

# Novel therapies in the treatment of Prader-Willi syndrome

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UNIVERSITY OF ZAGREB  
SCHOOL OF MEDICINE

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**Novel therapies in the Prader-Willi syndrome**

**GRADUATE THESIS**



Zagreb, 2020

This graduate thesis was made at the Department of Pediatrics, University Hospital Centre Zagreb, mentored by dr. sc. Mario Ćuk and was submitted for evaluation in the academic year 2019/2020.

**Abbreviations:**

ASD – autism spectrum disorder  
CFE – Caralluma fimbriata extract  
GH – growth hormone  
IQ – intelligence quotient  
IU – international units  
KO – knock-out  
OXT – oxytocin  
PWS – Prader Willi syndrome

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## SUMMARY

### **Novel therapies in the Prader-Willi syndrome**

**Petra Sulić**

Prader–Willi syndrome (PWS) is a rare and complex genetically determined neurodevelopmental disorder. PWS. It is caused by the loss of function of paternal genes in a particular region of chromosome 15q11.2-q13. It is the most common genetic cause of life-threatening obesity in humans. It occurs in 1 in 15,000 to 1 in 30,000 infants, and both sexes are affected equally. PWS is characterized by severe hypotonia and feeding difficulties in early infancy, followed by excessive eating and gradual development of morbid obesity in early childhood, together with a series of comorbidities including short stature, typical facial dysmorphism, psychomotor delay, behavioral abnormalities, and cognitive impairment. Obesity is the reason why this syndrome is related to many difficulties. When PWS was discovered, a child with PWS had small or no chance for survival. Today, the PWS diagnosis can be confirmed as early as in the first month of life using DNA testing (analysis). The cure for PWS is still not found; however, some medications and procedures can reduce the incidence of physical symptoms of the disease and significantly improve the quality of life. Up to date, several kinds of treatment have been used, such as growth hormone therapy, nutritive measures, sex hormone replacement, thyroid hormone replacement, and promotion of psychomotor development. Although no drugs have proven effective in controlling appetite and weight gain in PWS, there are reasons to be hopeful. Many anti-obesity medicines and other therapeutic modalities currently in clinical development, described in this paper, can offer new therapeutic options for individuals with PWS. The most promising results were noted in treatment with neuropeptide oxytocin, its analog carbetocin, and Indian cactus succulent *Caralluma fimbriata* extract (CFE). With early diagnosis and improved new treatment, the quality of life of individuals with PWS can be improved, severe health complications prevented, and life expectancy prolonged.

Keywords: Prader-Willi syndrome, hyperphagia, obesity, oxytocin, novel therapy

## SAŽETAK

### Nove terapije u Prader-Willi sindromu

**Petra Sulić**

Prader-Willi sindrom (PWS) je rijedak i složen genetički određen neurorazvojni poremećaj. Nastaje zbog gubitka funkcije očevih gena u određenoj regiji kromosoma 15q11.2-q13. Najčešći je genetski uzrok pretilosti opasne po život. Javlja se u 1 na 15 000 do 1 na 30 000 novorođenčadi te su oba spola podjednako pogođena. PWS karakteriziraju jaka hipotonija i poteškoće s hranjenjem u ranoj dojenačkoj dobi, praćeno prekomjernom prehranom i postupnim razvojem morbidne pretilosti u ranom djetinjstvu, zajedno s nizom komorbiditeta uključujući kratki rast, tipični faktor dismorfizma, psihomotorno kašnjenje, poremećaje ponašanja i oštećenje kognitivnih funkcija. Razlog povezanosti ovog sindroma s mnogim poteškoćama je pretilost. U vrijeme kada je PWS otkriven, šanse za preživljavanjem su bile male ili nikakve. Danas se dijagnoza PWS-a može potvrditi već u prvom mjesecu života primjenom DNK testiranja (analize). Lijek za PWS još uvijek nije pronađen, međutim postoje lijekovi i postupci koji mogu smanjiti učestalost fizičkih simptoma bolesti i tako uvelike poboljšati kvalitetu života. Danas postoji nekoliko vrsta liječenja, poput terapije hormonom rasta, prehrambenim mjerama, nadomještanjem spolnih hormona i hormona štitnjače, te promicanjem psihomotornog razvoja. Iako se do danas niti jedan lijek nije pokazao učinkovitim u kontroli apetita i regulacije povećanja tjelesne težine, nova istraživanjima ulijevaju nadu. Mnogi lijekovi i drugi terapijski modaliteti protiv pretilosti na kojima se trenutačno provode istraživanja će nadamo se ponuditi nove terapijske mogućnosti za osobe sa PWS-om.

