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Analysis of Phenotypic Features in a Large Cohort of Individuals with Prader-Willi syndrome: Differences between Gender, Molecular Type, Growth Hormone Exposure in Various Age Groups

THESIS

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for the degree of

MASTER OF SCIENCE

in Genetic Counseling

by

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ABSTRACT OF THE THESIS

Analysis of Phenotypic Features in a Large Cohort of Individuals with Prader-Willi syndrome: Differences between Gender, Molecular Type, Growth Hormone and Sex Hormone Use

By

Anna Leonenko

Master of Science in Genetic Counseling

University of California, Irvine, 2018

Professor Virginia E. Kimonis, MD, MRCP, Chair

Prader-Willi syndrome (PWS) is a rare and complex genetic condition. It is characterized by distinct phenotypical features, short stature and morbid obesity, growth hormone (GH) deficiency and hypogonadism. The purpose of this study (N=355 individuals) was to describe differences in PWS phenotypic features by gender, molecular PWS subtype (deletion vs. UPD), and the use of GH and sex hormone (SH) treatments.

Hypotheses include 1) Individuals with deletion versus UPD have an increased frequency of and more severe features, 2) Individuals treated with GH or SH have normalization of features, 3) The age at the GH or SH treatment initiation impacts the effects of treatment.

Individuals with deletions were found to have more frequent incidence of features such as: flat occiput, strabismus, almond shaped eyes, etc. They also tend to be heavier and with smaller head circumferences.

Individuals who were treated with GH had lower incidences of some phenotypic features such as: prominent occiput, almond shaped eyes, exotropia, etc. This study has also confirmed that PWS individuals who were treated with GH on average are taller, with lower BMIs, and longer hands and feet. Initiating GH treatment below the age 4 years also demonstrated lower

incidence rate of phenotypic features such as micrognathia, slit-like eyes, abdominal fat distribution, etc.

The effect of the SH treatment is less obvious and requires further investigation.

The results of this study support the benefit of GH treatment for individuals diagnosed with PWS and support the benefits of starting the GH treatment at a younger age.

INTRODUCTION

Background and Etiology of PWS

Prader-Willi syndrome (PWS) is a rare and complex genetic condition that affects individuals throughout their lifetime. PWS current prevalence is approximately 1 in 10,000 to 1 in 30,000. It affects both males, females and all races equally (Cassidy & Driscoll, 2009; Grugni et al, 2016). In the newborn and infant stages PWS is characterized with hypotonia (decreased muscle tone), short stature, failure to thrive, sleep apnea, feeding issues (poor suck) which also leads to poor weight gain. Starting from approximately 2 years of age the condition progresses to hyperphagia (excessive hunger), which can lead to excessive weight gain. PWS also presents itself with abnormal function of the endocrine system, which includes growth hormone (GH)/insulin-like growth factor I axis dysfunction, hypogonadism (decreased or absent function of gonads), hypothyroidism (underactive thyroid), premature adrenarche, and adrenal insufficiency (Cassidy & Driscoll, 2009; Unanue et al., 2007). Other major clinical manifestations include developmental delay, decreased levels of intelligence, behavioral problems such as frequent temper tantrums and psychiatric disorders such as psychosis, schizophrenia, manic-depressive and autism spectrum disorder features (Cassidy & Driscoll, 2009; Grugni et al., 2016). Individuals with PWS also develop distinctive physical and facial dysmorphic features which include short stature, small hands and feet, excessive body fat that often concentrates on the torso and around thighs, narrow forehead, deep set almond-shaped eyes (Weiss & Goodall, 2009), ophthalmic problems such as strabismus, decrease in visual acuity etc., hypopigmentation of hair, skin and the iris of the eye (Fox et al., 1999), and some individuals also manifest mild to severe scoliosis (Weiss & Goodall, 2009).

PWS is a genetic condition that is caused by lack of expression of genes that are located on the PWS-associated region on chromosome 15. Typically, humans have two copies of all of their chromosomes. One copy of each chromosome is inherited maternally and the other one paternally. PWS is caused by a lack of expression of *paternally* inherited imprinted genes that are located in the region 15q11.2-q13 (**Figure 1**). The majority of the genes in this region are involved in RNA and protein processing of neuroregulators and hormones (Bittel & Butler, 2005).

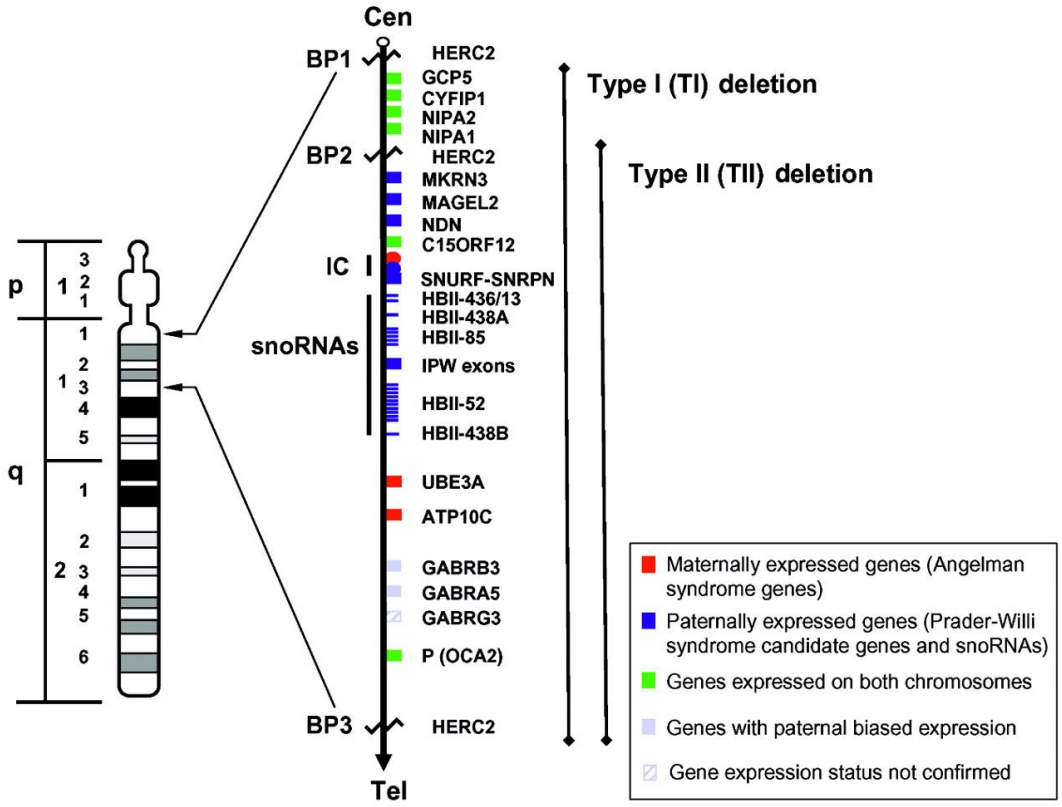

Figure 1. Chromosome 15 ideogram with representation of genes on 15q11.2-q13 region, and patterns of their expression.
[Reproduced with permission from *Pediatrics*, Vol. 118, Page e1277, ©2006 by American Academy of Pediatrics. Adapted from Bittel DC, Butler MG, *Expert Rev Mol Med*. 2005;7:1-20.]

There are currently three known types of molecular mechanisms that lead to lack of expression of paternally derived genes in 15q11.2-q13 region: deletion on the paternally derived chromosome 15, maternal uniparental disomy of chromosome 15 and defects in the imprinting region (**Figure 2**.).

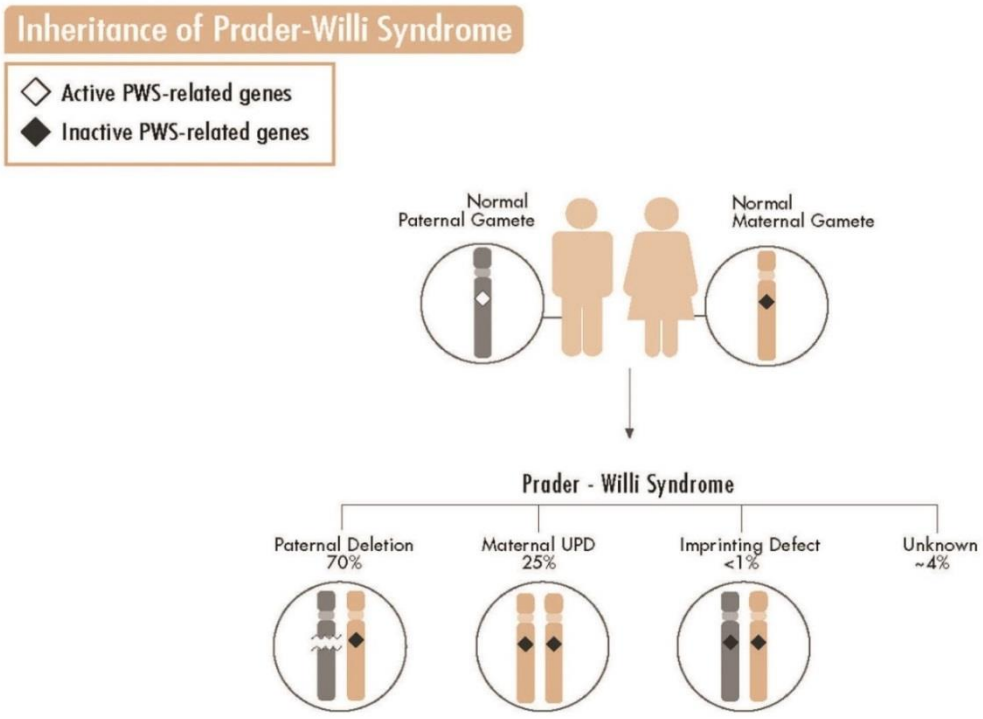

Figure 2. **Inheritance of Prader-Willi syndrome.** Molecular mechanisms of cause of PWS includes paternal interstitial deletion, maternal uniparental disomy, or an imprinting defect.

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Paternal Deletion

Approximately 70% of PWS cases are caused by the deletion in the PWS-associated region on chromosome 15 (Cassidy and Driscoll 2009). PWS deletion cases can be further subdivided into 2 deletion subgroups (Type I and Type II) (**Figure 1**.), depending on the location

of the breakpoints (BPs) and therefore the size of the interstitial deletion in the 15q11.2-15q13 region (Bittel & Butler, 2005). Type I subgroups deletions are larger and span from BP1 for approximately 5 megabases to the BP3 location. Type II subgroup deletions are smaller and span from BP2, which is located about 500 kilobases distal to BP1, up till BP3 (Bittel & Butler, 2005). The PWS-associated 15q11.2-15q13 region contains six maternally imprinted and only paternally expressed unique copy genes: MKRN3, MAGEL2, NECDIN, C15ORF2 and SNURF-SNRPN, and a family of 5 snoRNA (small nuclear non-coding RNAs) genes which are only paternally expressed (Holsen et al., 2009). Targeted knockout mice models for these genes were able to mimic some but not all clinical and biochemical features of humans with PWS. The MAGEL2-deficient mice have decreased alertness and mobility that is associated with decreased levels of sleep< and appetite-regulating hormone called orexin. The NECDIN-deficient mice have approximately 4 different phenotypes, including breathing problems similar to the ones observed in individuals with PWS. The SNRPN-knockout mice presented with signs of hypotonia and feeding difficulties. Abnormal changes in certain snoRNAs are associated with neurologic, cardiovascular, and oncologic diseases (Irizarry et al., 2016).

Maternal Uniparental Disomy (UPD)

The second most common known molecular cause of PWS, that accounts for approximately 27% of all PWS cases, is maternal uniparental disomy (UPD). Maternal UPD occurs when both copies of chromosome 15 are inherited from the mother (Cassidy & Driscoll, 2009).

One of the most common causes of chromosome 15 UPD is a failure of the two sister chromatids to separate properly into two daughter cells during meiosis I (Robinson et al., 1993). This process is known as nondisjunction, and it causes an abnormal distribution of chromosomes

in daughter cells. As a result, instead of having one copy of each chromosome in both daughter cells, one of them will contain both chromosome 15 copies, and the other one will have no copies of chromosome 15. After fertilization the cell with both copies of chromosome 15 will have trisomy 15 (three copies of chromosome 15) and the cell no copies of chromosome 15 will have monosomy 15 (only one copy of chromosome 15). If a second, "rescue", event occurs, it could lead to a loss of one of the extra chromosomes 15 in a trisomy. It can result in a cell with both copies of chromosome 15 having a maternal origin. In the case of monosomy, a single chromosome copy may duplicate itself, leading to UPD as well. For other examples of mechanisms that result in UPD please see Figure 3 (Shaffer et al., 2001).

