mat is increased in patients with Prader-Willi syndrome (PWS) born after medically assisted reproduction (MAR). It remains unknown whether the increase is primarily due to MAR procedure itself or advanced maternal childbearing ages as a predisposing factor for the disomic oocyte production.

[Methods and Results] We studied 122 naturally conceived PWS patients (PWS-NC group) and 13 MAR-conceived patients (PWS-MAR group). The relative frequency of disomic oocyte-mediated UPD(15)mat was significantly higher in PWS-MAR group than in PWS-NC group ($P=0.0045$), and the maternal childbearing ages were significantly higher in PWS-MAR group than in PWS-NC group ($P=0.0015$). However, there was no significant association between the occurrence of disomic oocyte-mediated UPD(15)mat and MAR, after adjusting for childbearing age ($P=0.25$). Consistent with this, while the frequency of ART-conceived livebirths was higher in the PWS patients than in the Japanese general population (6.4% vs. 1.1%, $P=0.00018$), the distribution of childbearing ages was significantly skewed to the increased ages in the PWS patients ($P<2.2\times 10^{-16}$).

[Conclusions] These results argue against a positive association of MAR procedure itself with the development of UPD(15)mat.

[Summary] We evaluated the effects of MAR and advanced maternal childbearing age in 135 Japanese PWS patients including 13 MAR-conceived patients. The present study suggests that MAR is unlikely to facilitate the disomic oocyte-mediated UPD(15)mat.

POSTER ABSTRACTS

P11 - Are frequent the Cholelithiasis in subjects with Prader Willi Syndrome?

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Introduction: The prevalence of cholelithiasis in general population is approximately 15%. Some of the many clinical features and therapeutic targets of subjects with (Prader Willi Syndrome) PWS are risk factors for the development of cholelithiasis in the general population including female gender, obesity, sudden weight loss, diabetes mellitus and family history of gallstones. The authors don't know other studies about cholelithiasis in people with PWS. The aim of this study is to identify risk factors and proportion of cholelithiasis in a sample of individuals with PWS. **Materials and Methods:** Observational and descriptive study based on sample of subjects in treatment monitoring in the SPINE Foundation date January 2016 in Buenos Aires, Argentina. Clinical data were tabulated and percentages and sample averages were obtained. Sudden drop in weight decline is defined as greater than or equal to 1.5 kg / week. **Results:** From a sample of 19 subjects with PWS, 36.84% (n=7) presented cholelithiasis during follow-up treatment. Most individuals (85.71%, n=6) had cholelithiasis at the beginning of the treatment and only one subject (8.33%) in its follow-up. Of the women in the sample (n=6), 50% had cholelithiasis (n=3); while the total number of men (n=13), only 30.76% (n=4) of the men in this sample presented this digestive pathology. The average age of subjects with PWS and cholelithiasis was 26.14 years (range 11-33 years) and an average of 23.42 years (range 11-35 years) subjects with PWS without cholelithiasis. All individuals with PWS with cholelithiasis were obese (n = 7) in our sample. Three subjects (42.85%) with cholelithiasis as a risk factor had the sudden drop in weight decline with a single case among subjects without cholelithiasis (8.33%). None of those with cholelithiasis had diabetes mellitus as a risk factor. While a smoker with PWS (5.26%) presented cholelithiasis, two smokers with PWS didn't suffer cholelithiasis (10.53%). Four subjects with PWS with cholelithiasis (21.05%) had a family history and only two people with PWS without cholelithiasis (10.53%) had family history of cholelithiasis. Our sample individuals who have not filed as diagnostic malabsorption syndrome, or hepatic cirrhosis, or cystic fibrosis or pregnancy or oral contraceptive use so that could not be valued as risk factors. **Conclusions:** Given the preliminary data of this reduced sample, the proportion of people with PWS with cholelithiasis appears to be greater than the reference values in the general population. The risk factors would be the same as for general population except the female sex perhaps with a less impact on people with PWS. Future research could clarify the prevalence, risk factors and their role in caring for people with PWS.

Summary: People with PWS could have greater proportion of bladder stones and bile ducts than general population. Given the potential clinical complications, this medical condition should be taken into account in clinical routine of these people.

POSTER ABSTRACTS

P12 - PRASOC project: Study of the cognitive capacities to process emotional and social informations in the Prader-Willi syndrome

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Background

Prader-Willi syndrome (PWS) is a rare genetic syndrome characterized by several behavioral and cognitive disorders as hyperphagia, mild intellectual disability and a dysexecutive syndrome. Some studies have showed a deficit to process emotional and social informations (Whittington & Holland, 2011). The aim of this research is to determine if the abilities to process emotional faces and words and to infer mental state are impaired in the PWS and if these deficits are linked to the intellectual level of the PWS.

Methods and Results

Thirty one PW adults ($M = 32.93$ years), 25 adults with an intellectual disability without a PWS ($M = 29.92$ years) and 29 adults without intellectual disability ($M = 27.41$ years) participated to this study. In the Experiment 1, participants performed a recognition task with emotional faces (fear, angry, joy, surprise, disgust, sadness or no emotion) and forms. A morphing procedure has modified the intensity of the expressed emotion (50% or 100%). In the Experiment 2, participants did an emotional Stroop task with positive, negative or neutral words and a food Stroop task with control or food words (low of high calories). In the Experiment 3, participants performed the TOM-15 to assess cognitive theory of mind.

Results of the Experiment 1 demonstrated that the two groups with intellectual disabilities have weaker performances than the control group but only for the face pictures, and revealed a specific deficit for angry and surprise faces in the PWS. Moreover, groups with an intellectual disability had more difficulty to recognize emotion at 50% rather than the control group. Results of Experiment 2 showed an emotional Stroop effect for negative words for the control and PW groups but not for group with an intellectual disability without PWS. A food Stroop effect was revealed only for participants with a PWS, specifically for stimuli low in calories. Finally, results of Experiment 3 showed a general impaired to the score at the TOM-15 for both groups with an intellectual disability.

Conclusions

Taken together, results of Experiments 1-2 not suggest an overall deficit to process emotional stimuli in the PWS but a specific deficit which was not always linked with the intellectual disability. Results of Experiment 3 showed difficulty to infer mental state to oneself or other people in the PWS, this time associated with the intellectual disability. These results can be associated with behaviour disorders observed in the PWS as the *temper* tantrum or the food regulation deficit.

Summary

In three experiments, we have study the abilities to process emotional and social stimuli in the PWS. People with a PWS not show an overall deficit. The PWS corresponds to a complex cognitive pattern. Some capacities seem to be comparable to those without intellectual disability but others similar to the group with an intellectual disability and finally some capacities seem to be specific to the PWS.

POSTER ABSTRACTS

P13 - French experience of assessment of hyperphagia in PWS patients

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Background: In 2007 Dykens et al. published a new questionnaire developed for PWS patients assessing hyperphagia. This questionnaire has never been explored in the French PWS population. We used this in our PWS population.

Methods and results:

We analysed all the questionnaires filled during a routine visit of children and adults with PWS on the French reference Centre from 2010 to 2015. Several variables (age, sex, genetic diagnosis, body mass index (BMI)) were recorded for each patient. Questionnaires with missing items were excluded (n=9). The whole population comprises 102 patients with 44% of male; the median age is 11.5 years ranging from 4 to 45 years. Eighty one patients were aged less than 20 years. The genetic subtype was deletion in 63.7% (9.8% type 1, 38.2% type2) and disomy in 34.3%, imprinting defect in 1% and others in 1%. According to International Obesity Task Force BMI charts, 37.3% patients had normal BMI, 23.5% were overweight, 23.5% were obese (BMI > IOTF 30) and 15.7% were excessive obese (BMI > IOTF 35).

We analysed the 3 subscores of hyperphagia questionnaire, hyperphagic behaviour, hyperphagic drive and hyperphagic severity in the whole population and in 4 age classes: ≤ 4 and ≥ 10 years (N=48); ≤ 11 and ≥ 19 years (N=33); ≤ 20 and ≥ 29 years (N=13) and ≥ 30 years (N=8). In the whole population, the median hyperphagic behaviour subscore was 10 ranging from 5 to 23, the median hyperphagic drive subscore was 10 ranging from 4 to 20 and the median hyperphagic severity subscore was 4 ranging from 2 to 9. The 3 subscores significantly increased with BMI with significantly higher scores in obese and excessively obese patients compared to those with normal BMI. The hyperphagic drive and severity subscores were significantly higher in the group of oldest patients (≥ 30 years) than in the youngest patients (≤ 4 and ≥ 10 years) but no other significant difference was observed regarding age. We did not observe difference according to sex in the whole population and in the different age classes. There was no difference between the patients with deletion and the patients with non-deletion. Among patients with deletion, patients with type 2 deletion have more severe subscores than patients with type 1 deletion (10 vs. 8.8 for hyperphagic behaviour, 10 vs. 7.5 for hyperphagic drive and 4 vs. 3 for hyperphagic severity, $p < 0.05$) although there is no significant difference in BMI repartition.

Conclusions: The hyperphagia questionnaire is an easy and interesting tool to characterise feeding behaviour in patients with PWS sensitive enough to identify differences in some genotype subtypes.

Summary: We assessed hyperphagic behaviour in 102 French patients with PWS using Dykens questionnaire. Scores were analysed according to sex, age, body mass index and genetic subtypes.

POSTER ABSTRACTS

P14 - Weight Management for Youth with Prader-Willi Syndrome: Delivery to Parents via Telemedicine

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Background. Although youth with Prader-Willi Syndrome (PWS) who receive a diet and activity program in a tightly controlled inpatient hospitalization setting experience significant weight loss, they are at risk for weight regain after they return home. However, interventions targeting weight maintenance for youth with PWS in the family and home environment are nonexistent. An interdisciplinary team of investigators from Duquesne University and the Children's Institute of Pittsburgh aims to address this tremendous unmet medical need among individuals with PWS.

Virtually all evidence-based approaches to treatment of childhood obesity are family-based. Furthermore, there is support for working exclusively with the parent for child weight management. This stands to reason as the home environment in general, as well as parent feeding in particular, contribute to childhood obesity. Additionally, a growing body of data indicates that behavioral weight management interventions can be successfully delivered via telemedicine. The goal of this pilot study is to demonstrate that telemedicine may be a practical and relatively low-cost way to deliver a behavioral intervention for child weight management directly to parents of youth with PWS in the home setting.

Methods and Results. The team plans to pilot an evidence-based, behavioral weight management intervention for delivery to parents of youth with PWS via telemedicine. Content is adapted to the unique needs of this patient population. Proposed treatment targets include 1) creating a healthier home environment; 2) parenting and parent feeding; and 3) compliance with diet and activity. The intervention consists of weekly contacts with parents over a three month period following child discharge, as well as tracking of child body weight with a wireless weight transmitting scale provided to the family. It could be delivered by providers interacting with youth and families in the inpatient milieu, such as a registered dietitian, exercise physiologist, psychologist, pharmacist, or nurse. The team has not yet evaluated the proposed intervention.