Najperspektivniji rezultati zabilježeni su u liječenju neuropeptidnim hormonom oksitocinom, ili njegovim analogom, karbetocinom i ekstraktom indijskog kaktusa *Caralluma fimbriata* (CFE). Uz ranu dijagnozu i uznapredovalo liječenje mogu se spriječiti ozbiljne zdravstvene komplikacije, poboljšati kvaliteta života i produžiti životni vijek osobe s PWS-om.

Ključne riječi: Prader-Willijev sindrom, hiperfagija, pretilost, oksitocin, nova terapija

## 1. INTRODUCTION

Prader–Willi syndrome (PWS), also known as Prader-Willi-Labhart syndrome, is a rare and complex genetically determined neurodevelopmental disorder. It is characterized by severe hypotonia and feeding difficulties in early infancy, followed by excessive eating and gradual development of morbid obesity in early childhood, together with a series of comorbidities including short stature, typical facial dysmorphism, psychomotor delay, behavioral abnormalities and cognitive impairment (1). PWS is caused due to genetic abnormalities that result in the absence of expression of one or more genes at the locus q11–q13 on chromosome 15 or by maternal uniparental disomy. In 1887, Langdon-Down described the first female child with probable PWS, manifested by mental impairment, short stature, hypogonadism, and obesity; he termed the condition polysarcia (2). Seventy years later, the Swiss pediatricians Prader, Labhart, and Willi first described a group of children with what subsequently came to be called PWS (3). In 1981, Ledbetter et al, identified microdeletions within chromosome 15 as the site for PWS (4).

Prader-Willi syndrome is the most common genetic cause of life-threatening obesity in humans (5). It occurs in 1 in 15,000 to 1 in 30,000 infants (6), and it is estimated that there are 350,000–400,000 people with this syndrome worldwide (5). Both sexes are affected equally (7). The prevalence of PWS is higher in populations referred for the key clinical features. One study reported PWS in 11 percent of infants referred for hypotonia (8). The prevalence of PWS among individuals with an intellectual disability is less than 1 percent (9).