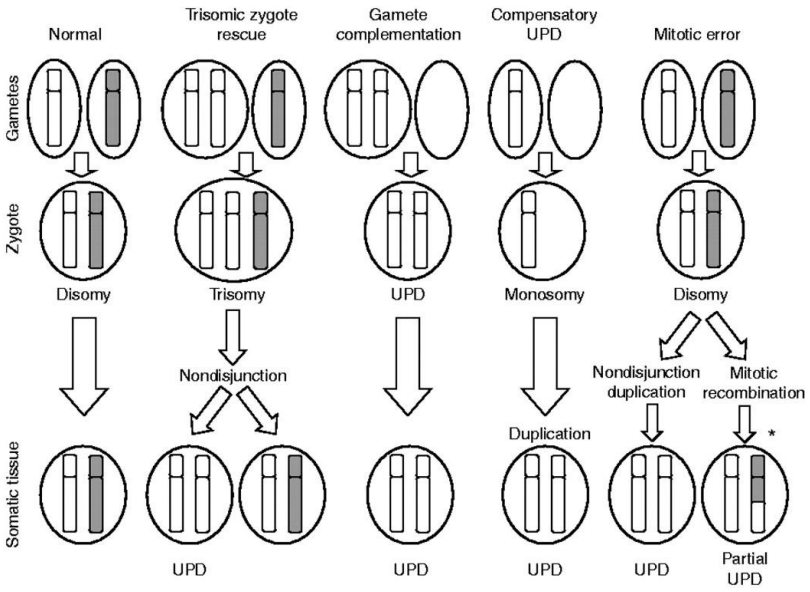

Figure 3. Mechanisms leading to uniparental disomy.
[Reproduced with permission from "New mechanisms involved in paternal 20q disomy associated with pseudohypoparathyroidism", Eur J Endocrinol. 2010 Dec;163(6):953-62. Adapted from "Kotzot D. Complex and segmental uniparental disomy (UPD): review and lessons from rare chromosomal complements. Journal of Medical Genetics200138497–507doi:10.1136/jmg.38.8.497]

Imprinting Defect

As mentioned previously, some genes should normally be active only when they are inherited from a father and others should normally be active only when inherited from a mother. Genomic imprinting can be defined as a phenomenon of a gene expression which is based on whether the expressed gene copy was inherited from a mother or from a father. During gametogenesis those genes that will be imprinted undergo methylation – attachment of methyl groups. Those epigenetic tags usually stay on imprinted genes, and epigenetically silence them, throughout the life of the organism. During gametogenesis another important process takes place. Before the imprint can be established, an imprint *reset* takes place. In the sperm all imprints, including the ones that came from mother, are erased and rewritten with paternal pattern. The same process is happening in the eggs, to ensure all eggs have maternal imprinting pattern (Reik 1989). Errors in genomic imprinting lead to specific conditions, such as PWS, depending on which chromosome is involved.

Imprinting error is the third cause of PWS and it is responsible for approximately 1-3% of PWS cases. An imprinting error in most cases is caused by a mutation of the imprinting control center (IC) in the paternally derived 15q11.2-q13 region (**Figure 1**)**.** If the father of PWS affected individual passed on his maternal copy of chromosome 15 with the IC error, the maternal imprint (coming from his mother) would still be in place. In other words, there was a failure to switch from maternal imprint to paternal imprint during the male gametogenesis. As a result, affected individual will have one maternal copy of chromosome 15 and one paternal copy of chromosome 15 with maternal imprint still present. (Gardner, Sutherland, & Shaffer, 2012; Cassidy & Driscoll, 2009).

Imprinting error in the IC could also be caused by a chromosomal balanced translocations or rearrangements that can result in deletion in the 15q11.2-q13 region (Butler, 2011).

Diagnosis of PWS

PWS is a clinically well-described multi-system syndrome. Some clinical features of PWS, such as obesity and low muscle tone, can also be present in normal obese individuals or overlap with other conditions such as Early-onset Morbid Obesity, Borjeson-Forssman-Lehmann syndrome, Cohen syndrome etc. Molecular and cytogenetic testing is required for a definitive diagnosis. Currently, in the majority of cases, PWS is diagnosed shortly after birth. Hypotonia and poor sucking prompts thorough investigation that in some individuals leads to PWS diagnosis. In some cases, however, PWS is not diagnosed until later in life. As mentioned previously, PWS has a heterogenous genetic etiology. In the past, the only technique that was used for PWS testing was the high-resolution chromosome analysis. However, use of molecular analysis techniques proved that high-resolution chromosome analysis has a high false positive and false negative results rate. Currently, there is a number of different diagnostic techniques used to test for PWS. One of them is the DNA methylation studies. In order to perform DNA methylation studies, the Southern blot hybridization method is used. This method is able to detect maternal-only or paternal-only methylation patterns using methylation-sensitive SNRPN or PW71B probes. After PWS is confirmed with maternal-only methylation pattern, the next step would be trying to distinguish which molecular mechanism is responsible for deletion versus UPD versus imprinting errors. Further tests such as fluorescence in situ hybridization (FISH) and/or polymerase chain reaction (PCR) are used. Other test used to diagnose PWS include chromosomal microarray (CMA), chromosome karyotyping, DNA polymorphism studies and multiplex ligation probe amplification (MLPA) (**Figure 4**.). Choosing most appropriate testing

techniques can correctly identify >99% of all cases of PWS (Smith et al., 2017; Glenn et al., 1997).

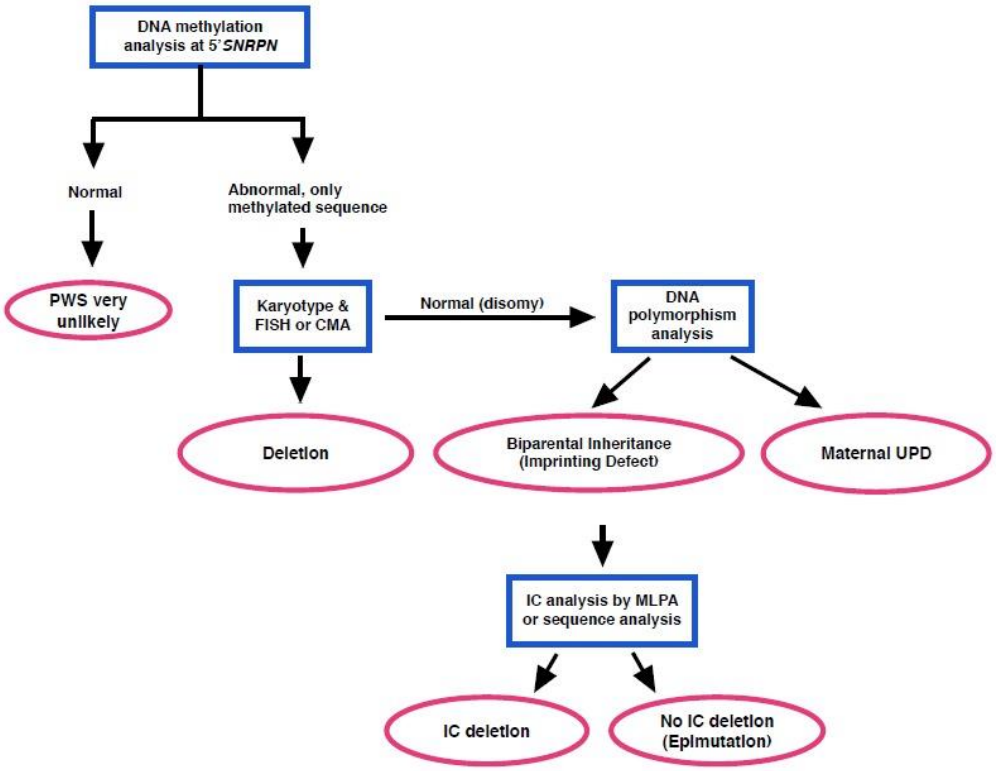

Figure 4. Proposed PWS comprehensive testing strategy.

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Distinct Facial and Physical Features

The distinctive facial and physical features in people with PWS were first described by Prof. A. Prader, Dr A. Labhart and Dr H. Willi in the Swiss Medical Weekly (Prader, et al. 1956). Dr B. Hall and Dr D. Smith included further comprehensive description in their

publication in the Journal of Pediatrics (Hall, et al. 1972). PWS facial features can include a small narrow bifrontal diameter, which can also have dolichocephaly associated with it, almond-shaped palpebral fissures, narrow nasal bridge, and thin upper lip with downturned corners of the mouth. Other physical features include small hands and feet, fat depositions that tend to accumulate on the torso and around thighs, hypoplastic genitalia, shorter stature than of individuals in general population of the same ethnic background, gender and age group (Miller et al. 2008; Stefan et al. 2005).

PWS Treatment Strategies

As of now, there is no cure for PWS, but there are treatments that can be used to help with PWS manifestations. During the infant period due to poor sucking reflex, bottles with uniquely designed nipples or feeding tubes can be used so that adequate nutrition is provided for the child. As the child gets older compulsive overeating habits take over. Overeating is often combined with lower than normal metabolic rate which results in an excessive weight gain. In order to prevent that, caloric intake needs to be restricted. Daily food intake, which should be based on height, weight, and BMI, should also be supervised. Having such a stringent diet control will help maintain a healthy weight and at the same time provide adequate energy source requirements (Bittel et al., 2005). Encouraging physical activity and receiving physical therapy, in conjunction with appropriate strict diet, may help improve muscle strength. Other therapies that proved to be successful with PWS individuals include occupational, speech, and language therapies (Goldstone et al. 2008).

Many controlled studies (Cassidy & Driscoll, 2009; Grugni et al, 2016; Brambilla et al., 1997; Hirsch et al., 2015; Longhi et al., 2015) have also shown benefits of GH and SH therapies.

Overall GH therapy effects include improvement of physical features and behavior issues that are attributed to PWS. It was also noted that replacement of sex hormones at puberty produces adequate secondary sexual characteristics. GH and SH replacement therapies, and the benefits and side effects of these therapies, are discussed below in greater detail.

Growth Hormone Treatment Benefits and Side Effects

GH therapy is now widely recognized as an effective treatment for people with PWS. Food and Drug Administration (FDA) in 2000 approved somatropin, injectable GH, as a suitable treatment for PWS manifestations (Irizarry et al., 2016). It is still, however, not used as a standard of care for *all* PWS individuals at *all* institutions. Some studies that report beneficial effects of GH therapy in individuals with PWS lack more long-term effect data and sufficient number of participants to show the true degree to which these effects alter the natural history of the disorder.

GH replacement therapy tends to be tolerated very well by individuals with PWS. There are however some noted side effects. Several studies showed some mild adverse reactions. Mogul *et al.* noted fluid retention and ankle edema in affected individuals (Mogul et al. 2008). Some individuals also experienced increased risk of developing diabetes when they were on GH therapy when compared to those who weren't (Herman-Bonert et al., 1995). Other studies note far more serious side effects that include respiratory issues, including frequent infections, obstructive sleep apnea due to soft tissue thickness that leads to adenoids and tonsils hypertrophy, and several cases of sudden death (Eiholzer et al., 2002; Van Vliet et al., 2004). All of those studies, however, conclude that benefits of GH therapy outweigh the possible side effects and PWS individuals should be under close observation of endocrinologists. As Grugni *et*

al. conclude in their 2016 study "further research is required to improve our understanding of the physiopathology of GH/IGF-I axis during the entire lifespan of PWS subjects" (Grugni et al., 2016).

Having GH deficiency places individuals with PWS at a higher risk for osteopenia and fractures. Longhi *et al*. did a cross-sectional study with 41 individuals with PWS and 46 healthy individuals to examine the modulating effect on bone of treatment with GH and sex steroids. The study noted that GH treatment had a positive effect and sex steroids a negative effect on bone size and strength. Bone strength was significantly reduced in PWS individuals who did not receive GH treatment and had been treated with sex steroids (Longhi et al., 2015).

Multiple studies (Cassidy & Driscoll, 2009; Grugni et al, 2016; Brambilla et al., 1997) noted that GH replacement therapy shows beneficial effects beyond just height. It has beneficial effects on facial features, cognition, behavioral phenotype, normalizes height and improves lean body mass of individuals with PWS. De Souza *et al*. from UCL Institute of Child Health (London, UK) conducted a study that for the first time provided objective analysis of GH replacement therapy on craniofacial features of PWS individuals. They analyzed 3D photogrammetric images of facial phenotypical features from 72 participants with PWS and 388 unaffected individuals using "dense surface modeling" and "shape signature techniques". Their study reports that adults with PWS who had never received GH replacement therapy displayed known characteristic PWS facial features, and facial growth was also significantly reduced, particularly in male participants. The study also demonstrated that GH supplementation lead to vertical facial growth of affected individuals to fall within the normal range. Lateral and periorbital face shape and nose shape differences in PWS children who received GH therapy, however, still remained significantly distinguishable in comparisons with age–sex matched,

unaffected individuals. The conclusion of this study is that the GH treatment normalizes vertical facial growth in PWS individuals, but not overall face shape. This study has a number of limitations, including a relatively small cohort of individuals with PWS. De Souza *et al*. (de Souza et al., 2013) also showed evidence that the age of initiation of GH therapy and the length of this treatment did not have statistically significant nor a consistent effect on the face shape in those affected with PWS (de Souza et al., 2013). They were unable to evaluate the effects of GH dosage on face shape changes as the data was insufficient. This study had its limitations such as having only 26 participants.