Conclusions. A pilot study would represent a critical first step toward a larger scale effort to evaluate and disseminate an effective intervention to parents and youth with PWS. Through this poster presentation, the team is seeking valuable input on the proposed intervention approach from patients, providers, and policy makers.

Summary. An intervention delivered to parents at home via telemedicine has a strong potential for preventing weight regain among youth with PWS following discharge from an inpatient hospitalization. Moreover, creating a continuum of care that extends from the hospital setting to the home environment may ultimately prove to be a cost-effective approach to increasing access to specialized services at home and decreasing the need for inpatient hospitalization.

POSTER ABSTRACTS

P15 - PWS & Self-Determination Helping Parents & Teachers to Enhance Child Resources to Grow in Autonomy

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BACKGROUND: Adults beliefs, attitudes, feelings and expectations about autonomy, play an important role in well-being and quality of the relationships of PWS people.

Questions to investigate:

What concept of autonomy have parents? Coincides with that of teachers?

What is the level of awareness about the strengths and the limitations caused by the disease?

Changes according to the age of the child?

In which areas are striving parents and teachers?

Signs for the future by parents and teachers.

Advice from experienced parents for parents with small children

METHODS AND RESULTS

Sample: 35 subjects (5 to 22 years) were evaluated through a specific Autonomy Questionnaire for Parents & Teachers and reports drawn during discussion groups.

Research areas: self-care, food relationship, social inclusion, learning, homework (modified for youngest in communication & language), move around the area (modified for youngest in motor skills), managing money, experiences away from family (modified for youngest in Autonomy/separation from adult).

Parents & teachers show different perceptions between the quantitative data and the description examples of life situations: rather positive assessment, denying dangers, reflect optimistic attitudes as opposed to limited autonomy in real situations.

CONCLUSIONS

The data underline the similarity in “parent-child” and “teacher-student” relationship, because it implies dependence and responsibility, along an acceptance and balance between the difference needs (protection, exploration) of child. Adults beliefs, involvement and priorities, play a decisive role in autonomy and well being of the child: providing a mental representation of himself, of others, of the world that will guide his behavior. The growth, also for caregivers, implies new knowledge, through various types of support and guidance, relationships that increase confident or vulnerability, that enhance trust or facilitate dependency.

There is a need for different levels of supervision by informed adults about PWS and aware of the specificity of the subject. By discussion of everyday situations, the group allows adults to deal realistically information about PWS: sharing not only limits and worries but also discoveries of positive aspects, providing ad hoc information, signs and advice on individual subjects. Thus creating the conditions for the development of a network of support for autonomy from the school and the family.

SUMMARY

This study underlines the influence of the beliefs of parents & teachers about autonomy: convictions are reflected on the perception of the syndrome, in the communication of the diagnosis and on educational styles. The study underlines also the importance of the adults role in enhancing and sustaining child with PWS to grow in autonomy, generalizing learnings from rehabilitation therapies in every day life.

POSTER ABSTRACTS

P16 - First results of the larger study on the evaluation of the impact of cognitive and executive abilities on socialization difficulties in childhood with PWS

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Background

Prader Willi Syndrome (PWS) is a very complex disorder. Patients and families deal with strong difficulties in daily life due to feeding and emotional disorder, with cognitive impairment. PW persons have been recently shown to display executive deficits which may explain behaviors like temper burst, repetitive behaviors and cognitive rigidity. We present here a study focused on the impact of emotional and food items on the executive processes by administrated a variant of the Stroop task, an emotional recognition task and a task of the theory of mind on children with PWS.

Methods and Results

Twenty children with PWS aged 6 to 17, mean age 11.0 (s.d.2.9) years and mean IQ 117.74 (s.d.10.9). Their performances were compared with a control group of 17 age matched children, mean age 11.0 (s.d.3.2) years with a mean IQ 67.30 (s.d.19.1). Three neuropsychological tests were used: a task of false beliefs to assess the theory of mind, a task for emotion faces recognition, and the emotional and food Stroop test to assess the treatment of emotional items.

On the Stroop, PWS children have a response time more important than the control group, as well on emotional Stroop ($p=.007$) than food Stroop ($p<.001$). They are significantly slower on the healthy and caloric food images than on the neutral ones without food. Interestingly we didn't find an effect of the food images on the response time in the control group. In the emotional Stroop, the control group is significantly slower both on positive and negative images than neutral ones, while in PWS group, the response time on the positive images is faster than on the negative ones. On the facial emotion recognition task as a whole, children with PWS made significantly more mistakes than the controls ($p<.001$). Nevertheless children with PWS tend to recognize anger and joy on the faces as well as controls. For other emotions, children with PWS failed more than controls. On the theory of mind test, the control group performs better than PWS group ($p<.001$), both in false belief and understanding of the story.

Conclusions

On the 3 tasks, the PWS group display lower competences than the control group. They are sensitive to any food items (healthy and caloric) which slows their reaction time. They are more sensitive to positive than negative images. They recognize the basics emotions (joy and anger) like control group while they fail more on the complex emotions. The theory of mind is largely deficient compared to control group.

Summary

This abstract presents the preliminary results of the PRASOC project on neuropsychological tasks with PWS children to assess some executive abilities in link with the emotional component. This study permits to apprehend the developmental pattern of cognitive capacities by comparing the results with a population without PWS.

POSTER ABSTRACTS

P17 - Manifestation of behavioral problems and BMI scores in persons with PWS by age groups, and daily living condition of adults with PWS in Japan

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The aim of this study was to assess the severity of maladaptive behaviors of persons with PWS and the relation between behavioral problems and age group in Japan. In addition, BMI scores by age group and daily living conditions in adulthood were investigated.

Questionnaires including the CBCL (Child Behavior Check List, Achenbach, 1991) were mailed to parents of persons with PWS. A total of 296 persons (52% male, 48% female) with PWS, aged 1 to 44 years, were rated by their parents using the CBCL. We divided these 296 persons into 7 age groups. The mean BMI scores increased markedly with age; group 1 ($n = 19$, aged 1-2) was 13.62, group 2 ($n = 43$, 3-5) was 14.88, group 3 ($n = 40$, 6-9) was 16.61, group 4 ($n = 54$, 10-14) was 20.85, group 5 ($n = 51$, 15-19) was 25.45, group 6 ($n = 59$, 20-29) was 34.55, and group 7 ($n = 30$, aged >30) was 37.87.

The total CBCL scores of the majority of children with PWS fell in the clinical range. Most of the CBCL subscale scores and the percentage of persons with maladaptive behaviors tended to increase with age. Delinquent and Aggressive behaviors were remarkable in two groups whose ages were 15-19 and in the 20s. However, mean scores on the CBCL Internalizing and Externalizing dimensions for the > 30 years group were both lower than those of the group in their 20s. On the CBCL Clinical scale for Delinquency, the >30 years group scored significantly lower than did the group in their 20s.

“Stealing outside” is the most serious problem especially for those with PWS. It is the most distressful problem for families. It is a problem for parents as their grown-up children can no longer use the social welfare transformation services to go to work or school in Japan. Parents, therefore, provide transportation for their grown-up children in order to prevent them from stealing. For the future, we plan to gather further information as to how parents deal with these problems, and how police or administration of justice deal with these matters in other countries. And the effective psychosocial intervention plan is needed.

In addition, these problems reduce QOL of persons with PWS. The percentage of nonworking adults with PWS was much higher than that of persons with intellectual disability. Approximately 90% of adults with PWS live with their parents in Japan. The remaining 10% of adults with PWS live in traditional style residential facilities for persons with intellectual disabilities. There are no group homes specialized for persons with PWS in Japan.

Summary: Percentage of persons with PWS revealed maladaptive behaviors tended to increase by age in Japan. Most of adults live with their parents and the percentage of them going to work is very low.

POSTER ABSTRACTS

P18 - Cerebellar hyperperfusion in PWS patients: the link for satiety?

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Background: Prader-Willi syndrome (PWS) is a rare, multisystem genetic disease leading to severe disabilities such as morbid obesity and behavioral and socialization problems. Imaging studies have shown structural and functional brain anomalies in these patients and we showed specific relative hyperperfusion in anterior cingulum and temporal gyrus.

Methods and results: Our analysis was performed in nine patients with PWS (six males, three females; mean age 16.4 years) who underwent positron emission tomography (PET) scanning with H₂¹⁵O as tracer to measure regional cerebral blood flow (rCBF). The images were compared with those from nine controls (six males, three females; mean age 21.2 years). In the patients with PWS, morphological magnetic resonance imaging (MRI) was also performed. WISC-III and CBCL evaluations were performed to characterize cognition and social skills.

The PET scans revealed relatively increased resting perfusion in brain regions in patients with PWS compared with controls principally in the cerebellum.

Conclusion: Our results demonstrate that complex networks impaired in PWS are involved in food intake and satiety which are also implicated in emotion, anxiety, executive functions and sensorial perceptions.

Summary: PWS is a rare genetic disease leading to morbid obesity. PET scanning have been done to compare nine patients with PWS to nine controls. Increasing resting perfusion in the cerebellum, involved in food intake and satiety, have been observed in patients with PWS.

POSTER ABSTRACTS

P19 - Psychosis in Prader-Willi syndrome

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Background: People with the UPD and IC genetic sub-type of PWS are at very high risk for psychotic illness (up to 60% in adults with UPD). This observation suggests that, rather than loss of paternal expression at 15q11-13, it is increased gene dosage of paternally imprinted/maternal expressed genes located on chromosome 15 that is associated with mental illness in PWS. However, at present the mechanism(s) are not understood hindering the development of specific treatments.

Methods & Results: A systematic literature review has been conducted to investigate potential mechanisms that could explain the high rate of psychosis in PWS. First, one of the strongest leads is the paternally imprinted gene UBE3A (15q11.2). An antisense transcript (AST) is produced, with a promotor located in the PWS imprinting centre, and binds to the UBE3A gene on the paternal chromosome, inhibiting its transcription. Thus, people with mUPD would not produce this AST, and its transcription would be altered in IC, resulting in both cases in increased dosage of the UBE3A gene product (an ubiquitin ligase protein); E6-AP, which is involved in GABA receptor recycling; NMDA receptor regulation; and arachidonic acid inflammatory pathway - all mechanisms that have been implicated in the aetiology of psychotic illness. Secondly, multiple studies in schizophrenia have demonstrated reduced expression of pre and post-synaptic markers of GABAergic neurotransmission and consequently a disrupted excitatory/inhibitory balance (Glutamate/GABA). Interestingly, three GABA receptor subunits are encoded at the PWS locus 15q12. Thirdly, alpha7 N-acetylcholine receptor, located at chromosomal locus 15q13.3 seems to be associated with schizophrenia in a least two ways: in acting on GABA metabolism and on inflammation through the cholinergic anti-inflammatory pathway: Fourthly, the immune system has been shown to play a major role in the aetiology of psychotic illness through the action on neuronal growth, connectivity and neurotransmitter balance and also, in rare cases, autoantibody activity against NMDA, potassium channel and GABA receptors. There have also been consistent reports of increased levels of pro-inflammatory cytokines and microglia inflammation associated with schizophrenia, involving brain inflammatory processes in psychosis. Lastly, cognitive profiles differ between Del and UPD, and similar cognitive and sensory impairments have been reported in UPD, in schizophrenia and in subjects at risk of psychosis, indicating that it is possible that this may be involved in the increased propensity of those with the UPD subtype to psychotic illness.

