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A Genotype-Phenotype Analysis of the Effects of Growth Hormone Treatment on Psychiatric Behavior in Prader-Willi Syndrome

THESIS

Submitted in partial satisfaction of the requirements
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by

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In dedication

to my extraordinary mother.

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ABSTRACT OF THE THESIS

A Genotype-Phenotype Analysis of the Effects of Growth Hormone Treatment on Psychiatric Behavior in Prader-Willi Syndrome

By

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Master of Science in Genetic Counseling

University of California, Irvine, 2019

Professor Virginia E. Kimonis, MD, MRCP, Chair

Prader-Willi syndrome (PWS) is a rare multisystemic disorder characterized by distinct physical, cognitive, behavioral, and psychiatric phenotypes. The purpose of this study was to describe the effects of growth hormone treatment (GHT) in 172 individuals on the following psychiatric categories: depressive disorders (depressed mood and anxiety), compulsions (skin picking, nail picking, compulsive counting, compulsive ordering, and plays with strings), and psychoses (visual hallucinations and delusions).

Hypotheses include 1) GHT contributes to a lower risk for psychiatric phenotypes in individuals with PWS, 2) Earlier age at initiation of GHT results in lower risk for psychiatric phenotypes, 3) Longer duration of GHT results in lower risk for psychiatric phenotypes, and 4) Risk of psychiatric outcomes associated with GHT differs for those with 15q11.2-q13 deletions versus those with maternal uniparental disomy (mUPD) of chromosome 15.

After controlling for the effects of confounding variables (psychiatric medication and age), the data suggests that growth hormone use is associated with an increased risk for delusions. Growth hormone use was also shown to have a greater effect on increased risk for

anxiety for those with mUPD than for those with deletions. Duration of GH treatment did not show a significant association with psychiatric phenotype. Age at growth hormone initiation could not be considered as a good measure because it was strongly correlated with age at visit 1.

As growth hormone treatment is currently the standard of care for individuals with Prader-Willi syndrome, having a better understanding of the psychiatric risks is important for management. Prospective studies in which all individuals start growth hormone at the same age are suggested to test these findings.

1 INTRODUCTION

1.1 Background

Prader-Willi syndrome (PWS) is a multisystemic neurogenetic disorder that affects 1/10,000-1/15,000 live births. It is found across all races and affects both genders equally (Cassidy, 1997). The clinical manifestations change dramatically with age. Infants present with severe hypotonia, feeding problems, poor weight gain, and overall failure to thrive. As the individual moves into early childhood, they develop hyperphagia leading to morbid obesity and the associated complications of diabetes, obstructive sleep apnea, and right-sided heart failure if diet is not controlled. Individuals with PWS develop hypothalamic dysfunction which may lead to endocrinopathies, including growth hormone deficiency, hypogonadism, and hypothyroidism. (Lukoshe et al, 2013). Death typically occurs in the fourth decade of life; however, with adequate management of eating behaviors, individuals with Prader-Willi syndrome can remain healthy into their seventh decade (McCandless, 2011).

The characteristic facial features of PWS include deep-set almond-shaped eyes, a narrow forehead and nasal bridge, strabismus, and a thin upper lip with down turned corners of the mouth. Other dysmorphic features include hypogonadism, cryptorchidism, short stature, small hands and feet, an abnormal body composition with excessive body fat that accumulates around the torso and thighs, scoliosis, and hypopigmentation of the eyes, skin, and hair (Cassidy et al, 2012).

In addition to having distinct physical features, PWS has a unique cognitive and behavioral profile. Affected individuals have delayed motor and language skills, learning disabilities, and an average IQ of 65 (Butler et al, 2006). They typically have severe behavioral problems and are stubborn, defiant, and easily frustrated. They are often quick to anger and have

a striking inability to control their emotions. They also tend to have mood disorders, obsessive tendencies, autistic traits, and are at high risk for developing psychoses in late adolescence or early adulthood (Zhang et al, 2013; Dykens and Shah, 2003).

1.2 Genetic Etiology

Prader-Willi syndrome is a genetically heterogeneous chromosomal disorder. It is caused by the absence of expression of imprinted genes in the paternally derived PWS region on chromosome 15 (15q11.2-q13). Imprinting is an epigenetic mechanism in which genes are differentially expressed depending on the parent-of-origin; one parental allele is methylated (silenced) and the other is expressed. The imprint persists through cycles of post-conceptional somatic mitoses, but resets when passed down to subsequent generations depending on the sex of the individual through whom it is transmitted. Humans typically inherit two copies of chromosome 15, one from their mother and one from their father (**Figure 1**). However, many of the genes in the PWS region of chromosome 15 are imprinted resulting in the expression of only the paternal allele and silencing of the maternal allele by hypermethylation. If the genes on the paternal PWS region are not expressed, the individual will be affected with Prader-Willi syndrome. There are three main molecular mechanisms by which the loss of the paternally expressed PWS region occurs: paternal interstitial deletion, maternal uniparental disomy, and imprinting center defects (Butler et al, 2009; Bittel and Butler, 2005; Cheon, 2016) **(Figure 2)**.

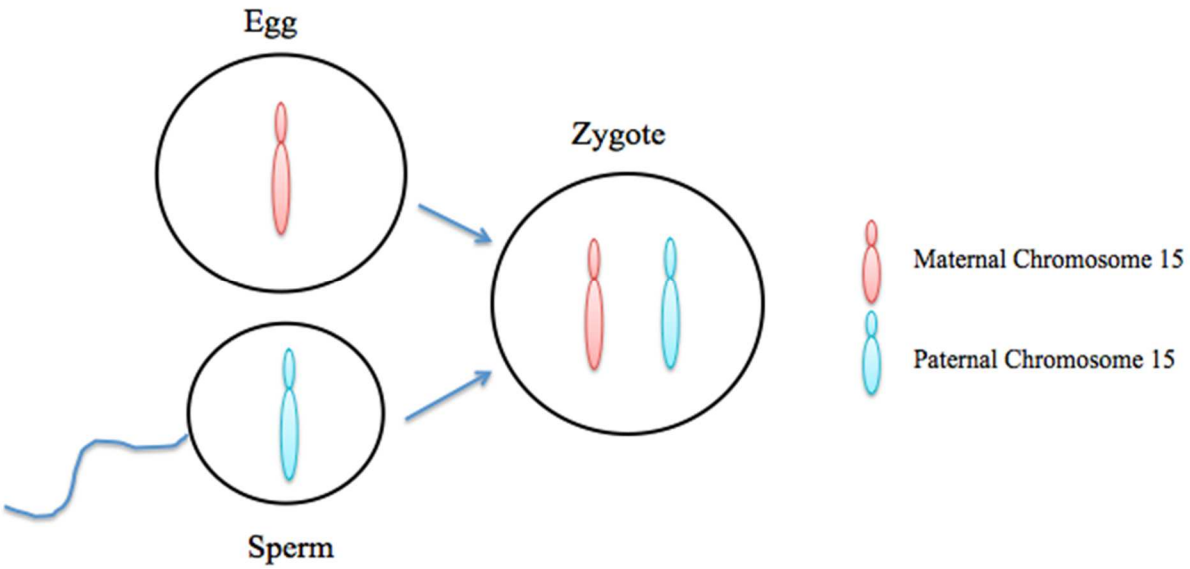

Figure 1. Normal Distribution of Chromosome 15 in Zygote Formation

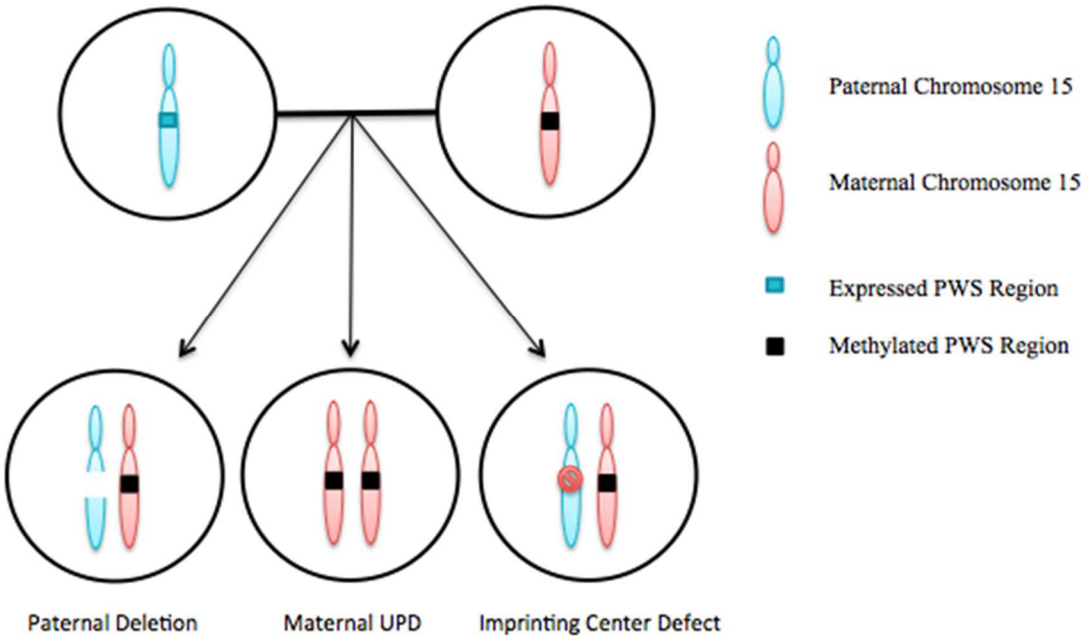

Figure 2. Inheritance of Prader-Willi syndrome
Molecular mechanisms that cause PWS include paternal interstitial deletion, maternal uniparental disomy, and imprinting center defects.

Paternal Deletion

The most common genetic etiology found in PWS is a paternal interstitial deletion of the 15q11.2-q13 region. This is the result of a *de novo* mutation and occurs in about 65%-75% of all PWS cases. Within the PWS region, there are three breakpoints that are involved in over 95% of deletion cases: proximal BP1, BP2, and a common distal BP3. These breakpoints result in two deletion subgroups: type 1 deletions involves the entire region from BP1 to BP3 and type 2 deletions involves the region from BP2 (located 500 kb distal to BP1) to BP3 (Sahoo et al, 2007). Flanking these breakpoints are large low-copy repeats that mediate misalignment of the PWS region during the cell replication and division process, predisposing it to structural changes (Amos-Landgraf et al, 1999).

Maternal Uniparental Disomy (mUPD)

The second most common genetic etiology of PWS is maternal uniparental disomy (mUPD) which is found in 20%-30% of all PWS cases and is most commonly associated with advanced maternal age due to the increased risk of nondisjunction at meiosis I. It occurs when both copies of chromosome 15 are maternally inherited. There are two forms of uniparental disomy: isodisomy and heterodisomy (Cassidy et al, 2012).

Uniparental Heterodisomy

Uniparental heterodisomy is the most common form of mUPD in Prader-Willi syndrome. It occurs as a result of a nondisjunction event where sister chromatids fail to separate into two daughter cells during meiosis I. This results in one daughter cell with both copies of chromosome 15 and the other daughter cell with no copies of chromosome 15 (Spence et al,

1988). If an abnormal maternal diploid gamete gets fertilized with a normal haploid paternal gamete, it will create an unviable trisomic conception. However, if the lone paternal copy is lost, it will result in a viable PWS zygote with two heterozygous copies inherited from the mother. This mechanism is known as "trisomy rescue" (Spence et al, 1988) (**Figure 3**).

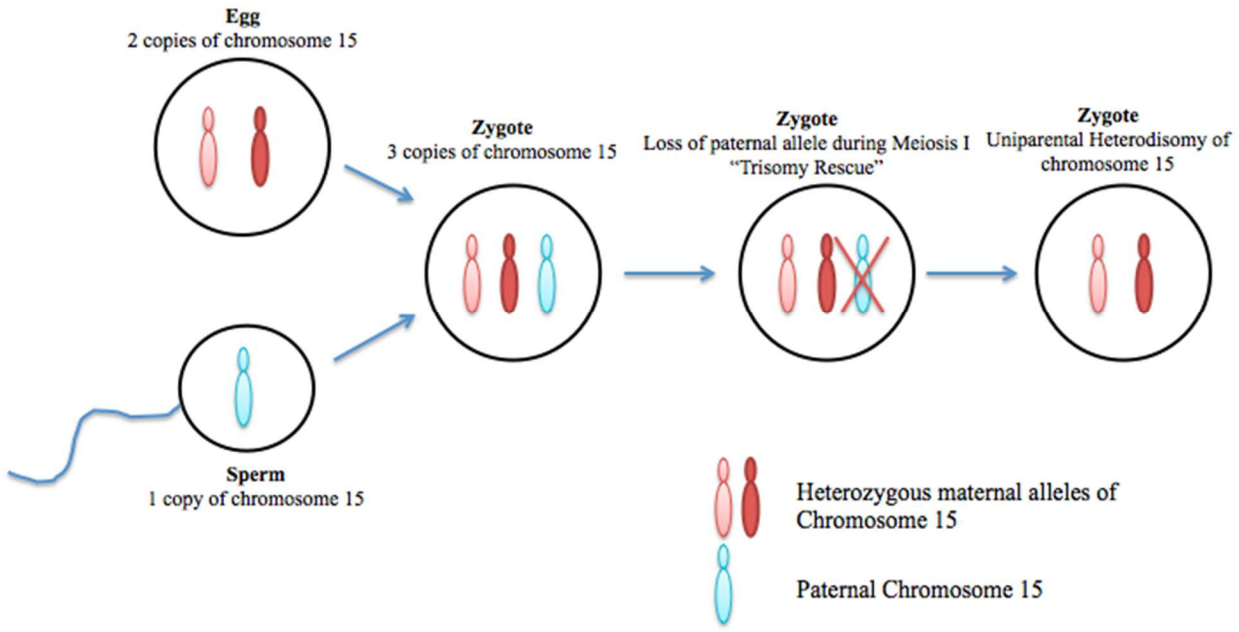

Figure 3. Uniparental Heterodisomy as a Result of Trisomy Rescue in Chromosome 15
During Meiosis I, the zygote has randomly eliminated the sole paternal copy, resulting in two maternally inherited copies and no paternally inherited copies.

Another possible mechanism of uniparental heterodisomy is gamete complementation. This occurs when a paternal gamete has no copies of a particular chromosome and it pairs with a maternal gamete containing two copies of the missing chromosome (Spence et al, 1988) (**Figure 4**).

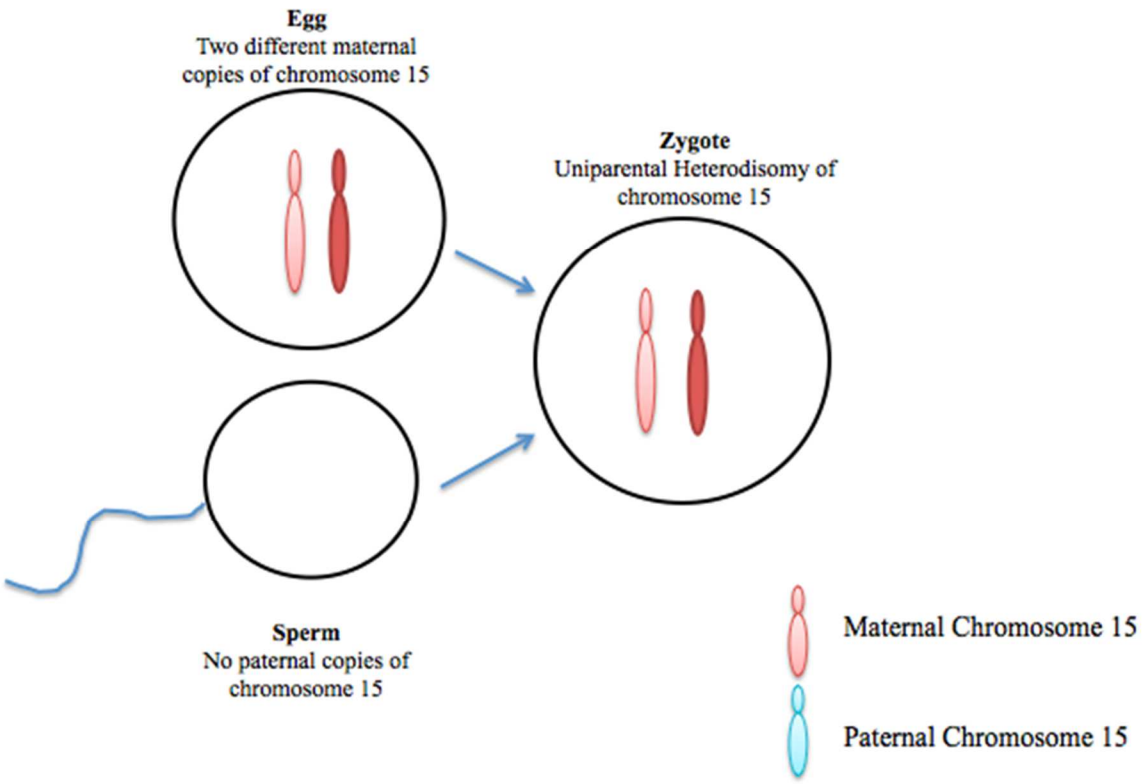

Figure 4. Uniparental Heterodisomy due to Gamete Complementation in Chromosome 15
During Meiosis I, the paternal gamete has no copies of chromosome 15 and pairs with the maternal gamete containing two copies of chromosome 15.

Uniparental Isodisomy

Uniparental isodisomy is a less common mechanism of mUPD in PWS. This occurs when an individual inherits two *identical* chromosome 15 homologs from their mother. This occurs during Meiosis II when the paternally inherited chromosome 15 is lost and the maternal chromosome 15 duplicates to replace the missing one. This is known as "monosomy rescue" and allows the embryo to survive; however, in the case of imprinted chromosomes where parent-of-origin matters, this can cause serious abnormalities (Shaffer et al, 2001) (**Figure 5**).