Several studies were able to consistently show that GH therapy increases short-term growth in children with PWS. The study conducted by Carrel *et al*. investigated the long-term effects. The team recruited and observed 21 children with PWS that were treated with GH and 27 children with PWS naïve to GH therapy. GH treatment prior to 2 years of age has been investigated to determine its beneficence. In this study they compared the difference in body fat, lean body mass, carbohydrate/lipid metabolism, and motor strength between those who had been started on growth hormone at either 6-32 months or aged 5-9 years. The conclusion was that if GH began prior to 2 years of age, it improves body composition, including body mass index (BMI), motor function, height and lipid profiles (Carrel et al., 2010). Three years later Wolfgram, Carrel and Allen published updated summary of this study, that confirmed and expanded reported benefits of GH therapy in children with PWS (Wolfgram et al., 2013). All of these studies show some moderate to strong effects of GH treatment. Most of these studies however, have relatively small cohorts and examine effect on a relatively small number of phenotypical features.

Sex Hormone Treatment Benefits and Side Effects

Hypogonadism, which in PWS cases is of hypothalamic origin, is prevalent in individuals with PWS. It manifests in PWS individuals as genital hypoplasia that is evident at birth and throughout lifetime, incomplete pubertal development, and infertility. Unilateral/bilateral cryptorchidism is present in 80–90% of males with PWS. Females generally have hypoplastic labia majora and labia minora, and clitoris. Precocious adrenarche occurs in both sexes in approximately 20% of PWS cases. The hypogonadism is of hypothalamic origin. Cassidy and Driscoll also note hypogonadotropism with decreased levels of testosterone or estrogen and decreased FSH and LH in both sexes (Cassidy et al. 2009). Sex hormone (SH) replacement therapy proves to help produce adequate secondary sexual characteristics. There were also several concerns noted with SH therapy. Testosterone replacement could possibly be causing behavior problems in males. This problem has been largely alleviated by daily use of the hormone patch or gel versus previously used monthly intramuscular injections. Using SH therapy in females raised concerns about hygiene issues with monthly menstruation and the increased risk of strokes with estrogen replacement. Cassidy and Driscoll also note the lack of well-designed sex hormone replacement studies. More research is desperately needed (Cassidy & Driscoll, 2009).

Currently most experts on PWS also agree that dosing and timing of SH replacement therapy should mirror normal pubertal development, but there is no consensus on a specific regimen or timing for SH induction. SH therapy must be individualized for each individual with PWS and the management of the therapy should be supervised by a pediatric endocrinologist (Hirsch et al., 2015).

Purpose of this Study

The purpose of this study was to describe differences in PWS phenotypic features, specifically physical and distinctive facial features, by gender, molecular type (deletion vs. maternal UPD), and the effect of exposure to GH and SH replacement treatment. A previous masters thesis study has been done in a small cohort of 64 participants with PWS which reported that 31 individuals who were treated with growth hormone were found to be on average taller, they had larger hands and feet, lower incidence rate of esotropia and also lower frequency of fair skin (St. John, 2010, unpublished data). This study expands on the previous research by analyzing a larger cohort (N=355) with longitudinal data on both GH treatment and phenotype for many research participants.

Statement of Hypothesis

Hypotheses include 1) Individuals with deletion versus UPD have an increased frequency of and more severe physical, and dysmorphic features associated with PWS, 2) Individuals treated with growth hormone versus not treated with growth hormone, and individuals treated with sex hormones (testosterone or estrogen) present with normalization of physical and dysmorphic features associated with PWS, 3) The younger the age of growth hormone and sex hormone treatment initiation the stronger the effect on physical and dysmorphic features associated with PWS.

MATERIALS AND METHODS

This study is an analysis of data collected in an observational study which included the comprehensive assessment of medical, behavioral and nutritional history, and phenotypical features of individuals with PWS or early-onset morbid obesity (EMO) which lasted from 2006 to 2014. The EMO individuals were excluded from this study. The collection of data was carried out through the use of the Rare Disease Clinical Research Network (RDCRN) Natural History PWS and Morbid Obesity Clinical Protocol (UCI IRB protocol 2007-5605), which was conducted at the University of California, Irvine by principal investigator Dr. Virginia Kimonis. The RDCRN also included four other main participants: University of Florida Health Science Center in Gainesville, Florida (Dr. Driscoll and Dr. Miller); Kansas' Children's Mercy Hospital and Kansas University Medical Center (Dr. Merlin Butler); Baylor College of Medicine in Houston, Texas (responsible for receiving and processing DNA samples in order to create a DNA and RNA repository), and Vanderbilt University Medical Center in Nashville, Tennessee (Dr. Elisabeth Dykens and Dr. Marshall Summar).

Participant Eligibility and Recruitment

All the centers recruited individuals with PWS and evaluated them with the same predetermined comprehensive assessment. The IRB approval number is HS#: 2007-5605. The centers recruited diverse age groups for PWS, which spanned from less than 1 year old to over 50 years old. All racial and ethnic, and both gender groups were eligible for participation. The inclusion criteria for the PWS group included a confirmed diagnosis of PWS and ages 0-60 years.

All the participants with PWS were required to have appropriate standard molecular and cytogenetic testing to confirm a diagnosis of PWS. This data was represented in three categories: deletion, UPD, or imprinting defects. PWS participants were recruited from local Genetic, Neurology and Endocrine clinics, the RDCRN website and from the newsletter and web page of the national parent support organization, Prader-Willi Syndrome Association of USA (PWSA-USA) and FPWR (Foundation for Prader Willi Research).

Informed Consent and Specific Procedures of the Study

The study was conducted in two phases – Part I and Part II. The first part involved consenting both of the participants' parents via telephone. This was done only for the review of medical records, which was necessary to determine eligibility into the study. The second part included procedural consent at the participant's initial visit. All participants and their parents/guardians received an explanation about the nature of the study and they were told that participation was entirely voluntary. For participating individuals who were 18 years and older, decision-making capacity was assessed by the attending physician. The consent form was also translated into the appropriate language for non-English speaking subjects.

The observational study involved collecting data on comprehensive assessment of medical, behavioral and nutritional history, and clinical features of individuals with PWS. During all the visits RDCRN forms were used to record participants' data. During the initial visit and at following visits, individuals also had the following activities done: 1) signed consent; 2) initial history form; 3) diet history; 4) a physical exam and an impression examination by the physician; 5) current history form; 6) photographs taken; 7) blood sample obtained of the participants in order to create a DNA and RNA repository to enable further genetic studies (only done at one of the visits); 8) a Dual-Energy X-Ray Absorptiometry (DEXA) scan for body fat

measurement; 9) a Kaufman Brief Intelligence Test, 2nd edition (KBIT2) ("Pearson Education," 2013); 10) a Behavior Assessment System for Children, 2nd edition (BASC-2) ("Pearson Education," 2013) for the parent and the participant (this was assisted by the physician and the study coordinator if participant was able to read). The Teacher BASC form was passed to the teacher (only applicable if participant was at school-age). Participants were followed for up to 6 years. For participants >16 years, follow up occurred every 2 years. Some participants had only one, initial baseline visit, others had several visits during the course of the study. Only a few participants had overall 5 visits. All participants were compensated $50/visit for travel expenses.

Each participant was assigned a study identification number prior to the first initial baseline visit. This number was associated with the visit date, specific study location code and subject identification. All the data obtained from the study was coded and deidentified to be shared with the Data Management and Coordinating Center (DMCC) and RDCRN for loading into the database. Data collection for the RDCRN was accomplished with online electronic case report forms. Using encrypted communication links, on-line forms were developed that contained the requisite data fields.

RDCRN Database

The initial goal was to enroll and longitudinally follow a minimum of 200 individuals with PWS and 100 with EMO as described in the original proposal of the study. This was subsequently increased to 300 participants. Up until August 2013, at which time the RDCRN data analyzed in this study was captured, the RDCRN database contained a total of 386 enrolled individuals with PWS or early morbid obesity who were being studied by the various enrolling centers. Demographic, medical, educational, and familial surveys were completed for each PWS individual in the database. Data from these 386 individuals was de-identified and made available to this study by the Data Management and Coordinating Center (DMCC), a center that not only stores and manages the RDCRN data but also plays an active role in the design of clinical protocols and analyses of the RDCRN studies. Data collected from the DMCC on behalf of the RDCRN included the participant data records explained from above, as well as: date-of-birth, gender, PWS genotype sub-type (deletion, UPD or imprinting defect), growth hormone and sex hormone treatment information (which included medication log and date of treatment initiation and cessation). Other parameters such as height, weight, and BMI, were also collected at each visit. Specific behavior information and whether or not the individual required a feeding tube were also collected.

For this study, data containing 661 entries following these visits was extracted from the above described RDCRN database. EMO participants were excluded from this dataset, giving a final number of 355 participants with confirmed PWS. This dataset in Microsoft Excel format contained data from multiple visits for multiple subjects. The data was transferred into SPSS Statistics software for analysis (Yang et al., 2011). The data was transformed, to create one

record per subject in our study database. Four individual records had to be removed as they proven to be complete duplicates of already existing records.

This study was focused on the age of the GH and SH treatment initiation, the length of the treatment and its effect on phenotypical features of individuals with PWS. PWS participants with imprinting center defects (N=11) were excluded from the analysis of PWS molecular subtype on phenotypical features. PWS participants with missing information on GH exposure (N=3) were excluded from the analysis of GH exposure effect on phenotypical features.

Age at GH or SH treatment initiation or cessation was coded using following rules: 1) if a participant was exposed to GH or SH treatment but the age of initiation was missing, their age of GH treatment initiation was coded as the age at the visit when GH treatment exposure was first noted; 2) data from the column with age of GH or SH treatment cessation was not used if the participant stated being on GH or SH treatment at, and beyond, the age stated in that column; 3) if participant had GH or SH treatment initiated, ceased the treatment and eventually re-initiated the treatment, the initial age when GH or SH treatment started was used as the age of initiation.

One of the participants (unique ID: 103168) gender was originally noted as “female” in the original dataset. After further evaluation it was decided to change this participant’s gender to “male”: there was no data on female secondary sexual characteristics and instead, there was data present for male secondary sexual characteristics. Review of other participants did not show any other inconsistencies. There is a research paper (Butler et al., 2018) that explores the demographic data of the 355 PWS participants of the original HS#: 2007-5605 study. Their findings indicate that overall number of female versus male participants (female=197, male=158) is different from what was found in this study (female=195, male=160). The reason for this

difference in gender distribution within these studies is currently unknown. This potentially could be a human error in the data entry or transmission of data in either of studies.

A new set of variables was also introduced to the dataset. One of the variables indicated presence of the phenotypical feature if it was noted as present during at least one of the visits. Other three introduced variables indicated age groups: age group depending on the age at the first visit, age group depending on the age at the GH treatment initiation and age at the age group depending on the age of the SH treatment initiation. All three variables had the following five age sub-groups: 0 - 1, 1 - 4, 4 - 12, 12 - 21 and 21 – 70 years of age.

If a variable was a continuous variable, such as height, weight, head circumference etc., a percentile variable was introduced to each visit (percentiles of measurements normalized by the age). The World Health Organization (WHO) growth charts were used for the percentile calculations: http://www.who.int/childgrowth/standards/en (The WHO Child Growth Standards), http://www.who.int/growthref/en (The WHO Growth reference data for 5-19 years). In order to compare percentiles of physical measurements, data from the individuals' last follow-up visit was used.

Data Analysis

Study participants were subdivided into groups to be compared based on four variables: gender, molecular type and use of GH and/or SH. These subgroups were compared with respect to phenotype characteristics.

The data was summarized using mean and standard deviation (SD) for continuous variables, such as height, weight, head circumference etc. Groups subdivided by gender, PWS genotype and GH/SH use were compared using two-group t-test for continuous variables and

chi-square test for categorical variables. The statistical analyses were accomplished using SPSS Statistics software (Yang et al., 2011). The significance reported is the nominal significance and it was not corrected for multiple comparisons.

RESULTS

Study Participants

The total number of study participants with PWS was 355 (Table 1). Gender distribution was as follows: 160 males (45.1%) and 195 females (59.2%). The distribution of PWS participants genotype was: 217 (61.1%) with deletions, 127 (35.8%) with UPD and 11 (3.1%) with imprinting problem.

Overall, 289 participants (81.4%) had received growth hormone treatment. Out of 160 males, 137 (85.6%) had growth hormone treatment at some point in their lives. Out of 195 female participants, 152 (77.9%) received this treatment. There was no significant difference between males and females with respect to the number of individuals who received the GH treatment ($p=0.180$). Distribution of growth hormone treatment based on the PWS molecular subtype was following: 179 out of 217 (82.5%) individuals with deletion, 103 out of 127 (81.1%) individuals with UPD and 7 out of 11 (63.6%) individuals with imprinting errors were treated with growth hormone at some point in their lives (See Table 1).