Conclusions: we propose that a gene dosage effect results in the high propensity to develop psychosis in UPD, but the brain mechanisms that are underpin this risk are unknown. Drawing from the PWS and general psychosis literature, we have proposed specific mechanisms.

Summary: we outline potential pathways involved in the development of psychosis in PWS, that we argue are a consequence of an increased dosage of paternally imprinted/maternal expressed genes on chromosome. Identifying the underlying brain mechanism would inform treatment developments.

POSTER ABSTRACTS

P20 - Relationship between electrodermal activity and behavioural responses to inhibition and working memory updating tasks in Prader-Willi syndrome: A sub-section of the PRASOC project.

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Background Prader-Willi Syndrome (PWS) is characterized by cognitive, neurological and physiological disorders. As a multi-level symptomatology disorder, one path towards understanding the links between endophenotypical levels is to consider the concomitance of psychological and physiological factors. This study aimed at exploring the links between the activity of the sympathetic nervous system with electrodermal activity (EDA) and behavioral responses to executive tasks in an event-related experimental design.

Methods and Results The performance of 30 adults with PWS was compared to 30 aged-matched healthy participants on two computerized executive tasks assessing inhibition and working memory updating. Behavioural and EDA data were recorded and both were related to specific stimulation events in order to isolate their impact on psychological and physiological measures. Compared to controls, adults with PWS exhibited general delayed behavioral responses associated with a lower tonic EDA component and surprisingly earlier and weaker skin conductance responses. Correlational analyses revealed links between electrodermal and behavioural measures in the two groups. However, PWS participants showed a lower number of these links as compared to controls.

Conclusion These results show that abnormal EDA is associated with cognitive impairment in PWS. Furthermore, results indicate that PWS group showed weaker psychophysiological associations when eliciting executive functions and open up perspectives for further investigation of these associations in this disorder.

Summary The present study investigated the relationship between electrodermal and behavioural responses to executive tasks among adults with PWS. Results highlight abnormal electrodermal activity associated with cognitive impairment in this syndrome and weaker psychophysiological associations as compared to healthy age-matched individuals.

POSTER ABSTRACTS

P21 - Muscle development and function in a *Magel2* mouse model of Prader-Willi syndrome

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Background: Prader-Willi syndrome (PWS) is a multigene disorder commonly associated with hyperphagia and obesity. Children with PWS have increased fat mass and decreased lean mass before the onset of hyperphagia and obesity. Severe hypotonia and reduced muscle strength are typically present in PWS infants. Inactivating mutations in one PWS candidate gene, *MAGEL2*, cause a Prader-Willi-like syndrome (Schaaf-Yang syndrome) with neonatal hypotonia and joint contractures, highlighting the importance of loss of *MAGEL2* in PWS phenotypes. The process of autophagy is essential in maintaining musculoskeletal homeostasis, and increased or decreased autophagy can lead to muscle atrophy. Autophagic markers such as ubiquitin (Ub), p62/SQSTM1 (p62) and microtubule associated protein 1-light chain 3 (LC3) are hypothesized to be connected to the MAGE family of proteins that includes *MAGEL2*, suggesting that loss of *MAGEL2* could modulate autophagy in muscle.

Methods and Results: Expression of *Magel2* was detected in the murine nervous system and in developing muscle, connective tissue and bone. Adult mice lacking *Magel2* had reduced muscle mass, increased fat mass and decreased bone mass compared to wild-type mice. Immunohistochemistry (IHC), immunoblotting and real time RT-PCR (qRT-PCR) were used to determine whether loss of *Magel2* affects the accumulation of autophagic markers or expression of genes associated with muscle atrophy, in both neonatal and adult mice. The p62 positive aggregates were increased in muscle from *Magel2* mice and atrophy genes were up-regulated.

Conclusions: Abnormal autophagic processes likely contribute to muscle atrophy in mice lacking *Magel2*. Further studies are needed to determine how the inactivation of *Magel2* causes muscle phenotypes at a cellular level. Our mouse strain carrying loss of *Magel2* provides a model for muscular dysfunction in Prader-Willi syndrome.

Summary: One of the symptoms of PWS is poor muscle tone, which could be caused by disruption in the autophagy process that regulates skeletal muscle. Autophagy is disrupted and atrophy is increased in muscle from mice lacking *Magel2*, one of the PWS candidate genes. We propose a mechanism by which loss of *MAGEL2* causes hypotonia in children with PWS.

POSTER ABSTRACTS

P22 - Altered gaze behavior during face processing in patients with Prader-Willi syndrome

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Background : A large body of neuropsychological studies present evidence that Prader-Willi syndrome (PWS) have deficit in social communication. The difficulties of PWS with relationships with others are probably related to abnormal processing of human face. Mixed results have been reported on how PWS integrate face information. During a Benton Face Recognition Task, some studies report a near-normal performance level while others report an abnormal face recognition scores in agreement with previous studies revealing an impairment of PWS to attribute emotional or mental states from human facial expressions. In order to progress in our comprehension of social impairment in PWS we investigated the oculomotor behavior of PWS engaged in a face recognition task.

Methods: 28 patients were recruited from the national reference center for PWS to perform a face recognition task based either on the identity or the emotion (happiness, sadness and fear) of children faces presented on a computer screen. After the presentation of a sample face, the patients have to select among two other faces, which picture is the same subject or which face contains the same emotional expression. During the test gaze behavior was recorded using a classical eye-tracker device (Mirametrix, Tobii).

Results: Compared to a group of 14 typically developing (TD) subjects, PWS are about 2 times slower to execute the task, reaching high performance levels which are nevertheless significantly lower than in TD (83 vs 96% respectively). A fine analysis of gaze behavior (%fixation time, FT) revealed that PWS tend to gaze predominantly the mouth of the sample face (50% FT) while TD fixate mainly the eyes (50% FT). Similarly, when searching for the matching face, PWS fixate equally the eyes and the mouth/nose regions (51 and 49% FT respectively) while TD subjects are predominantly on the eyes (68% FT). Preliminary analysis revealed also different scanning strategy when exploring the 2 faces.

Conclusions. Our results confirm previous studies of a specific deficit of PWS in face discrimination in spite of the weak difficult of the task. Further, PWS show altered eye gaze pattern avoiding the eye region which content most of the social information embedded in a face. Such altered face gaze in PWS participates probably to the social communication deficit characteristic of PWS.

Summary. In the present study we show clearly that PWS present an altered oculomotor exploration of human faces that contribute to their deficit in face processing, including emotional recognition.

POSTER ABSTRACTS

P23 - Role of the Prader-Willi Syndrome protein MAGEL2 in intracellular pathways regulating leptin receptor processing.

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Background: Children with Prader-Willi syndrome have neonatal feeding difficulties, developmental delay and excessive appetite. Loss of MAGEL2 causes a neurodevelopmental disorder (Schaaf-Yang syndrome) and may contribute to obesity in children with Prader-Willi Syndrome who lack MAGEL2 and other genes. MAGEL2 is essential in neurons that sense levels of the adipose tissue-derived hormone leptin. The MAGEL2 protein is important for recycling or degradation of proteins in the brain and interacts with and modifies the activity of E3 ubiquitin ligases. RNF41 is a E3 ubiquitin ligase that associates with a ubiquitin-specific protease (USP8). Together with USP8, RNF41 regulates the recycling of the leptin receptor by targeting it either for degradation or for recycling to the cell membrane. We hypothesized that MAGEL2 normally regulates the interaction between RNF41 and USP8, and that loss of this regulation could impair leptin response pathways in the brain in children with PWS.

Methods: Human U2OS cells were transfected with recombinant constructs encoding epitope tagged versions of MAGEL2 and wild type and mutant forms of RNF41. Immunofluorescence was used to visualize the co-localization of different forms of RNF41 with MAGEL2 in intracellular compartments. We expressed recombinant MAGEL2, RNF41 and USP8 in combinations in human U2OS cells and examined the relative abundance of each protein in the presence or absence of the other components of the complex.

Results: Abundance of RNF41 was lower when MAGEL2 was co-expressed, and abundance of MAGEL2 was lower when RNF41 was co-expressed. This suggests that MAGEL2 and RNF41 proteins destabilize each other inside the cell. Abundance of RNF- SQ (ligase defective mutant) was higher, while abundance of RNF-AE (USP8 binding mutant) was lower when MAGEL2 was co-expressed. Preliminary results suggest that MAGEL2 modifies the ability of RNF41 to auto-ubiquitinate or to ubiquitinate MAGEL2. Expression of all three RNF41 forms was higher when co-transfected with USP8, but co-transfection with MAGEL2 reversed the stabilizing effect of USP8.

Conclusion: Our results suggest that MAGEL2 could modify the activity of the RNF41-USP8 ubiquitination complex in leptin sensing neurons, providing a possible mechanism for dysregulation of leptin sensing in neurons in children with Prader-Willi syndrome.

Summary: This study will help to understand how MAGEL2 interacts with the network of genes and proteins that normally control body weight. We will investigate the mechanism by which loss of MAGEL2 could increase appetite and obesity in PWS, and children with inactivating mutations in MAGEL2 alone.

POSTER ABSTRACTS

P24 - Elucidating the neurobehavioral phenotypes of *Magel2*-null mice

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Background: Prader-Willi syndrome (PWS) is a neurodevelopmental disorder characterized by a variety of symptoms, including a complex behavioral profile with temper tantrums, stubbornness, controlling and manipulative behavior, obsessive-compulsive characteristics, and difficulty with changes in routine. A subset of individuals with PWS meet criteria for autism spectrum disorder (ASD), and the prevalence of ASD among molecularly confirmed cases of PWS with loss of the paternal copy of chromosome 15q11-q13 has been estimated as 27%, well above the rate of 1% in the general population. *MAGEL2* is one of five protein-coding genes in the PWS-critical domain on chromosome 15q11. Six individuals with truncating variants of *MAGEL2* have been reported, all of which carry a diagnosis of ASD based on DSM-IV/V criteria and clinical assessment by an expert physician. Since this time, we have identified an additional 40 individuals including 3 families each with multiple affected individuals, expressing a wide spectrum of neurodevelopmental phenotypes, including a characteristic behavioral profile including impulsiveness, compulsiveness, manipulation, and stubbornness, and ASD. These findings highlight that ASD is a common phenotype among individuals with loss-of-function *MAGEL2* mutations.