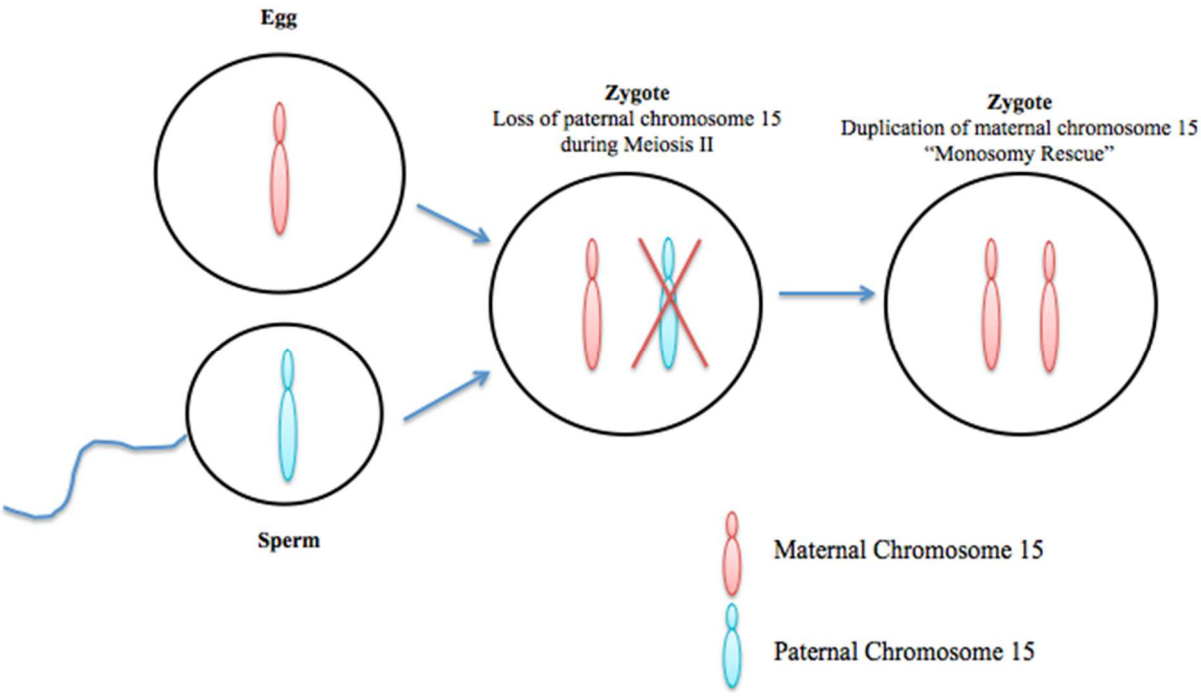

Figure 5. Uniparental Isodisomy via Post-Zygotic Error in Chromosome 15
During Meiosis II, the paternal chromosome is lost and the maternal chromosome is duplicated, resulting in two identical maternal chromosomes.

Another possible mechanism is monosomic conception with subsequent chromosome gain. This occurs when the paternal gamete has no copies of a specific chromosome and it pairs with a normal maternal haploid gamete, followed by a post-zygotic chromosome duplication of the maternal chromosome 15 to replace the missing chromosome (Spence et al, 1988) (**Figure 6**).

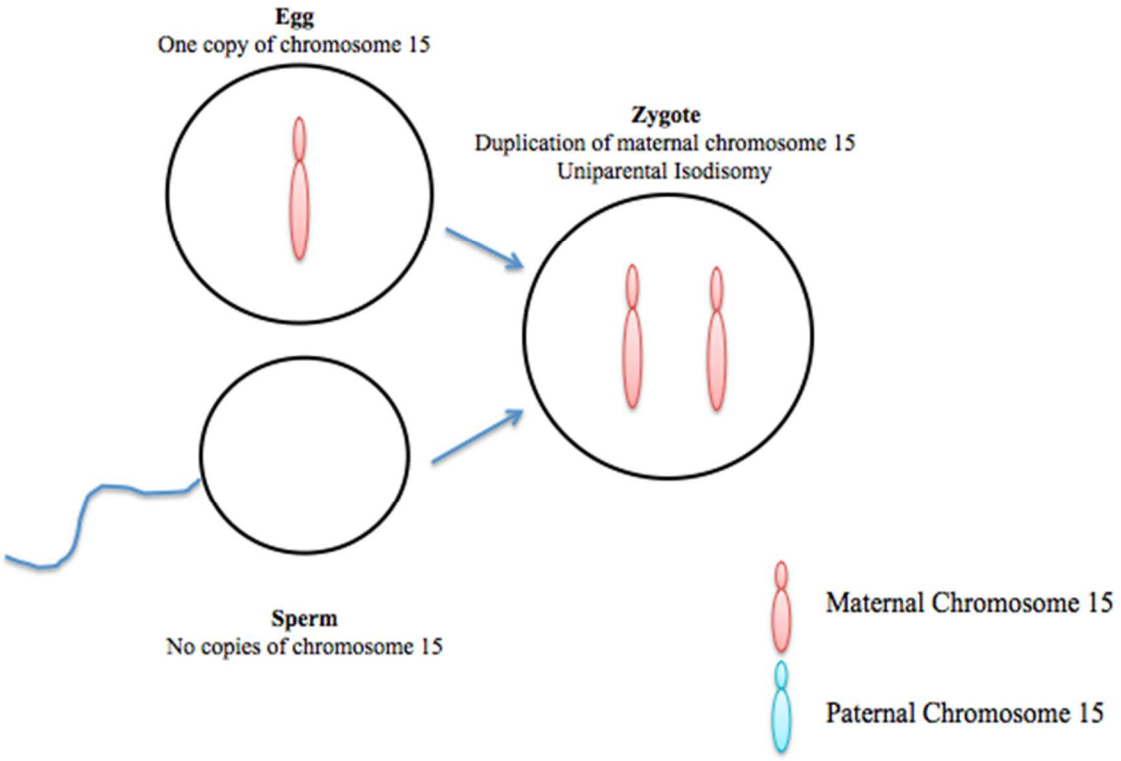

Figure 6. Uniparental Isodisomy as a Result of Monosomic Conception with Subsequent Chromosome Gain in Chromosome 15
During Meiosis II, the paternal gamete has no copies of chromosome 15 and it pairs with the normal maternal gamete with one copy of chromosome 15, followed by post-zygotic duplication of the maternal chromosome.

Imprinting Center Defect

Approximately 1%-3% of Prader-Willi syndrome cases are the result of an imprinting center defect. Imprinting is an epigenetic mechanism in which gene expression is dependent on the parent-of-origin (Angulo et al, 2015). During gametogenesis, the imprinting center (PWS/AS-IC) on chromosome 15 controls whether imprinted genes are methylated (silenced) or expressed. The maternal chromosome region of 15q11–q13 is typically methylated, while the paternal chromosomal region 15q11-q13 typically lacks methylation of the PWS-SRO

(Horsthemke et al, 2008). An individual must receive an expressed paternal imprinted region on chromosome 15 as two inactive maternal copies of this region will not compensate for the loss of the paternal region. During gametogenesis, the existing parental imprints are reset. Methylation is reversible so normally when imprinted genes are passed down, the existing imprints are erased and new imprints are made according to the parent-of-origin. If an imprinting center mutation interferes with the resetting process, an unaffected father's maternally inherited allele may be passed down to their offspring with the methylation still intact. In these cases, patients have apparently normal chromosomes of biparental origin; however, the PWS region is fully methylated on both copies of chromosome 15 resulting in the PWS phenotype (Ohta et al, 1999; Buiting et al, 1995).

1.3 Diagnosis of PWS

Early diagnosis and management of Prader-Willi syndrome can allow for proactive management of diet and exercise and early treatment, thus preventing associated co-morbidities (Mahmoud, 2018). Although clinical diagnostic criteria have been validated, several of the clinical features of PWS are present in obese individuals without the disorder so genetic testing is required to establish a definitive diagnosis. In general, PWS should be considered in any infant with significant hypotonia and poor feeding. In older children, the diagnosis should be considered when there is impaired satiety for food with central obesity and global developmental delay (Grechi et al, 2012).

First-line testing for Prader-Willi syndrome is DNA methylation analysis to determine abnormal parent-specific imprinting within the PWS region of chromosome 15. Southern blot

hybridization method is used to detect maternal and paternal methylation patterns using methylation-sensitive SNRPN or PW71B probes. Over 99% of cases can be detected and parental DNA samples are not required in order to determine the parental origin (Cassidy et al, 2012). Normal individuals have a methylated maternal PWS region and an unmethylated paternal PWS region, whereas individuals with PWS have only the maternally methylated allele. Although it is an effective first-line test, DNA methylation analysis lacks the ability to distinguish between molecular subgroups so once the diagnosis of PWS is established, further testing is indicated to determine prognosis. To identify deletions, the most common cause of PWS, fluorescence *in situ* hybridization (FISH) using SNRPN probes can be obtained, however increasingly chromosomal microarray (CMA) or SNP (single nucleotide polymorphism) array is a preferred test in conjunction with methylation studies. If SNP array does not detect deletions (70%), or maternal isodisomy (15%). The possibility of maternal uniparental heterodisomy is investigated by DNA polymorphism analysis on chromosome 15 using blood samples from the patient and his or her parents. If biparental inheritance of chromosome 15 is confirmed in the presence of abnormal methylation and normal FISH or SNP array results, the mechanism responsible for PWS is assumed to be an imprinting center defect (approximately 5%). In order to determine if the defect is due to an epigenetic mutation, one in which DNA is normal but the imprint is abnormal, or an inherited microdeletion in the imprinting center, sequence analysis at the PWS-SRO or MS-MLPA assay must be performed (Horsthemke et al, 2008). Karyotype and chromosomal microarray may also be used to detect rare instances of translocations or other chromosome rearrangements which may increase the risk of aneuploidy resulting in mUPD (Cassidy et al, 2012; Horsthemke et al, 2008; McCandless, 2011) **(Figure 7)**.

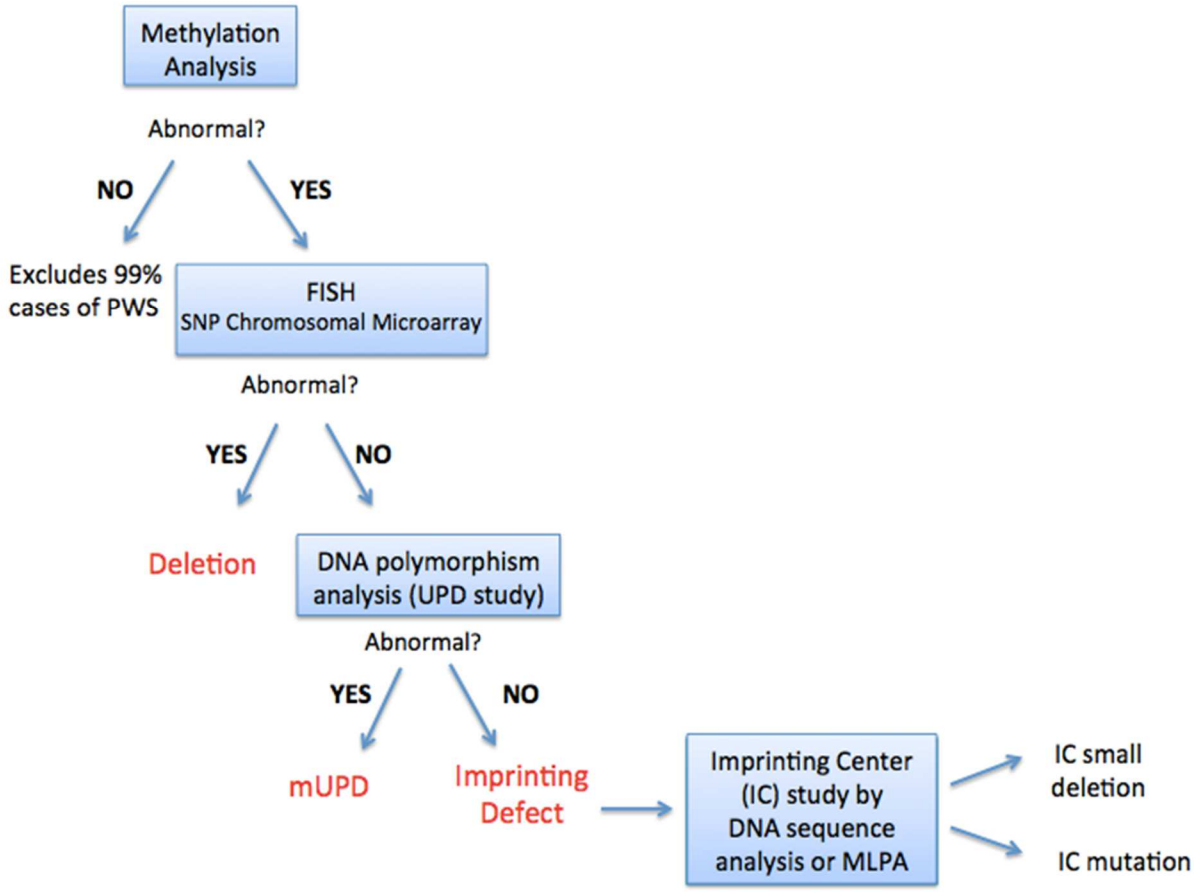

Figure 7. Flowchart of the recommended testing strategy for PWS.

1.4 Phenotype and Natural History

The Prader-Willi syndrome phenotype varies according to the stage of the disorder. Throughout their lifespan, individuals with PWS present with seven distinct nutritional phases, five main phases and two subphases (Miller et al, 2011).

The Fetus

Phase 0 occurs while *in utero* and it is characterized by a lack of fetal movement as well as growth restriction. Babies born full-term have a birth weight and BMI about 15-20% less than

their siblings (Miller et al, 2011). Rates of induced labor and cesarean section are significantly higher compared to the general population (Singh et al, 2018).

The Infant

Phase 1a begins at birth and lasts around 9 months. In infancy, the most consistent clinical feature is severe hypotonia, which causes decreased movements, weak or absent cry, and poor reflexes, including a weak uncoordinated suck that leads to feeding difficulties and failure to thrive. Cryptorchidism and a hypoplastic penis and scrotum are often present in males and hypogenitalism in females (Butler et al, 2006). Infants have severely decreased appetite, show little or no evidence of being hungry, and do not cry for food or get excited at feeding time. (Miller et al, 2011). Although infants with PWS experience poor weight gain, they have excess body fat. Lean body mass measurements are decreased, correlating with a 30% lower energy expenditure as compared to healthy individuals (Bekx et al, 2003). Early milestones are typically reached at double the normal age (Cassidy et al, 2012). At around 9 to 25 months, the infant transitions to **Phase 1b** where they develop a normal appetite and no longer need assisted feedings. They grow steadily along their growth curve with normal feeding; however, they continue to experience global developmental delay (Miller et al, 2011).

The Child

Phase 2a occurs between 2.1 and 4.5 years. Weight begins to increase without an increase in appetite or excessive calories. The child starts to cross growth curve centile lines and will become obese if given the standard recommended daily allowance for calories (Miller et al, 2011). Infants and toddlers with PWS tend to be sweet and affectionate, but with this change in

eating pattern, children start to show significant maladaptive behavior, oppositional tendencies, an overreaction to frustration, and perseveration in thought and speech, the first psychiatric manifestation seen in PWS (Cataletto et al, 2011). As the child moves into **Phase 2b** around 4.5 to 8 years old, their weight begins to increase with an increase in appetite. They frequently ask food-related questions and they become very concerned about the next meal or snack. They will eat more food than a typical child if allowed, but they can still feel full and will stop eating voluntarily. When they reach about 8 years old, they enter **Phase 3** and become hyperphagic and rarely feel full. They are constantly thinking about their next meal and will awaken from sleep thinking about food. Temper tantrums and "meltdowns" related to food occur frequently. They will steal food or money to pay for food and are often dishonest about what they have eaten. They often eat food from the garbage and other inedible sources (e.g., dog food, frozen food, crayons, etc.). If left unsupervised, they will gain a considerable amount of weight over a short period of time so food typically needs to be locked up. Affected individuals need to be placed on a diet that is approximately 50-70% of the Recommended Daily Allowance to maintain a healthy weight (Miller et al, 2011).

The Adolescent

Phase 3 hyperphagia continues into adolescence. Normal puberty is absent or delayed in both males and females and has generally been attributed to hypothalamic dysfunction. The low levels of gonadotropin released by the brain result in hypogonadism and hypogenitalism and become especially evident during adolescence. Behavior and learning problems may become more prominent and temper tantrums are usually food-related. Psychiatric symptoms might occur in adolescence, mostly psychosis and affective disorders (Butler et al, 2006).

The Adult

Many adults remain in Phase 3 for the rest of their lives. Typical behavior problems include temper-tantrums, obsessive-compulsive symptoms, perseveration in conversation, stubbornness, and acute psychosis (Butler et al, 2006;). Adults with PWS typically desire to live independently; however, their eating behaviors and cognitive deficits typically preclude normal adult independent living. For males not treated with growth hormone, the average adult height is 61 inches and the average shoe size is 5. For females not treated with growth hormone, the average adult height is 58 inches and the average shoe size is 3. Complications of severe obesity, such as diabetes mellitus type II or respiratory insufficiency frequently occur and may lead to an early death. If severe obesity can be avoided, patients with PWS may have a reasonable life expectancy (Butler et al, 2006). In adulthood, some individuals reach **Phase 4**, the final nutritional phase. In this phase, appetite is no longer insatiable and individuals can feel full. Appetite can fluctuate, but the key component is a noticeable improvement in control of appetite compared to when they were younger. Most adults, however, will never enter this phase (Miller et al, 2011).

1.5 Genotype-Phenotype Correlations

Although the phenotypic features of PWS can be found in individuals of all molecular subtypes, there are significant differences in the frequency and severity.

Compared to individuals with mUPD, those with paternal deletion are likely to have more severe behavioral problems, such as self-injury, food-stealing, and compulsive behavior such as skin picking. They are also more likely to have speech articulation deficits as well as high pain tolerance. Individuals with deletions tend to have a particular strength with visual-perceptive

skills and have an unusual skill with jigsaw puzzles (Butler et al, 2006). They are more likely to have hyperpigmented skin, eyes, and hair which may be due to the location of the non-imprinted OCA2 gene, responsible for melanin production. Individuals with the deletion produce less melanin because they have only one copy of the gene, whereas individuals with mUPD still have two functional copies (Robinson et al, 1991). Individuals with the larger Type 1 deletion tend to have more compulsions and poorer adaptive behavior, intellectual ability, and academic achievement than individuals with Type 2 deletions (Butler et al, 2017).

Relative to individuals with deletions, patients with maternal mUPD are less likely to have the typical PWS facial features and less likely to present with certain behaviors like skin-picking. They are less likely to have articulation problems, a high pain threshold, and skill with jigsaw puzzles. Individuals with mUPD are more likely to have significantly higher verbal IQ scores and to develop psychotic disorders (Roof et al, 2000). Psychiatric illness in individuals with mUPD may be more severe possibly due to the configuration of imprinted genes (Soni et al, 2007). Post-term deliveries are also more common in those with mUPD (Butler, 2009).

1.6 Psychiatric Disorders

Individuals with Prader-Willi syndrome carry a high risk of psychiatric comorbidities. While the general population risk for psychiatric disorders is less than 3.5%, the risk is 60% for individuals with PWS due to mUPD and 20% in individuals with with PWS due to deletions (Lukoshe et al, 2013; Perälä et al, 2007). These psychiatric disorders include affective disorders, compulsions, autistic disorders, and psychosis. Whittington et al (2018) suggests that, although there are common symptoms, the mental and behavioral deviations in genetic syndromes such as PWS differ in etiology as compared to the general population. Therefore, diagnostic labels must be applied with care as they may lead to inappropriate treatment.