Out of 355 participants (including both males and females), 84 (23.7%) had received sex hormone treatment. Out of 160 males, 47 (29.4%) received sex hormone treatment. Out of 195 female participants, 37 (19.0%) received this treatment. There was a significant difference between males and females with respect to the number of individuals who received the SH treatment ($p =0.022$). Distribution of sex hormone treatment based on the PWS molecular

subtype was following: 54 out of 217 (24.9%) individuals with deletion, 26 out of 127 (20.5%) individuals with UPD and 4 out of 11 (36.4 %) individuals with imprinting errors were treated with sex hormone at some point in their lives (See Table 1).

Data presented in Table 2 shows the age groups of all participants based on the age recorded at their initial visit. This data demonstrates a higher number of participants who belong to a younger age groups: 56.3% were below 12 years of age. The rest of the age group distributions is as follows: 21.4% of participants were in their teens and 22.3% were adults above 21 years of age (See Table 2).

The Table 3 data represents the distribution of all participants amongst the recruiting centers: Kansas' Children's Mercy Hospital and Kansas University Medical Center recruited 53 participants (14.9%), University of California at Irvine recruited 40 individuals (11.3%), University of Florida Health Sciences Center recruited 100 participants (28.2%) and Vanderbilt University Medical Center recruited 162 (45.6%) individuals taking part in the study (See Table 3). Data in Table 4 demonstrates participants' cumulative number of visits. All participants had a visit 1 (N=355), 190 individuals had a visit 2, etc. (See Table 4).

Table 1: Demographics of this study

		N	%
Total Cohort		355	100.0
Gender			
	Male	160	45.1
	Female	195	54.9
Genotype			
	Deletion	217	61.1
	UPD	127	35.8
	Imprinting	11	3.1
GH Use			
	Yes	289	81.4
	No	63	17.7
	Missing data	3	0.8
GH by Gender			
	Males: GH	137	85.6
	Males: No GH	22	13.8
	Missing	1	0.6
	Females: GH	152	77.9
	Females: No GH	41	21.0
	Missing	2	1.0
	Male vs Female GH	Chi square: p=0.180	
GH by Genotype			
	Deletion: GH	179	82.5
	Deletion: No GH	35	16.1
	Missing	3	1.4
	Total	217	100.0
	UPD: GH	103	81.1
	UPD: No GH	24	18.9
	Missing	0	0.0
	Total	127	100.0
	Imprinting: GH	7	63.6
	Imprinting: No GH	4	36.4
	Missing	0	0.0
	Total	11	100.0
SH Use			
	Yes	84	23.7
	No	271	76.3
	Missing data	0	0.0
	Total	355	100.0

SH by Gender			
	Males: T	47	29.4
	Males: No T	113	70.6
	Total	160	100
	Females: E	37	19.0
	Females: No E	158	81.0
	Total	195	100.0
	Male vs Female SH	Chi square: p=0.022	
SH by Genotype			
	Deletion: SH	54	24.9
	Deletion: No SH	163	75.1
	Missing	0	0.0
	Total	217	100.0
	UPD: SH	26	20.5
	UPD: No SH	101	79.5
	Missing	0	0.0
	Total	127	100.0
	Imprinting: SH	4	36.4
	Imprinting: No SH	7	63.6
	Missing	0	0.0
	Total	11	100.0

Table 2: Age groups of participants with PWS

Age groups			
	Years	**Frequency**	**%**
1	0 - 1	20	5.6
2	1 - 4	52	14.6
3	4 - 12	128	36.1
4	12 - 21	76	21.4
5	21 - 70	79	22.3
Total		**355**	**100**

Based on the age recorded at the initial baseline visit

Table 3: Participating institutions

Name of the institution:	Number of participants	
	Frequency	%
Children's Mercy Hospital	3	0.8
Kansas University Medical Center	50	14.1
University of California at Irvine	40	11.3
University of Florida Health Sciences Center	100	28.2
Vanderbilt University Medical Center	162	45.6
Total	**355**	**100**

Number of PWS participants per institution that participated in the data collection.

Table 4: Number of visits

Number of visits					
Visit number	**1**	**2**	**3**	**4**	**5**
Frequency	355	190	83	26	2

This table shows how many participants had one visit (N=355), two visits (N=190) etc.

Comparison by Gender

The first analyses compared the study participants' phenotypical features based on their gender. The results of these analyses are presented in Table 5 and Table 6. Variables highlighted in green are showing suggestive differences (defined as $p<0.05$).

Suggestive differences were found between females and males ($p<0.05$) for presence of flat occiput ($p=0.006$), bitemporal narrowing ($p=0.010$), hypotelorism ($p=0.022$), flat philtrum ($p=0.047$), short neck ($p=0.035$) and short 5th finger ($p=0.031$). Other suggestive differences were found between females and males ($p<0.05$) for presence of height difference ($p=0.033$), right hand ($p=<0.005$) and right foot ($p=0.004$) lengths.

Table 5: Physical Features by Gender

	Female		Male		Chi-Square
	N	%	N	%	p-value
Head:					
Head Prominent Occiput					0.115
YES	53	27.2	32	20.0	
NO	142	72.8	128	80.0	
Head Flat Occiput					0.006
YES	46	23.6	59	36.9	
NO	149	76.4	101	63.1	
Head Round Face					0.931
YES	63	32.3	51	31.9	
NO	132	67.7	109	68.1	
Head Bitemporal Narrowing					0.010
YES	135	69.2	130	81.3	
NO	60	30.8	30	18.8	
Head Craniosynostosis					0.179*
YES	1	0.5	4	2.5	
NO	194	99.5	156	97.5	
Normal Hair Color					0.437
YES	165	84.6	140	87.5	
NO	30	15.4	20	12.5	
Hypopigmented Hair Color					0.281
YES	66	33.8	63	39.4	
NO	129	66.2	97	60.6	
Hyperpigmented Hair Color					0.291
YES	9	4.6	4	2.5	
NO	186	95.4	156	97.5	
Chin: Micrognathia					0.530
YES	52	26.7	38	23.8	
NO	143	73.3	122	76.3	
Chin: Prognathia					0.417
YES	18	9.2	19	11.9	
NO	177	90.8	141	88.1	
Chin: Retrognathia					0.615
YES	12	6.2	12	7.5	
NO	183	93.8	148	92.5	

Eyes: Almond Shaped Eyes					0.081
YES	132	67.7	94	58.8	
NO	63	32.3	66	41.3	
Eyes: Slit-like					0.400
YES	21	10.8	13	8.1	
NO	174	89.2	147	91.9	
Eyes: Strabismus					0.356
YES	94	48.2	85	53.1	
NO	101	51.8	75	46.9	
Eyes: Esotropia					0.888
YES	79	40.5	66	41.3	
NO	116	59.5	94	58.8	
Eyes: Exotropia					0.806
YES	16	8.2	12	7.5	
NO	179	91.8	148	92.5	
Eyes: Ptosis					0.158
YES	28	14.4	32	20.0	
NO	167	85.6	128	80.0	
Eyes: Epicanthal folds					0.757
YES	59	30.3	46	28.7	
NO	136	69.7	114	71.3	
Eyes: Hypertelorism					0.880
YES	15	7.7	13	8.1	
NO	180	92.3	147	91.9	
Eyes: Hypotelorism					0.022
YES	19	9.7	29	18.1	
NO	176	90.3	131	81.9	
Eyes: Telecanthus					0.248
YES	16	8.2	19	11.9	
NO	179	91.8	141	88.1	
Eyes: Upslanting					0.132
YES	55	28.2	34	21.3	
NO	140	71.8	126	78.8	
Eyes: Downslanting					0.246
YES	17	8.7	20	12.5	
NO	178	91.3	140	87.5	
Nose Bridge: broad					0.091
YES	43	22.1	24	15.0	
NO	152	77.9	136	85.0	
Nose Bridge: narrow					0.112
YES	61	31.3	63	39.4	
NO	134	68.7	97	60.6	
Nose Bridge: flat					0.591

YES	23	11.8	16	10.0	
NO	172	88.2	144	90.0	
Nose Tip: Anteverted					0.064
YES	43	22.1	23	14.4	
NO	152	77.9	137	85.6	
Nose Tip: Flat					0.164
YES	26	13.3	30	18.8	
NO	169	86.7	130	81.3	
Nose nares: hypoplastic					0.291
YES	43	22.1	43	26.9	
NO	152	77.9	117	73.1	
Nose “JC”					0.585
YES	14	7.2	14	8.8	
NO	181	92.8	146	91.3	
Mouth: philtrum flat					0.047
YES	77	39.5	80	50.0	
NO	118	60.5	80	50.0	
Mouth: philtrum abnormal ridges					0.291
YES	9	4.6	4	2.5	
NO	186	95.4	156	97.5	
Mouth: Upper lip downturned					0.486
YES	64	32.8	47	29.4	
NO	131	67.2	113	70.6	
Ears: Pits					0.130*
YES	4	2.1	0	0	
NO	191	97.9	160	100.0	
Ears: Tags					0.331
YES	1	0.5	3	1.9	
NO	194	99.5	157	98.1	
Ears: Low Set					0.120
YES	39	20.0	22	13.8	
NO	156	80.0	138	86.3	
Ears: Posteriorly Angulated					0.228
YES	22	11.3	12	7.5	
NO	173	88.7	148	92.5	
Neck:					
Neck: Short					0.035
YES	73	37.4	43	26.9	
NO	122	62.6	117	73.1	
Neck: Broad					0.182
YES	44	22.6	27	16.9	
NO	151	77.4	133	83.1	

Chest:					
Chest: Pectus Excavatum					0.577
YES	31	15.9	29	18.1	
NO	164	84.1	131	81.9	
Chest: Pectus Carinatum					0.126
YES	4	2.1	8	5.0	
NO	191	97.9	152	95.0	
Abdomen: Distribution of Fat					
Primarily Abdominal (Central)					0. 579
YES	119	61.0	93	58.1	
NO	76	39.0	67	41.9	
Primarily hips/thighs					0.721
YES	89	45.6	70	43.8	
NO	106	54.4	90	56.3	
Back:					
Scoliosis					0.333
YES	116	59.5	87	54.4	
NO	79	40.5	73	45.6	
Kyphosis					0.065
YES	50	25.6	28	17.5	
NO	145	74.4	132	82.5	
Lordosis					0.247
YES	66	33.8	45	28.1	
NO	129	66.2	115	71.9	
Sacral dimple					0.248
YES	8	4.1	11	6.9	
NO	187	95.9	149	93.1	
Extremities:					
Fourth Metacarpal Short					0.208
YES	54	27.7	35	21.9	
NO	141	72.3	125	78.1	
5th finger short					0.031
YES	80	41.0	48	30.0	
NO	115	59.0	112	70.0	
5th finger clinodactyly					0.643
YES	100	51.3	86	53.8	
NO	95	48.7	74	46.3	
Skin: Cafe au lait spots					0.928
YES	31	15.9	26	16.3	
NO	164	84.1	134	83.8	

*Fisher's exact test

Table 6: Physical Measurements: Comparison between Female and Male participants

	Female		Male		t-test
	N	Mean (SD)	N	Mean (SD)	p-value
Height %ile	150	39.3 (33.4)	125	48.1 (34.4)	0.033
Weight %ile	184	71.4 (30.5)	156	69.9 (34.2)	0.671
BMI (kg/m^2)	184	26.1 (10.0)	152	25.6 (10.9)	0.663
Head circumference %ile	173	58.3 (34.5)	144	55.9 (32.4)	0.526
Right Hand length %ile	171	30.3 (29.9)	143	44.8 (32.2)	<0.005
Right Foot length %ile	171	19.8 (22.2)	130	28.3 (28.0)	0.004

This is a comparison of physical measurements based on gender. Data was used from the individuals' last follow-up visit. The percentiles of the measurements normalized by age were used for height, weight, head circumference, hand and foot lengths.

Comparison by Molecular Subtype (Deletion vs. UPD)

The second analyses compared the study participants' phenotypical features based on PWS molecular subtypes: UPD versus deletion. The results of these analyses are presented in Table 7, Table 8 and Table 9. Variables highlighted in green are showing suggestive differences (defined as $p<0.05$).

Suggestive differences were found between UPD and deletion groups ($p<0.05$) for presence of flat occiput ($p=0.002$), normal hair color ($p=0.001$), hypopigmented hair color ($p<0.050$), almond shaped eyes ($p=0.045$), strabismus ($p=0.019$), downslanting eyes ($p=0.041$), narrow nose bridge ($p=0.010$), flat nose bridge ($p=0.032$), abnormal philtrum ridges ($p=0.037$), posteriorly angulated ears ($p=0.019$), short 5th finger ($p=0.024$).