Methods and Results: *Magel2*-null mice (C57BL/6-*Magel2*^{tm1Stw/J}) have been developed. They harbor a maternally inherited imprinted wildtype allele and a paternally inherited *Magel2-lacZ* knock-in allele that abolishes endogenous *Magel2* gene function. These *Magel2* null mice were found to exhibit neonatal growth retardation, excessive weight gain after weaning, and increased adiposity with altered metabolism in adulthood, thereby recapitulating some of the core features of the PWS phenotype. The same mice have reduced fertility in both males and females through extended breeding intervals and early reproductive decline and termination. We performed a battery of behavioral assays on 20 male and 20 female *Magel2*-null mice and their wildtype littermate counterparts.

Conclusions: Our studies suggest an altered social phenotype for mice with *Magel2* mutations, in particular a deficit in appreciation of social novelty. Patients diagnosed with PWS have been described as being able to engage in social interactions and making friends, however, there are noted deficits in the amount of time spent with friends and interpersonal communication, which may have some similarity to what we see in this mouse model.

Summary: The investigation of autistic behaviors caused by *MAGEL2* loss-of-function may help us understand a subset of behavioral phenotypes seen in PWS in general.

POSTER ABSTRACTS

P25 - Interactions between the Prader-Willi syndrome candidate protein MAGEL2 and neuronal proteins

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Background: Inactivating mutations in the *MAGEL2* gene cause a neurodevelopmental disorder that includes intellectual disability and autism spectrum disorder (Schaaf-Yang syndrome). *MAGEL2* is one of the genes inactivated in Prader-Willi syndrome, which shares many clinical findings with Schaaf-Yang syndrome. Loss of *Magel2* in mice affects brain development and function, disrupts circadian cycles and causes other phenotypes that recapitulate symptoms of PWS. Mice lacking *Magel2* have decreased brain volume in certain areas as well as altered brain chemistry. However, little is known about the role *MAGEL2* plays in brain function and development. Identifying the proteins that interact with *MAGEL2* in neuronal cells will allow us to connect the underlying molecular processes that *MAGEL2* is involved in with neurodevelopmental deficiencies in Prader-Willi syndrome and associated disorders.

Methods and Results: A cell line derived from neurons in the mouse hypothalamus (GT1-7) and a human osteosarcoma cell line (U2OS) both endogenously express the *Magel2/MAGEL2* gene. Recombinant epitope-tagged *MAGEL2* can be expressed in these cell lines and used to identify novel interactions. Complexes containing *MAGEL2* and associated proteins are purified biochemically to identify the interacting proteins. The effects of over-expression of *MAGEL2* or loss of expression of *MAGEL2* on levels of these interacting proteins will be examined.

Conclusions: These studies will provide additional insight into the cellular role of *MAGEL2*, and how *MAGEL2* participates in normal brain development and function.

Summary: *MAGEL2* is one of the genes that is inactivated in Prader-Willi syndrome. Loss of *MAGEL2* contributes to autism spectrum disorder in children with Prader-Willi or Schaaf-Yang syndrome. Through our studies of the *MAGEL2* protein network, we will better understand why brain cells need *MAGEL2* for their function.

POSTER ABSTRACTS

P26 - The Impact of Hyperphagia and Food Restriction on Siblings of Individuals with Prader-Willi Syndrome

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Background Having an individual in a family with PWS impacts the entire family system. The behavioral phenotype and hyperphagia are usually the most difficult and stressful aspects of the disorder to manage. Siblings are often highly involved in almost all aspects of care for their brother/sister with PWS and are immersed in the environment; thus it is important to understand their perspectives on an in-depth basis of how this has impacted their eating patterns, their feelings towards themselves and their families, and outlook towards their own futures.

Methods and Results Using a qualitative approach and a constant comparative thematic analysis, twenty-four semi-structured telephone interviews were conducted with siblings over the age of 18 of individuals with PWS. Five major themes emerged including: “Environmental and dietary restrictions”; “Positive/Negative Feelings”; “Comfort with friendships”; “Supportive relationships”; “Sense of responsibility.”

Conclusions Feelings of ambivalence towards their home environment as well as a sense of normalization towards the behaviors of their sibling with PWS was a common experience exemplified by participants. Stress surrounding strict eating regimens resulted in siblings having both positive and negative eating habits. Participants also noted that the accessibility and utilization of support groups was reduced. Moreover, participants identified that there is a present and/or future perception of responsibility to care for their brother/sister with PWS. Next steps include determining when siblings would best benefit from support along with enabling them to feel more adequate in the caregiving role. Additionally, allowing typically developing children more time with their families may reduce negative feelings towards their sibling with PWS.

Summary Siblings of individuals with PWS are greatly affected by being present in the environment with their brother/sister and are largely involved in their care. The need for more support and early professional involvement for siblings was a major finding of this study. Providing siblings with more accessible resources may allow for less negative experiences and more positive outcomes.

POSTER ABSTRACTS

P27 - The role of MAGEL2, a protein implicated in Prader-Willi syndrome, in the regulation of RING-zinc finger-type E3 ubiquitin ligases

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Background. Prader-Willi syndrome (PWS) is a genetic disorder of the nervous and endocrine systems characterized by developmental disabilities, obesity, excessive daytime sleepiness and night-time wakening, and autism spectrum disorder. One of the genes inactivated in PWS is *MAGEL2*, and mutations in *MAGEL2* alone cause the neurodevelopmental disorder Schaaf-Yang syndrome. The exact cellular role of *MAGEL2* remains to be elucidated. MAGE proteins interact with RING-zinc finger-type E3 ubiquitin ligases and enhance their activity. E3 ubiquitin ligases participate in protein ubiquitylation, a cellular process in which substrate proteins are recognized, ubiquitylated, and targeted for proteosomal degradation. Protein ubiquitylation is implicated in the regulation of diverse processes including cell cycle, intracellular signaling, and transcription. TRIM32 is an E3 ligase that may interact with *MAGEL2* and has been implicated in other disorders such as Bardet-Biedl intellectual disability/obesity syndrome and Limb-girdle muscular dystrophy type 2H, which overlap clinically with findings in PWS.

Methods and Results. To determine if *MAGEL2* affects the E3 ligase TRIM32, constructs encoding the wildtype and mutant forms were co-transfected in U2OS cells and analysed by immunoblotting. *MAGEL2* affects the abundance of TRIM32 in U2OS cells, with increased TRIM32 levels on co-transfection with *MAGEL2*. *MAGEL2* also affects the abundance of mutant TRIM32, different from the effect of *MAGEL2* on the wildtype TRIM32. By immunofluorescence analysis, we found that *MAGEL2* co-localizes with TRIM32 in the perinuclear region. Additional co-localization experiments will be performed with mutant forms of TRIM32. We will investigate whether these proteins directly interact. Finally, we will determine if *MAGEL2* affects the regulation of TRIM32 and the ubiquitination levels of its substrates.

Conclusions. *MAGEL2* may interact with TRIM32 and could affect its function in protein ubiquitylation. The results from this project will elucidate the role of protein ubiquitylation in cellular processes that can contribute to PWS symptoms. We will further understand how loss of *MAGEL2* in children with Schaaf-Yang syndrome or PWS contributes to these complex disorders.

Summary. My project will investigate the role that *MAGEL2*, a protein implicated in PWS, has within the cell. Understanding the function of *MAGEL2* will shed light on how the loss of *MAGEL2* contributes to neurodevelopmental disorders including PWS.

POSTER ABSTRACTS

P28 - Characteristics of change triggered temper outbursts in children with PWS and ASD and how these may impact on the efficacy of behavioural intervention strategies

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Background

Temper outbursts are prevalent in individuals with PWS and are often triggered by unexpected changes to routines or plans. However, such outbursts are also common in individuals with several other neurodevelopmental disorders, including those with a diagnosis of autism spectrum disorder (ASD). We compared the profile of temper outbursts in children with PWS to that in children with ASD. We examined whether differences in the temper outburst profile predicted differences in the outcomes of two caregiver led intervention strategies aiming to reduce change triggered outbursts.

Methods and results

Thirteen 7-15 year olds with PWS – taking part in a larger study involving 60 children evidencing temper outbursts following changes – were individually matched for age to children with ASD (mean ages: 10.70; 10.76 yrs). Caregivers participated in a structured/semi-structured interview on children's outbursts; completed a web-based outburst diary over a 6 month baseline; and are currently using either a change signalling intervention to reliably warn children of forthcoming changes; or a planning ahead intervention to reduce children's exposure to unexpected changes.

As reported at interview, on average, children with PWS showed more frequent temper outbursts than those with ASD (closer to daily vs. weekly). For seven children with PWS and six with ASD, 60% or more of their temper outbursts were reported to be triggered by changes. Whilst outbursts had similar durations when triggered by changes or by other events in children with PWS; change triggered outbursts in children with ASD were generally shorter. The most commonly reported outburst components in children with PWS included indicators of heightened emotional arousal but this was not the case for children with ASD. Data on behavioural change associated with each of the intervention strategies will be discussed.

Conclusions

Change triggered temper outbursts can be a problem for children PWS and ASD, however subtle differences appear to exist in the profile of these outbursts. Some of these differences may be relevant for the expected efficacy of different behavioural intervention strategies that target outbursts.

Summary

Temper outbursts (tantrums) were compared in children with PWS or autism spectrum disorder before and during use of one of two helping strategies. Helping strategies were led by caregivers and aimed to reduce outbursts that follow changes to routines or plans by making such changes more predictable, or by reducing the quantity of changes. Characteristics of outbursts may be important to help us predict which helping strategies may be most effective.

POSTER ABSTRACTS

P29 - Beloranib Improves Hyperphagia-Related Behavior: Results from a Phase 3, Randomized, Placebo-Controlled Clinical Trial of Beloranib in Patients with Prader-Willi Syndrome

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Background: Prader-Willi syndrome (PWS) is the leading genetic cause of life-threatening obesity. The insatiable hunger characteristic of PWS negatively impacts quality of life for patients and their caregivers and can cause choking, stomach rupture, gastric necrosis, and other severe complications. Beloranib inhibits MetAp2, an enzyme that modulates key cellular processes that control metabolism, lipid synthesis, and fat storage, and was recently shown to reduce body weight and hyperphagia in a study in 17 patients with PWS.