Affective Disorders

Affective disorders are characterized by extremes or dramatic changes in mood and include depression, bipolar (fluctuating mania and depression), and anxiety disorder. Beardsmore et al (1998) found that 17.4% of adults with Prader-Willi syndrome met formal criteria for affective disorders.

Compulsions

Compulsions, repetitive behaviors that a person is driven to perform in response to obsessional thought, are also highly associated with PWS. These behaviors serve to reduce distress but are time-consuming and significantly interfere with normal functioning. In addition to compulsive food seeking behaviors, individuals with Prader-Willi syndrome have an increased risk of nonfood-related compulsive behaviors such as hoarding, ordering and arranging, concerns

with symmetry and exactness, rewriting, and need to tell, know, or ask (Dykens, 1996). Individuals with Type 1 deletions tend to have more compulsions, especially having to do with cleaning and hygiene, while individuals with Type 2 deletions have more academic compulsions such as rereading and rewriting (Sinnema et al, 2011). Individuals with deletions have higher rates of compulsive self injurious behaviors like skin-picking and nail picking when compared to those with mUPD (Krefft et al, 2014).

Autism Spectrum Disorders

Autism spectrum disorders are also highly prevalent in individuals with PWS due to mUPD. It affects 15% of the general population, but 37.7% of individuals with mUPD and 18.5% with deletions are affected (Bennett et al, 2015). Autism spectrum disorders include social communication problems, perseverative thinking, and stereotypic behavior (Mukherjee, 2017).

Psychotic Disorders

The risk of developing psychosis is also highly increased in individuals with PWS. It is characterized by symptoms such as confusion, auditory and visual hallucinations, delusions, and paranoid behavior and onset is typically in late adolescence or early adulthood. Boer et al (2002) found that 62% of individuals with PWS due to mUPD had symptoms indicative of psychotic illness, which increased to 100% over the age of 28 years. Only 8% of individuals with PWS due to deletions had psychosis and that increased to 11% over the age of 28.

Possible Etiologies

The underlying cause of these psychiatric disorders is unknown, but similarities between the brain abnormalities seen in individuals with Prader-Willi syndrome mUPD type with those seen in non-PWS individuals with psychiatric disorders have been reported, suggesting common genetic pathways (Lukoshe et al, 2013).

Individuals with Prader-Willi syndrome due to both deletions and mUPD were shown to have a smaller brain stem and white matter volume compared with healthy controls, indicating early deviations in prenatal brain development. The brainstem is responsible for several basal bodily functions that have been reported to be impaired in PWS, such as pain perception, respiratory regulation, and sleep cycle. Children with deletions, however, have an overall smaller, but proportionately developed brain, while children with mUPD had a significantly increased surface cerebral spinal fluid (CSF) and pronounced enlargement of the lateral ventricles compared to healthy controls. Patients with schizophrenia, young children with 22q11.2 deletions who are at high risk of schizophrenia, and adolescents with bipolar disorder are widely reported to have enlarged ventricles. Given that children with mUPD have an elevated risk of psychotic illness, these findings suggest that ventricular enlargement may be part of a predisposition for psychotic disease (Lukoshe et al, 2013; Campbell et al, 2006; Edmiston et al, 2011).

Cortical thickness, which is determined primarily through neuronal migration and pruning of ineffective synapses, is shown to be normal in children with deletions, but thickened in children with mUPD (Lukoshe, 2013) Increased cortical thickness has also been reported in non-PWS children with autism (Hill et al, 2003). Knowing that ASD traits are very common in

children with mUPD, impaired pruning might, therefore, underlie ASD symptoms in children with mUPD.

Although both individuals with deletions and mUPD show impaired brain development, the atypical neurodevelopmental trajectory seen in children with mUPD is similar to brain findings in non-PWS children with schizophrenia or autism, possibly explaining the higher incidence of psychiatric disorders in that population.

1.7 Treatment for Prader-Willi Syndrome

There is no cure for Prader-Willi syndrome so treatment is based on the individual's symptoms. Infants with PWS tend to have feeding problems and poor suck so nipples/feeders may be used to ensure adequate nutrition. In childhood, individuals with PWS develop an insatiable appetite so food intake must be restricted to help prevent obesity. To address poor muscle tone, physical and occupational therapy may help improve muscle mass as well as GHT (see below). Sleep disturbances are common so individuals with PWS should be evaluated and treated based on their particular disorder. Many people with PWS have psychiatric and behavioral problems and may benefit from therapy and medication. Hypogonadism is a common finding in PWS and presents as genital hypoplasia, incomplete pubertal development, and infertility. It is a result of hypothalamic dysfunction and can be treated with sex hormone therapy. The characteristic feature of short stature can be treated with growth hormone therapy which has also been shown to improve other physical, behavioral, and cognitive features associated with Prader-Willi syndrome (Driscoll et al, 2017).

Growth Hormone Treatment

Growth hormone deficiency (GHD) is the most commonly reported endocrinopathy in Prader-Willi patients. Burman et al (2001) reported that at least 15 studies have documented GHD in 40-100% of children with PWS. It is accompanied by low insulin like growth factor 1 (IGF-1) levels which distinguish it from GHD observed due to nutritional obesity (Carrel and Allen, 2018). Recombinant Growth Hormone Therapy for Prader-Willi syndrome was approved in the United States in 2000 and has since been widely recognized as a beneficial treatment for the multiple morbidities associated with PWS (Grugni et al, 2016). However, despite the significant health benefits of growth hormone, there remains concern for potential adverse effects such as insulin resistance, lower extremity edema, and lymphoid hyperplasia resulting in worsened sleep-disordered breathing (Vogt and Emerick, 2015). There have been reports of sudden death in patients on growth hormone treatment, although they have not been proven to be the result of the GHT (Bakker et al, 2007).

GHT has been found to improve linear growth, body composition and lean muscle mass, metabolism, motor development, energy expenditure, bone mineral density (BMD), and cardiovascular health across all ages of PWS patients. Significant improvements in physical strength, agility, and exercise capacity have been reported which helps to reduce the risk of obesity (Butler et al, 2006).

In addition to the physical improvements, growth hormone therapy has also has been shown to improve behavior and cognitive ability in individuals with Prader-Willi syndrome (Siemensma et al, 2011; Hoybye et al 2005; Myers et al, 2007). Randomized studies looking at GHT in infants and toddlers with PWS found significant improvements in motor development as

well as other markers of development, such as language and cognitive ability (Festen et al, 2008). Characteristic feature in PWS of impaired growth hormone and insulin-like growth factor (IGF-I) and it is thought to be implicated in the cognitive, behavioral, and psychiatric phenotype of the condition as they are important neurochemicals involving brain and axonal growth and myelination. Growth hormone therapy is thought to strengthen neuronal signaling, long-term potentiation, and plasticity in hippocampal and other brain regions, thus improving brain growth and resulting in improved cognition (Lukoshe et al, 2013).

Sex Hormone Treatment

Hypogonadism is a common feature of individuals with Prader Willi syndrome. It occurs in both sexes and manifests as genital hypoplasia, incomplete pubertal development, and infertility. In one study of 84 individuals with PWS ages 2-35 years, 100% of males had cryptorchidism, 76% of males had small testes, and 69% had scrotal hypoplasia. Of the females, 76% had labia minora and/or clitoral hypoplasia, and 56% had primary amenorrhea (Crino et al, 2003). Sex hormone replacement therapy is effective in producing adequate secondary sexual characteristics, however, it is controversial because of the possible role of testosterone replacement in behavior problems in males and the role of estrogen replacement in the risk of stroke and hygiene concerns related to menstruation in females (Goldstone et al, 2008).

1.8 Genetic Counseling

The recurrence risk in future pregnancies depends on the genetic etiology of the affected individual. Most families have a recurrence risk of less than 1%; however, certain etiologies confer a risk of up to 100% (Butler et al, 2006).

Most 15q11.2-q13 deletions are due to interstitial *de novo* paternal deletions with a recurrence risk of less than 1%. Maternal uniparental disomy (mUPD) is also typically *de novo* with a recurrence risk of less than 1%. Advanced maternal age is also a risk factor for maternal uniparental disomy of chromosome 15 because of increased meiosis 1 errors (Cassidy et al, 2012). Rarely, the deletion is the result of a chromosomal rearrangement which could have occurred *de novo* in the father's gamete, or the father may be the carrier of a balanced rearrangement (translocation or inversion). It is also theoretically possible that the mother carries a 15/15 Robertsonian translocation which would result in a 100% risk. If the individual with PWS has a normal karyotype, then it can be assumed that the mother does not have a Robertsonian translocation, however, the paternal karyotype should still be ordered.

Approximately 85% of imprinting center defects have a *de novo* epigenetic mutation with a recurrence risk of less than 1%. Imprinting center defects due to a microdeletion make up the remaining 15% and approximately half of those are inherited with a 50% recurrence risk. Therefore, fathers of children with imprinting center defects should have DNA methylation and dosing analysis to determine whether they carry the microdeletion. If the microdeletion was not found to be a familial deletion, the recurrence risk would vary between <1%-50% depending on the presence or amount of paternal germline mosaicism (Camprubi et al., 2007).

1.9 Purpose of Study

The purpose of this study was to explore any association between growth hormone use and psychiatric behaviors of individuals with Prader-Willi syndrome. The prevalence of nine psychiatric behaviors (depressed mood, anxiety, skin picking, nail picking, compulsive counting, compulsive ordering, plays with strings, visual hallucinations, and delusions) were compared between growth hormone users and non-growth hormone users and dosage effect was assessed by analyzing ages of GH initiation and duration of GH treatment. The effects of GH use depending on genotype was also investigated.

The majority of studies looking at the effects of growth hormone have focused on the physical and metabolic improvement and some have documented beneficial effects of GHT on cognition and behavior (Bridges, 2014). There have been few such studies, however, specifically focused on psychiatric disorders. The existing studies have failed to report significant differences due to limitations such as small sample size, short-term treatment, low levels of baseline symptoms, large age spans making it difficult to compare differences across age groups, and young study participants. This study would be analyzing a large cohort with a wider range of treatment duration, it will account for age at initiation, and it will be excluding patients under age 14 as the onset of psychiatric disorders is typically after puberty (Siennema et al, 2011). As behavioral and psychiatric problems interfere with the quality of life in adolescents and adults with Prader-Willi syndrome, a better understanding of the impact of growth hormone could help guide management and improve prognosis.

1.10 Hypotheses

1. Growth hormone treatment contributes to a lower risk for psychiatric phenotypes in individuals with Prader-Willi syndrome.
2. Earlier age at initiation of GH treatment results in lower risk for psychiatric phenotypes.
3. Longer duration of GH treatment results in lower risk for psychiatric phenotypes.
4. The risk of psychiatric outcomes associated with GH use differs for those with deletions versus those with mUPD.

2 MATERIALS AND METHODS

2.1 Background

An observational study was conducted through the Rare Disease Clinical Research Network's (RDCRN) Natural History PWS and Morbid Obesity Clinical Protocol (IRB protocol 2007-5605) from 2006 to 2014. Data was collected on 355 patients with Prader-Willi syndrome through a comprehensive assessment of medical, behavioral and nutritional history, and clinical features. The study was conducted at five centers initially University of California, Irvine under Dr. Virginia Kimonis and Dr. June-Anne Gold, University of Florida Health Science Center in Gainesville, Florida under Dr. Driscoll and Dr. Miller, Children's Mercy Hospital in Kansas City, Kansas under Dr. Merlin Butler, Baylor College of Medicine in Houston, Texas under Dr. Arthur Beaudet, and Vanderbilt University Medical Center in Nashville, Tennessee under Dr.

Elisabeth Dykens and Dr. Marshall Summar. All of the data collected was managed by the Data Management Coordinating Center (DMCC) in Tampa, Florida.

2.2 Participant Eligibility and Recruitment

Participants were recruited from all age groups (infants to adults) for both PWS and EMO, with some bias towards recruiting the younger ages in order to capture nutritional data while in each phase. All races and genders were eligible for participation. Inclusion criteria for the PWS group included molecular confirmation of PWS and being between the ages of 0 and 60 years old.

All PWS participants were required to have appropriate molecular and cytogenetic testing to confirm a diagnosis of PWS (e.g. karyotype, FISH 15, DNA methylation, DNA polymorphism studies when necessary) and were categorized into the appropriate molecular group (e.g. deletion, uniparental disomy, and imprint defect). PWS participants were recruited from the Genetics, Neurology, and Endocrine clinics, the national parent support organization (Prader-Willi Syndrome Association of the USA, PWSA-USA), and the RDCRN website.

2.3 Informed Consent and Specific Procedure

Informed consent was obtained in two phases. In Part I, consent was obtained from both parents of the patient via telephone in order to access patient medical records to determine eligibility. In Part II, procedural consent was obtained at the first study visit. For subjects 7+ years old, decision-making capacity was assessed by the attending physician and those who were

lacking were excluded from the study. The English version of the consent form was translated into the appropriate languages for non-English speaking subjects.

At each visit, the following RDCRN forms were used to record participant data and intervention of the following activities: 1) signed consent; 2) initial history form; 3) diet history; 4) physical exam; 5) impression examination by the physician; 6) current history form; 7) photographs; 8) blood for DNA and submission to the Coriell repository ("Coriell Institute," 2013) to enable further genetic studies; 9) Dual-Energy X-Ray Absorptiometry (DEXA) scan for body fat measurement; 10) Kaufman Brief Intelligence Test, 2nd edition (KBIT2) ("Pearson Education," 2013); 11) Behavioral Assessment for Children, 2nd edition (BASC-2) ("Pearson Education," 2013) for the parent and the participant (assisted by physician and study coordinator if participant to read). If applicable, the family was asked to take the Teacher BASC form to the teacher. Participants were followed for 5 or more years while funds were available with an annual visit for patients 3 years of age and under and biannual visits for all others. Participants were compensated $50 for each visit for travel costs.

Prior to the baseline visit, each participant was assigned a study identification number. All the data obtained from the study sites was coded, de-identified, and shared with the Data Management and Coordinating Center (DMCC) and RDCRN.

2.4 RDCRN Database

The RDCRN database contains de-identified data from 355 subjects with Prader-Willi Syndrome (PWS) and 36 subjects with early-onset morbid obesity (EMO) who were monitored by four different clinical sites: University of California, Irvine, CA; Kansas University Medical

Center, Kansa City, KS; Vanderbilt University, Nashville, TN; and University of Florida, Gainesville, FL. All data from this registry was managed by the Data Management and Coordinating Center (DMCC) in Tampa, Florida.

For this study, EMO participants and participants under the age of 14 by the end of the study were excluded from the dataset leaving 176 participants with a confirmed diagnosis of PWS. The information compiled included participant ID, growth hormone treatment status, sex hormone treatment status, age at growth hormone initiation and termination, psychiatric medications, gender, race, PWS subtype, institution, number of visits, age at each visit, and the presence of the following psychiatric phenotypes at each visit: depressed mood, anxiety, skin picking, nail picking, compulsive counting, compulsive ordering, plays with strings, visual hallucinations, auditory hallucinations, and delusions.

2.5 Data Analysis

Study participants are described with respect to demographics, genotype, use of medications and prevalence of psychiatric phenotypes using descriptive statistics. For categorical variables (growth hormone use, psychiatric medication use, sex hormone use, and genotype), data are described by frequencies and percents, and for continuous variables (age at first visit, age at GH initiation, and GH duration) data are described using mean and standard deviation. Associations between use of GH and psychiatric phenotype were explored using Pearson Chi-Square tests. To further explore associations between GH use, other exposures, and the risk of psychiatric outcome, univariate and multivariate logistic regression analyses were employed. Multivariate logistic regression was used to control for other independent risk factors

and possible confounding exposures when a univariate analysis suggested some association between GH and psychiatric outcome with significance $p<0.15$. Age at growth hormone initiation was strongly correlated with age at visit 1 ($r=0.837$) making it uninformative as an independent contributor to outcome risk, therefore, it was dropped from multivariate analyses (see table). Sex hormone use was not shown to be associated with any of the outcomes, so this variable was also dropped from multivariate analyses. To determine whether growth hormone had a different effect on outcome risk for different genotypes, an interaction between GH and genotype (deletions vs. mUPD) was added to the multivariate model. The following interaction variables were created: GH*Del (DEL=1 and mUPD=0) for phenotypes which showed a positive association with the deletion subtype and GH*UPD (UPD=1 and DEL=0) for phenotypes which showed a positive association with the mUPD subtype. Statistical analyses were performed using SPSS Statistics Software.

3 RESULTS

3.1 Demographics

A total of 172 participants were included in this study. The study sample is described in terms of sex, race, recruiting institution, number of visits, genotype, growth hormone use, sex hormone use, psychiatric medication use, age at first visit, age at growth hormone initiation, and growth hormone duration. Out of the 172 participants, 94 (54.7%) were female and 78 (45.3%) were male. 120 (69.8%) had received growth hormone treatment and 52 (30.2%) had not. The genetic etiology of the participants was as follows: 107 (62.2%) deletions, 57 (33.1%) maternal mUPD, and 8 (4.7%) imprinting center defects. Racial distribution was 147 (85.5%) White, 9 (5.2%) Latino, 3 (1.7%) Asian, 7 (4.1%) Black, 5 (2.9%) Native American, and 1 (0.6%) was unknown. Age at the first visit was subdivided into 4 groups approximately representing quartiles: 55 (32.0%) were 8-13 years old, 35 (20.3%) were 14-18 years old, 43 (25.0%) were 19-26 years old, and 39 (22.7%) were 27-62 years old. The mean age for all study participants at visit 1 was 21.8 (SD=11.4) (see Table 1).

3.2 Medication Use

There were no significant differences between GH users and non-users of GH with respect to gender ($p=0.127$), genotype ($p=0.404$), use of psychiatric medications ($p=0.217$), sex hormone treatment ($p=0.328$), testosterone use ($p=0.780$), estrogen use ($p=0.357$), or race ($p=0.272$) (see Table 2). However, those who used GH were significantly younger at visit 1 than those who did not use GH ($p<0.001$; 90.9% were $\leq$13, 85.7% were 14-18, 65.1% were 19-26,

and 30.8% were ≥27) (see Table 2 and Figure 8). The mean age at first visit for GH users was 18.1 (SD=8.2). For non-users of GH, the mean age at first visit was 30.3 (SD=13.1) (see Table 3). Those who used psychiatric medication were significantly older at visit 1 than those who did not use psychiatric medication ($p<0.001$; 16.4% were ≤13, 34.3% were 14-18, 39.5% were 19-26, and 59% were ≥27) (see Table 4 and Figure 9). The mean age at first visit for users of psychiatric medication was 25.9 (SD=12.2). For non-users of psychiatric medication, the mean age at first visit was 19.4 (SD=10.3) (see Table 5).