## **2. ETIOLOGY AND GENETICS**

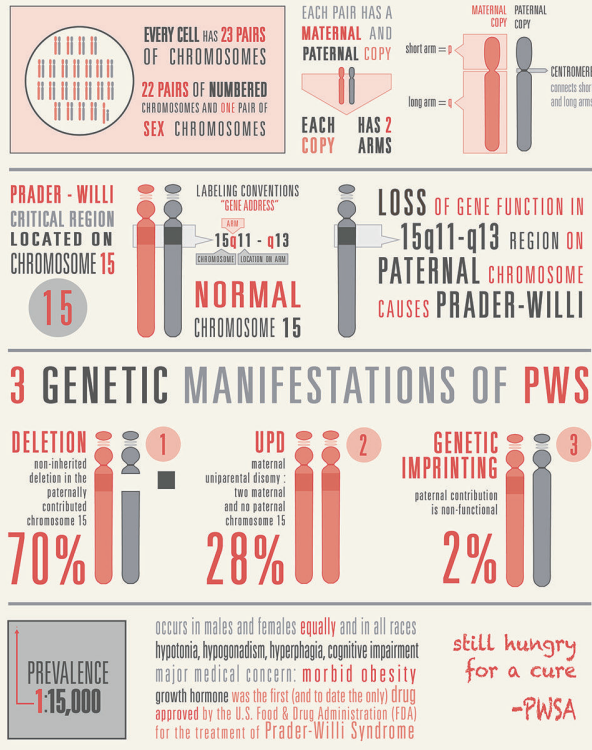
### **2.1. ETIOLOGY**

The etiology of the disease is still unknown, and the vast majority of cases occur sporadically, although multiple cases in the same family have been reported (10).

### **2.2. GENETICS**

Prader-Willi syndrome, along with Angelman syndrome, is particularly important because it represents the first evidence of genomic imprinting, meaning that the expression of the gene depends on the gender of the parent donating the gene (5, 11). Deletion of paternal 15q11.2-13 accounts for 70 percent of cases, while 28 percent of cases arise from maternal uniparental disomy (Figure 1). In the most recent series, the proportion caused by maternal uniparental disomy was as high as 50 percent, which was attributed to advanced maternal age as well as advanced molecular diagnostic techniques (12,13). Defects in the imprinting center, mostly epimutations, cause approximately 2 percent of cases, while less than 0.5 percent is caused by deletions. In extremely rare cases, PWS can also be caused by balanced translocation (14). The association between the type of genetic defect and the phenotypic features has been established. Individuals with deletions generally have more distinct physical features, more severe behavioral problems, and lower intelligence quotients (IQs) than individuals with PWS caused by uniparental disomy. Nevertheless, individuals with uniparental disomy are more likely to exhibit autistic-like behaviors and psychosis (1,15). Determining the type of mutation is important for establishing the diagnosis of PWS as well as for establishing the risk of recurrence in future pregnancies. The risk of recurrence is low if the affected child has deletion, maternal disomy, or an imprinting defect without a deletion, less than 1 percent. Contrary, two rare molecular defects, a deletion of the imprinting control center and parental chromosomal rearrangement, are associated with a higher recurrence risk; 50 and 25 percent, respectively (1,16).

# GENETIC CAUSES OF PRADER-WILLI SYNDROME



**Figure 1.** Genetic causes of Prader-Willi syndrome.

(taken from <https://www.pwsausa.org/>)

### **3. MOLECULAR PATHOGENESIS**

The major manifestations of PWS are caused by paternal deficiency for SNORD116-1 (HBII-85) “small nucleolar RNA” (snoRNA) cluster (17,18). Small nucleolar RNAs (snoRNAs) are a class of small RNA molecules that primarily guide chemical modifications of other RNAs, mainly ribosomal RNAs, transfer RNAs, and small nuclear RNAs. The modifications include methylation, which is the mechanism for sex-specific imprinting. It has not yet been established by which precise mechanisms HBII-85 deletions cause the clinical features of PWS.

## **4. CLINICAL FEATURES**

### **4.1. PRENATAL**

Affected pregnancies often exhibit reduced fetal activity, polyhydramnios, breech positioning, small size for gestational age, and asymmetrical intrauterine growth (increased head:abdomen circumference ratio) (19). Third-semester ultrasounds may show unusual positioning of the fetal hands and feet, with flexed wrists and dorsi-extended feet with flexed toes (20). The majority of the patients are born by caesarean section (21).

## 4.2. INFANCY

Newborns with PWS have lower birth weight and length compared to siblings by 15 to 20 percent. One of the hallmark features of this disorder is neonatal hypotonia, which is also an important clue to initiate diagnostic testing. The profound hypotonia can lead to asphyxia. In addition, lethargy is also present with reduced spontaneous arousal, weak crying, and weak reflexes (22,23). An important early symptom is an infant's inability to suck, which leads to that nearly all infants with PWS need help with feeding. Failure to thrive due to hypotonia and difficulty feeding usually recede between 6 and 12 months of age. Another common feature is genital hypoplasia (e.g. cryptorchidism, clitoral hypoplasia, or scrotal hypoplasia). Hypopigmentation of the skin, eyes (iris), and hair relative to the familial background are present in 30 to 50 percent of patients (24,25) (Figure 2).