Methods and Results: The effect of beloranib on hyperphagia-related behavior was investigated in bestPWS, a 26-week, Phase 3, randomized, placebo-controlled clinical trial of biweekly beloranib in patients with PWS and total score of ≥ 13 on the PWS-specific Hyperphagia Questionnaire for Clinical Trials (HQ-CT), a retrospective 9-item questionnaire, each item scored 0-4 (total score 0-36), reduction in score indicates improvement. Baseline characteristics: (mean \pm SD) 20 \pm 6 y, BMI 40.0 \pm 10.1 kg/m², and HQ-CT total score 16.9 \pm 6.6. After 26 weeks, the placebo-adjusted reduction in HQ-CT total score was -6.3 \pm 1.7 and -7.0 \pm 1.7 for 1.8 and 2.4 mg beloranib (both $p < 0.001$). Beloranib-treated patients had improvements vs placebo in all 9 HQ-CT items. Both beloranib groups had significant reductions vs placebo in HQ-CT total score at Week 4, the earliest point measured. There were more HQ-CT responders (identified using the anchor-based threshold of ≥ 7.7 derived from the Caregiver Global Impression of Change) in the 1.8 mg (36%; $p < 0.05$) and 2.4 mg beloranib (51%; $p < 0.001$) groups vs placebo (12%). Larger proportions of beloranib- vs placebo-treated patients reached a threshold reduction in HQ-CT total score of at least 20%, 30%, 40%, and 50%. The most common AEs were injection site bruising, aggression, and hyperphagia; only injection site bruising was more frequent with beloranib vs placebo. The incidence of serious AEs was low and similar across treatment groups. Six beloranib-treated patients withdrew due to AEs during the randomized treatment period (4 psychiatric AEs, 1 injection site pain, 1 pulmonary embolism resulting in death).

Conclusions: Beloranib treatment resulted in statistically and clinically significant improvements hyperphagia-related behavior in patients with PWS.

Summary: This clinical trial investigated the effect of beloranib on body weight and hyperphagia in patients with PWS. Beloranib-treated patients had greater improvements in hyperphagia-related behavior vs placebo as early as Week 4. AEs were generally consistent with the patient population and prior clinical trials.

POSTER ABSTRACTS

P30 - Parental approaches regarding prenatal diagnosis and termination of pregnancy in Prader Willi syndrome

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Background: The field of prenatal diagnosis has recently developed tremendously resulting in serious questions and ethical dilemmas such as the option of termination of pregnancy (TOP) in cases with severe outcome. The decisions concerning TOP and prenatal tests is influenced by factors such as: religion, gestational age, socio-economic status and years of education.

Prader Willi Syndrome (PWS) is a complex neurogenetic syndrome with high morbidity and mortality due to severe medical and behavioral problems along the life span. PWS was rarely diagnosed prenatally, lately, we documented that PWS have a unique prenatal phenotype and when any combination of SGA or asymmetric intra- uterine growth, polyhydramnios, and diminished fetal movements is present there is an indication for prenatal methylation testing. In this study we tried to evaluate the attitudes of parents of individuals with PWS to prenatal diagnosis (PND) and TOP under different circumstances.

Methods All consecutive parents of individuals with PWS who attended the National Israeli PWS multidisciplinary clinic between March-September 2015 were interviewed, using a semi structured questionnaire about their attitude towards PND and TOP. The interviews were done by a single physician (VTG) who treats and follows all families.

Results: Eighty five parents were recruited, no one refused. 31F/26M (67%) were in favor of invasive prenatal tests. The rest agreed to non- invasive tests only. None opposed all PND tests. 17F/21M (44%) were in favor of TOP, 17F/14M agreed to TOP under certain conditions (36.5%) and 8F/7M were against TOP (17.6%). The parental attitude correlated with religion status: M ($p < 0.000$, F $p < 0.025$, mothers years of education ($p < 0.001$), mother's working status ($p < 0.001$) and for the fathers- child's age ($p < 0.008$). Couples had similar attitude regarding PND and TOP. No correlations were found with gender, PWS genetic subtype, age of the parents at the birth of the child with PWS or at time of interview.

Conclusions: Most parents of individuals with PWS think that PND is needed before and during pregnancy including invasive tests. Being secular or religious had the most influential effect on the decision of TOP. Unexpectedly, age of the disabled child does not affect parental decision.

Summary: In families with a child with PWS all parents are in favor of PNT even if they are against TOP. Religion is the most significant factor in the decision of TOP. When giving a prenatal consultation physicians and other counselors should be attentive and empathic to the perspective of families and guide them according to their principles

POSTER ABSTRACTS

P31 - Correlation of Five Distinct Characteristics of Prader-Willi Syndrome: A Four Year Case Study on a Daily (School Days) Basis.

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Objective: Persons with Prader-Willi Syndrome (PWS) manifest an abundance of physical and behavioral characteristics of variable degree. The occurrence and severity of these characteristics can rarely be predicted undermining the persons' independency. We closely monitor the prominent symptoms of the case study and describe their possible interrelation/correlation. Moreover, we investigate the possible impact of the sex hormones. Our objective is to inspire new ways of observing and therefore researching the symptomatology of PWS.

Methods: The index person is an 11' year old girl diagnosed with PWS, m-UPD also presenting Autistic Spectrum Disorder (ASD). The PWS profile of the index case, in short, includes hypotonia, hypothyroidism, precocious puberty, lean body mass and IQ 58 (WISC-III).

We sorted out the more distinct symptoms of our index case based on frequency, functional impairment and, not yet, apparent correlation between them. Attention deficit, aggressiveness, daily sleepiness, cuticle picking and food seeking were selected.

We then defined the time period during the day, for the symptoms to be monitored, based on stability of the time frame and the environment, as well as the longest possible daily length of observation along one year period. School hours met all the previous criteria.

Subsequently, we created a detailed 0-10 measuring scale for each symptom. All data are collected by the same psychologist on a daily basis.

Finally, we will implement sex hormones' curves over the curves of the symptoms in order to clarify their relation since there are several behavioral indications that a full circle of 28 days is implicated.

Results: The observation of this index case revealed a strong correlation between symptoms' scale and long, school holidays, both preceding and following them. There is an apparent escalation of most symptoms as the school week progresses. All symptoms, except cuticle picking, slightly attenuate over the years. A preliminary analysis of the data shows that aggressiveness is negatively related to attention deficit, cuticle picking is positively correlated to attention deficit and food seeking to daily sleepiness.

Conclusions: A full report of index case findings will be available for the conference along with the results of the sex hormones impact on the symptoms. However, the preliminary analysis indicates new, interesting interrelations between the specific symptoms and therefore we suggest a wide, similar research.

Summary: The physical and behavioral characteristics of persons with PWS can rarely be predicted undermining their independency. A daily (school days), four year long documentation and analysis of five distinct characteristics -attention deficit, aggressiveness, daily sleepiness, cuticle picking and food seeking- of an index case with PWS was set with the preliminary results to indicate new, interesting interrelations between the specific symptoms. Final findings will be presented at the conference.

POSTER ABSTRACTS

P32 - Determining the Mathematical Profile of a Child with Prader-Willi Syndrome and Applying the Constructivism Model as a Learning Tool

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Background: Review of literature on Prader-Willi Syndrome (PWS) shows intense research activity concerning its medical dimension. However, few studies have been conducted in terms of the cognitive and pedagogical dimension of the syndrome, and even fewer studies on those individuals' mathematical skills. The population used in the few studies conducted up to day has been adults and not children. Failure in mathematical skills acquisition constitutes the most striking characteristic in the cognitive profile of individuals with PWS, and especially of type m-UPD.

Objective: The current case study aims at: a) evaluating the mathematical profile of a child with PWS (m-UPD) from the 1st grade up to the 4th grade of primary school and b) investigating the extent to which application of the constructivism model of learning is effective regarding acquisition of mathematical skills and improvement of motivation toward mathematics.

Methods: The case study is an 11-year-old girl diagnosed with PWS, m-UPD, at one year of age and Autistic Spectrum Disorder (ASD) by the age of 4. Initially, information from WISC-III, from diagnostic interviews in Number Sense by Denvir & Bibby and from Mann-Suitter Developmental Arithmetic Inventory in 1st grade of elementary school was used for determining the child's profile in mathematics. Then, an individualized program of intervention was constructed and applied on the basis of the principles of the constructivism model. In terms of investigating motivation, a questionnaire was completed by the mother, the parallel support of the child and the special education teacher, in the beginning and the end of the study period.

Results: Data processing from tests used shows that: a) the child's profile in mathematics presented in specific areas similarities to what has been mentioned in literature regarding adults with the syndrome, b) the constructivism model of learning improved the child's performance although intelligence quotient decreased. Improvement refers to areas of mathematical readiness, number sense, place value, arithmetic calculations, problem solving and measurements (time, money, weight, length), and c) the child's motivation toward mathematics was positively differentiated.

Conclusions: The educational model of active learning positively influenced the research subject's performance in mathematics. The results are encouraging and could lead us in a change of perspective toward children with PWS and mathematics. We suggest further research in the pedagogical sector.

Summary: Through a number of tests, the current study examines performance in mathematics of a child with PWS, m-UPD, during the 1st grade of elementary school. "Learning by doing" is selected as method of treating difficulties in mathematics while in the 4th grade the child is reexamined. The results of the method are encouraging in terms of child's performance in and motivation toward mathematics.

POSTER ABSTRACTS

P33 - Abnormal body composition phenotype in children with Prader-Willi Syndrome.

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Background: Prader-Willi Syndrome (PWS) is a genetic syndrome associated with unfavorable alterations in body composition such as reduced lean mass and increased adipose tissue. Detailed analysis of skeletal muscle mass and adipose tissue distribution (subcutaneous, visceral and intermuscular) in PWS children has not been conducted. The aim of this study was to describe skeletal muscle mass and adipose tissue distribution in PWS children.

Methods and Results: Anthropometric measures and an abdominal T1-weighted magnetic resonance imaging were performed in PWS and control group. Multiple images from the top of the liver to the top of femoral heads were analyzed using Slice-O-Matic. Subcutaneous adipose tissue (SAT) was identified using the “watershed” tool. Visceral adipose tissue (VAT), intermuscular adipose tissue (IMAT), and skeletal muscle mass (SM) were identified using a threshold technique. IMAT was manually identified. Volume (cm³) of each tissue was automatically computed, and then converted to liters. Total adipose tissue volume (TAT) was calculated as a sum of SAT+VAT+IMAT volumes. Mann-Whitney test was used to describe between-group comparisons, and Spearman rank correlations to measure associations between variables. All data is presented as medians. PWS group (N=16) and controls (N=17) had similar age (10.5; 12.8 years; $p=0.127$) and BMI z-scores (0.49; 0.22; $p=0.382$). WC z-score was significantly higher in PWS (0.69) compared to control (-0.04) ($p=0.028$). No significant differences were observed in SAT, VAT, IMAT and TAT volumes between PWS and controls. Skeletal muscle mass was significantly lower in PWS (1.53 liters) compared to controls (3.11 liters) ($p=0.008$), and remained different when corrected for height (m²) ($p=0.023$). In PWS, VAT/SAT was significantly lower (0.37) compared to control (0.50) ($p=0.014$), and was negatively correlated with WC z-score (-0.680; $p=0.004$). In contrast, TAT/SM was significantly higher (PWS=2.25; Control=1.18; $p=0.000$) as well as IMAT/SM (PWS=0.16; Control= 0.08; $p=0.000$). A strong positive correlation between TAT/SM and BMI z-score (0.656; $p=0.006$) was noted in PWS.