3.3 Age at GH Initiation

Age at growth hormone initiation was subdivided into four groups approximately representing quartiles: 29 (25.2%) were 0-2 years old, 27 (23.5%) were 3-6 years old, 31 (27.0%) were 7-11 years old, and 28 (24.3%) were 12-49 years old. Age at GH initiation was unknown for four of the participants. The mean age of growth hormone initiation was 8.6 (SD=8.4) (see Table 1).

Age at first visit was also strongly correlated with age at GH initiation ($r=0.837$, $p<0.0001$). As age at first visit increased so did age of GH initiation. Among participants ages 8-13 at visit 1, 57.1% started GH by age 2 and 77.5% started GH by age 6. For those aged 19 or older, none started GH by age 2 and only 3.7% started GH by age 6 (see Table 9 and Figure 11). Therefore, age at growth hormone initiation was found to be a poor measure of dosage and it was dropped from the analysis.

3.4 GH Duration

GH duration was subdivided into four groups: 32 (28.3%) were on GH for 0-3 years, 31 (27.4%) were on GH for 4-9 years, 35 (31.0%) were on GH for 10-12 years, and 15 (13.3%) were on GH for 13-19 years. GH duration was unknown for seven of the participants. The mean duration of growth hormone treatment was 7.7 (SD=4.6) years (see Table 1).

3.5 Psychiatric Outcomes

Psychiatric symptoms were by guardian report. 50 (30.7%, n=163) reported depressed mood, 104 (63.0%, n=165) reported anxiety, 127 (74.7%, n=170) reported skin picking, 61 (45.9%, n=133) reported nail picking, 28 (16.9%, n=166) reported compulsive counting, 68 (41.0%, n=166) reported compulsive ordering, 34 (20.0%, n=168) played with strings, 6 (3.7%, n=164) reported visual hallucinations, and 11 (6.4%, n=164) reported delusions. Not every participant responded to each question about psychiatric behavior so the total number of responses varies by phenotype. Out of the 52 participants who did not use growth hormone, the number of responses for each psychiatric behavior varied from 40 to 52. Out of the 120 participants who used growth hormone, the number of responses ranged from 133 to 170. The number of individuals with each phenotype is recorded over the total number of individuals who responded (see Table 6).

Users of GH were significantly more likely to have anxiety compared to those who never used GH (68.4% vs 51%, p=0.032). Users of GH were less likely to report depressed mood than non-users (27.2% vs 38.3%, p=0.141) and more likely to have delusions (8% vs 2%, p=0.103),

however, neither result reached statistical significance. There were no significant differences between GH users and non-users of GH with respect to prevalence of skin picking (p=0.112), nail picking (p=0.610), compulsive counting (p=0.798), compulsive ordering (p=0.473), plays with strings (p=0.893), and visual hallucinations (p=0.437) (see Table 6 and Figure 10).

Older participants were significantly more likely to skin pick (60% of 8-13 year olds vs 79% of 14-18 year olds vs 83% of 19-26 year olds vs 82% of 27-62 year olds, p=0.024). There were no significant differences between age at first visit with respect to prevalence of depressed mood (p=0.068), anxiety (p=0.097), nail picking (p=0.841), compulsive counting (p=0.764), compulsive ordering (p=0.442), plays with strings (p=0.444), visual hallucinations (p=0.262), and delusions (p=0.276) (see Table 10).

Age at GH initiation showed an inconsistent association with psychiatric phenotype. Anxiety decreased with increasing age; skin picking showed little change in frequency across age groups; depressed mood increased with increasing age; and the other psychiatric phenotypes did not show any significant associations (see Table 11).

3.6 Genotype-Phenotype Associations

Participants with mUPD were significantly more likely to have anxiety compared to those who had deletions (74.1% vs 57.3%, p=0.038) and participants with deletions were significantly more likely to skin pick compared to those who mUPD (81.9% vs 63.2%, p=0.008). There were no significant differences between genotypes (deletion vs. mUPD) with respect to prevalence of depressed mood (p=0.979), nail picking (p=0.299), compulsive counting (p=0.687), compulsive

ordering (p=0.741), plays with strings (p=0.663), visual hallucinations (p=0.212), and delusions (p=0.523) (see Table 7).

The association between GH use and psychiatric behavior was significant for increased anxiety for the deletions genotype (p=0.007). The association between GH use and anxiety for mUPD subtype alone was not significant (p=0.547). When restricting by genotype, associations between GH use and psychiatric behavior were non-significant for both deletions and mUPD for the following psychiatric symptoms: depressed mood (p=0.064; p=0.322), skin picking (p=0.066; p=0.244), nail picking (p=0.548; p=0.717), compulsive counting (p=0.488; p=0.101), compulsive ordering (p=0.986; p=0.634), plays with strings (p=0.348; p=0.622), visual hallucinations (p=0.383; p=0.925), and delusions (p=0.158; p=0.658) (see Table 8).

Table 1. Descriptive Data

		N	%
Total Cohort		172	100.0
Sex			
	Male	78	45.3
	Female	94	54.7
Race			
	White	147	85.5
	Latino	9	5.2
	Asian	3	1.7
	Black	7	4.1
	Native American	5	2.9
	Unknown	1	0.6
Participating Institution			
	Kansas University Medical Center	24	14.0
	Children's Mercy Hospital	3	1.7
	University of Florida Health Sciences Center	26	15.1
	University of California at Irvine	17	9.9
	Vanderbilt University Medical Center	102	59.3

Number of Visits			
	1	172	100.0
	2	86	50.0
	3	25	14.5
	4	2	1.2
Genotype			
	Deletion	107	62.2
	UPD	57	33.1
	Imprinting Center Defect	8	4.7
Growth Hormone Treatment			
	Yes	120	69.8
	No	52	30.2
	Total	172	100.0
Sex Hormone Treatment			
	Yes	70	40.9
	No	101	59.1
	Total	171	100.0
Psychiatric Medication			
	Yes	61	35.5
	No	111	64.5
	Total	172	100.0

Age at Visit 1 (years)			
Mean: 21.8 Standard Deviation: 11.4	8-13	55	32.0
	14-18	35	20.3
	19-26	43	25.0
	27-62	39	22.7
	Total	172	100.0
Age of GH Initiation (years)			
Mean: 8.6 Standard Deviation: 8.4	0-2	29	16.9
	3-6	27	15.7
	7-11	31	18.0
	12-49	28	16.3
	Total	115	100
GH Duration (years)			
Mean: 7.7 Standard Deviation: 4.6	0-3	32	25.2
	4-9	31	23.5
	10-12	35	27.0
	13-19	15	24.3
	Total	113	100.0

Table 2. Descriptive data by Growth Hormone Use

		Growth Hormone Use						
		No		Yes		Total		Pearson Chi Square P-value
		N	%	N	%	N	%	
Sex	Male	19	24.4	59	75.6	78	45.3	0.127
	Female	33	35.1	61	64.9	94	54.7	
Genotype	Deletion	29	27.1	78	72.9	107	65.2	0.404
	UPD	19	33.3	38	66.7	57	34.8	
Psychiatric Medication	No	30	27.0	81	73.0	111	64.5	0.217
	Yes	22	36.1	39	63.9	61	35.5	
Sex Hormone Treatment	No	33	32.7	68	67.3	101	59.1	0.328
	Yes	18	25.7	52	74.3	70	40.9	
Testosterone	No	11	25.6	32	74.4	43	55.8	0.780
	Yes	8	22.9	27	77.1	34	44.2	
Estrogen	No	22	37.9	36	62.1	58	63.0	0.357
	Yes	10	28.6	25	71.4	34	37.0	
Age Group at Visit 1	8-13	5	9.1	50	90.9	55	32.0	<0.001
	14-18	5	14.3	30	85.7	35	20.3	
	19-26	15	34.9	28	65.1	43	25.0	
	27-62	27	69.2	12	30.8	39	22.7	

Recruiting Institution	Kansas University Medical Center	9	37.5	15	62.5	24	14.0	0.174*
	Children's Mercy Hospital	3	100	0	0	3	1.7	
	University of Florida Health Sciences Center	3	11.5	23	88.5	26	15.1	
	University of California at Irvine	5	29.4	12	70.6	17	9.9	
	Vanderbilt University Medical Center	32	31.4	70	68.6	102	59.3	
Race	White	43	29.3	104	70.7	147	85.5	0.272
	Latino	3	33.3	6	66.7	9	5.2	
	Asian	0	0	3	100	3	1.7	
	Black	4	57.1	3	42.9	7	4.1	
	Native American	1	20.0	4	80.0	5	2.9	
	Unknown	1	100	0	0	1	0.6	

*Chi-square test excludes Children's Mercy Hospital due to limited sample size.

Table 3. Growth Hormone Use by Age at Visit 1

	No GH				**GH**				**t-test**
	N	Mean	SD	Std. Error Mean	N	Mean	SD	Std. Error Mean	p-value
Age at Visit 1 (cont.)	52	30.3	13.1	1.8	120	18.1	8.2	0.7	<0.001

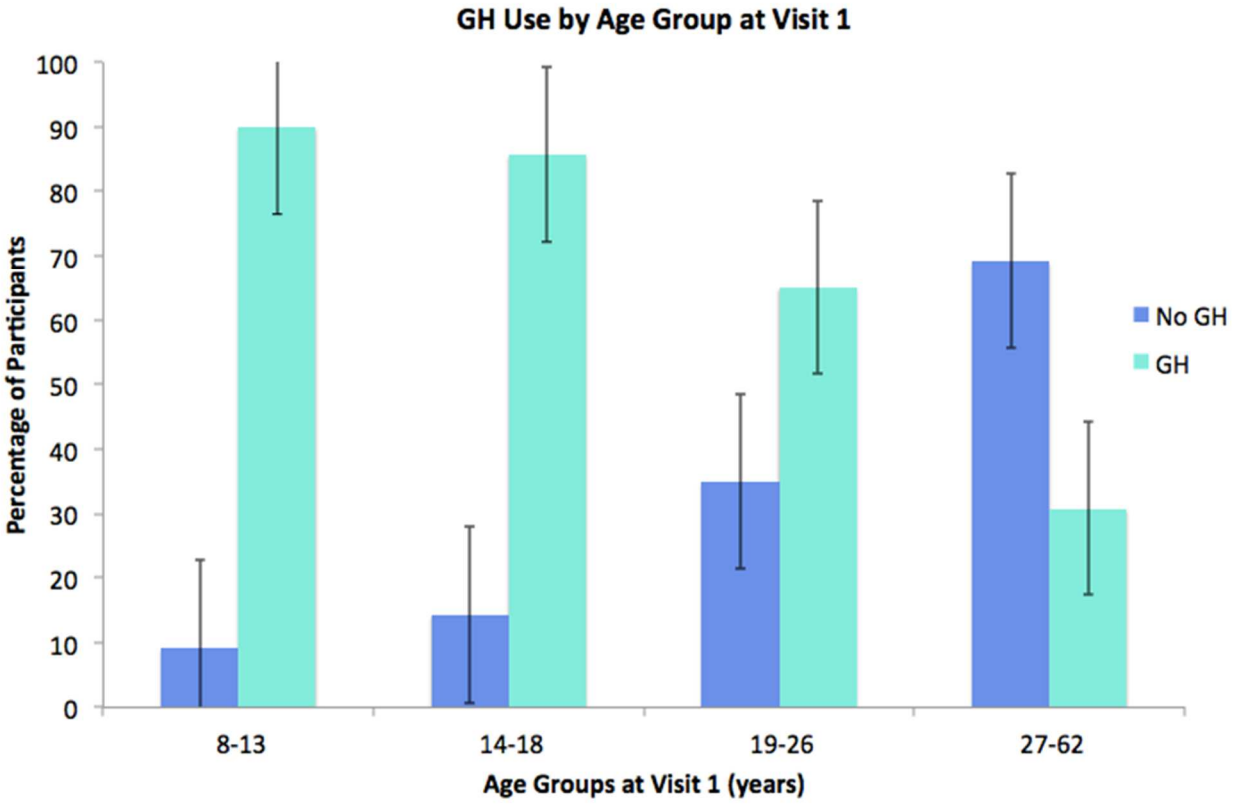

Figure 8. Growth Hormone Use by Age Group at Visit 1
Individuals who used GH were significantly younger at visit 1 than those who did not use GH ($p<0.001$; 90.9% were $\leq$13, 85.7% were 14-18, 65.1% were 19-26, and 30.8% were $\geq$27).

Table 4. Psychiatric Medication Use by Age at Visit 1 (categorical)

		Psychiatric Medication Use						
		No		Yes		Total		Pearson Chi Square P-value
		N	%	N	%	N	%	
Age Group at Visit 1	8-13	46	83.6	9	16.4	55	32.0	<0.001
	14-18	23	65.7	12	34.3	35	20.3	
	19-26	26	60.5	17	39.5	43	25.0	
	27-62	16	41.0	23	59.0	39	22.7	

Table 5. Psychiatric Medication Use by Age at Visit 1 (continuous)

	No Psychiatric Meds				Psychiatric Meds				t-test
	N	Mean	SD	Std. Error Mean	N	Mean	SD	Std. Error Mean	p-value
Age at Visit 1 (continuous)	111	19.4	10.3	0.97	61	25.9	12.2	1.56	<0.001

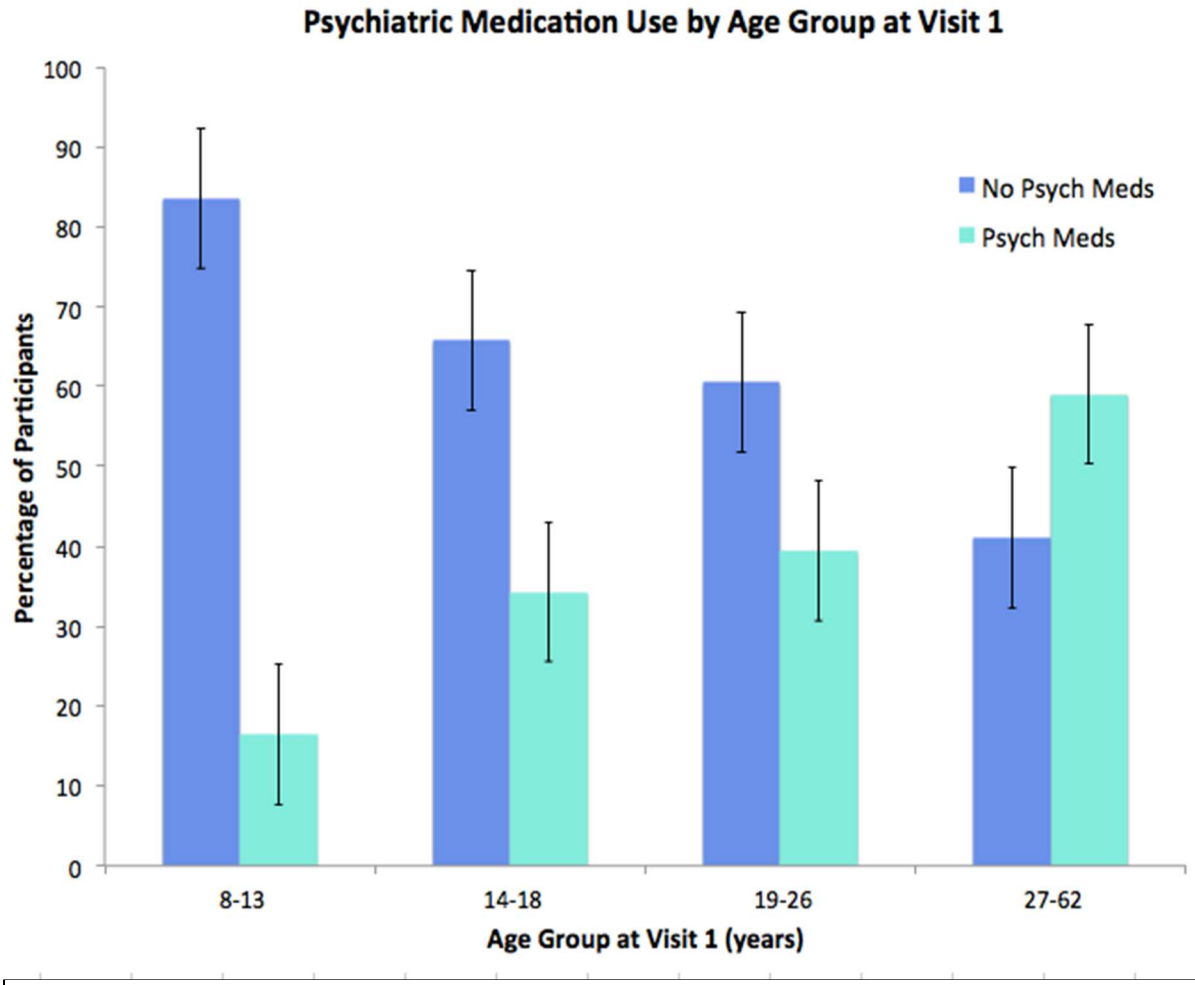

Figure 9. Psychiatric Medication Use by Age Group at Visit 1
Individuals who used psychiatric medication were significantly older at visit 1 than those who did not use psychiatric medication ($p<0.001$).