**Figure 2.** Infant with PWS  
(taken from <http://www.pws.hr/>)

### 4.3. EARLY CHILDHOOD

As the infants grow into toddlers and children, poor feeding and feeding support is replaced with compulsive overeating. Between ages 3 and 6, symptoms of hyperphagia start to manifest with the progressive development of obesity. At that time, access to food needs to be restricted, and caloric intake reduced, often to 60 percent of the caloric requirement of comparably sized children without the syndrome. Body composition is abnormal, with reduced lean body mass and increased fat mass as compared with normal and obese controls (26, 27). Short stature is present during childhood, and most patients with PWS have growth hormone (GH) deficiency. Toddlers with PWS demonstrate late acquisition of major motor milestones. At the age of 12 months, they start to sit on their own and usually begin to walk around the age of two. At 36 months of age, they adopt a dozen words, and intellectual limitation becomes apparent by school age.

#### 4.4. LATE CHILDHOOD AND ADOLESCENCE

Secondary sexual characteristics are generally delayed, or incomplete, however pubic and axillary hair may arise prematurely due to adrenarche. Testicular descent can occur late as adolescence, and menarche even later, some cases reporting in the late 20s or even 30s. Other complications of obesity like cor pulmonale, diabetes mellitus, sleep apnea, and atherosclerosis are common. Hypogonadism causing osteoporosis, and behavioral issues are often recorded. Up to 25 percent of patients with PWS have epilepsy, usually focal (e.g., Staring spells) (28). Scoliosis is seen frequently, with a reported prevalence of 37 percent, of which 13 percent require brace treatment surgery (29) (Figure 3).



**Figure 3.** Adolescent with PWS

(patient Albina Velija, taken with permission from parents and dr. sc. Mario Ćuk)

#### 4.5. ADULTHOOD

Individuals with PWS almost never reach a normal average height in adulthood. Constant monitoring of food intake and comorbidities present make them not able to live independently. Most of them live in a family home with constant parental care or in an institutional accommodation. In the past, many of the deaths were attributable to obesity, and its complications (e.g., cardiovascular problems, diabetes mellitus, and sleep apnea) and survival after age 50 was uncommon (30). Life expectancy has somewhat improved due to advances in the care of the patients. One case series describes 12 individuals older than 50 years of age (31). Most of these individuals had experienced a decline in physical and psychological function with advancing age. A separate case series of 26 individuals older than 40 years of age found evidence of dementia in four (15 percent) (32). All four patients with early onset dementia were female, had a long history of psychotic illness, and had maternal uniparental disomy (UPD).