Conclusions: PWS children exhibited reduced SM compared to controls, but similar measures of abdominal adipose tissue. The decreased ratio of VAT/SAT suggests a positive metabolic pattern of adipose tissue distribution. However, the greater TAT/SM and IMAT/SM ratios in PWS illustrates that skeletal muscle plays a driving role in their unique body composition phenotype; it might be associated with poor motor development and function.

Summary: This study describes skeletal muscle mass and fat distribution in PWS children. Results show that PWS children have similar amounts of fat in the abdomen, but reduced quantity of skeletal muscle mass compared to healthy children. These findings might explain their difficulties with coordination, balance and posture.

POSTER ABSTRACTS

P34 - Methylene tetrahydrofolate reductase (MTHFR) polymorphisms, genetic subtype, psychiatric diagnosis and symptom severity in PWS adults residing in group homes

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Background: PWS is associated with a high incidence of mood disorders that vary according to genetic subtype, symptom severity, and age of onset. Risk for psychosis is increased in both genotypes. In the general population, MTHFR polymorphisms are associated with psychiatric disorder (depression, schizophrenia, mood disorder with psychosis and rapid cycling) particularly when serum levels of folate are low or homocysteine is high. In this study the prevalence of MTHFR polymorphisms, and serum levels of homocysteine, folate and vitamin B12 were ascertained among adults with PWS residing in a group home system where diet was strictly controlled. Results were then correlated with serum levels, PWS genetic subtype, psychiatric disorder and indicators of severity.

Methods: Adults with PWS residing at the Prader-Willi Homes of Oconomowoc (PWHO) received psychiatric diagnoses based upon clinical evaluation and DSM IV TR criteria. Clinical severity was determined by the most severe diagnosis, cumulative diagnostic complexity, number of medications needed to stabilize, and number of incident reports in the preceding 6 months. MTHFR gene polymorphisms (677CT, 1298AC), serum folate, homocysteine and vitamin B12 levels were obtained. Genetic subtype of PWS was previously determined by MS-MLPA.

Results: There were 28 adults; 14 males and 14 females. Distribution of MTHFR alleles was not statistically different from population norms. MTHFR polymorphisms, 677CT (7), 677TT (3), 1298AC (8), and 1298CC (4), were mutually independent, except in one case of compound heterozygosity. Distribution of PWS genetic subtypes was not representative: DEL I (5), DEL II (6), mUPD segmental isodisomy (6), mUPD heterodisomy (8), and IC DEL (3). PWS genetic subtype varied independently from MTHFR polymorphisms, but there were some gene interactions with serum levels associated with folate metabolism. No one was folate deficient, but differences among genetic subtypes was significant ($p < 0.029$) with higher folate level in IC DEL. Serum homocysteine levels were not elevated but varied with PWS genetic subtype ($p < 0.068$) and were inversely proportional to serum vitamin B12 ($p < 0.023$) and folate levels. Serum vitamin B12 levels were elevated in 1/3 of the cohort among individuals with 677CT (UPD > DEL), 1298AC (UPD) and 1298CC (DEL). Individuals with 677CT or 677TT polymorphisms had lower mean number of incident reports, especially in DEL condition. **Conclusions:** In this case series, MTHFR polymorphisms among PWS adults did not relate to genetic subtype. Serum homocysteine, vitamin B12 and folate levels did vary by genetic subtype but were not related to psychiatric symptom severity. However, the presence of the 677T allele was associated the *lowest* number of incident reports. The literature suggests that the T allele is related to *increased* sensitivity to a stressful environment. If an incident report can be interpreted as an indicator of the behavioral response to the environment, then the T allele may confer a *protective* effect in the consistency and stability of the PWS milieu. Elevated serum vitamin B12 levels without exogenous supplementation were seen in 1/3 of this cohort. Further exploration of the regulation of folate metabolism in PWS is warranted.

Summary: In this cohort, the distribution of MTHFR polymorphisms varied independently of PWS genetic subtype. There were some interesting gene interactions with products of folate metabolism. The presence of the MTHFR 677T allele is a recognized risk factor for depression in a stressful context, but it may offer a protective effect in a controlled environment like the PWS milieu.

POSTER ABSTRACTS

P35 - Early Diagnosis of Prader-Willi syndrome delays onset of obesity

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Introduction/Background: Prader-Willi syndrome (PWS), affecting 1/15,000 individuals, is a genetic disease characterized by lack of expression of genes on the paternal chromosome 15q11-q13 region with 70 percent caused by paternal deletions, 28 percent due to uniparental maternal disomy and 2 percent caused by an imprinting defect. Clinical features include neonatal hypotonia, poor suck, feeding difficulty, failure to thrive, hypogonadism, characteristic dysmorphic features, mild intellectual disability, growth hormone deficiency with short stature, small hands and feet, and later development of excessive hunger and obesity and distinctive behavioral characteristics with temper tantrums, outbursts and self-injury. Growth hormone replacement has revolutionized the stature and body composition in individuals with PWS who have started treatment early. Despite significant advances in the diagnostic genetic testing in individuals with PWS, the mean age for diagnosis continues to lag behind. We hypothesized that early diagnosis and treatment of PWS reduces the risk of obesity and associated co-morbidities.

Methods and Results: The long term data accrued from 351 Prader Willi patients recruited for the 9 year NIH funded RDCRN (Rare Diseases Clinical Research Network) natural history studies was analyzed for age of diagnosis versus obesity and co-morbidities. Because of large variability in age of diagnosis, the age of diagnosis was categorized into 3 categories (< 1 yr, ≥ 1 yr and < 3 yrs, and ≥ 3 yrs). Three variables from the Natural History Form: Age child first became heavy according to your physician, Age first developed an increased appetite, and Age first started to seek food, were analyzed by the Cox proportional hazards model. The earliest reported age for each of these variables was used as the endpoint age for each variable. In addition to age of diagnosis (categorized), race (white vs non-white), gender, and PWS type were included as covariates in the Cox model.

The mean age for diagnosis was delayed in a large group of subjects with 84 (25%) diagnosed with PWS ≥ 3 yrs, 41 (12%) between 1 and 3 yrs and 208 (62%) < 1 yr (range from 1 month to 47 yrs old). Both the age of diagnosis (p<.001) and race (p=.018) were significant factors for the age when child first became heavy. The estimated median ages for when the child first became heavy was 10 years for age of diagnosis < 1 yr, 6 yrs for age of diagnosis between 1 – 3 yrs, and 4 yrs for age of diagnosis greater than 3 yrs. Non-white individuals exhibited earlier age of first becoming heavy than whites. Age of diagnosis was not related to Age of development of an increased appetite, and Age of first starting to seek food,

Conclusion: It is critical for PWS to be detected early in order to avoid costly invasive diagnostic work-ups and permit early dietary and growth hormone treatment to prevent obesity. We propose that molecular technology currently exists to perform newborn screening to detect patients early to prevent obesity and associated co-morbidities.

Summary: Early diagnosis and treatment of PWS delays the onset of obesity but not the onset of hyperphagia or food seeking.

POSTER ABSTRACTS

P36 - An oligonucleotide changes alternative splicing of the serotonin receptor 2C, reduces food intake in mice and could substitute SNORD115

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Background

The Prader-Willi critical region contains five C/D box snoRNAs (SNORDs) (107, 109, 64, 115 and 116) that are not expressed in PWS subjects. The 47 copies of SNORD115 are almost identical and promote alternative exon inclusion of the serotonin receptor 2C pre-mRNA. The serotonin 2C receptor regulates food uptake and its activity is controlled by alternative pre-mRNA splicing and editing. Loss of SNORD115 leads to alternative exon skipping, which is predicted to generate a truncated receptor protein isoform. cDNAs encoding this truncated receptor are located inside the cell, not at the cell membrane. They heterodimerize with the full-length serotonin 2C receptor.

Method and Results

We raised an antiserum specific for the truncated serotonin receptor 2C (RNA1) and showed that the expected protein is expressed in mouse, rat and human. We developed an oligonucleotide that promotes exon inclusion, which increases the ratio of full-length to truncated receptor protein isoform. The oligonucleotide works in the low nanomolar concentration and its intronic binding site is unique in the human genome. Decreasing the amount of truncated receptor results in the accumulation of full-length, constitutively active receptor at the cell surface, suggesting that the ratio of full-length and truncated receptor controls the surface localization of the full-length receptor. After injection into the third ventricle of mice, the oligonucleotide accumulates in the arcuate nucleus, where it changes alternative splicing of the serotonin 2C receptor leading to an increase of the full-length, non-edited receptor. This increase of the constitutively active receptor results in an increase of pro-opiomelanocortin (POMC) expression. Oligonucleotide injection reduced food intake in wild-type mice. This effect is most likely due to an increase in alpha melanocortin that is generated from POMC, as the oligo affects ob/ob mice, but not melanocortin receptor 4 knock out mice. Unexpectedly, the oligonucleotide crossed the blood brain barrier and its systemic delivery reduced food intake in wild-type mice.

Conclusions

The physiological effect of the oligonucleotide suggests that a truncated splice variant regulates the activity of the serotonin 2C receptor, indicating that therapies aimed to change pre-mRNA processing could be useful to treat the hyperphagia, characteristic for Prader-Willi syndrome.

Summary

The truncated serotonin receptor 2C isoform has an important biological function: It sequesters the full-length receptor inside the cell, which switches off the serotonin signaling. Through heterodimerization, it could affect other receptor systems as well. These properties could explain why the loss of SNORD115 in PWS creates a syndrome, affecting multiple receptor systems. Loss of SNORD115 can be substituted with an oligonucleotide.

Reference: Zhang, Z., Shen, M., Gresch, P., Ghamari-Langroudi, M., Rabchevsky, A.G., Emeson, R.B. and Stamm, S. (2016) Oligonucleotide-induced alternative splicing of serotonin 2C receptor reduces food intake. *Embo Mol. Med.*, in press.

POSTER ABSTRACTS

P37 - Von Economo neurons (VENs) in post mortem brains in PWS: a preliminary report

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Background: VENs are large spindle cell neurons located in layer V of the fronto-insular (FI) and anterior cingulate cortex (ACC) of human brain. VENs appear bilaterally in the 8th month of gestation but proliferate selectively in the right hemisphere. The number of VENs at age 4 is comparable to adult numbers. Their putative function is to relay fast information necessary to ascertain social salience, self-awareness, and physiological state. They may also maintain balance in the autonomic nervous system that is essential for homeostasis. Recently, the role of VENs and pyramidal neurons in psychiatric illness has been explored in conditions of autism, schizophrenia, bipolar disorder, and frontotemporal dementia (FTD). This preliminary report describes the number, location, and morphology of VENs in ACC and insula of post mortem brains in PWS.