Other suggestive differences were found between UPD and deletion subgroups ($p<0.05$) for presence of weight difference ($p=0.024$), head circumference ($p=0.003$) and the age of menarche for females ($p=0.012$). The age group distribution between UPD and deletion demonstrates a small suggestive difference ($p<0.05$) for higher number of younger participants (before the age of 12 years old) with UPD (64.5%) versus deletion (52.9%), ($p=0.036$).

Table 7: Physical Features by Molecular Subtype

	Deletion		UPD		Chi-Square
	N	%	N	%	p-value
Head:					
Head Prominent Occiput					0.281
YES	47	21.7	34	26.8	
NO	170	78.3	93	73.2	
Head Flat Occiput					0.002
YES	77	35.5	25	19.7	
NO	140	64.5	102	80.3	
Head Round Face					0.157
YES	74	34.1	34	26.8	
NO	143	65.9	93	73.2	
Head Bitemporal Narrowing					0.054
YES	169	77.9	87	68.5	
NO	48	22.1	40	31.5	
Head Craniosynostosis					0.886
YES	3	1.4	2	1.6	
NO	214	98.6	125	98.4	
Normal Hair Color					0.001
YES	175	80.6	119	93.7	
NO	42	19.4	8	6.3	
Hypopigmented Hair Color					<0.050
YES	104	47.9	22	17.3	
NO	113	52.1	105	82.7	
Hyperpigmented Hair Color					0.197
YES	6	2.8	7	5.5	
NO	211	97.2	120	94.5	
Chin: Micrognathia					0.210
YES	50	23.0	37	29.1	
NO	167	77.0	90	70.9	
Chin: Prognathia					0.690
YES	21	9.7	14	11.0	
NO	196	90.3	113	89.0	
Chin: Retrognathia					0.820
YES	14	6.5	9	7.1	
NO	203	93.5	118	92.9	
Eyes: Almond Shaped Eyes					0.045
YES	148	68.2	73	57.5	
NO	69	31.8	54	42.5	

Eyes: Slit-like					0.064
YES	25	11.5	7	5.5	
NO	192	88.5	120	94.5	
Eyes: Strabismus					0.019
YES	98	45.2	74	58.3	
NO	119	54.8	53	41.7	
Eyes: Esotropia					0.659
YES	87	40.1	54	42.5	
NO	130	59.9	73	57.5	
Eyes: Exotropia					0.094
YES	13	6.0	14	11.0	
NO	204	94.0	113	89.0	
Eyes: Ptosis					0.636
YES	35	16.1	23	18.1	
NO	182	83.9	104	81.9	
Eyes: Epicanthal folds					0.560
YES	68	31.3	36	28.3	
NO	149	68.7	91	71.7	
Eyes: Hypertelorism					0.688
YES	18	8.3	9	7.1	
NO	199	91.7	118	92.9	
Eyes: Hypotelorism					0.646
YES	27	12.4	18	14.2	
NO	190	87.6	109	85.8	
Eyes: Telecanthus					0.690
YES	21	9.7	14	11.0	
NO	196	90.3	113	89.0	
Eyes: Upslanting					0.325
YES	60	27.6	29	22.8	
NO	157	72.4	98	77.2	
Eyes: Downslanting					0.041
YES	16	7.4	18	14.2	
NO	201	92.6	109	85.8	
Nose Bridge: broad					0.153
YES	36	16.6	29	22.8	
NO	181	83.4	98	77.2	
Nose Bridge: narrow					0.010
YES	86	39.6	33	26.0	
NO	131	60.4	94	74.0	
Nose Bridge: flat					0.032
YES	30	13.8	8	6.3	
NO	187	86.2	119	93.7	

Nose Tip: Anteverted					0.209
YES	36	16.6	28	22.0	
NO	181	83.4	99	78.0	
Nose Tip: Flat					0.427
YES	36	16.6	17	13.4	
NO	181	83.4	110	86.6	
Nose nares: hypoplastic					0.167
YES	57	26.3	25	19.7	
NO	160	73.7	102	80.3	
Nose “JC”					0.099
YES	21	9.7	6	4.7	
NO	196	90.3	121	95.3	
Mouth: philtrum flat					0.068
YES	104	47.9	48	37.8	
NO	113	52.1	79	62.2	
Mouth: philtrum abnormal ridges					0.037
YES	11	5.1	1	0.8	
NO	206	94.9	126	99.2	
Mouth: Upper lip downturned					0.086
YES	61	28.1	47	37.0	
NO	156	71.9	80	63.0	
Ears: Pits					0.619
YES	3	1.4	1	0.8	
NO	214	98.6	126	99.2	
Ears: Tags					0.619
YES	3	1.4	1	0.8	
NO	214	98.6	126	99.2	
Ears: Low Set					0.688
YES	34	15.7	22	17.3	
NO	183	84.3	105	82.7	
Ears: Posteriorly Angulated					0.019
YES	13	6.0	17	13.4	
NO	204	94.0	110	86.6	
Neck:					
Neck: Short					0.739
YES	68	31.3	42	33.1	
NO	149	68.7	85	66.9	
Neck: Broad					0.595
YES	41	18.9	27	21.3	
NO	176	81.1	100	78.7	

Chest:					
Chest: Pectus Excavatum					0.314
YES	32	14.7	24	18.9	
NO	185	85.3	103	81.1	
Chest: Pectus Carinatum					0.551
YES	6	2.8	5	3.9	
NO	211	97.2	122	96.1	
Abdomen: Distribution of Fat					
Primarily Abdominal (Central)					0.598
YES	127	58.5	78	61.4	
NO	90	41.5	49	38.6	
Primarily hips/thighs					0.848
YES	98	45.2	56	44.1	
NO	119	54.8	71	55.9	
Back:					
Scoliosis					0.713
YES	31	14.3	20	15.7	
NO	186	85.7	107	84.3	
Kyphosis					0.738
YES	46	21.2	25	19.7	
NO	171	78.8	102	80.3	
Lordosis					0.451
YES	65	30.0	43	33.9	
NO	152	70.0	84	66.1	
Sacral dimple					0.994
YES	12	5.5	7	5.5	
NO	205	94.5	120	94.5	
GU:					
Male:					
Male: scrotum hypoplastic					0.739
YES	50	50.0	28	52.8	
NO	50	50.0	25	47.2	
Male: scrotum rugation poor					0.490
YES	34	34.0	21	39.6	
NO	66	66.0	32	60.4	
Male: scrotum bifid					0.962
YES	2	2.0	1	1.9	
NO	98	98.0	52	98.1	
Male: scrotum pigmentation poor					0.814
YES	21	21.0	12	22.6	
NO	79	79.0	41	77.4	

Female:					
Female: labia majora normal					0.722
YES	32	27.4	22	29.7	
NO	85	72.6	52	70.3	
Female: labia minora normal					0.544
YES	11	9.4	9	12.2	
NO	106	90.6	65	87.8	
Female: labia minora hypoplastic					0.526
YES	53	45.3	37	50.0	
NO	64	54.7	37	50.0	
Female: labia minora hyperplastic					0.317
YES	1	0.9	2	2,7	
NO	116	99.1	72	97.3	
Female: clitoris hypoplastic					0.531
YES	48	41.0	27	36.5	
NO	69	59.0	47	63.5	
Female: clitoris hyperplastic					0.425
YES	1	0.9	0	0	
NO	116	99.1	74	100.0	
Extremities:					
Fourth Metacarpal Short					0.138
YES	60	27.6	26	20.5	
NO	157	72.4	101	79.5	
5th finger short					0.024
YES	86	39.6	35	27.6	
NO	131	60.4	92	72.4	
5th finger clinodactyly					0.281
YES	119	54.8	62	48.8	
NO	98	45.2	65	51.2	
Skin: Cafe au lait spots					0.063
YES	28	12.9	26	20.5	
NO	189	87.1	101	79.5	

Table 8: Physical Measurements: Comparison between the UPD and Deletion Subgroups

	UPD		Deletion		t-test
	N	Mean (SD)	N	Mean (SD)	p-value
Height %ile	109	45.0 (34.8)	160	42.2 (33.6)	0.510
Weight %ile	123	65.1 (34.5)	207	73.7 (30.9)	0.024
BMI (kg/m^2)	116	24.6 (9.2)	209	26.3 (10.7)	0.137
Head circumference %ile	115	63.9 (30.5)	192	52.1 (34.3)	0.003
Right Hand length %ile	114	36.5 (32.7)	190	37.6 (31.3)	0.773
Right Foot length %ile	106	23.1 (26.0)	185	23.8 (25.3)	0.841
Male: penis length %ile	18	20.9 (21.9)	31	18.9 (20.8)	0.750
Male: penis diameter (cm)	20	2.1 (1.1)	32	2.5 (1.6)	0.321
Female: age of menarche (yrs)	8	12.7 (1.5)	11	14.9 (1.9)	0.012
Female: average cycle length (days)	6	3.3 (1.5)	13	3.7 (1.9)	0.674

This is a comparison of the physical measurements based on PWS genotype (UPD vs deletion). Data was used from the individuals' last follow-up visit. The percentiles of the measurements normalized by age were used for height, weight, head circumference, hand and foot lengths, and penis length. Individuals with Imprinting Defects (N=11) were excluded from this analysis

Table 9: Genotype distribution based on the Age Group

Age groups		UPD		Deletion	
	Years	**Frequency**	**%**	**Frequency**	**%**
1	0 - 1	7	5.5	13	5.9
2	1 - 4	22	17.3	30	13.8
3	4 - 12	53	41.7	72	33.2
4	12 - 21	22	17.3	50	23.0
5	21 - 70	23	18.1	52	23.9
Total		**127**	**100**	**217**	**100**

Individuals with Imprinting Defects (N=11) were excluded from this analysis

Comparison by GH Treatment

The following analyses compared the study participants' phenotypical features based on GH treatment versus never treated with GH. The results of these analyses are presented in Table 10 and Table 11. Variables highlighted in green are showing suggestive differences (defined as $p<0.05$).

Suggestive differences were found between individuals who had GH treatment versus the ones who were never treated with GH ($p<0.05$) for presence of prominent head occiput ($p=0.004$), round face ($p=0.001$), hyperpigmented hair color ($p=0.0003$), almond shaped eyes ($p=0.005$), esotropia ($p=0.012$), hypotelorism ($p=0.026$), narrow nose bridge ($p=0.020$), "JC" nose ($p=0.026$), upper lip downturned ($p=0.001$), short and broad neck (both have $p=0.0003$), pectus excavatum ($p=0.0003$), kyphosis ($p=0.00002$), lordosis ($p=0.018$), short fourth metacarpal ($p=0.014$), short 5th finger ($p=0.002$), 5th finger clinodactyly ($p=0.002$), hypoplastic labia minora ($p=0.005$) and hypoplastic clitoris ($p=0.044$) in females.

Other suggestive differences were found between individuals who had GH treatment versus the ones who were never treated with GH ($p<0.05$) for presence of height difference ($p<0.05$), BMI ($p<0.05$), right hand and right foot lengths ($p<0.05$). The age group distribution between individuals who had GH treatment versus the ones who were never treated with GH demonstrates a suggestive difference ($p<0.05$) for higher number of younger participants (before the age of 12 years old) who had GH treatment (64.3%) versus the number of younger participants among those who were never treated with GH (22.1%).