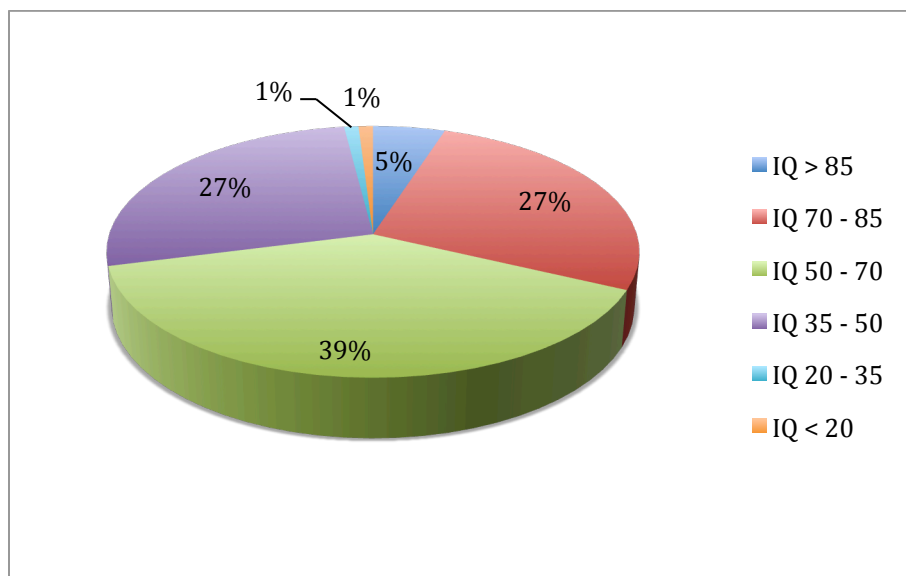
#### 4.6. PHYSICAL APPEARANCE

Individuals with PWS have prominent nasal bridge, small hands and feet with tapering of fingers, soft and easily bruised skin, high narrow forehead, excess fat (especially in the central portion of the body), thin upper lip, almond-shaped eyes. They frequently pick their skin and have stretch marks. Lack of complete sexual development accompanies delayed motor development.



#### 4.7. NEUROCOGNITIVE DISABILITY

Individuals with PWS have varying levels of intellectual disability. Most individuals (50 to 65 percent) fall within the mild/borderline/low average intelligence range (33,34) (Figure 4). Children with PWS show an unusual cognitive profile, often strong in visual organization and perception (reading and vocabulary), but their spoken language is generally poorer. Auditory information processing and sequential processing are relatively poor, as are arithmetic and writing skills, visual and auditory short-term memory, and auditory attention span (35). PWS may be associated with psychosis (36).



**Figure 4.** Display of IQ in PWS. (taken from Curfs LM, Fryns JP. Prader-Willi syndrome: a review with special attention to the cognitive and behavioral profile. Birth defects original article series. 1992;28(1):99-104.)

#### 4.8. BEHAVIORAL DEFECTS

The majority of PWS patients are associated with a constant, insatiable, extreme appetite, which persists no matter how much patients eat. The strict limit of patients' access to food is needed, usually by installing locks on refrigerators and cabinets where the food is stored. It is hypothesized that the genetic abnormalities in chromosome 15 disrupt the normal functioning of the hypothalamus (37). Hypothalamic arcuate nucleus regulates many basic processes, including appetite, links extreme hunger and PWS. Nerve cells that produce oxytocin, a hormone thought to contribute to satiety, is found to be abnormal in PWS patients. Patients with PWS have high ghrelin levels, which are thought to directly contribute to the increased appetite, hyperphagia, and obesity seen in this syndrome (38). The main mental health difficulties experienced by people with PWS include compulsive behavior (usually manifested in skin picking) and anxiety (35,39). Psychiatric symptoms like hallucinations, paranoia, and depression, have been described in some cases (35) and affect about 5–10% of young adults (37). Typically, 70–90% of affected individuals develop behavioral patterns in early childhood (34). Aspects of these patterns can include stubbornness, temper tantrums, controlling and manipulative behavior, difficulty with change in routine, and compulsive-like behaviors. Psychiatric and behavioral problems are the most common cause of hospitalization (40).

## 4.9. ENDOCRINOLOGIC DEFECTS

### 4.9.1. GROWTH HORMONE DEFICIENCY

Short stature is often observed during early childhood, and without recombinant human growth hormone therapy, the final height in men is on average 155 cm, and 148 cm in women. The lack of pubertal growth momentum due to hypogonadism and hypothyroidism also contributes to the reduced growth (41).

### 4.9.2. HYPOTHYROIDISM

Twenty to thirty percent of children under the age of two have central hypothyroidism with low levels of free thyroxine and normal or decreased levels of thyroid-stimulating hormone (42).