Table 6. Psychiatric Phenotype by Growth Hormone Use

	No GH		GH		Total		Chi-Square p-value
Phenotype	**N**	**%**	**N**	**%**	**N**	**%**	
Depressed Mood (N=50)	19/49	38.8	31/114	27.2	50/163	30.7	0.141
Anxiety (N=104)	26/51	51.0	78/114	68.4	104/165	63.0	0.032
Skin Picking (N=127)	43/52	82.7	84/118	71.2	127/170	74.7	0.112
Nail Picking (N=61)	17/40	42.5	44/93	47.3	61/133	45.9	0.610
Compulsive Counting (N=28)	9/50	18.0	19/116	16.4	28/166	16.9	0.798
Compulsive Ordering (N=68)	18/49	36.7	50/117	42.7	68/166	41.0	0.473
Plays with Strings (N=34)	10/51	19.6	24/117	20.5	34/168	20.2	0.893
Visual Hallucinations (N=6)	1/51	2.0	5/113	4.4	6/164	3.7	0.437
Delusions (N=11)	1/51	2.0	10/113	8.8	11/164	6.7	0.103

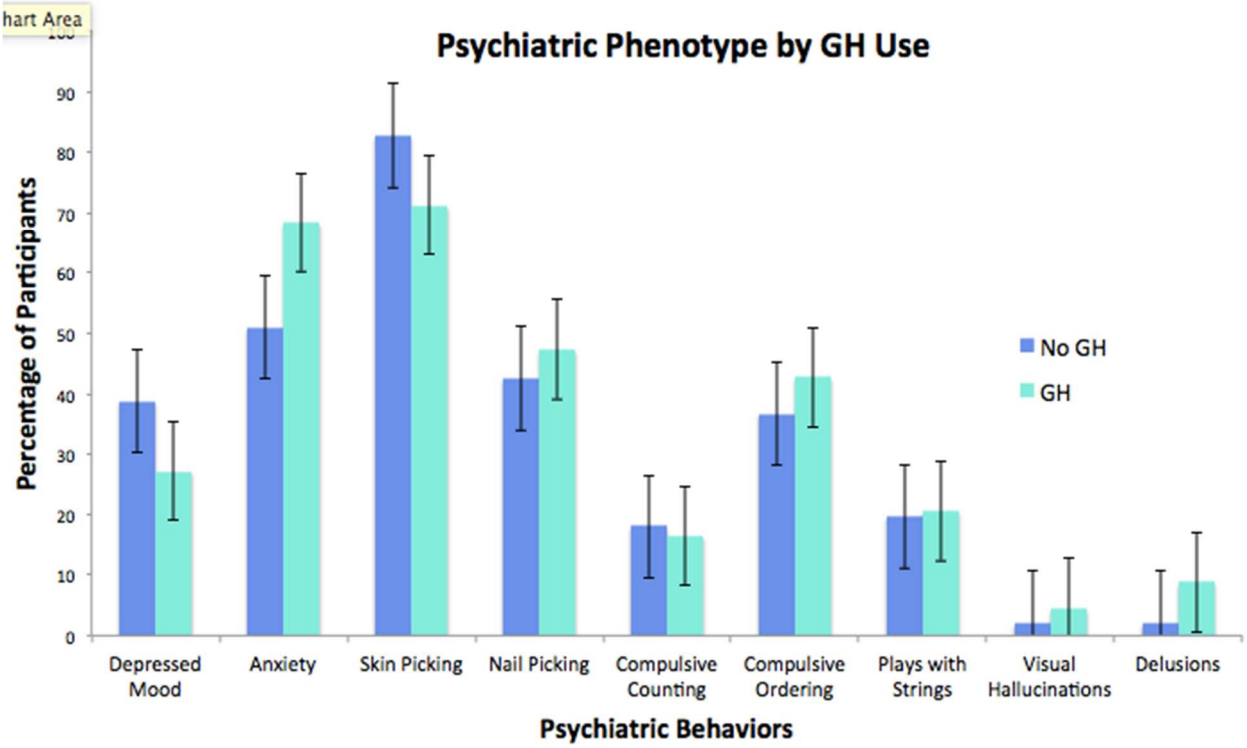

Figure 10. Psychiatric Behavior by GH Use
Number of GH users and non-users of GH who reported the presence of each psychiatric behavior at their first visit. Only *anxiety* showed a statistically significant difference between GH users and non-users of GH (p=0.032).

Table 7. Psychiatric Phenotype by Deletion and mUPD Subtypes

	Deletions		UPD		
	N	**%**	**N**	**%**	**p-value**
Depressed Mood (N=47/155, 30.3%)					
Yes (n=47)	31/102	30.4	16/53	30.2	0.979
No (n=108)	71/102	69.6	37/53	69.8	
Anxiety (N=99/157, 63.1%)					
Yes (n=99)	59/103	57.3	40/54	74.1	0.038
No (n=58)	44/103	42.7	14/54	25.9	
Skin Picking (N=122/162, 75.3%)					
Yes (n=122)	86/105	81.9	36/57	63.2	0.008
No (n=40)	19/105	18.1	21/57	36.8	
Nail Picking (N=58/127, 45.7%)					
Yes (n=58)	42/86	48.8	16/41	39.0	0.299
No (n=69)	44/86	51.2	25/41	61.0	
Compulsive Counting (N=28/158, 17.7%)					
Yes (n=28)	19/102	18.6	9/56	16.1	0.687
No (n=130)	83/102	81.4	47/56	83.9	
Compulsive Ordering (N=66/158, 41.8%)					
Yes (n=66)	44/103	42.7	22/55	40.0	0.741
No (n=92)	59/103	57.3	33/55	60.0	

Plays with Strings (N=31/160, 19.4%)					
Yes (n=31)	21/103	20.4	10/57	17.5	0.663
No (n=129)	82/103	79.6	47/57	82.5	
Visual Hallucinations (N=5/156, 3.2%)					
Yes (n=5)	2/103	1.9	3/53	5.7	0.212
No (n=151)	101/103	98.1	50/53	94.3	
Delusions (N=9/156, 5.8%)					
Yes (n=9)	5/102	4.9	4/54	7.4	0.523
No (n=147)	97/102	95.1	50/54	92.6	

Table 8. Psychiatric Phenotype by Growth Hormone Use for Deletion and mUPD Subtypes

	Deletions (n=107)						**UPD** (n=57)					
	No GH (N=29, 27%)		**GH** (N=78, 73%)		**Total**	**P-value**	**No GH** (N=19, 33.3%)		**GH** (N=38, 66.7%)		**Total**	**P-value**
	N	**%**	**N**	**%**	**N**		**N**	**%**	**N**	**%**	**N**	
Depressed Mood (N=47/155, 30.3%)												
Yes (n=47)	12	38.7	19	61.3	31	0.064	7	43.8	9	56.3	16	0.322
No (n=108)	15	21.1	56	78.9	71		11	29.7	26	70.3	37	

Anxiety (N=99/157, 63.1%)												
Yes (n=99)	10	16.9	49	83.1	59	0.007	15	37.5	25	62.5	40	0.547
No (n=58)	18	40.9	26	59.1	44		4	28.6	10	71.4	14	
Skin Picking (N=122/162, 75.3%)												
Yes (n=122)	27	31.4	59	68.6	86	0.066	14	38.9	22	61.1	36	0.244
No (n=40)	2	10.5	17	89.5	19		5	23.8	16	76.2	21	
Nail Picking (N=58/127, 45.7%)												
Yes (n=58)	10	23.8	32	76.2	42	0.548	6	37.5	10	62.5	16	0.717
No (n=69)	13	29.5	31	70.5	44		8	32.0	17	68.0	25	
Compulsive Counting (N=28/158, 17.7%)												
Yes (n=28)	4	21.1	15	78.9	19	0.488	5	55.6	4	44.4	9	0.101
No (n=130)	24	28.9	59	71.1	83		13	27.7	34	72.3	47	
Compulsive Ordering (N=66/158, 41.8%)												
Yes (n=66)	12	27.3	32	72.7	44	0.986	6	27.3	16	72.7	22	0.634
No (n=92)	16	27.1	43	72.9	59		11	33.3	22	66.7	33	
Plays with Strings (N=31/160, 19.4%)												
Yes (n=31)	4	19.0	17	81.0	21	0.348	4	40.0	6	60.0	10	0.622
No (n=129)	24	29.3	58	70.7	82		15	31.9	32	68.1	47	
Visual Hallucinations (N=5/156, 3.2%)												

Yes (n=5)	0	0	2	100	2	0.383	1	33.3	2	66.7	3	0.925
No (n=151)	28	27.7	73	72.3	101		18	36.0	32	64.0	50	
Delusions (N=9/156, 5.8%)												
Yes (n=9)	0	0	5	100	5	0.158	1	25.0	3	75.0	4	0.658
No (n=147)	28	28.9	69	71.1	97		18	36.0	32	64.0	50	

Table 9. Age at Visit 1 by Age at GH Initiation

Growth Hormone Cohort (n=115)		**Age at Visit 1**							
		8-13 years (N=49, 42.6%)		**14-18 years** (N=29, 25.2%)		**19-26 years** (N=27, 23.5%)		**27-62 years** (N=10, 8.7%)	
		N	%	N	%	N	%	N	%
Age of GH Initiation	**0-2 years** (N=29, 25.2%)	28	57.1	1	3.4	0	0	0	0
	3-6 years (N=27, 23.5%)	10	20.4	16	55.2	1	3.7	0	0
	7-11 years (N=31, 27%)	10	20.4	5	17.2	15	55.6	1	10.0
	12-49 years (N=28, 24.3%)	1	2.0	7	24.1	11	40.7	9	90.0

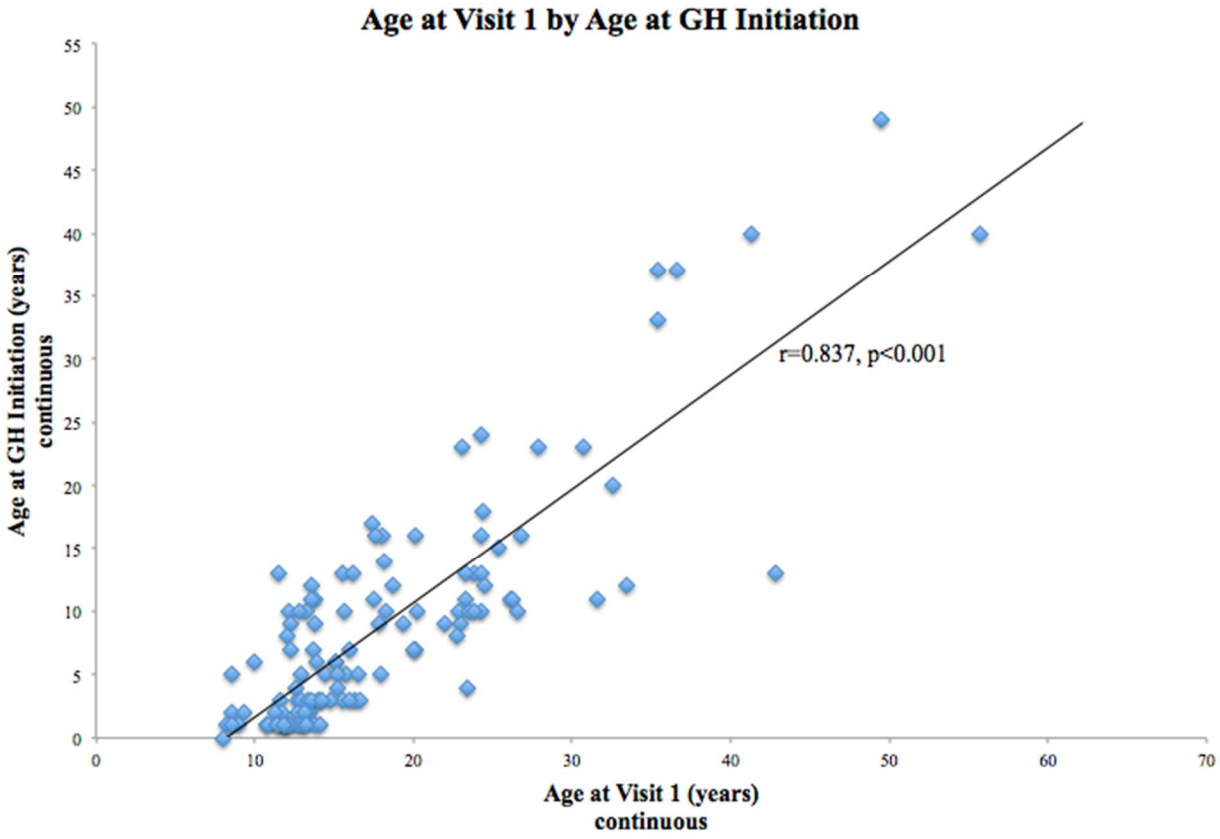

Figure 11. Age at Visit 1 by Age at GH Initiation
Age at visit 1 was strongly correlated with age at GH initiation (r=0.837, p<0.0001). As age at visit 1 increased so did age of GH initiation.

Table 10. Psychiatric Phenotype by Age at Visit 1

	Age at Visit 1								
	8-13 years (N=55,, 32.4%)		**14-18 years** (N=34, 20.0%)		**19-26 years** (N=42, 24.7%)		**27-62 years** (N=39, 22.9%)		
	N	%	N	%	N	%	N	%	p-value
Depressed Mood (N=50/163, 30.7%)									
Yes (n=50)	9	18.0	11	22.0	15	30.0	15	30.0	0.068
No (n=113)	44	38.9	21	18.6	25	22.1	23	20.4	
Anxiety (N=104/165, 63.0%)									
Yes (n=104)	35	33.7	25	24.0	25	24.0	19	18.3	0.097
No (n=61)	18	29.5	7	11.5	17	27.9	19	31.1	
Skin Picking (N=127/170, 74.7%)									
Yes (n=127)	33	26.0	27	21.3	35	27.6	32	25.2	0.024
No (n=43)	22	51.2	7	16.3	7	16.3	7	16.3	
Nail Picking (N=61/133, 45.9%)									
Yes (n=61)	20	32.8	12	19.7	16	26.2	13	21.3	0.841
No (n=72)	19	26.4	15	20.8	19	26.4	19	26.4	
Compulsive Counting (N=28/166, 16.9%)									
Yes (n=28)	9	32.1	6	21.4	5	17.9	8	28.6	0.764
No (n=138)	45	32.6	27	19.6	36	26.1	30	21.7	
Compulsive Ordering (N=68/166, 41.0%)									
Yes (n=68)	26	38.2	14	20.6	16	23.5	12	17.6	0.442
No (n=98)	27	27.6	20	20.4	26	26.5	25	25.5	

Plays with Strings (N=34/168, 20.2%)									
Yes (n=34)	14	41.2	8	23.5	6	17.6	6	17.6	0.444
No (n=134)	40	29.9	26	19.4	35	26.1	33	24.6	
Visual Hallucinations (N=6/164, 3.7%)									
Yes (n=6)	4	66.7	1	16.7	0	0	1	16.7	0.262
No (n=158)	49	31.0	31	19.6	42	26.6	36	22.8	
Delusions (N=11/164, 6.7%)									
Yes (n=11)	3	27.3	2	18.2	1	9.1	5	45.5	0.276
No (n=153)	49	32.0	30	19.6	41	26.8	33	21.6	

Table 11. Psychiatric Behavior by Age at Initiation

	Age at Initiation (years)								
	0-2		**3-6**		**7-11**		**12-49**		
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**p-value**
Depressed Mood (N=28/109, 25.7%)									
Yes (n=28)	5	17.9	7	25.0	6	21.4	10	35.7	0.372
No (n=81)	22	27.2	18	22.2	24	29.6	17	21.0	
Anxiety (N=75/109, 68.8%)									
Yes (n=75)	23	30.7	15	20.0	22	29.3	15	20.0	0.079
No (n=34)	4	11.8	10	29.4	8	23.5	12	35.3	

Skin Picking (N=80/113, 70.8%)									
Yes (n=80)	16	20.0	18	22.5	23	28.7	23	28.7	0.077
No (n=33)	13	39.4	9	27.3	7	21.2	4	12.1	
Nail Picking (N=43/90, 47.8%)									
Yes (n=43)	13	30.2	12	27.9	9	20.9	9	20.9	0.613
No (n=47)	12	25.5	9	19.1	14	29.8	12	25.5	
Compulsive Counting (N=18/112, 16.1%)									
Yes (n=18)	8	44.4	2	11.1	4	22.2	4	22.2	0.213
No (n=94)	21	22.3	25	26.6	25	26.6	23	24.5	
Compulsive Ordering (N=48/113, 42.5%)									
Yes (n=48)	15	31.3	10	20.8	12	25.0	11	22.9	0.711
No (n=65)	14	21.5	16	24.6	18	27.7	17	26.2	
Plays with Strings (23/113, 20.4%)									
Yes (n=23)	9	39.1	5	21.7	7	30.4	2	8.7	0.168
No (n=90)	20	22.2	22	24.4	23	25.6	25	27.8	
Visual Hallucinations (5/108, 4.6%)									
Yes (n=5)	2	40.0	1	20.0	2	40.0	0	0	0.564
No (n=103)	25	24.3	24	23.3	28	27.2	26	25.2	
Delusions (8/108, 7.4%)									
Yes (n=5)	3	37.5	2	25.0	0	0.0	3	37.5	0.308
No (n=103)	23	23.0	23	23.0	30	30.0	24	24.0	

3.7 Depressed Mood

In the univariate logistic regression analysis, GH use showed a decreased, though non-significant association with depressed mood (OR=0.590, 95% CI: 0.291-1.196; p=0.143). The following covariates were also associated with depressed mood in the univariate analyses: age at visit 1 (OR=1.024, 95% CI: 0.996-1.053; p=0.098) and use of psychiatric medications (OR=2.645, 95% CI: 1.325-5.281; p=0.006) and they were included in the multivariate analysis. After controlling for these covariates, GH use did not show a significant association with depressed mood (OR=0.676, 95% CI: 0.291-1.567; p=0.361).

There was a non-significant association with higher rates of depressed mood (OR=1.084, 95% CI: 0.509-2.307; p=0.835) in the deletions subtype vs the mUPD subtype after adjusting for age at first visit, use of psychiatric medications and use of GH (see Table 12).

3.8 Anxiety

In the univariate logistic regression analyses, GH use was significantly associated with increased anxiety (OR=2.083, 95% CI: 1.059-4.907; p=0.033). The following covariates were also associated with anxiety with $p<0.1$ and were thus included in the multivariate analysis: age at visit 1 (OR=0.972, 95% CI: 0.945-0.999; p=0.041) and psychiatric medication use (OR=2.816, 95% CI: 1.364-5.816; p=0.005). After controlling for these covariates, GH was no longer significantly associated with a higher prevalence of anxiety as in the univariate analysis (OR=1.681, 95% CI: 0.75-3.767; p=0.207).

In the univariate analysis, increasing duration of GH use was significantly associated with increased anxiety (OR=2.083, 95% CI: 1.059-4.907; p=0.005). The following covariates were included in the multivariate analysis: GH use (OR=2.083, 95% CI: 1.059-4.907; p=0.33), age at visit 1 (OR=0.972, 95% CI: 0.945-0.999; p=0.041) and psychiatric medication use (OR=2.816, 95% CI: 1.364-5.816; p=0.005). After adjusting for the covariates, longer duration of GH use was associated with increased risk of anxiety (OR=1.093, 95% CI: 0.998-1.198), however, it did not reach statistical significance (p=0.054).