Table 10: Physical Features by GH Use

	GH		No GH		Chi-Square
	N	%	N	%	p-value
Head:					
Head Prominent Occiput					0.004
YES	36	13.7	16	29.6	
NO	227	86.3	38	70.4	
Head Flat Occiput					0.103
YES	73	27.8	21	38.9	
NO	190	72.2	33	61.1	
Head Round Face					0.001
YES	72	27.4	27	50.0	
NO	191	72.6	27	50.0	
Head Bitemporal Narrowing					0.906
YES	192	73.0	39	72.2	
NO	71	27.0	15	27.8	
Head Craniosynostosis					0.430
YES	3	1.1	9	14.3	
NO	260	98.9	54	85.7	
Normal Hair Color					0.941
YES	223	84.8	46	85.2	
NO	40	15.2	8	14.8	
Hypopigmented Hair Color					0.057
YES	99	37.6	13	24.1	
NO	164	62.4	41	75.9	
Hyperpigmented Hair Color					0.0003
YES	6	2.3	7	13.0	
NO	257	97.7	47	87.0	
Chin: Micrognathia					0.089
YES	59	22.4	18	33.3	
NO	204	77.6	36	66.7	
Chin: Prognathia					0.571
YES	26	9.9	4	7.4	
NO	237	90.1	50	92.6	
Chin: Retrognathia					0.716
YES	16	6.1	4	7.4	
NO	247	93.9	50	92.6	
Eyes: Almond Shaped Eyes					0.005
YES	156	59.3	43	79.6	
NO	107	40.7	11	20.4	

Eyes: Slit-like					0.975
YES	24	9.1	5	9.3	
NO	239	90.9	49	90.7	
Eyes: Strabismus					0.357
YES	135	51.3	24	44.4	
NO	128	48.7	30	55.6	
Eyes: Esotropia					0.012
YES	122	46.4	15	27.8	
NO	141	53.6	39	72.2	
Eyes: Exotropia					0.533
YES	18	6.8	5	9.3	
NO	245	93.2	49	90.7	
Eyes: Ptosis					0.780
YES	43	16.3	8	14.8	
NO	220	83.7	46	85.2	
Eyes: Epicanthal folds					0.151
YES	79	30.0	11	20.4	
NO	184	70.0	43	79.6	
Eyes: Hypertelorism					0.343
YES	19	7.2	2	3.7	
NO	244	92.8	52	96.3	
Eyes: Hypotelorism					0.026
YES	29	11.0	12	22.2	
NO	234	89.0	42	77.8	
Eyes: Telecanthus					0.519
YES	27	10.3	4	7.4	
NO	236	89.7	50	92.6	
Eyes: Upslanting					0.793
YES	59	22.4	13	24.1	
NO	204	77.6	41	75.9	
Eyes: Downslanting					0.955
YES	25	9.5	5	9.3	
NO	238	90.5	49	90.7	
Nose Bridge: broad					0.068
YES	41	15.6	14	25.9	
NO	222	84.4	40	74.1	
Nose Bridge: narrow					0.020
YES	79	30.0	25	46.3	
NO	184	70.0	29	53.7	
Nose Bridge: flat					0.442
YES	25	9.5	7	13.0	
NO	238	90.5	47	87.0	
Nose Tip: Anteverted					0.991
YES	44	16.7	9	16.7	
NO	219	83.3	45	83.3	

Nose Tip: Flat					0.787
YES	40	15.2	9	16.7	
NO	223	84.8	45	83.3	
Nose nares: hypoplastic					0.102
YES	60	22.8	18	33.3	
NO	203	77.2	36	66.7	
Nose “JC”					0.026
YES	19	7.2	9	16.7	
NO	244	92.8	45	83.3	
Mouth: philtrum flat					0.317
YES	112	42.6	27	50.0	
NO	151	57.4	27	50.0	
Mouth: philtrum abnormal ridges					0.268
YES	7	2.7	3	5.6	
NO	256	97.3	51	94.4	
Mouth: Upper lip downturned					0.001
YES	68	23.5	26	48.1	
NO	195	67.5	28	51.9	
Ears: Pits					0.451
YES	2	0.8	1	1.9	
NO	261	99.2	53	98.1	
Ears: Tags					0.362
YES	4	1.5	0	0.0	
NO	259	98.5	54	100.0	
Ears: Low Set					0.832
YES	42	16.0	8	14.8	
NO	221	84.0	46	85.2	
Ears: Posteriorly Angulated					0.583
YES	23	8.7	6	11.1	
NO	240	91.3	48	88.9	
Neck:					
Neck: Short					0.0003
YES	63	24.0	35	64.8	
NO	200	76.0	19	35.2	
Neck: Broad					0.0003
YES	38	14.4	19	35.2	
NO	225	85.6	35	64.8	
Chest:					
Chest: Pectus Excavatum					0.0003
YES	52	19.8	0	0.0	
NO	211	80.2	54	100.0	

Chest: Pectus Carinatum					0.187
YES	6	2.3	3	5.6	
NO	257	97.7	51	94.4	
Abdomen: Distribution of Fat					
Primarily Abdominal (Central)					0.086
YES	152	57.8	38	70.4	
NO	111	42.2	16	29.6	
Primarily hips/thighs					0.338
YES	121	46.0	21	38.9	
NO	142	54.0	33	61.1	
Back:					
Scoliosis					0.422
YES	152	57.8	28	51.9	
NO	111	42.2	26	48.1	
Kyphosis					0.00002
YES	44	16.7	23	42.6	
NO	219	83.3	31	57.4	
Lordosis					0.018
YES	92	35.0	10	18.5	
NO	171	65.0	44	81.5	
Sacral dimple					0.240
YES	10	3.8	4	7.4	
NO	253	96.2	50	92.6	
GU:					
Male:					
Male: scrotum hypoplastic					0.390
YES	66	48.2	9	40.9	
NO	71	51.8	13	59.1	
Male: scrotum rugation poor					0.613
YES	46	33.6	9	40.9	
NO	91	66.4	13	59.1	
Male: scrotum bifid					0.774
YES	3	2.2	0	0.0	
NO	134	97.8	22	100.0	
Male: scrotum pigmentation poor					0.338
YES	26	19.0	7	31.8	
NO	111	81.0	15	68.2	

Female:					
Female: labia majora normal					0.080
YES	48	31.6	6	14.6	
NO	104	68.4	35	85.4	
Female: labia minora normal					0.088
YES	17	11.2	2	4.9	
NO	135	88.8	39	95.1	
Female: labia minora hypoplastic					0.005
YES	79	52.0	11	26.8	
NO	73	48.0	30	73.2	
Female: labia minora hyperplastic					0.650
YES	3	2.0	0	0.0	
NO	149	98.0	41	100.0	
Female: clitoris hypoplastic					0.044
YES	64	42.1	11	26.8	
NO	88	57.9	30	73.2	
Female: clitoris hyperplastic					0.867
YES	1	0.7	0	0.0	
NO	151	99.3	41	100.0	
Extremities:					
Fourth Metacarpal Short					0.014
YES	56	21.3	20	37.0	
NO	207	78.7	34	63.0	
5th finger short					0.002
YES	78	29.7	28	51.9	
NO	185	70.3	26	48.1	
5th finger clinodactyly					0.002
YES	145	55.1	17	31.5	
NO	118	44.9	37	68.5	
Skin: Cafe au lait spots					0.057
YES	47	17.9	4	7.4	
NO	263	82.1	50	92.6	

Table 11: Physical Measurements: Comparison between the participants treated with GH and not treated with GH.

	GH		No GH		t-test
	N	Mean (SD)	N	Mean (SD)	p-value
Height %ile	238	47.4 (32.8)	36	17.4 (30.9)	<0.05
Weight %ile	277	69.8 (32.1)	60	73.6 (33.2)	0.413
BMI (kg/m^2)	276	24.2 (9.9)	57	34.2 (8.6)	<0.05
Head circumference %ile	260	59.0 (32.9)	55	50.8(35.1)	0.099
Right Hand length %ile	256	41.2 (31.8)	55	17.9 (24.2)	<0.05
Right Foot length %ile	246	25.9 (26.9)	53	12.4 (8.6)	<0.05
Male: penis length %ile	42	21.0 (21.4)	7	11.7 (17.3)	0.283
Male: penis diameter (cm)	45	2.4 (1.4)	7	2.4 (1.2)	0.911
Female: age of menarche (yrs)	15	13.8 (2.0)	3	15.5 (1.3)	0.196
Female: average cycle length (days)	14	3.5 (1.7)	3	3.5 (1.5)	1.000

This is a comparison of the physical measurements based on individuals with GH treatment exposure and individuals who did not have GH treatment exposure. Data was used from the individuals' last follow-up visit. The percentiles of the measurements normalized by age were used for height, weight, head circumference, hand and foot lengths, and penis length.

Table 12: GH intake based on the Age Group

Age groups		GH		No GH	
	Years	**Frequency**	**%**	**Frequency**	**%**
1	0 - 1	17	5.9	3	5.1
2	1 - 4	51	17.6	1	1.7
3	4 - 12	118	40.8	9	15.3
4	12 - 21	65	22.5	10	16.9
5	21 - 70	38	13.1	36	61.0
Total		**289**	**100**	**63**	**100**

Individuals with missing information on GH intake (N=3) were excluded from this analysis. The age at the initial baseline visit was used for this table.

Effect of GH Treatment After Adjusting for Age of GH Treatment Initiation

The following analyses compared the study participants' phenotypical features based on the age of the GH treatment initiation. All the participants who were ever on GH treatment were subdivided into five age groups: from birth to 1 year of age, from 1 year to 4 years, from 4 years to 12 years, from 12 years to 21 years and from 21 years to the oldest participant who was 49 years old at the initiation of the GH treatment. The results of these analyses are presented in Table 13, Table 14 and Table 15. Variables highlighted in green are showing suggestive differences (defined as $p<0.05$).

Suggestive differences were found between the age groups ($p<0.05$) for presence of micrognathia ($p=0.039$), slit-like eyes ($p=0.025$), narrow nose bridge ($p=0.013$), pectus excavatum ($p<0.05$), kyphosis ($p<0.05$), primarily abdominal distribution of fat ($p<0.05$), hypoplastic scrotum ($p=0.021$), and short 5th finger ($p=0.026$).

Table 13: GH intake based on the age of the GH treatment initiation

Age groups		GH	
	Years	**Frequency**	**%**
1	0 - 1	121	41.7
2	1 - 4	84	29.2
3	4 - 12	52	18.1
4	12 - 21	19	6.6
5	21 - 70	13	4.5
Total		**289**	**100**

Table 14: Physical Features compared by the age of GH treatment initiation

Age at the start of the GH treatment (years)	0 - 1		1 - 4		4 - 12		12 - 21		21 - 70		Chi-Square
	N	%	N	%	N	%	N	%	N	%	p-value
Head:											
Head Prominent Occiput	15	13.9	12	15.8	5	10.2	2	11.8	2	15.4	0.928
Head Flat Occiput	34	31.5	19	25.0	15	30.6	2	11.8	3	23.1	0.471
Head Round Face	25	23.1	23	30.3	18	36.7	5	29.4	1	7.7	0.198
Head Bitemporal Narrowing	81	75.0	54	71.1	32	65.3	13	76.5	12	92.3	0.355
Head Craniosynostosis	3	2.8	0	0.0	0	0.0	0	0.0	0	0.0	0.360
Normal Hair Color	90	83.3	66	86.8	45	91.8	11	64.7	11	84.6	0.106
Hypopigmented Hair Color	50	46.3	23	30.3	15	30.6	8	47.1	3	23.1	0.089
Hyperpigmented Hair Color	2	1.9	1	1.3	2	4.1	0	0.0	1	7.7	0.521
Chin: Micrognathia	24	22.2	18	23.7	5	10.2	6	35.3	6	46.2	0.039
Chin: Prognathia	11	10.2	5	6.6	8	16.3	2	11.8	0	0.0	0.317
Chin: Retrognathia	7	6.5	6	7.9	3	6.1	0	0.0	0	0.0	0.661
Eyes: Almond Shaped Eyes	61	56.5	46	60.5	31	63.3	10	58.8	8	61.5	0.945
Eyes: Slit-like	5	4.6	5	6.6	8	16.3	4	23.5	2	15.4	0.025
Eyes: Strabismus	61	56.5	40	52.6	22	44.9	7	41.2	5	38.5	0.467
Eyes: Esotropia	53	49.1	40	52.6	18	36.7	6	35.3	5	38.5	0.341
Eyes: Exotropia	6	5.6	3	3.9	4	8.2	4	23.5	1	7.7	0.065
Eyes: Ptosis	15	13.9	11	14.5	10	20.4	3	17.6	4	30.8	0.515
Eyes: Epicanthal folds	38	35.2	22	28.9	11	22.4	5	29.4	3	23.1	0.549
Eyes: Hypertelorism	8	7.4	7	9.2	3	6.1	1	5.9	0	0.0	0.809
Eyes: Hypotelorism	9	8.3	11	14.5	7	14.3	2	11.8	0	0.0	0.424

Eyes: Telecanthus	12	11.1	7	9.2	6	12.2	1	5.9	1	7.7	0.934
Eyes: Upslanting	23	21.3	16	21.1	10	20.4	7	41.2	3	23.1	0.446
Eyes: Downslanting	11	10.2	6	7.9	5	10.2	1	5.9	2	15.4	0.895
Nose Bridge: broad	18	16.7	15	19.7	7	14.3	1	5.9	0	0.0	0.312
Nose Bridge: narrow	22	20.4	22	28.9	22	44.9	7	41.2	6	46.2	0.013
Nose Bridge: flat	15	13.9	8	10.5	1	2.0	1	5.9	0	0.0	0.121
Nose Tip: Anteverted	21	19.4	12	15.8	9	18.4	0	0.0	2	15.4	0.387
Nose Tip: Flat	23	21.3	10	13.2	4	8.2	3	17.6	0	0.0	0.105
Nose nares: hypoplastic	22	20.4	15	19.7	17	34.7	5	29.4	1	7.7	0.146
Nose “JC”	7	6.5	4	5.3	6	12.2	1	5.9	1	7.7	0.659
Mouth: philtrum flat	42	38.9	33	43.4	25	51.0	6	35.3	6	46.2	0.647
Mouth: philtrum abnormal ridges	4	3.7	3	3.9	0	0.0	0	0.0	0	0.0	0.542
Mouth: Upper lip downturned	35	32.4	16	21.1	11	22.4	5	29.4	1	7.7	0.201
Ears: Pits	2	1.9	0	0.0	0	0.0	0	0.0	0	0.0	0.576
Ears: Tags	4	3.7	0	0.0	0	0.0	0	0.0	0	0.0	0.212
Ears: Low Set	20	18.5	12	15.8	8	16.3	1	5.9	1	7.7	0.648
Ears: Posteriorly Angulated	14	13.0	4	5.3	4	8.2	0	0.0	1	7.7	0.264
Neck:											
Neck: Short	19	17.6	19	25.0	14	28.6	5	29.4	6	46.2	0.146
Neck: Broad	10	9.3	14	18.4	8	16.3	5	29.4	1	7.7	0.135
Chest:											
Chest: Pectus Excavatum	36	33.3	14	18.4	2	4.1	0	0.0	0	0.0	<0.05
Chest: Pectus Carinatum	2	1.9	1	1.3	1	2.0	2	11.8	0	0.0	0.108
Abdomen: Distribution of Fat											
Primarily Abdominal (Central)	44	40.7	53	69.7	32	65.3	11	64.7	12	92.3	<0.05