Methods: The NIH NeuroBioBank provided 20 post mortem brains with PWS. Clinical information on 11 cases included age, gender, genetics, BMI, medical history, psychiatric history, and neuropathology. Areas of interest (ACC and FI) were dissected as a block from the right hemisphere of the brain. Each tissue block was prepared, sectioned, stained and examined to clarify cytoarchitectural boundaries of regions of interest. VENs were identified and their densities, location, morphology and spatial orientation were documented.

Results: To date, 7 brains have been examined. Stereological analysis, immunohistochemical testing, clinical correlation and genetic studies have not been performed as yet. In the ACC the VENs are numerous in the area around the genu of the corpus callosum. Some samples showed large pyramidal cells, which are indicative of the cingulate motor field, but they are positioned more posteriorly than typical for the ACC. VEN density is typically distributed in a ventro-dorsal and antero-posterior gradient. There were more VENs in the ACC than in the insula, comparable to what has been seen in autism. The cells in the ACC appear to be normal in morphology and orientation. But in the insula, the VENs are not distributed properly in regard to their spatial orientation; some were actually oblique. Pyramidal cells were also in disarray. Further, VENs were found in the mid and posterior insula, where they are not usually encountered. The functional significance of VENs in the posterior insula is not known, but it appears to be a finding unique to PWS.

Conclusions: In this preliminary overview of the ACC and insula in post mortem brains of PWS persons, the apparent density, morphology and spatial orientation of VENs and pyramidal cells is intact. In contrast to the ACC, the number, morphology, spatial orientation and distribution of VENs in FI is abnormal, and more comparable to what has been reported in autism. The presence of VENs in the posterior insula is a finding unique to PWS. Because VENs probably differentiate from pyramidal cells, their distribution and orientation may be affected by abnormalities in brain folding during development. Abnormal insular closure has been reported in PWS.

Summary: This is the first examination of the ACC and FI in post mortem brains in PWS. These very preliminary findings indicate that the number, morphology, spatial orientation and distribution of VENs in insular cortex are abnormal. In the ACC, both VENs and pyramidal cells appear to be more typical, but additional quantitative and stereologic assessment has yet to be performed.

POSTER ABSTRACTS

P38 - Functional and therapeutical investigation of the oxytocin system in the *Magel2*-deficient mouse model.

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Background: Prader-Willi syndrome is a complex disorder including severe feeding disturbances learning disabilities, behavioral and social disturbances. PWS involves several contiguous genes, including the *MAGEL2* gene. Recently, pathogenic mutations of *MAGEL2* have been reported in several patients causing autism and PWS-like phenotypes. These results underline the major role of *MAGEL2* in PWS.

Compelling evidence coming from humans and animal studies suggests that a deficit in the neuropeptide oxytocin (OT) plays a central role in the pathogenesis of PWS. OT, for its central role in the regulation of social behavior, has been proposed as a treatment for several neuropsychiatric disorders characterized by deficits in the social domain. Preliminary clinical trials have been carried out in adult patients and the results are controversial. However, it is conceivable that a treatment with OT early in life, when the plastic capacity of the brain is at a maximum and the social and behavioral dysfunctions are not consolidated, could produce longer-lasting effects. Thus, an early OT administration could act as a true curative intervention by exploiting the immature state of OT-system and the higher plastic capacity of the brain.

Results: We have created a mouse model deficient for *Magel2*. This mouse model presents a similar PWS phenotype with a deficiency in early feeding (from birth) and later on, in adulthood, alterations in social behavior and cognition. Restricted production of several bioactive neuropeptides including OT was detected in the hypothalamus of the mutant newborns. In such mutants, a single subcutaneous injection of OT after birth rescues the feeding onset and suckling phenotypes allowing all mutant pups to survive. Our study indicates for the first time that *Magel2* and the OT/OT- receptor system play a pivotal role in feeding behaviour at birth. Because the phenotype of *Magel2* deficient murine neonates mimics the feeding deficiency observed in PW newborns, rescue of the murine phenotype by OT injection opens a promising avenue for better therapeutic care of the PW syndrome at birth. Importantly, we have also shown that a daily administration of OT during the first week after birth cures alterations in social behavior and cognition in the adult *Magel2*-deficient mice, revealing a long term effect of this early OT acute treatment.

Conclusion: The *Magel2*-deficient mouse model is particularly pertinent for understanding the mechanisms of rescue of OT and for optimizing OT-based therapeutic strategies for the early feeding behavior, social and cognition alterations in PWS. Currently we are investigating those questions and we will present our ongoing studies.

Summary

Of great relevance for PWS, our team found that a postnatal administration of OT restores a normal suckling activity and cures alterations in social behavior and cognition in the *Magel2*-deficient mice, making this model particularly pertinent for understanding the mechanisms of rescue of OT and for optimizing OT-based therapeutic strategies for this neurodevelopmental disorder.

POSTER ABSTRACTS

P39 - An in vitro model of Prader-Willi Syndrome by generation of hypothalamic neurons and genome editing of induced pluripotent stem cells.

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Background

Prader-Willi syndrome (PWS) is a disorder of genomic imprinting that is caused by the absence of a normal paternal contribution to chromosome 15q11-q13. Most other cases of PWS result from uniparental disomy of the maternal chromosome 15. Recently the PWS critical region (PWSCR) has been narrowed to an ~91kb region encompassing several non-coding RNAs including a cluster of box C/D snoRNAs (SNORD116). PWS is characterized by neonatal hypotonia and failure to thrive followed by hyperphagia, obesity, short stature, dysregulated sleep, infertility and distinctive behavioral problems. These clinical manifestations are consistent with dysfunction of the hypothalamus, a ventral diencephalon structure implicated in the control of the endocrine system.

Methods and Results

In order to differentiate in hypothalamic neurons from several PWS-specific and normal control induced pluripotent stem cells (iPSC) lines, we modified existing protocols involving activation of the Sonic Hedgehog pathway for both monolayer and 3-dimensional spheroid neuronal differentiation. We compared the ability of control and PWS-iPSC derived neurons to adopt hypothalamic cell fates by assaying for gene and neuropeptide expression. We observed a marked increase in the gene expression of NKX2.1, OTP, RAX and POMC for the hypothalamic enrichment relative to our standard protocol. These findings suggest that our normal and PWS-specific iPSC lines can be differentiated into hypothalamic neurons, particularly, those of the arcuate nucleus (ARC) region.

In order to gain an understanding of how the loss of the SNORD116 cluster contributes to PWS phenotypic abnormalities, we used CRISPR/Cas9 to engineer isogenic pairs of human hESCs that differ exclusively at the SNORD116 cluster. We confirmed the loss of expression of the SNORD116 cluster in the isogenic deletion line by qRT-PCR and RNA fluorescence hybridization. We will differentiate isogenic normal and SNORD116 deletion lines into hypothalamic neurons in order to identify novel cellular and molecular targets of the SNORD116 regulation.

Conclusions

We have generated hypothalamic neurons from different PWS-specific iPSC lines to better model PWS and elucidate a cellular phenotype of PWS and to define the critical role of the SNORD116 cluster.

Summary

Our goal is to produce improved stem cell models of PWS. To do this, we are generating hypothalamic neurons and engineering a deletion of the SNORD116 cluster.

POSTER ABSTRACTS

P40 - Truncating Mutations of *MAGEL2*, a Gene within the Prader-Willi Locus, Are Responsible for Fetal Akinesia and Arthrogryposis

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Background: Arthrogryposis multiplex congenita (AMC) is characterized by the presence of multiple joint contractures resulting from reduced or absent fetal movement. The difficulty in establishing a genetic diagnosis for non-syndromic AMC individuals is due to the high genetic heterogeneity and/or to some not yet identified disease causing genes.

Methods and Results: Here, we report two unrelated families affected by lethal non syndromic arthrogryposis. By genetic mapping and whole-exome sequencing in a multiplex family, a heterozygous truncating *MAGEL2* mutation leading to frameshift and a premature stop codon (c.1996delC, p.Gln666Serfs*36) and inherited from the non-affected father was identified in the probands. In another family, a distinct heterozygous truncating mutation leading to frameshift (c.2118delT, p.Leu708Trpfs*7) and occurring *de novo* on the paternal allele of *MAGEL2* was identified in the affected individual. In both families, RNA analysis identified the mutated paternal *MAGEL2* transcripts only in affected individuals. Exome sequencing targeted to AMC genes including *MAGEL2* allowed identifying additional families.

Conclusions: *MAGEL2* is one of the paternally expressed genes within the Prader-Willi syndrome (PWS) locus. PWS has been reported in fetuses with a phenotype similar to that reported here. In these cases, paternal deletion or maternal uniparental disomy of 15q11 were identified suggesting that PWS should be considered in fetuses with such phenotype. Here we report that truncating mutations in *MAGEL2* are also responsible for this condition. *MAGEL2* mutations have been recently reported in affected individuals with features resembling PWS and called Schaaf-Yang syndrome. These data revealed that intragenic mutations of *MAGEL2* result in a large clinical spectrum ranging from severe fetal phenotype (our report) to syndromic intellectual disability or autism (Schaaf-Yang syndrome). These data strongly support the view that *MAGEL2* is a PWS-determining gene. In the absence of paternal deletion or maternal uniparental disomy of 15q11, search for intragenic mutations on the paternal allele of *MAGEL2* should be proposed in fetuses with reduced movements, polyhydramnios and distal arthrogryposis, newborns with severe undiagnosed central hypotonia or in children when PWS is suspected clinically.

Summary: *MAGEL2* mutations have been recently reported in affected individuals with features resembling PWS (Schaaf-Yang syndrome). Here we report that truncating mutations in *MAGEL2* are also responsible for fetal akinesia and arthrogryposis suggesting that *MAGEL2* is a PWS-determining gene.

POSTER ABSTRACTS

P41 - Brain and Behaviour in PWS

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Background: The diverse characteristics of PWS, as well as previous research, suggest widespread atypical brain development and functioning in PWS and aberrant activity across distributed neural networks. It is likely that the difficulties with emotional regulation and behaviour may be associated with abnormalities in the limbic cortico-striatal-thalamic circuitry, implicated in emotional cognition, regulation and lability. Moreover, many of these are areas which have been most consistently implicated in the literature regarding neural structure and function in PWS.

Methods & results: 20 participants with PWS, aged between 19-28 years, completed measures of cognition and behaviour, and underwent an MRI scan consisting of the following sequences: multiparameter mapping (MPM), resting state fMRI, and DTI. MRI data for comparison was available for 40 typically-developing controls, matched 2-to-1 for age and gender. A VBM analysis of grey matter morphology found a number of areas of increased volume in PWS, which were widespread across frontal, parietal, cingulate and temporal areas, extending into the more superior and anterior regions of the occipital lobe. Ventromedial and wider orbitofrontal areas showed decreased volume, as did the right lateral PFC and areas of the bilateral medial temporal lobes, bilateral temporal poles, and posterior parietal into occipital cortex. Within the PWS group itself, greater severity of maladaptive behaviours was associated with reduced volume in the left insula and bilateral cingulate. Data concerning cortical thickness and resting state functional connectivity will also be presented.