In univariate analyses, there was a significantly higher prevalence of anxiety in the mUPD subtype vs the deletion subtype (OR=2.131, 95% CI: 1.034-4.391; p=0.040). The following covariates were included in the multivariate analysis: GH use (p=0.033), age at visit 1 (p=0.041), psychiatric medication use (p=0.005), and mUPD genotype (p=0.040). In addition, an interaction variable for the interaction between GH use and genotype was included. *After adjusting for these covariates, a significant interaction between GH use and genotype was observed (p=0.036) suggesting that 1) GHT is associated with increased risk for anxiety (OR=2.733, 95% CI: 1.006-7.426, p=0.049), 2) Individuals with mUPD have a higher risk for anxiety than those with deletions (OR=7.567, 95% CI: 1.781-32.146, p=0.006), and 3) GHT has a greater effect on increased risk for anxiety for those with mUPD than for those with deletions (p=0.036) (see Table 13). Relative to those with deletion who did not use GH, those with mUPD who used GH had a 3.25 fold increased risk in anxiety, whereas those with deletions who used GH had a 2.73 fold increased risk in anxiety (see Table 13 and Table 21).*

3.9 Skin Picking

In the univariate logistic regression analyses, GH use showed a decreased, though non-significant association with skin picking (OR=0.517, 95% CI: 0.227-1.176; p=0.116). The following covariates were included in the multivariate analysis: Age at visit 1 (OR=1.036, 95% CI: 0.999-1.075; p=0.059) and psychiatric medication use (OR=2.143, 95% CI: 0.971-4.731; p=0.059). After controlling for these covariates, GH use did not show a significant association with skin picking (OR=0.675, 95% CI: 0.267-1.704; p=0.405).

The association between duration of GH use and prevalence of skin picking did not reach statistical significance (OR=0.517, 95% CI: 0.227-1.176; p=0.116). The following covariates were included in the multivariate analysis: GH use (OR=0.517; 95% CI: 0.227-1.176; p=0.116), age at visit 1 (OR=1.036, 95% CI: 0.999-1.075; p=0.059), and psychiatric medication use (OR=2.143, 95% CI: 0.971-4.731; p=0.059). After controlling for these covariates, GH duration still did not show a significant association with skin picking (OR=0.952, 95% CI: 0.872-1.039; p=0.266).

In the univariate analysis, there was a significantly higher prevalence of skin picking in the deletions subtype vs the mUPD subtype (OR=2.640, 95% CI: 1.269-5.492; p=0.009). The following covariates were included in the multivariate analysis: GH use (OR=0.517, 95% CI: 0.227-1.176; p=0.116), age at visit 1 (OR=1.036, 95% CI: 0.999-1.075; p=0.059), psychiatric medication use (OR=2.143, 95% CI: 0.971-4.731; p=0.059), and deletion genotype (OR=2.640, 95% CI: 1.269-5.492; p=0.009). After adjusting for covariates, there was no significant association with GHT and increased or decreased skin picking. However, the deletion genotype was associated with a higher risk of skin picking than the mUPD genotype (1.43 fold vs 0.50 fold; p=0.007) (see Table 14).

3.10 Nail Picking

In the univariate logistic regression analyses, a significant association was not observed between GH use and nail picking (OR=1.215, 95% CI: 0.575-2.565; p=0.61). There was a non-significant association of GH use with higher rates of nail picking in those with deletions vs those with mUPD (OR=1.491, 95% CI: 0.700-3.179; p=0.300) (see Table 15).

3.11 Compulsive Counting

In the univariate logistic regression analysis, a significant association was not observed between GH use and compulsive counting (OR=0.892, 95% CI: 0.373-2.137; p=0.798). There was a non-significant association of GH use with higher rates of compulsive counting in those with deletions vs those with mUPD (OR=1.195, 95% CI: 0.501-2.854; p=0.688) (see Table 16).

3.12 Compulsive Ordering

In the univariate logistic regression analyses, a significant association was not observed between GH use and compulsive ordering (OR=1.285, 95% CI: 0.647-2.554; p=0.474). There was a non-significant association of GH use with higher rates of compulsive ordering in those with deletions vs those with mUPD (OR=0.894, 95% CI: 0.459-1.740; p=0.741) (see Table 17).

3.13 Plays with Strings

In the univariate logistic regression analyses, a significant association was not observed between GH use and plays with strings (OR=1.058, 95% CI: 0.464-2.412; p=0.893). There was

a non-significant association of GH use with higher rates of plays with strings in those with deletions vs those with mUPD (OR=1.204, 95% CI: 0.523-2.772; p=0.663) (see Table 18).

3.14 Visual Hallucinations

In the univariate logistic regression analysis, a significant association was not observed between GH use and visual hallucinations (OR=2.315, 95% CI: 0.263-20.336; p=0.449). There was a non-significant association of GH use with a higher prevalence of visual hallucinations in the mUPD subtype vs the deletion subtype (OR=3.030, 95% CI: 0.490-18.720; p=0.233) (see Table 19).

3.15 Delusions

In the univariate analysis, GH use was not significantly associated with delusions (OR=4.85, 95% CI: 0.605-38.981; p=0.137). The following covariates were included in the multivariate analysis: psychiatric medication use (OR=3.5, 95% CI: 0.979-12.508; p=0.054) and age at visit 1 (OR=1.023, 95% CI: 0.976-1.072; p=0.344). *After controlling for these covariates, GH use was significantly associated with increased risk of delusions (OR=14.013, 95% CI: 1.262-155.638; p=0.032).*

The following covariates were included in the multivariate analysis: GH use (OR=4.854, 95% CI: 0.605-38.981; p=0.137), psychiatric medication use (OR=3.5, 95% CI: 0.979-12.508; p=0.054), and age at visit 1 (OR=1.023, 95% CI: 0.976-1.072; p=0.344). After controlling for covariates, GH duration did not show a significant association with delusions (OR=0.999, 95% CI: 0.874-1.143; p=0.991).

In univariate analyses, there was a non-significant association with higher prevalence of delusions in the mUPD subtype vs the deletion subtype (OR=1.552, 95% CI: 0.339-6.037; p=0.526) (see Table 20).

3.16 Summary of Results

In summary, GH use was significantly associated with increased risk for delusions (OR=14.013, p=0.022) and anxiety (OR=3.25, p=0.036). The results suggest that GH use increases risk for anxiety significantly more for those with mUPD than for those with deletions (3.25 fold vs 2.73 fold).

Table 12. Depressed Mood: Univariate and Multivariate Analyses

Dependent Variable: DEPRESSED MOOD (n=50)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	-0.528	0.361	0.143	0.590	0.291	1.196
Age at V1	0.023	0.014	0.098	1.024	0.996	1.053
Psych Meds	0.973	0.353	0.006	2.645	1.325	5.281
GH Duration (cont.)	-0.013	0.033	0.706	0.987	0.925	1.054
Genotype (DEL)	0.010	0.369	0.979	1.010	0.490	2.079
MULTIVARIATE ANALYSES						
GH Use	-0.392	0.429	0.361	0.676	0.291	1.567
Age at V1	0.006	0.018	0.749	1.006	0.972	1.041
Psych Meds	0.908	0.371	0.014	2.478	1.199	5.124
GH Use	-0.585	0.439	0.182	0.557	0.236	1.315
Genotype (Del)	0.080	0.386	0.835	1.084	0.509	2.307
Psych Meds	0.803	0.384	0.036	2.233	1.053	4.735
Age at V1	0.009	0.018	0.599	1.009	0.975	1.045

Table 13. Anxiety: Univariate and Multivariate Analyses

Dependent Variable: ANXIETY (n=104)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	0.734	0.345	0.033	2.083	1.059	4.907
Age at V1	-0.029	0.014	0.041	0.972	0.945	0.999
Psych Meds	1.035	0.37	0.005	2.816	1.364	5.816
GH Duration (cont.)	0.097	0.035	0.005	1.102	1.030	1.179
Genotype (mUPD)	0.756	0.369	0.040	2.131	1.034	4.391
MULTIVARIATE ANALYSES						
GH Use	0.519	0.412	0.207	1.681	0.75	3.767
Age at V1	-0.039	0.018	0.029	0.962	0.928	0.996
Psych Meds	1.478	0.425	0.001	4.386	1.906	10.095
GH Use	-0.014	0.490	0.977	0.986	0.377	2.577
Age at V1	-0.034	0.018	0.055	0.966	0.933	1.001
Psych Meds	1.508	0.428	0.000	4.520	1.954	10.453
GH Duration	0.089	0.046	0.054	1.093	0.998	1.198
GH	0.457	0.431	0.271	1.608	0.690	3.744
Age at V1	-0.036	0.018	0.045	0.964	0.931	0.999
Psych Meds	1.332	0.427	0.002	3.789	1.641	8.748
Genotype (mUPD)	0.782	0.392	0.046	2.185	1.014	4.710

GH Use	1.005	0.510	0.049	2.733	1.006	7.426
Age at V1	-0.042	0.019	0.025	0.959	0.924	0.995
Psych Meds	1.355	0.439	0.002	3.879	1.640	9.174
UPD	2.024	0.738	0.006	7.567	1.781	32.146
GH* mUPD	-1.849	0.880	0.036	0.157	0.028	0.883
GH Use	0.537	0.572	0.0348	1.711	0.557	5.249
Age at V1	-0.038	0.019	0.044	0.963	0.928	0.999
Psych Meds	1.399	0.444	0.002	4.052	1.699	9.665
UPD	2.005	0.734	0.006	7.424	1.760	31.309
GH* mUPD	-1.885	0.882	0.033	0.152	0.027	0.855
GH Duration	0.082	0.046	0.077	1.086	0.991	1.189

Table 14. Skin Picking: Univariate and Multivariate Analyses

Dependent Variable: SKIN PICKING (n=127)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	-0.66	0.419	0.116	0.517	0.227	1.176
Age at V1 (cont.)	0.035	0.019	0.059	1.036	0.999	1.075
Psych Meds	0.762	0.404	0.059	2.143	0.971	4.731
GH Duration (cont.)	-0.068	0.034	0.043	0.934	0.875	0.998
Genotype (DEL)	0.971	0.374	0.009	2.640	1.269	5.492
MULTIVARIATE ANALYSES						
GH Use	-0.393	0.472	0.405	0.675	0.267	1.704
Age at V1 (cont.)	0.02	0.021	0.339	1.02	0.979	1.063
Psych Meds	0.619	0.42	0.14	1.858	0.816	4.231
GH Use	-0.051	0.574	0.929	0.950	0.309	2.927
Age at V1 (cont.)	0.017	0.021	0.426	1.017	0.976	1.060
Psych Meds	0.630	0.421	0.134	1.878	0.823	4.284
GH Duration (cont.)	-0.050	0.045	0.266	0.952	0.872	1.039

GH Use	-0.688	0.530	0.194	0.503	0.178	1.420
Age at V1 (cont.)	0.020	0.023	0.382	1.020	0.976	1.067
Psych Meds	0.537	0.441	0.223	1.710	0.721	4.056
Genotype (DEL)	1.045	0.390	0.007	2.842	1.322	6.108
GH Use	-0.275	0.700	0.695	0.760	0.193	2.996
Age at V1 (cont.)	0.023	0.023	0.327	1.023	0.978	1.070
Psych Meds	0.554	0.440	0.208	1.740	0.735	4.121
Genotype (DEL)	1.746	0.917	0.057	5.730	0.951	34.542
GH*DEL	-0.879	1.019	0.388	0.415	0.056	3.059

Table 15. Nail Picking: Univariate and Multivariate Analyses

Dependent Variable: NAIL PICKING (n=61)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	0.195	0.381	0.61	1.215	0.575	2.565
Age at V1 (cont.)	-0.004	0.015	0.796	0.996	0.967	1.026
Psych Meds	0.028	0.377	0.941	1.028	0.491	2.151
GH Duration (cont.)	0.028	0.033	0.396	1.029	0.964	1.098
Genotype (DEL)	0.400	0.386	0.300	1.491	0.700	3.179

Table 16. Compulsive Counting: Univariate and Multivariate Analyses

Dependent Variable: COMPULSIVE COUNTING (n=28)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	-0.114	0.445	0.798	0.892	0.373	2.137
Age at V1 (cont.)	0.013	0.017	0.56	0.454	0.979	1.048
Psych Meds	0.225	0.427	0.597	1.253	0.543	2.891
GH Duration (cont.)	-0.006	0.040	0.873	0.994	0.919	1.075
Genotype (DEL)	0.179	0.444	0.688	1.195	0.501	2.854

Table 17. Compulsive Ordering: Univariate and Multivariate Analyses

Dependent Variable: COMPULSIVE ORDERING (n=68)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	0.251	0.35	0.474	1.285	0.647	2.554
Age at V1 (cont.)	-0.016	0.015	0.275	0.984	0.957	1.013
Psych Meds	-0.376	0.338	0.267	0.687	0.354	1.332
GH Duration (cont.)	0.021	0.224	0.491	1.021	0.962	1.083

Genotype (DEL)	0.112	0.340	0.741	1.119	0.575	2.177

Table 18. Plays with Strings: Univariate and Multivariate Analyses

Dependent Variable: PLAYS WITH STRINGS (n=34)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	0.056	0.42	0.893	1.058	0.464	2.412
Age at V1 (cont.)	-0.013	0.018	0.473	0.987	0.953	1.023
Psych Meds	-0.325	0.417	0.436	0.723	0.319	1.636
GH Duration (cont.)	0.069	0.036	0.055	1.072	0.998	1.151
Genotype (DEL)	0.185	0.426	0.663	1.204	0.523	2.772
MULTIVARIATE ANALYSES						
GH Use	-1.004	0.664	0.130	0.366	0.100	1.346
GH Duration (cont.)	0.121	0.053	0.023	1.128	1.017	1.251
Psych Meds	-0.283	0.440	0.520	0.753	0.318	1.784
Age at V1 (cont.)	-0.005	0.021	0.798	0.995	0.954	1.037

Table 19. Visual Hallucinations: Univariate and Multivariate Analyses

Dependent Variable: VISUAL HALLUCINATIONS (n=6)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	0.839	1.109	0.449	2.315	0.263	20.336
Age at V1 (cont.)	-0.077	0.063	0.222	0.926	0.819	1.047
Psych Meds	0.655	0.834	0.432	1.926	0.376	9.866
GH Duration (cont.)	0.032	0.078	0.687	1.032	0.885	1.203
Genotype (mUPD)	1.109	0.929	0.233	3.030	0.490	18.720

Table 20. Delusions: Univariate and Multivariate Analyses

Dependent Variable: DELUSIONS (n=11)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
GH Use	1.58	1.063	0.137	4.854	0.605	38.981
Age at V1 (cont.)	0.023	0.024	0.344	1.023	0.976	1.072
Psych Meds	1.253	0.65	0.054	3.5	0.979	12.508
GH Duration (cont.)	0.036	0.059	0.537	1.037	0.924	1.164
Genotype (mUPD)	0.440	0.693	0.526	1.552	0.339	6.037

MULTIVARIATE ANALYSES						
GH Use	2.64	1.228	0.032	14.013	1.262	155.638
Age at V1 (cont.)	0.054	0.03	0.077	1.055	0.994	1.12
Psych Meds	1.228	0.675	0.069	3.414	0.909	12.818
GH Use	2.644	1.272	0.038	14.066	1.162	170.275
GH Duration (cont.)	-0.001	0.069	0.991	0.999	0.874	1.143
Psych Meds	1.227	0.676	0.069	3.412	0.908	12.830
Age at V1 (cont.)	0.054	0.031	0.083	1.055	0.993	1.121
GH Use	3.064	1.339	0.022	21.418	1.553	295.392
Age at V1 (cont.)	0.081	0.034	0.017	1.085	1.015	1.160
Psych Meds	1.328	0.766	0.083	3.774	0.842	16.925
Genotype (mUPD)	1.005	0.783	0.200	2.731	0.588	12.674

Table 21. Coefficients of the Anxiety Analysis in Table 11

Model	b(GH)	b(UPD)	b(GH+UPD)	b(GH*UPD)	b(GH+UPD+ GH*UPD)	GH=1, UPD=1 OR(GH+UPD)	GH=1, UPD=0 OR(GH=DEL)
GH + Genotype + GH*Genotype	1.005	2.024	3.029	-1.849	1.18	3.25	2.73

4 DISCUSSION

The purpose of this study was to determine the association between growth hormone use and psychiatric phenotype using data from the Rare Disease Clinical Research Network's Natural History PWS and Morbid Obesity study, the largest Prader-Willi study known to date. Three categories of psychiatric symptoms were analyzed: affective symptoms (depressed mood and anxiety), compulsive symptoms (skin picking, nail picking, compulsive counting, compulsive ordering, and plays with strings), and psychotic symptoms (visual hallucinations and delusions). For those on growth hormone, the association between duration of treatment and age at growth hormone initiation was assessed. Lastly, the possibility of an interaction between growth hormone treatment and genotype (deletion vs mUPD) was explored. The following four hypotheses were investigated: 1) Growth hormone treatment contributes to a lower risk for psychiatric phenotypes in individuals with Prader-Willi syndrome, 2) Earlier age at initiation of GH treatment results in lower risk for psychiatric phenotypes, 3) Longer duration of GH treatment results in lower risk for psychiatric phenotypes, and 4) Risk of psychiatric outcomes associated with GH use differs for those with deletions versus those with mUPD.

4.1 GH Use and Psychiatric Phenotype

Based on reports that growth hormone may improve cognition and behavior in individuals with PWS, the primary hypothesis speculated that growth hormone use would contribute to a decreased risk of psychiatric behaviors. This hypothesis was not supported by the data. After adjusting for confounding variables, delusions was the only outcome that had a significant association with growth hormone use and it suggested that growth hormone use was

associated with a 14.0 times **increased** risk of delusions. The association of GH use with an increased risk of delusions is contrary to the study hypothesis. While this finding may be true, it may also be due to chance or other confounding variables that were not captured by the study. Additionally, out of 172 participants in the study, only 11 reported delusions which explains the wide confidence interval and increases the possibility that this is a chance finding.

4.2 Age at GH Initiation and Psychiatric Phenotype

Age at GH initiation was intended to be a measure of dosage, however, it was too strongly correlated with age ($r=0.837$, $p<0.001$) to clearly interpret. This is largely due to the fact that GHT was FDA approved for PWS patients in 2000 and it took time for it to be adopted as the gold standard of treatment. In other words, younger individuals had a greater chance of initiating GHT at an earlier age if born around 2000. While individuals born prior to this could not have received GHT at a younger age as the treatment was not available. Age of GH initiation was, therefore, dropped from the analysis.

4.3 GH Duration and Psychiatric Phenotype

It was hypothesized that if growth hormone has an association with outcome then dosage would also have an effect. As the delusions phenotype was the only psychiatric outcome that had a statistically significant association with GH use, GH duration was expected to support that association. This hypothesis was not supported by the data. Although it is possible that growth hormone use is associated with delusions while duration of treatment does not have an effect, the

finding of a dosage effect typically supports the association between exposure and outcome. The lack of an association with GH duration may suggest that the association of GH use and increased risk of delusions is a chance finding especially as it only became significant after adjusting for confounding variables like psychiatric medications. Prior to adjustment it was not a significant finding. It is also possible that dosage truly does have an effect on outcome, but that duration of treatment is not a good stand alone measure of dosage. The type of growth hormone treatment and dosage of each treatment was not included in the analysis. Another possibility is that GH duration truly is associated with the outcome, but the sample was not large enough to produce a statistically significant association.