Primarily hips/thighs	44	40.7	36	47.4	27	55.1	10	58.8	4	30.8	0.264
Back:											
Scoliosis	55	50.9	47	61.8	35	71.4	10	58.8	5	38.5	0.08
Kyphosis	5	4.6	11	14.5	17	34.7	4	23.5	7	53.8	<0.05
Lordosis	41	38.0	31	40.8	13	26.5	5	29.4	2	15.4	0.238
Sacral dimple	6	5.6	1	1.3	1	2.0	2	11.8	0	0.0	0.194
GU:											
Male: scrotum hypoplastic	37	34.3	11	14.5	9	18.4	5	29.4	2	15.4	0.021
Male: scrotum rugation poor	19	17.6	10	13.2	6	12.2	3	17.6	1	7.7	0.789
Male: scrotum bifid	1	0.9	0	0.0	1	2.0	0	0.0	0	0.0	0.751
Male: scrotum pigmentation poor	10	9.3	3	3.9	3	3.9	3	3.9	3	3.9	0.309
Female: labia majora normal	21	19.4	14	18.4	6	12.2	2	11.8	4	30.8	0.531
Female: labia minora normal	6	5.6	5	6.6	2	4.1	0	0.0	3	23.1	0.088
Female: labia minora hypoplastic	36	33.3	24	31.6	9	18.4	3	17.6	3	23.1	0.265
Female: labia minora hyperplastic	2	1.9	1	1.3	0	0.0	0	0.0	0	0.0	0.841
Female: clitoris hypoplastic	27	25.0	20	26.3	7	14.3	2	11.8	4	30.8	0.343
Female: clitoris hyperplastic	1	0.9	0	0.0	0	0.0	0	0.0	0	0.0	0.837
Extremities:											
Fourth Metacarpal Short	21	19.4	12	15.8	13	26.5	6	35.3	4	30.8	0.279
5th finger short	23	21.3	24	31.6	23	46.9	4	23.5	4	30.8	0.026
5th finger clinodactyly	65	60.2	39	51.3	26	53.1	7	41.2	8	61.5	0.525
Skin: Cafe au lait spots	23	21.3	15	19.7	5	10.2	3	17.6	1	7.7	0.416

Table 15: Physical Features compared by the age of GH treatment initiation between two age groups (0-1 and 1-4).

Age at the start of the GH treatment (years)	Primarily Abdominal (Central) Distribution of Fat: YES	Primarily Abdominal (Central) Distribution of Fat: NO	Chi-Square (p-value)
0-1	44 (36.4%)	77 (63.6%)	0.000163
1-4	53 (63.1%)	31 (36.9%)	
Age at the start of the GH treatment (years)	**Kyphosis YES**	**Kyphosis NO**	**Chi-Square (p-value)**
0-1	5 (4.1%)	116 (95.9%)	0.0186
1-4	11 (13.1%)	73 (86.9%)	
Age at the start of the GH treatment (years)	**Pectus Excavatum: YES**	**Pectus Excavatum: NO**	**Chi-Square (p-value)**
0-1	36 (29.8%)	85 (70.2%)	0.0319
1-4	14 (16.7%)	70 (83.3%)	
Age at the start of the GH treatment (years)	**Male: scrotum hypoplastic YES**	**Male: scrotum hypoplastic NO**	**Chi-Square (p-value)**
0-1	37 (30.6.3%)	84 (69.4%)	0.0036
1-4	11 (13.1%)	73 (86.9%)	

Comparison by Sex Hormone (SH) Treatment

The following analyses compared the study participants' phenotypical features, more specifically genitourinary system (GU) features, based on SH treatment versus never treated with SH. The results of these analyses are presented in Table 16 and Table 17. Variables highlighted in green are showing suggestive differences (defined as $p<0.05$).

Suggestive differences were found between females who had SH treatment and the ones that did not have SH treatment ($p<0.05$) for presence of normal labia majora ($p=0.024$).

Other suggestive differences were found between males who had SH treatment versus the ones who were never treated with SH ($p<0.05$) for presence of BMI ($p<0.05$).

Suggestive differences were found between females who had SH treatment versus the ones who were never treated with SH ($p<0.05$) for presence of height ($p=0.013$), BMI ($p<0.05$), right hand length ($p=0.001$) and the age of menarche ($p=0.043$).

Table 16: Physical Features by Sex Hormone (SH) Use

	SH		No SH		Chi-Square
	N	%	N	%	p-value
GU:					
Male: Testosterone					
Male: scrotum hypoplastic					0.783
YES	23	48.9	58	51.3	
NO	24	51.1	55	48.7	
Male: scrotum rugation poor					0.431
YES	14	29.8	41	36.3	
NO	33	70.2	72	63.7	
Male: scrotum bifid					0.207*
YES	2	4.3	1	0.9	
NO	45	95.7	112	99.1	
Male: scrotum pigmentation poor					0.156
YES	13	27.7	20	17.7	
NO	34	72.3	93	82.3	
Female: Estrogen					
Female: labia majora normal					0.024
YES	16	43.2	39	24.7	
NO	21	56.8	119	75.3	
Female: labia minora normal					0.546*
YES	5	13.5	15	9.5	
NO	32	86.5	144	90.5	
Female: labia minora hypoplastic					0.103
YES	13	35.1	79	50.0	
NO	24	64.9	79	50.0	
Female: labia minora hyperplastic					1.000*
YES	0	0.0	3	1.9	
NO	37	100.0	155	98.1	
Female: clitoris hypoplastic					0.329
YES	12	32.4	65	41.1	
NO	25	67.6	93	58.9	

Female: clitoris hyperplastic					1.000*
YES	0	0.0	1	0.6	
NO	37	100.0	157	99.4	

*Fisher's Exact Test: some cells have expected count < 5.

Table 17: Physical Measurements: Comparison between the participants treated with Sex Hormone (SH) and not treated with SH.

	SH		No SH		t-test
	N	Mean (SD)	N	Mean (SD)	p-value
Male:					
Height %ile	26	38.0 (33.4)	99	50.8 (34.2)	0.093
Weight %ile	46	72.6 (36.6)	110	68.7 (33.2)	0.522
BMI (kg/m^2)	45	30.3 (10.8)	107	23.7 (10.3)	<0.05
Head circumference %ile	43	63.9 (34.9)	101	52.5 (30.8)	0.054
Right Hand length %ile	42	44.8 (35.2)	101	44.9 (31.0)	0.991
Right Foot length %ile	40	32.6 (28.4)	90	26.3 (27.8)	0.245
Male: penis length %ile	14	17.6 (18.4)	35	20.5 (22.1)	0.673
Male: penis diameter (cm)	13	2.2 (1.1)	40	2.4 (1.5)	0.741
Female:					
Height %ile	22	23.1 (27.9)	128	42.1 (33.5)	0.013
Weight %ile	34	71.7 (30.0)	150	71.3 (30.7)	0.939
BMI (kg/m^2)	34	32.4 (9.9)	150	24.7 (9.5)	<0.05
Head circumference %ile	32	56.8 (38.0)	141	58.7 (33.8)	0.781
Right Hand length %ile	32	16.9 (23.7)	139	33.4 (30.4)	0.001
Right Foot length %ile	34	19.5 (21.0)	137	19.8 (22.6)	0.946
Female: age of menarche (yrs)	14	14.5 (1.9)	5	12.5 (1.3)	0.043
Female: average cycle length (days)	13	3.8 (1.6)	6	3.1 (2.1)	0.445

This is a comparison of the physical measurements based on individuals with SH treatment exposure and individuals who did not have SH treatment exposure. Data was used from the individuals' last follow-up visit. The percentiles of the measurements normalized by age were used for height, weight, head circumference, hand and foot lengths, and penis length.

Effect of SH Treatment After Adjusting for Age of SH Treatment Initiation

The following analyses compared the study participants' phenotypical features based on the age of the SH treatment initiation. All the participants were subdivided into five age groups: from birth to 1 year of age, from 1 year up to 4 years, from 4 years to 12 years, from 12 years up to 21 years and from 21 years up to the oldest participant who was 55 years old at the initiation of the SH treatment. The results of these analyses are presented in Table 18 and Table 19. Variables highlighted in green are showing suggestive differences (defined as $p<0.05$).

Suggestive difference was found between the age groups ($p<0.05$) for presence of poor scrotum pigmentation in males ($p=0.040$).

Table 18: SH intake based on the age of the SH treatment initiation

Age groups		SH	
	Years	**Frequency**	**%**
1	0 - 1	10	11.9
2	1 - 4	6	7.1
3	4 - 12	5	6.0
4	12 - 21	42	50
5	21 - 70	21	25
Total		**84**	**100**

Table 19: Physical Features compared by the age of SH treatment initiation

Age at the start of the SH treatment	0 - 1		1 - 4		4 - 12		12 - 21		21 - 70		Chi-Square
	N	%	N	%	N	%	N	%	N	%	p-value
GU:											
Male: scrotum hypoplastic											**0.569**
YES	6	66.7	2	33.3	2	50.0	7	38.9	6	60.0	
NO	3	33.3	4	66.7	2	50.0	11	61.1	4	40.0	
Male: scrotum rugation poor											**0.086**
YES	5	55.6	0	0.0	2	50.0	3	16.7	4	40.0	
NO	4	44.4	6	100.0	2	50.0	15	83.3	6	60.0	
Male: scrotum bifid											**0.735**
YES	1	11.1	0	0.0	0	0.0	1	5.6	0	0.0	
NO	8	88.9	6	100.0	4	100.0	17	94.4	10	100.0	
Male: scrotum pigmentation poor											**0.040**
YES	5	55.6	0	0.0	2	50.0	2	11.1	4	40.0	
NO	4	44.4	6	100.0	2	50.0	16	88.9	6	60.0	
Female: labia majora normal											**0.413**
YES	1	100.0	0	0.0	1	100.0	10	41.7	4	36.4	
NO	0	0.0	0	0.0	0	0.0	14	58.3	7	63.6	

Female: labia minora normal											**0.910**
YES	0	0.0	0	0.0	0	0.0	3	12.5	2	18.2	
NO	1	100.0	0	0.0	1	100.0	21	87.5	9	81.8	
Female: labia minora hypoplastic											**0.259**
YES	1	100.0	0	0.0	1	100.0	8	33.3	3	27.3	
NO	0	0.0	0	0.0	0	0.0	16	66.7	8	72.7	
Female: labia minora hyperplastic											**n/a**
YES	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
NO	1	100.0	0	0.0	1	100.0	24	100.0	11	100.0	
Female: clitoris hypoplastic											**0.220**
YES	1	100.0	0	0.0	1	100.0	7	29.2	3	27.3	
NO	0	0.0	0	0.0	0	0.0	17	70.8	8	72.7	
Female: clitoris hyperplastic											**n/a**
YES	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
NO	1	100.0	0	0.0	1	100.0	24	100.0	11	100.0	

DISCUSSION

The aim of this study was to describe differences in PWS phenotypic features, specifically various physical features, by gender, molecular type (deletion vs. maternal UPD), and the use of GH and SH treatments. Hypotheses include 1) Individuals with deletion versus UPD have an increased frequency of and more severe physical, and dysmorphic features associated with PWS, 2) Individuals treated with growth hormone (versus not treated with growth hormone), and individuals treated with sex hormones (testosterone or estrogen) present with normalization of physical and dysmorphic features associated with PWS, 3) The younger the age of growth hormone and sex hormone treatment initiation the stronger the effect on physical and dysmorphic features associated with PWS.

This study's analysis is based on one of the largest known datasets of phenotypical features of PWS individuals. The benefit of having a large and diverse cohort, including participants' age and gender differences, allows to successfully study the correlation between PWS genotype and PWS phenotype. Having participants who were treated with GH and/or SH, and having participants who did not undergo these treatments, also allows study of the possible effects of these treatments on physical characteristics.