### 4.9.3. HYPOGONADISM

Hypogonadism is a consistent feature of both males and females with PWS. Genital hypoplasia is evident at birth. In females, it manifests as clitoral and labia minora hypoplasia and can easily be overlooked on physical examination. Males commonly have cryptorchidism, a poorly rugated, under pigmented, hypoplastic scrotum, and may have a small penis. Unilateral or bilateral cryptorchidism is present in 80-90% of males' (22).

### 4.9.4. ADRENAL INSUFFICIENCY

Central adrenal insufficiency occurs in PWS, but the frequency is unclear. Children and adults with PWS are at risk for adrenal insufficiency due to the generalized hypothalamic dysfunction. Depending on the frequency of the stimulation test applied, the incidence of central adrenal insufficiency is 14-60%. There are no clear recommendations on when and how to assess the adrenal gland function or when to start the treatment.

#### 4.10. OPHTHALMOLOGIC DEFECTS

Strabismus is commonly associated with PWS. In one study, over 50 percent of patients had strabismus, mainly esotropia (43).

#### 4.11. SCOLIOSIS

About 40–80% of patients with PWS have scoliosis whose prevalence increases with age as a result of obesity, prolonged paravertebral muscle hypotonia, and bone dysplasia (22). Growth hormone therapy does not worsen scoliosis, and their association has not been confirmed, but it is recommended that an orthopedic examination be performed before the start of therapy and, if necessary, an X-ray of the spine performed (44). Scoliosis is one of the risk factors for nocturnal hypoventilation with obesity and hypotension of the respiratory muscles (45).

## 5. DIAGNOSTICS

Until recently, PWS was mostly diagnosed at puberty. Nowadays, the diagnosis can be made in the first month of life by DNA genetic analysis, which allows for early intervention as well as early prescribing of growth hormone.

### 5.1. MOLECULAR DIAGNOSTICS

The genetic test of choice is DNA methylation analysis of the critical region for Prader-Willi syndrome, which confirms the diagnosis in 99% of patients. Methylation analysis is performed using Southern blot or PCR specific for methylation, at the 5' end of the SNURF-SNRPN locus, or at the promoter and first exon of this gene, i.e., the PWS-SRO region. Since methylation analysis does not reveal molecular etiology, further testing will determine molecular genetic subtype. In 65-75% of patients, fluorescent in situ hybridization (FISH) will confirm the deletion of the paternal chromosome in the 15q11,2-q13 region. For more reliable prognostic information and genetic counseling on the risk of recurrence of the same disease in the family, it is essential to identify the molecular etiology as accurately as possible.

### 5.2. CLINICAL DIAGNOSTICS

The diagnosis of PWS is suspected in patients who have characteristic clinical features and is confirmed by genetic testing. Clinical diagnostic criteria were developed in 1993 (46) (Table 1) containing 8 major and 11 minor criteria, each worth 1 and 1/2 points, respectively. In order to make a clinical diagnosis according to this criteria, for age up to three years, 5 points are needed, of which 4 from the group of large criteria, and in those older than three years 8 points are needed, 5 of which from the group of large criteria. However, now that definitive testing is available, it is appropriate to use less rigid clinical criteria to determine who should undergo genetic testing.

**Table 1.** Clinical diagnostic criteria for Prader-Willi syndrome (from Holm VA, Cassidy SB, Butler MG, et al. Prader-Willi syndrome: consensus diagnostic criteria. *Pediatrics* 1993; 91:398.)