4.4 Interaction of GH use with Genotype and Psychiatric Phenotype

In univariate analyses of associations between genotype and psychiatric disorders, mUPD genotype was significantly associated with higher risk for anxiety. mUPD was also associated with higher risk for visual hallucinations and delusions, however, the associations did not reach statistical significance. The deletions genotype was significantly associated with higher risk for skin picking and non-significantly associated with higher risk for nail picking, compulsive counting, compulsive ordering, and depressed mood.

These findings support the literature that reports that those with mUPD have greater vulnerability for developing psychoses and those with deletions have higher rates of developing compulsions (Krefft et al, 2014). However, for the results that were non-significant, it is uncertain if they are truly consistent. Based on the reports of psychiatric differences due to genotype, it was hypothesized that growth hormone would have a different effect on the deletion

versus mUPD subtypes. This hypothesis was supported by the data, which suggested that GH use has a greater effect on increased risk for anxiety for those with mUPD than for those with deletions. While this finding may be true, it may also be due to chance or other confounding variables that were not captured by the study.

4.5 Strengths and Limitations of the Study

While this study had several strengths, there were also many limitations. Strengths included the large sample size and the amount of information gathered on each patient which allowed for the evaluation of several possible confounding variables. Another strength was that the diagnosis of PWS for all participants was confirmed by molecular testing.

A major limitation of the study was that psychiatric information was by guardian report. These self-reported diagnoses may not be as reliable as those made through a formal psychiatric evaluation. Psychiatric disorders are highly stigmatized which may lead to under-reporting of symptoms (Takayanagi et al, 2014). Stigma on mental health disorders has been reported to vary among individuals and families from different races and cultures (Anglin et al, 2006). Wong et al (2017) found that regardless of racial and ethnic group, most individuals believe those with mental illness experience high levels of prejudice and discrimination, however, Asian Americans and Latinos were found to hold more negative views of mental illness than African Americans and whites. Substantial differences existed for Latinos depending on what language they chose for their interviews with those interviewed in Spanish seeming to experience lower levels of stigma. Using language as a measure of acculturation showed that the degree to which Latinos adopt US cultural norms was also an important factor for predicting levels of stigma associated

with mental health (Wong et al, 2017). Differences in reporting by race and/or culture was not assessed in the analyses in this study as 85% of study participants were white; therefore, an important confounding factor for psychiatric behavior reporting was missed.

Other possible confounding variables that were not controlled for were socio-economic status and sleep disturbances. It is estimated that children and adolescents with low socio-economic status are up to three times more likely to develop mental health problems than their peers from families with high socio-economic status (Reiss et al, 2019). Access to growth hormone treatment is also associated with socio-economic status as it is expensive and not all families have equal access due to variable health care coverage (Dykens et al, 2017). Sleep disturbances are also very common in individuals with Prader-Willi syndrome and sleep is one of the most important psychophysiological processes to promote healthy brain function and mental health, possibly making it a confounding variable (Baglioni et al, 2017).

The study took place across five institutions and inter-investigator reporting variability must be noted due to differences in data collection and areas of expertise. For example, approximately 59% of participants were seen at Vanderbilt University which has research interests in psychiatric disorders.

Another major limitation of the study was that the exact age of onset of psychiatric symptoms was not recorded. This information would be important in determining when symptoms started in relation to growth hormone use. Additionally, age at growth hormone initiation and GH duration was not well documented in the study making it extremely difficult to accuratlely evaluate these variables. As mentioned earlier, the mental deviations observed in PWS are similar but differ in etiology to those seen in the general population. (Whittington et al,

2018). Therefore, information gathered may not have been specific to the differences present in individuals with PWS.

4.6 Future Studies

Psychiatric behaviors are complex and are often due to a combination of several genetic and environmental factors (Tsuang et al, 2004). Measuring the effects of one exposure on psychiatric outcome is therefore complicated and one must attempt to eliminate the effects of all other confounding variables. To address the limitations of this study, future studies must address the racial, culture, and socioeconomic background of each patient. Information on sleep disturbances should also be obtained as they contribute to a higher rate of psychiatric symptoms. A better family history evaluation of the psychiatric manifestations within the families is also recommended as psychiatric disorders are highly heritable. There was no standardization of the family history taking process and it may have varied due to investigator bias between the study sites. To address the potential problem of underreporting of symptoms, the presence of psychiatric behaviors should be evaluated by a medical professional. This should include an evaluation of each participant's medical records for authentication rather than family reporting. Another important limitation of this study was the limited information on growth hormone medication as well as other types of medication. An accurate record of medication type, age at initiation, dosage, and duration are important items to capture in a future study. Information on the age of onset of psychiatric symptoms as well as duration and frequency of episodes should also be included in the analyses. Increasing the sample size to include more adolescent and adult individuals would also be beneficial to give the study more statistical power.

4.6 Conclusions

The purpose of this study was to describe the association between growth hormone use and the following psychiatric symptoms: depressed mood, anxiety, skin picking, nail picking, compulsive counting, compulsive ordering, plays with strings, visual hallucinations, and delusions using a data set of 172 participants.

Growth Hormone Use: The data suggests that there is a statistically significant association between growth hormone use and increased risk for delusions, however, the results are based on only 9 cases with delusions so this association may be a chance finding.

Duration of Growth Hormone: The data does not show a statistically significant association between duration of growth hormone use on any of the psychiatric outcomes.

Age at Initiation of Growth Hormone: The data suggests age at initiation, as captured in this study, is a poor measure of growth hormone dosage.

Interaction of Growth Hormone on Genotype: The data suggests that there is an interaction between GHT and genotype in their influence on risk for anxiety. After including the interaction in the analysis, GH use was associated with an increased risk for anxiety, and those with mUPD who used GH had a greater risk for anxiety than those with deletion who used GH.

4.7 Summary

Individuals with Prader-Willi syndrome have a high risk of psychiatric comorbidities that interfere with their quality of life. Growth hormone is a standard treatment in the management of Prader-Willi syndrome to improve body composition and some studies have shown that growth hormone contributes to improvements in cognition and behavior. The purpose of this study was to investigate the association of GH use and nine psychiatric behaviors (depressed mood, anxiety, skin picking, nail picking, compulsive counting, compulsive ordering, plays with strings, visual hallucinations, and delusions). According to this data set, 1) GH use has a significant association with increased risk of delusions, 2) GH use has a significant association with increased risk for anxiety for both mUPD and deletion genotypes, and 3) GH use has a greater effect on increased risk for anxiety for those with mUPD over those with deletions. None of these findings have not been previously documented, therefore, further exploration of these results are indicated.

Abbreviations: PWS, Prader-Willi syndrome; GH, growth hormone; GHT, growth hormone treatment; mUPD, maternal uniparental disomy; IGF-1, insulin like growth factor 1; ASD, autism spectrum disorder; EMO, Early-onset morbid obesity; FDA, Food and Drug Administration

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6 APPENDIX A

DEMOGRAPHICS AND DIAGNOSIS

RDN Participant ID:		Date of Registration: (dd mmm yyyy)	
Local Subject ID:		Status	
Site ID:		Date of Visit	

***To the parent/caretaker**: Please complete the history section of this form (pages 1-19) to the best of your ability. One of the research professionals will review your answers with you at a later time. Please answer only those questions for which you are certain of your answers. Thank you!*

Date____/____/_____ (dd/mmm/yyyy)
Person Completing Form: (Last)_____ (First) _____(MI) _____
Relationship to Patient: _____

Patient Name: (Last)_____ (First) _____ (MI) _____

Date of Birth: ___/___ /_____(dd/mmm/yyyy) **Current age**_____years

Sex: O Male O Female

Race/Ethnicity (indicate all that apply):

Race: (select as many as apply)	Ethnicity:
O American Indian/Alaskan Native O Asian O Black/African American O Hawaiian Native/Pacific Islander O White O Unknown or not reported O Refused	Hispanic/Latino: O YesO No O Unknown O Refused

PWS Diagnosis (if applicable**):**

PWS type (indicate one): O Deletion O Uniparental Disomy (mUPD) O Imprinting Defect (ID) O Other (please describe) _____

Age when diagnosed _____years
Doctor/Hospital/Lab_____
(i.e., *Who made the diagnosis? Where did the diagnosis occur?)*

Tests used to diagnose PWS (indicate all that apply):

□ FISH 15 Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Deletion positive? O Yes O No
Doctor/Hospital/Lab_____

□ Chromosomal Analysis Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Deletion positive? O Yes O No
Doctor/Hospital/Lab_____

□ DNA Methylation Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Positive for PWS? O Yes O No
Doctor/Hospital/Lab_____

□ DNA Polymorphism studies Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Deletion positive? O Yes O No
Doctor/Hospital/Lab_____

□ Other (please describe) Date ____/___/_____ (mm/dd/yyyy)
Test name _____
Results _____
Doctor/Hospital/Lab_____

□ We (the primary caretakers) do not know £

Not Prader-Willi Syndrome, but "Prader-Willi-like" and/or Obesity
(if applicable, indicate all that apply):

□ Chromosomal Analysis Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Results normal? O Yes O No No
If results abnormal, please describe:_____
_____
Doctor/Hospital/Lab_____

□ FISH Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Results normal? O Yes O No
If results abnormal, please describe:_____
_____
Doctor/Hospital/Lab_____

□ DNA Methylation Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Positive for PWS? O Yes O No
Doctor/Hospital/Lab_____

□ Leptin Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Results normal? O Yes O No
If results abnormal, please describe:_____
_____
Doctor/Hospital/Lab_____

□ Fragile X Test done? O Yes O No
Date ____/___/_____ (mm/dd/yyyy)
Results normal? O Yes O No
If results abnormal, please describe:_____
_____
Doctor/Hospital/Lab_____

□ Other Date ____/___/_____ (mm/dd/yyyy)
Test name: _____

Results normal? O Yes O No
If results abnormal, please describe:_____
_____
Doctor/Hospital/Lab_____

BEHAVIOR HISTORY BASELINE FORM

RDN Participant ID:		Date of Registration: (dd mmm yyyy)	
Local Subject ID:		Status	
Site ID:		Date of Visit	

1. Does your child currently exhibit any of the following behaviors?

		If not currently, have they ever?	If yes, at what age?
a. Skin picking	O YES O NO	O YES O NO	from_____to_____years
b. Nail picking	O YES O NO	O YES O NO	from_____ to _____years
c. Nail biting	O YES O NO	O YES O NO	from_____ to _____years
d. Self-mutilation	O YES O NO	O YES O NO	from_____ to _____years
e. Hoarding/saving (not food)	O YES O NO	O YES O NO	from _____ to _____years
f. Food seeking	O YES O NO	O YES O NO	from _____ to _____years
g. Food hiding	O YES O NO	O YES O NO	from _____ to _____years
h. Compulsive ordering/arranging	O YES O NO	O YES O NO	from _____ to _____years
i. Compulsive counting	O YES O NO	O YES O NO	from _____ to _____years
j. Plays with strings	O YES O NO	O YES O NO	from _____ to _____ years

2. Describe your child's skin picking. (*please indicate one*)

O None
O Until the skin is red/irritated
O Until the skin bleeds
O It takes longer than 2 months for lesions to heal

How would you rate your child's skin picking? (*please indicate one*)

O Not a problem
O Mild
O Moderate
O Severe

3. If your child has a skin-picking problem, have you tried any medication or intervention?

O YES O NO O Not applicable

a. If yes, did this medication or intervention help? O YES O NO

Please describe:

_____

4. Does your child currently exhibit any of the following behaviors?

		If not currently, have they ever?	If yes, at what age?
a. Screaming/yelling	O YES O NO	O YES O NO	from ____ to_____ years
b. Throwing objects	O YES O NO	O YES O NO	from ____ to_____ years
c. Aggressive/violent actions (*e.g., hitting/biting*)	O YES O NO	O YES O NO	from ____ to_____ years

d. Foul language	O YES	O NO	O YES O NO	from ____ to_____ years
e. Destructive behavior	O YES	O NO	O YES O NO	from ____ to_____ years
f. Threatens to hurt others	O YES	O NO	O YES O NO	from ____ to_____ years
g. Tantrums	O YES	O NO	O YES O NO	from ____ to_____ years
h. Sexual acting out/ inappropriate sexual behaviors	O YES	O NO	O YES O NO	from ____ to ____- years

5. Does your child currently exhibit any of the following behaviors?

		If not currently, have they ever?	If yes, at what age?
a. Depressed mood	O YES O NO	O YES O NO	from _____ to ____ years
b. Anxiety	O YES O NO	O YES O NO	from _____ to ____years
c. Thoughts of hurting him/herself	O YES O NO	O YES O NO	from _____ to ____ years
d. Cries easily	O YES O NO	O YES O NO	from _____ to ____ years
e. Visual hallucinations (seeing things not there)	O YES O NO	O YES O NO	from _____ to ____ years
f. Auditory hallucinations (hearing voices)	O YES O NO	O YES O NO	from _____ to ____ years
g. Delusions?	O YES O NO	O YES O NO	from _____ to ____ years

6. Has your child ever seen any of the following professionals for behavior problems?

		If yes, at what age?	Currently seeing?	
a. Mental Health Counselor	O YES O NO	_____years	O YES	O NO
b. Psychiatrist	O YESO NO	_____years	O YES	O NO
c. Psychologist	O YES O NO	_____years	O YES	O NO
d. Social Worker	O YES O NO	_____years	O YES	O NO
e. Applied Behavior Analyst	O YES O NO	_____years	O YES	O NO

MEDICATION HISTORY FORM

RDN Participant ID:		Date of Registration: (dd mmm yyyy)	
Local Subject ID:		Status	
Site ID:		Date of Visit	

Growth Hormone:

1. Is your child currently on growth hormone? O Yes O No
2. What type of growth hormone? O Not Applicable

Current dose _____ mg per day

3. If not currently, has your child ever been on growth hormone in the past? O Yes O No
4. How old was your child when he/she started growth hormone?

_____ years O Not applicable

5. How old was your child when he/she stopped growth hormone?

_____ years O Not applicable

6. If growth hormone was stopped, why was it discontinued? (Indicate all that apply)

Not applicable

□ Side effects (describe) _____

□ Due to age/ had stopped growing

□ Child's decision

□ Behavioral problems

□ Insurance would not pay

□ Research study completed

□ Other (describe) _____

Sex Hormones:

Males Only: O Not applicable

7. Is your child currently on testosterone? O Yes O No
8. If not on currently, has your child ever been on testosterone in the past? O Yes O No
9. If applicable, how old was your child when he started testosterone? _____ years
10. If applicable, how old was your child when he stopped testosterone? _____ years

11. What type of administration of the testosterone? (check box)

Type:	Drug name:	Dose
□ Injection:	OTestosterone Cypionate	mg IM q2-4 weeks_____
□ Patch:	O Androderm	mg per day_____
□ Gel:	O Androgel O Testim	grams per day _____
□ Other:	O_____O_____	grams per day_____

12. If testosterone was stopped, why was it discontinued? (Indicate all that apply) O Not applicable

□ Behavioral problems

□ Other side effects (describe) _____

□ Child's decision

□ Other (describe)_____

Females Only: O Not applicable

13. Is your child currently on estrogen? O Yes O No

14. If not on currently, has your child ever been on estrogen in the past? O Yes O No

15. How old was your child when she started estrogen?

_____ years O Not applicable

16. How old was your child when she stopped estrogen?

_____ years O Not applicable

17. If estrogen was stopped, why was it discontinued?

Not applicable

Behavioral problems

Other side effects (describe) _____

Child's decision

Other (describe) _____

7 APPENDIX B

Dependent Variable: DEPRESSED MOOD (n=50)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	0.023	0.014	0.098	1.024	0.996	1.053
GH Use	-0.528	0.361	0.143	0.590	0.291	1.196
Age at GH Initiation	0.017	0.024	0.475	1.018	0.97	1.067
Psych Meds	0.973	0.353	0.006	2.6	1.325	5.3
Genotype (DEL)	0.010	0.369	0.979	1.010	0.490	2.079
Sex of Participant	-0.071	0.341	0.836	0.932	0.477	1.818
Sex Hormones	0.350	0.344	0.309	1.420	0.723	2.788
GH Duration (cont.)	-0.013	0.033	0.706	0.987	0.925	1.054
MULTIVARIATE ANALYSES						
GH Use	-0.310	0.416	0.456	0.733	0.324	1.658
Age at V1	0.018	0.016	0.284	1.018	0.986	1.051

GH Use	-0.461	0.370	0.213	0.631	0.305	1.303
Psych Meds	0.942	0.355	0.008	2.565	1.279	5.144
GH Use	-0.392	0.429	0.361	0.676	0.291	1.567
Age at V1	0.006	0.018	0.749	1.006	0.972	1.041
Psych Meds	0.908	0.371	0.014	2.478	1.199	5.124
GH Use	-0.585	0.439	0.182	0.557	0.236	1.315
Genotype (Del)	0.080	0.386	0.835	1.084	0.509	2.307
Psych Meds	0.803	0.384	0.036	2.233	1.053	4.735
Age at V1	0.009	0.018	0.599	1.009	0.975	1.045
GH*DEL	-0.411	0.797	0.606	0.663	0.139	3.161
Genotype (DEL)	0.358	0.637	0.574	1.430	0.411	4.981
GH Use	-0.435	0.636	0.493	0.647	0.186	2.249
Psych Meds	0.879	0.369	0.017	2.408	1.167	4.967

Dependent Variable: ANXIETY (n=104)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	-0.029	0.014	0.041	0.972	0.945	0.999
GH Use	0.734	0.345	0.033	2.083	1.059	4.907
Age at GH Init.	-0.06	0.026	0.021	0.942	0.895	0.991
Psych Meds	1.035	0.37	0.005	2.816	1.364	5.816
Genotype (mUPD)	0.756	0.369	0.040	2.131	1.034	4.391
Sex of Participant	-0.394	0.327	0.228	0.674	0.355	1.280
Sex Hormones	-0.419	0.329	0.204	0.658	0.345	1.255
GH Duration	0.090	0.047	0.058	1.094	0.997	1.201
MULTIVARIATE ANALYSES						
GH Use	0.511	0.394	0.194	1.667	0.771	3.606
Age at V1 (cont.)	-0.019	0.016	0.239	0.981	0.951	1.013
GH Use	0.925	0.367	0.012	2.521	1.229	5.172