A previous master's thesis study has been done using the RDCRN database data overlapping with this study. The main differences between these studies include using a slightly larger cohort of 355 individuals with PWS versus 330, and having more longitudinal data. The purpose of that study was to describe differences in PWS phenotype, specifically behaviors and some physical features, by gender, molecular PWS subtype (UPD vs deletion) and the use of growth hormone treatment. The study reported differences in PWS phenotype by molecular subtype and by use of GH treatment, but not by gender. Those on GH treatment differed from

those not taking GH with respect to some of their physical features, including Body Mass Index (BMI), and improved behavioral patterns ($p<0.0005$). The investigator concluded that results support the benefit of GH treatment for patients diagnosed with PWS (Heidi D. Swanson-Fellows, 2013, UCI, unpublished data).

Comparison by Gender

This study demonstrates that for the majority of physical features and physical measurements of PWS participants there is not a significant difference in frequency based on gender. There were, however, some physical features that reached statistical significance: males were found to have flat occiput, bitemporal narrowing, hypotelorism, and flat philtrum more frequently than females. On the other hand, females were found to have short necks and short 5th finger more frequently than males. Other findings also suggest that males were on average taller with bigger hands and feet.

Comparison by Molecular Subtype (Deletion vs. UPD)

The second analyses compared the study participants' phenotypical features based on molecular subtypes: UPD versus deletion to test the first hypothesis that individuals with deletion when compared to UPD have an increased frequency of and more severe physical and dysmorphic features associated with PWS. This study found that individuals with deletion have more frequent incidence of flat occiput, hypopigmented hair color, almond shaped eyes, narrow nose bridge, flat nose bridge, abnormal philtrum ridges and short 5th finger. Individuals with UPD, however, demonstrated to have more frequent incidence of normal hair color, strabismus, downslanting eyes and posteriorly angulated ears.

This study has also shown that individuals with deletions on average are heavier, with smaller head circumferences. Female participants also demonstrated a slightly older age for menarche.

This study does not support findings of a previous study that suggested individuals with deletion have higher chance for hypoplastic genitalia (Lin, Lin et al. 2007), smaller foot length and narrow bifrontal diameter. Narrow bifrontal diameter ($p=0.054$) and slit-like eyes ($p=0.064$) in individuals with deletions did not meet significance criteria in this data set, however, differences are in the direction expected based on previous studies (Butler et al., 1986; Lin, Lin et al. 2007). Since the Imprinting Defects cohort had a small number of participants, it was not included in this analysis.

Differences by GH Use

The next step in this study's analysis was looking into possible significant difference between individuals who had GH treatment, versus the ones who never had treatment done to test the second hypothesis that individuals treated with growth hormone, and individuals treated with sex hormones (testosterone or estrogen) present with normalization of physical and dysmorphic features associated with PWS versus those not treated.

Results of this study suggest that individuals who were treated with GH, have less frequent incidence of prominent head occiput, round face, hyperpigmented hair color, almond shaped eyes. Individuals who were treated with GH also have higher incidence rate of esotropia, but less incidences of exotropia, less incidence of hypotelorism, narrow nose bridge, "JC" nose, downturned upper lip, fewer incidences of short and broad neck, kyphosis, short fourth metacarpal and short 5th finger. Data also demonstrates that individuals who were treated with

GH, however, have higher incidence rate of pectus excavatum, lordosis, 5th finger clinodactyly, and of females having hypoplastic genitalia.

This study has also shown that PWS individuals overall who were treated with GH on average are taller, with lower BMIs and longer hands and feet.

Differences by SH Use

The next step in this study's analysis was looking into possible significant difference between individuals who had SH treatment, versus the ones who never had this treatment done. Results of this study suggest that individuals who were treated with SH are more likely to have normal labia majora.

This study has also shown that males who were treated with SH on average have higher BMIs. Females who were treated with SH on average are shorter have higher BMIs, smaller hand length and the age of menarche is also increased.

Differences in age of GH and SH treatment initiation

One of the final steps in this study's analysis was looking into possible significant difference between individuals who had GH treatment initiated at a younger age (from birth to 1 year and from 1 year to 4 years of age), versus GH treatment initiated at an adult stage of life (from 21 years of age and older). This analysis was done to test the third hypothesis that the younger the age of growth hormone treatment initiation, the stronger the effect on physical and dysmorphic features associated with PWS.

Results of this study suggest that individuals who had GH treatment initiated at a younger age (from birth to 1 year and from 1 year to 4 years of age) have less frequent incidence of

micrognathia, slit-like eyes, narrow nose bridge, primarily abdominal distribution of fat, kyphosis and short fifth finger. Individuals who had GH treatment initiated at a younger age (from birth to 1 year and from 1 year to 4 years of age), also have more frequent incidence of hypoplastic scrotum in male participants.

There was no significant difference between starting GH prior to 1 year of age versus starting between 1 to 4 years of age for micrognathia, slit-like eyes and narrow nose bridge. There were, however, some results that suggest that starting GH prior to age of 1 years old demonstrates less frequent incidence of primarily abdominal distribution of fat and kyphosis. Results also demonstrate that starting GH prior to age 1 increase incidence of pectus excavatum and hypoplastic scrotum in male participants.

The second final step in this study's analysis was looking into possible significant difference between individuals who had SH treatment initiated at a younger age (from 12 years to 21 years of age), versus the ones who had SH treatment initiated at an adult stage of life (from 21 years of age and older). This analysis was done to test the third hypothesis that the younger the age of sex hormone treatment initiation, the stronger the effect on physical and dysmorphic features associated with PWS.

Results suggest that starting SH treatment during the pre-pubescent years (from 12 years to 21 years of age) increases incidence of poor scrotum pigmentation in male participants.

Study Strengths and Limitations

This study contains many strengths and a number of limiting components. The first and most important strength of this study is the size of the cohort (N=355). PWS is relatively rare condition and historically it has been extremely difficult to obtain larger numbers of participants.

Having large number of participants increases statistical power of the study and demonstrates better population-representativeness.

One of the biggest limitations of this study is that dysmorphology evaluation, which includes appraisal of physical and facial features, was conducted by multiple clinical geneticist in several different centers. Having multiple evaluators for all study participants raises the possibility of inter-evaluator differences and raises the possibility of variability of results. It is important to note, however, that all evaluations were performed by the same clinical geneticists at each site with vast dysmorphology expertise.

Clinical geneticists completing physical evaluations were not blinded to study participant's molecular diagnosis and growth hormone treatment status. This means that evaluations were conducted with the knowledge of participant's PWS type, based on their genotype, and whether or not each participant was or was not on growth hormone treatment. It is highly unlikely that this may have increased the possibility of observer biases, however, there is a possibility that evaluators might have had prior expectations of the findings based on that knowledge.

This study was conducted at five different centers with multiple evaluators. Even though it is not anticipated for there to be a lack of consistency in the setting in which the evaluations were performed and data was entered, this still potentially increases the chance of entry errors and inter evaluator differences. All those performing the clinical measurements and phenotypic descriptions were experienced clinical geneticists who are experts in PWS.

The cohort of participants who had sex hormone treatment was small and the analysis performed was limited. There was no comparative analysis done on PWS participants who had just sex hormone treatment or just growth hormone treatment. There was also no separate

comparative analysis between these groups to see what the effect these hormones can have on physical features separately from each other.

Future Studies

The design of future studies could address and find efficient solutions to the limitations of this study. For example, one of the future considerations could include a re-design of PWS dysmorphology evaluation guidelines that could help in minimizing the possibility of inter-evaluator differences.

The second future consideration could also include evaluators completing physical evaluations being blinded to study participant's molecular diagnosis and growth hormone treatment status to eliminate the small possibility of observer biases.

Other future consideration is to expand the dataset by recruiting more participants to the study that will provide more statistical power to detect differences between groups.

Future studies could also include exploring the differences in phenotypical features and GH/SH treatment effects between participants' various ethnic groups.

There can also be a future study comparing the effect of growth hormone and sex hormone treatment on phenotypical features separate from each other.

There is also data available on PWS participants' waist circumferences, hip circumferences and the size of testes of male participants for each visit. Future study could include analysis of growth hormone and sex hormone effects on these features. Standardized growth charts should be used for the percentile calculation prior to analysis.

Conclusions

This study was designed to describe differences in PWS phenotypic features, specifically physical features, by gender, molecular type (deletion vs. maternal UPD), and the use of GH and SH treatment. This study of 355 individuals with PWS is one of the largest known PWS cohorts.

Gender: The results suggest there are some statistically significant phenotypical differences between males and females with PWS, which include flat occiput, bitemporal narrowing, hypotelorism, and flat philtrum which occur more frequently males. Males also tend to be taller with bigger hands and feet. Females, on the other hand, have short necks and short 5th finger occurring more frequently.

UPD vs Deletion: The results suggest that individuals with deletion have more frequent incidence of flat occiput, hypopigmented hair color, almond shaped eyes, narrow nose bridge, flat nose bridge, abnormal philtrum ridges and short 5th finger. Individuals with deletions also tend to be heavier and smaller head circumferences. Female participants with deletions also demonstrated a slightly older age for menarche. Individuals with UPD have more frequent incidence of normal hair color, strabismus, downslanting eyes and posteriorly angulated ears.

These results suggest the possibility of accepting the first hypothesis that individuals with deletions versus UPD have more physical and dysmorphic features associated with PWS and they tend to be more severe.

GH/No GH: The results suggest that individuals who were treated with GH, have less frequent incidence of prominent head occiput, round face, hyperpigmented hair color, almond shaped eyes, exotropia, hypotelorism, narrow nose bridge, "JC" nose, downturned upper lip, short neck, broad neck, kyphosis, short fourth metacarpal and short 5th finger. Individuals who were treated with GH have higher incidence rate of pectus excavatum, lordosis, 5th finger

clinodactyly, and of females having hypoplastic genitalia. This study has also confirmed that PWS individuals who were treated with GH on average are taller, with lower BMIs, and longer hands and feet.

These results suggest the possibility of accepting the second hypothesis that individuals treated with growth hormone (versus not treated with growth hormone) present with normalization of physical and dysmorphic features associated with PWS. However, some features associated with PWS did not demonstrate normalization with the GH treatment exposure.

SH/No SH: The results suggest that females who were treated with SH are more likely to have normal labia majora. This partially supports findings that were previously described (Cassidy et al. 2009).

This study has also shown that males who were treated with SH on average have higher BMIs. Females who were treated with SH on average are shorter have higher BMIs, smaller hand length and the age of menarche is also increased.

These results suggest the possibility of accepting the second part of the second hypothesis that individuals treated with sex hormones (testosterone or estrogen) present with normalization of some physical and dysmorphic features associated with PWS. Although some features associated with PWS did not demonstrate normalization with the GH treatment exposure.

Age at GH initiation: The results of this study suggest that individuals who had GH treatment initiated at a younger age (from birth to 4 years of age) versus GH treatment initiation to an adult, have less frequent incidence of micrognathia, slit-like eyes, narrow nose bridge, primarily abdominal distribution of fat, kyphosis and short fifth finger. Individuals who

had GH treatment initiated at a younger age also have more frequent incidence of hypoplastic scrotum in male participants.

There was no significant difference between starting GH prior to 1 year of age versus starting between 1 to 4 years of age for micrognathia, slit-like eyes and narrow nose bridge. The results also suggest that starting GH prior to age of 1 years old versus between 1 to 4 years old, demonstrates less frequent incidence of primarily abdominal distribution of fat and kyphosis. Results also demonstrate that starting GH prior to age 1 increase incidence of pectus excavatum and hypoplastic scrotum in male participants.

These results suggest the possibility of accepting the third hypothesis that the younger the age of growth hormone treatment initiation the stronger the effect on physical and dysmorphic features associated with PWS. Although some features associated with PWS did not demonstrate normalization with the GH treatment exposure.

Age at SH initiation: The results of this study suggest that males who had SH treatment initiated at a younger age (between 12 and 21 years of age), versus the ones who had SH treatment initiated as adults (from 21 years of age and older) increases incidence of poor scrotum pigmentation in male participants.

These results suggest the possibility of rejecting the second part of the third hypothesis that the younger the age of sex hormone treatment initiation the stronger the effect on physical and dysmorphic features associated with PWS.

Summary: Individuals with PWS are known to have growth hormone (GH) deficiency and hypogonadism. It has been previously proven that using GH treatment and sex hormone (SH) treatment displayed normalization of PWS manifestations in many individuals with this condition. These treatment options have been recommended as a standard of care for patients

with PWS but unfortunately it hasn't been implemented in healthcare management of many individuals with PWS (Grugni et al, 2016). The purpose of this study was to describe differences in PWS phenotypic features, specifically physical features, by gender, molecular type (deletion vs. maternal UPD), and the use of GH and SH treatment. This study has documented statistically significant differences in phenotypic features by molecular type, gender and by use of GH and/or SH treatments. This study also documented statistically significant differences/benefit in GH initiation at a young age. These findings confirm and extend previously suggested benefits of GH and SH treatments, and the benefit of initiating GH treatment as early as possible.

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