Conclusions: Widespread morphological abnormalities of both increased and decreased volume were found in those with PWS compared to a typically-developing control group in areas previously reported to show atypical anatomy or function in PWS and related behaviours, although the direction of findings has often been inconsistent. In particular, areas involved in the cortico-striatal-thalamic loops implicated in emotional and behavioural regulation or prominent connections and input areas were highlighted. Volume in the cingulate and insula cortices, in particular, were associated with severity of maladaptive behaviour in PWS, and are widely implicated in emotional processing and interoception and within circuitry involved in emotional state regulation. The largely bilateral nature of the morphological differences indicates an early and systemic biological basis to developmental abnormalities.

Summary: The array of difficulties experienced by people with PWS suggests widespread differences in the way the brain develops. This study compared young adults with PWS with typically-developing peers, finding differences across the brain suggestive of early biological alterations in development. We propose that these structural differences and their interconnections underpin the behavioural abnormalities of PWS.

POSTER ABSTRACTS

P42 - The development of resistance to change in individuals with PWS

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Background

Many individuals with PWS demonstrate a strong resistance to change and unexpected changes are a common trigger for temper outbursts. Experimental work by our group has provided some evidence that increased exposure to certain routines without change may accentuate the difficulties experienced following changes to those routines. Here, we aimed to examine the relationships between resistance to change and varying levels of exposure to routines over the life course.

Methods and results

The caregivers of 10 individuals with PWS (5-23 years) participated in a structured/semi-structured interview in which they used a five-point Likert-type scale to rate the level of routines and structure individuals had been exposed to at different stages of their lives. Life stages were anchored to notable events to facilitate recall (e.g. before primary school, during primary school). Caregivers also rated individuals' current resistance to change and the developmental pattern of resistance to change at each life stage compared to others. Open ended questions on routines and resistance to change were included. Interviews demonstrated acceptable inter-rater and inter-informant reliability. Descriptive, correlational and comparative analyses converged to suggest that increased exposure to rigid routines during the primary school life phase may be associated with increased resistance to change later in life. However, in individuals with already established resistance to change, structured routines were associated with less disruptive behaviour.

Conclusions

Practice with flexibility via less rigid routines may be particularly important during primary school years, when children's cognitive control processes – including task switching, which has been linked to one's ability to cope with change – develop rapidly. Strategies that increase flexibility in routines during the primary school years may reduce the development of subsequent resistance to change. However, careful research is needed to understand how to increase flexibility in routines during early life without negatively impacting on children's current behaviour and wellbeing.

Summary

Interviews with caregivers of people with PWS suggested that children who experience more rigid routines during primary school years may go on to develop greater difficulty with change later in life. However, structure and routine can be very helpful for people with PWS who already find change difficult. It should be possible to develop early prevention strategies based on this information but more careful research is needed to find a feasible approach.

POSTER ABSTRACTS

P43 - Oxytocin neurons promote arousal in a mouse model of PWS

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Background. Daytime sleepiness, disrupted sleep, and cataplexy-like falling episodes are common in Prader-Willi Syndrome (PWS). The cause of these symptoms is unknown, but it may involve reduced orexin signaling. The orexin neuropeptides activate the oxytocin neurons, and oxytocin activates the orexin neurons. We hypothesize that this positive feedback loop normally plays an essential role in promoting wakefulness and regulating sleep. Furthermore, patients with PWS have fewer oxytocin neurons, and we predict that low oxytocin tone reduces orexin signaling, resulting in daytime sleepiness, abnormal REM sleep, and cataplexy.

Methods and Results. Using optogenetics and EEG, EMG, and video recordings, we examined sleep/wake behavior in wild type, orexin knockout, and MAGEL2 null mice, a model of PWS. We selectively expressed channelrhodopsin in oxytocin neurons of the PVH and targeted optical fibers to illuminate the oxytocin fibers of the orexin field.

Conclusion. In all three lines of mice, activation of oxytocin nerve terminals wakes mice from sleep and increases the amount of wake during the day.

Summary. These findings demonstrate that oxytocin can increase arousal, most likely by activating the orexin neurons. This suggests that therapies that enhance oxytocin signaling may help people with PWS better maintain wakefulness throughout the day.

Support: Foundation for Prader-Willi Research

POSTER ABSTRACTS

P44 - Abnormal Videofluoroscopic Swallow Studies (VFSS) in Infants with Prader-Willi Syndrome Indicate a High Rate of Silent Aspiration

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Background: Prader-Willi Syndrome (PWS), due to loss of expression from genes within the PWS imprinted region at chromosome 15q11.2-13, is characterized by hypotonia and feeding intolerance in infancy with later development of hyperphagia and obesity. Growth hormone improves tone, body composition, and height and can be started in infancy. Morbidity and mortality in PWS include those secondary to hyperphagia and respiratory illness as well as a 17% reported incidence of sudden death in childhood. Choking is a known hazard with a 34% reported incidence. Despite well-described feeding intolerance in infants with PWS, there are no published reports of formal swallow studies. We hypothesize that VFSS will diagnose pathology missed by clinical observation and may help determine feeding safety in PWS infants.

Methods and Results: VFSS results of infants followed in the interdisciplinary SCH PWS clinic between October 2014 - April 2016 were reviewed. The study was approved by the SCH IRB.

Six infants with genetically confirmed PWS underwent 10 VFSS (age: 3 weeks-15 months; gender: male 4, female 2; subtypes: deletion 3, uniparental disomy 2, imprinting defect 1). One patient received 5 studies over 14 months. Of all the studies, 100% indicated oropharyngeal phase dysphagia with abnormal pharyngeal clearance in 80% (5 infants). 100% showed silent aspiration with thin liquids, 60% with thickened liquids, 20% with purees. 60% were done while the infant was on growth hormone. Average age of growth hormone initiation was 2.5 months. The infant with multiple studies showed improvement over time, but still had an abnormal VFSS at 15 months old.

Conclusion: VFSS showed oropharyngeal phase dysphagia and silent aspiration in all infants which may have been undiagnosed with only clinical observation. Abnormalities were present despite early initiation of growth hormone. Careful consideration should be made before starting oral feeds in infants with PWS, and VFSS can be a useful clinical tool in this decision. Swallow dysfunction may be a contributor to morbidity in PWS. Further longitudinal studies are needed to characterize swallowing function in PWS over time.

Summary: Infants with PWS who had VFSS done to evaluate swallowing function were found to have a high rate of abnormalities, including silent aspiration and poor clearance of food after swallow. These characteristics can increase risk of choking as well as lung disease from aspiration and may have been undiagnosed without the VFSS. It is important to consider these risks in evaluation of safety for oral feeds in infants with PWS.

POSTER ABSTRACTS

P45 - Role of the Endocannabinoid System in Prader-Willi Syndrome

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Background: Extreme obesity is a core phenotypic feature of Prader-Willi syndrome (PWS). Among the numerous metabolic regulators, the endocannabinoid (eCB) system is critically involved in the control of feeding, body weight, and metabolism, and globally acting cannabinoid-1 receptor (CB₁R) blockade reverses obesity both in animals and humans. However, due to their neuropsychiatric side effects, they are no longer considered as a valid treatment for obesity in humans.

Methods and Results: Using an established mouse model for obesity in PWS, *Magel2*-null mice, we measured the expression of CB₁R as well as the endogenous levels of the main eCBs, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). We then determined the anti-obesity efficacy of the peripherally restricted CB₁R antagonist, JD5037, in obese female and male *Magel2*-null mice. To assess the relevance of our findings to humans, we measured eCB levels in the serum of two cohorts of individuals with PWS and their age- and gender-matched healthy controls.

Increased both hypothalamic and peripheral (circulating and adipose) eCB tone were found in *Magel2*-null mice. Daily oral treatment of obese *Magel2*-null mice and their controls with JD5037 (3 mg/kg/d for 28 days) resulted in significant and comparable reductions in body weight, food intake, hyperglycemia, hyperinsulinemia, liver injury and dyslipidemia in both mutant and control mice. Human patients with PWS from Israel and the North America showed increased levels of 2-AG, but not AEA.

Conclusions: Dysregulation of the eCB/CB₁R system may contribute to obesity in *Magel2*-null mice and humans with PWS. Our findings with JD5037 in *Magel2*-null mice may provide the rationale for clinical testing of peripherally restricted CB₁R antagonists for the treatment of obesity in PWS, while avoiding the risk of psychiatric side effects.

Summary: Using a pre-clinical animal model for PWS and human data, our results document for the first time the contribution of the eCB system to the metabolic phenotype associated with PWS.

POSTER ABSTRACTS

P46 - Discovery of Dysphagia in Children and Adults with Prader-Willi Syndrome

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Background: The need for the Heimlich maneuver, death from choking (defined as asphyxiation by a food bolus), and fatal pulmonary infections occur in a disproportionately high number of persons with PWS. The widely-held belief is that eating behaviors and silent aspiration are primarily responsible for these events; yet, dysphagia (swallowing impairment) can also cause choking and lung infection. To date, no investigation had directly examined swallowing function in persons with PWS. Therefore, the purpose of this research project was to determine if subclinical (without symptoms) dysphagia (swallowing impairments) and/or risk factors for aspiration/airway invasion are present in persons with PWS. We also sought to determine how eating behaviors contribute to choking and aspiration risk.

Methods and Results: Simultaneous videofluoroscopy and nasal respiratory signals were used to record swallowing function and breathing/swallowing coordination in 30 participants with PWS. Subjects drank thin liquid and ate barium cookies under two randomized conditions: 1. Controlled (cues to swallow and standardized bolus sizes) 2. Spontaneous (no cues or control of rate of consumption, and no bolus size control). The results showed that persons with PWS can have multiple, sometimes severe, symptoms of dysphagia and important risk factors for aspiration. Overall, the cohort showed disturbances in timing between the oral and pharyngeal phases, incomplete pharyngeal and esophageal clearance, and impaired coordination of swallowing with the respiratory cycle. None of the participants showed any sensory response to their dysphagia such as attempting to clear residue or coughing, thus verifying the lack of overt symptoms. There were no statistically significant differences between the two conditions; however, the spontaneous condition did not elicit rapid eating or large bolus sizes in the majority of the participants.

Conclusions: We conclude that choking events and some pulmonary infections in children and adults with PWS may be related, in part, to the underlying asymptomatic dysphagia that our experiment revealed. Based upon these findings, it is recommended that persons with PWS should receive videofluoroscopic swallowing evaluations that include examination of the esophagus.

Summary: Persons with PWS tend to have a higher than typical rate of choking deaths and fatal lung infections. We used x-rays to determine that swallowing impairments, such as food sticking in the throat and esophagus and other risk factors for aspiration, can be present even though there are no symptoms.

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