Psych Meds	1.188	0.386	0.002	3.28	1.538	6.995
GH Use	0.519	0.412	0.207	1.681	0.75	3.767
Age at V1 (cont.)	-0.039	0.018	0.029	0.962	0.928	0.996
Psych Meds	1.478	0.425	0.001	4.386	1.906	10.095
GH Use	0.685	0.366	0.061	1.984	0.968	4.066
Genotype (mUPD)	0.832	0.377	0.027	2.298	1.098	4.809
Genotype (mUPD)	0.759	0.376	0.043	2.137	1.023	4.465
Psych Meds	0.939	0.379	0.013	2.556	1.215	5.378
GH Use	0.861	0.386	0.026	2.366	1.111	5.040
Genotype (mUPD)	0.841	0.385	0.029	2.319	1.091	4.930
Psych Meds	1.075	0.393	0.006	2.929	1.355	6.332
GH	0.457	0.431	0.271	1.608	0.690	3.744

Age at V1 (cont.)	-0.036	0.018	0.045	0.964	0.931	0.999
Psych Meds	1.332	0.427	0.002	3.789	1.641	8.748
Genotype (mUPD)	0.782	0.392	0.046	2.185	1.014	4.710
GH Use	0.475	0.431	0.271	1.608	0.690	3.744
Genotype (mUPD)	0.782	0.392	0.046	2.185	1.014	4.710
Psych Meds	1.332	0.427	0.002	3.789	1.641	8.748
Age at V1 (cont.)	-0.036	0.018	0.045	0.964	0.931	0.999
Age at V1	-0.024	0.045	0.592	0.976	0.894	1.066
Age at GH Init	-0.040	0.045	0.368	0.961	0.88	1.049
Age at V1 (cont.)	-0.017	0.047	0.713	0.983	0.896	1.078
Age at GH Initiation (cont.)	-0.068	0.048	0.157	0.934	0.85	1.026
Psych Meds	1.254	0.567	0.027	3.505	1.154	10.644
GH Use	0.475	0.431	0.271	1.608	0.690	3.744

Genotype (mUPD)	0.782	0.392	0.046	2.185	1.014	4.710
Psych Meds	1.332	0.427	0.002	3.789	1.641	8.748
Age at V1 (cont.)	-0.036	0.018	0.045	0.964	0.931	0.999
Genotype (mUPD)	0.759	0.394	0.054	2.136	0.988	4.619
Psych Meds	1.374	0.431	0.001	3.952	1.699	9.190
Age at V1 (cont.)	-0.032	0.017	0.064	0.969	0.937	1.002
GH Duration (cont.)	0.081	0.040	0.043	1.084	1.003	1.173
Genotype (mUPD)	0.175	0.493	0.723	1.191	0.453	3.127
Psych Meds	1.211	0.572	0.034	3.357	1.094	10.307
Age at V1 (cont.)	-0.017	0.048	0.722	0.983	0.896	1.079
GH Initiation	-0.065	0.048	0.174	0.937	0.853	1.029
Genotype (mUPD)	0.349	0.471	0.459	1.418	0.563	3.571
GH Duration (cont.)	0.083	0.048	0.081	1.087	0.990	1.193

Psych Meds	0.669	0.501	0.182	1.952	0.732	5.209
GH Duration	0.092	0.048	0.055	1.097	0.998	1.205
Age at V1 (con.t)	-0.051	0.026	0.051	0.951	0.904	1.000
GH Duration	0.067	0.049	0.170	1.069	0.972	1.176
Age at V1 (cont.)	-0.063	0.028	0.025	0.939	0.889	0.992
Psych Meds	0.968	0.541	0.074	2.633	0.912	7.606
GH Duration	0.064	0.050	0.204	1.066	0.966	1.177
GH Use	-0.014	0.490	0.977	0.986	0.377	2.577
Age at V1 (cont.)	-0.034	0.018	0.055	0.966	0.933	1.001
Psych Meds	1.508	0.428	0.000	4.520	1.954	10.453
GH Duration (cont.)	0.089	0.046	0.054	1.093	0.998	1.198
GH*UPD	-1.556	0.839	0.064	0.211	0.041	1.093
Genotype	1.884	0.706	0.008	6.581	1.650	26.245

(mUPD)						
GH Use	1.377	0.485	0.005	3.961	1.530	10.253
Psych Meds	1.061	0.403	0.008	2.890	1.312	6.366
GH Use	1.005	0.510	0.049	2.733	1.006	7.426
Age at V1 (cont.)	-0.042	0.019	0.025	0.959	0.924	0.995
Psych Meds	1.355	0.439	0.002	3.879	1.640	9.174
Genotype (mUPD)	2.024	0.738	0.006	7.567	1.781	32.146
GH* mUPD	-1.849	0.880	0.036	0.157	0.028	0.883
GH Use	0.537	0.572	0.0348	1.711	0.557	5.249
Age at V1 (cont.)	-0.038	0.019	0.044	0.963	0.928	0.999
Psych Meds	1.399	0.444	0.002	4.052	1.699	9.665
Genotype (mUPD)	2.005	0.734	0.006	7.424	1.760	31.309
GH* mUPD	-1.885	0.882	0.033	0.152	0.027	0.855
GH Duration	0.082	0.046	0.077	1.086	0.991	1.189

Dependent Variable: SKIN PICKING (n=127)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1 (cont.)	0.035	0.019	0.059	1.036	0.999	1.075
GH Use	-0.66	0.419	0.116	0.517	0.227	1.176
Age at GH Initiation (cont.)	0.036	0.03	0.221	1.037	0.978	1.1
Psych Meds	0.762	0.404	0.059	2.143	0.971	4.731
Genotype (DEL)	0.971	0.374	0.009	2.640	1.269	5.492
Sex of Participant	-0.187	0.357	0.601	0.830	0.412	1.669
Sex Hormones	0.039	0.361	0.913	1.040	0.513	2.110
GH Duration	-0.065	0.047	0.169	0.937	0.854	1.028
MULTIVARIATE ANALYSES						
GH Use	-0.345	0.472	0.464	0.708	0.281	1.785
Age at V1 (cont.)	0.028	0.021	0.174	1.029	0.988	1.072
GH Use	-0.603	0.423	0.155	0.547	0.239	1.255
Psych Meds	0.718	0.407	0.078	2.05	0.923	4.552

GH Use	-0.393	0.472	0.405	0.675	0.267	1.704
Age at V1 (cont.)	0.02	0.021	0.339	1.02	0.979	1.063
Psych Meds	0.619	0.42	0.14	1.858	0.816	4.231
GH Use	-0.982	0.471	0.037	0.374	0.149	0.942
Genotype (DEL)	1.053	0.383	0.006	2.867	1.353	6.073
GH Use	-0.909	0.474	0.055	0.403	0.159	1.021
Genotype (DEL)	1.088	0.387	0.005	2.970	1.390	6.343
Psych Meds	0.630	0.427	0.140	1.877	0.813	4.334
GH Use	-0.051	0.574	0.929	0.950	0.309	2.927
Age at V1 (cont.)	0.017	0.021	0.426	1.017	0.976	1.060
Psych Meds	0.630	0.421	0.134	1.878	0.823	4.284
GH Duration (cont.)	-0.050	0.045	0.266	0.952	0.872	1.039
GH Use	-0.688	0.530	0.194	0.503	0.178	1.420
Age at V1 (cont.)	0.020	0.023	0.382	1.020	0.976	1.067

Psych Meds	0.537	0.441	0.223	1.710	0.721	4.056
Genotype (DEL)	1.045	0.390	0.007	2.842	1.322	6.108
GH Use	-0.275	0.700	0.695	0.760	0.193	2.996
Age at V1 (cont.)	0.023	0.023	0.327	1.023	0.978	1.070
Psych Meds	0.554	0.440	0.208	1.740	0.735	4.121
Genotype (DEL)	1.746	0.917	0.057	5.730	0.951	34.542
GH*DEL	-0.879	1.019	0.388	0.415	0.056	3.059
GH Use	-0.660	0.530	0.213	0.517	0.183	1.461
Genotype (DEL)	0.997	0.386	0.010	2.711	1.273	5.775
Age at V1	0.027	0.023	0.229	1.028	0.983	1.075
Genotype (DEL)	1.027	0.380	0.007	2.793	1.326	5.883
Psych Meds	0.720	0.421	0.087	2.055	0.900	4.692
GH*DEL	-0.742	1.004	0.460	0.476	0.067	3.406
Genotype (DEL)	1.684	0.908	0.063	5.389	0.910	31.916
GH Use	-0.591	0.626	0.346	0.554	0.162	1.890
Psych Meds	0.649	0.428	0.129	1.913	0.827	4.424

Dependent Variable: NAIL PICKING (n=61)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	-0.004	0.015	0.796	0.996	0.967	1.026
GH Use	0.195	0.381	0.61	1.215	0.575	2.565
Age at GH Init.	-0.062	0.034	0.067	0.94	0.88	1.004
Psych Meds	0.028	0.377	0.941	1.028	0.491	2.151
Genotype (DEL)	0.400	0.386	0.300	1.491	0.700	3.179
Sex of Participant	0.297	0.350	0.396	1.346	0.678	2.674
Sex Hormones	-0.085	0.353	0.809	0.918	0.460	1.834
GH Duration (cont.)	0.028	0.033	0.396	1.029	0.964	1.098
MULTIVARIATE ANALYSES						
GH Duration	-0.019	0.056	0.738	0.982	0.880	1.095
Age at GH Init	-0.068	0.039	0.083	0.934	0.865	1.009
Age at GH init.	-0.118	0.057	0.039	0.888	0.794	0.994
Age at V1	0.063	0.051	0.212	1.065	0.965	1.177

GH Duration	-0.035	0.057	0.539	0.965	0.863	1.080
Age at GH Init.	-0.136	0.065	0.037	0.873	0.769	0.992
Age at V1	0.071	0.053	0.183	1.073	0.967	1.191

Dependent Variable: COMPULSIVE COUNTING (n=28)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	0.013	0.017	0.56	0.454	0.979	1.048
GH Use	-0.114	0.445	0.798	0.892	0.373	2.137
Age at GH Initiation (cont.)	-0.024	0.036	0.501	0.976	0.911	1.047
Psych Meds	0.225	0.427	0.597	1.253	0.543	2.891
Genotype (DEL)	0.179	0.444	0.688	1.195	0.501	2.854
Sex of Participant	-0.405	0.416	0.330	0.667	0.295	1.507
Sex Hormones	-0.408	0.440	0.354	0.665	0.281	1.575
GH Duration (cont.)	-0.006	0.040	0.873	0.994	0.919	1.075

MULTIVARIATE ANALYSES						
GH Use	0.058	0.512	0.91	1.060	0.388	2.893
Age at V1	0.014	0.02	0.479	1.014	0.975	1.054

Dependent Variable: COMPULSIVE ORDERING (n=68)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	-0.016	0.015	0.275	0.984	0.957	1.013
GH Use	0.251	0.35	0.474	1.285	0.647	2.554
Age at GH Initiation (cont.)	0.000	0.023	0.985	1.000	0.957	1.046
Psych Meds	-0.376	0.338	0.267	0.687	0.354	1.332
Genotype (mUPD)	0.112	0.340	0.741	1.119	0.575	2.177
Sex of Participant	-0.028	0.317	0.930	0.973	0.522	1.810
Sex Hormones	0.101	0.321	0.754	1.106	0.590	2.074
GH Duration (cont.)	0.021	0.224	0.491	1.021	0.962	1.083

Dependent Variable: PLAYS WITH STRINGS (n=34)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	-0.013	0.018	0.473	0.987	0.953	1.023
GH Use	0.056	0.42	0.893	1.058	0.464	2.412
Age at GH Initiation (cont.)	-0.050	0.038	0.186	0.952	0.884	1.024
Psych Meds	-0.325	0.417	0.436	0.723	0.319	1.636
Genotype (DEL)	0.185	0.426	0.663	1.204	0.523	2.772
Sex of Participant	-0.419	0.385	0.278	0.658	0.309	1.401
Sex Hormones	-0.765	0.426	0.073	0.466	0.202	2.073
GH Duration (cont.)	0.069	0.036	0.055	1.072	0.998	1.151
MULTIVARIATE ANALYSES						
GH Duration	0.193	0.065	0.003	1.213	1.067	1.379
Sex Hormones	-0.980	0.547	0.074	0.375	0.128	1.098
GH Duration	0.197	0.069	0.004	1.218	1.064	1.394
Sex Hormones	-1.007	0.567	0.076	0.365	0.120	1.111

Age of GH Initiation (cont.)	0.007	0.039	0.851	1.007	0.934	1.087
GH Duration	0.196	0.069	0.005	1.216	1.063	1.392
Sex Hormones	-1.020	.0569	0.073	0.361	0.1188	1.100
Age of GH Init	0.005	0.039	0.895	1.005	0.931	1.085
Psych Meds	0.161	0.561	0.774	1.175	0.391	3.529
GH Duration	0.256	0.095	0.007	1.292	1.073	1.556
Sex Hormones	-0.984	0.574	0.087	0.374	0.121	1.152
Age of GH Init	0.111	0.105	0.288	1.118	0.910	1.373
Psych Meds	0.089	0.569	0.876	1.093	0.359	3.330
Age at V1	-0.107	0.099	0.277	0.898	0.740	1.090
GH Duration	0.263	0.098	0.007	1.301	1.073	1.577
Sex Hormones	-0.779	0.583	0.182	0.459	0.146	1.439
Age of GH Initiation (cont.)	0.123	0.106	0.244	1.131	0.919	1.392
Psych Meds	-0.057	0.599	0.925	0.945	0.292	3.057

Age at V1	-0.124	0.101	0.218	0.883	0.724	1.076
Genotype (DEL)	0.710	0.583	0.224	2.033	0.648	6.378
GH Use	-1.004	0.664	0.130	0.366	0.100	1.346
GH Duration (cont.)	0.121	0.053	0.023	1.128	1.017	1.251
Psych Meds	-0.283	0.440	0.520	0.753	0.318	1.784
Age at V1 (cont.)	-0.005	0.021	0.798	0.995	0.954	1.037

Dependent Variable: VISUAL HALLUCINATIONS (n=6)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1	-0.077	0.063	0.222	0.926	0.819	1.047
GH Use	0.839	1.109	0.449	2.315	0.263	20.336
Age at GH Init.	-0.078	0.091	0.386	0.925	0.774	1.104
Psych Meds	0.655	0.834	0.432	1.926	0.376	9.866
Genotype (mUPD)	1.109	0.929	0.233	3.030	0.490	18.720
Sex of Participant	0.515	0.881	0.558	1.674	0.298	9.407

Sex Hormones	-18.473	4985.324	0.997	0.000	0.000	
GH Duration (cont.)	0.032	0.078	0.687	1.032	0.885	1.203

Dependent Variable: DELUSIONS (n=11)						
					95% CI	
Independent Variable	**Coefficient (B)**	**SE (coefficient)**	**Significance (p)**	**Exp(B)**	**Lower**	**Upper**
UNIVARIATE ANALYSES						
Age at V1 (cont.)	0.023	0.024	0.344	1.023	0.976	1.072
GH Use	1.58	1.063	0.137	4.854	0.605	38.981
Age at GH Init.iation (cont.)	0.039	0.033	0.242	1.04	0.974	1.109
Psych Meds	1.253	0.65	0.054	3.5	0.979	12.508
Genotype (mUPD)	0.440	0.693	0.526	1.552	0.339	6.037
Sex of Participant	-0.379	0.627	0.545	0.685	0.200	2.339
Sex Hormones	0.610	0.628	0.331	1.840	0.538	6.299
GH Duration (cont.)	0.036	0.059	0.537	1.037	0.924	1.164
MULTIVARIATE ANALYSES						
GH Use	1.781	1.074	0.097	5.935	0.723	48.718
Psych Meds	1.411	0.66	0.033	4.100	1.124	14.954

GH Use	2.525	1.203	0.036	12.489	1.182	132.013
Age at V1 (cont.)	0.061	0.029	0.033	1.063	1.005	1.124
GH Use	2.64	1.228	0.032	14.013	1.262	155.638
Psych Meds	1.228	0.675	0.069	3.414	0.909	12.818
Age at V1 (cont.)	0.054	0.03	0.077	1.055	0.994	1.12
Psych Meds	1.308	0.655	0.046	3.699	1.024	13.361
GH Duration (cont.)	0.049	0.059	0.409	1.050	0.935	1.178
Age at V1 (cont.)	0.034	0.026	0.186	1.034	0.984	1.088
GH Duration (cont.)	0.068	0.063	0.277	1.070	0.947	1.210
GH Use	2.644	1.272	0.038	14.066	1.162	170.275
GH Duration (cont.)	-0.001	0.069	0.991	0.999	0.874	1.143
Psych Meds	1.227	0.676	0.069	3.412	0.908	12.830

Age at V1 (cont.)	0.054	0.031	0.083	1.055	0.993	1.121
GH Use	3.064	1.339	0.022	21.418	1.553	295.392
Age at V1 (cont.)	0.081	0.034	0.017	1.085	1.015	1.160
Psych Meds	1.328	0.766	0.083	3.774	0.842	16.925
Genotype (mUPD)	1.005	0.783	0.200	2.731	0.588	12.674

8 Appendix C

August 22, 2018

VIRGINIA E. KIMONIS
PEDIATRICS

RE: HS# 2007-5605 *Prader-Willi Syndrome and Early-onset Morbid Obesity Natural History Clinical Protocol*

Modification Application # 24259

The following modification(s) for the human subjects research protocol referenced above has/have been reviewed and approved by Human Research Protections Staff, on behalf of the UC Irvine Institutional Review Board (UCI IRB). Below is a summary of the approved changes requested via modification application number 24259**:

Change in Personnel:
Add: ANDREA MONTES as RP
Reason: Andrea Montes, BA, is a researcher who graduated from Scripps College in 2011 with a degree in Human Biology. She will be involved in data analysis and interpretation of research data and assist in manuscript preparation under the supervision of Dr. Kimonis.

**Changes to approved protocols may not be made without prior approval. All changes proposed in the modification application may not have been approved. Review the above summary of approved changes and the approved documents released with this letter. If a requested change does not appear in the summary above or in the revised documents, the change was not approved. Please consult with an IRB Administrator for further information.

Note: If the approved modification(s) includes changes to the informed consent document, the approved stamped consent document will be released with this letter. Please discontinue use of any previous versions of the informed consent document and use only the most updated version for enrollment of all new subjects. Questions concerning registration of this study or approval of this modification request may be directed to the UC Irvine Office of Research, 141 Innovation Drive, Suite 250, Irvine CA 92697-7600; 949-824-6068 or 949-824-2125 (biomedical committee) or 949-824-6662 (social-behavioral committee).

Level of Review: Administrative Review

William Kettler
IRB Analyst
Approval Issued: 8/15/2018
Expiration Date: 1/16/2019

UCI (FWA) 00004071, Approved: January 31, 2003