Major criteria (1 point)	Minor criteria (1/2 points)
Neonatal and infantile central hypotonia with poor suck	Decreased fetal movement or infantile lethargy
Feeding problems in infancy with a need for special feeding techniques and poor weight gain/failure to thrive	Characteristic behavior problems
Excessive or rapid weight gain after 12 months but before 6 years of age	Sleep disturbance or sleep apnea
Characteristic facial features	Short stature for genetic background by age 15
Hypogonadism - genital hypoplasia/delayed or incomplete gonadal maturation	Hypopigmentation—fair skin and hair compared to family
Global developmental delay	Small hands and/or feet for height age
Hyperphagia/food foraging/obsession with food	Narrow hands with straight ulnar border
Deletion 5q11–13 or other cytogenetic/molecular abnormality of the Prader-Willi chromosome region, including maternal disomy	Eye abnormalities (esotropia, myopia)
	Thick viscous saliva
	Speech articulation defects
	Skin picking

## 6. TREATMENT TO DATE

The cure for PWS is still not found; however, some medications and procedures can reduce the incidence of physical symptoms of the disease and greatly improve the quality of life. Patient care requires a multidisciplinary approach involving professionals from various fields such as a primary pediatrician, geneticist, endocrinologist, nutritionist, physiatrist, speech therapist, psychologist, psychiatrist, and other subspecialists to prevent the development of severe obesity and treat associated morbidities.

### 6.1. NUTRITIVE MEASURES

During the first months of life, special feeding techniques are usually used due to severe hypotonia, which interferes with sucking and swallowing and prevents weight gain. Placement of a nasogastric tube or the use of special pacifiers are the most commonly used techniques. Frequent weight checks help adjust infant's diet to maintain a proper weight gain. However, this condition improves significantly over several months, and solid food intake becomes regular (47). From the moment the diagnosis is confirmed, without waiting for the development of a typical clinical picture, it is necessary to start with restrictions and strict monitoring of the diet since severe obesity is one of the biggest problems associated with this syndrome. Better control of obesity is also contributed by daily physical activity appropriate to age and degree of obesity by increasing muscle mass and strength and energy expenditure.

### 6.2. GROWTH HORMONE THERAPY

Children with PWS and growth failure are candidates for treatment with recombinant growth hormone (GH). Growth failure is typically defined by the standards used for children without PWS as decreased height velocity or decreased height compared to the mid-parental height prediction. The optimal time to introduce GH therapy is not defined, but early initiation (e.g., before two years of age) improves clinical outcomes, and most experts suggest it (48). The therapy is started after an extensive check of the child's health condition and in agreement with the pediatric endocrinologist and patients' family. The initial dose is 0.5 mg / m<sup>2</sup> / day with a gradual increase to 1 mg / m<sup>2</sup> subcutaneously in the evening (49,50). Many studies have investigated the effect of GH administration on

linear growth, body composition, and bone density in children with PWS, and virtually all have demonstrated beneficial effects (51-54). The positive effect of GH therapy in patients with PWS is manifested by accelerated growth, greater muscle strength, reduced fat content, and improved functioning of the entire metabolism, which ultimately greatly improves the quality of life of patients' (55,56). GH also improves the lipid profile and measures of physical function (57-59), and several studies suggest that it also has benefits on cognitive and motor development (60,61). The response to GH in children with PWS is greatest during the first 12 months of therapy (48). Some studies report a connection between GH therapy and an increased number of sudden deaths in treated individuals due to tonsillar and surrounding soft tissue hypertrophy, which may cause obstructive sleep apnea. Therefore, before the introduction of GH therapy, it is necessary to perform an all-night polysomnographic recording to assess sleep-disordered breathing and to repeat the recordings in the first year of treatment, and later only according to clinical indications (45, 62). However, other studies have not confirmed the connection, and there is currently no direct evidence of a causal relationship between growth hormone and respiratory problems in individuals with PWS (63).

### 6.3. THYROID HORMONE REPLACEMENT

Hypothyroidism is more common at an early age, and despite the neonatal screening, thyroid function should be monitored. Monitoring should start in the third month of life by determining free thyroxin and thyroid stimulating hormone and then repeated once a year, especially in patients on GH therapy. In case of hypothyroidism, levothyroxine supplementation of 1–2 µg / kg per day is introduced (64,65).

### 6.4. SEX HORMONE REPLACEMENT

In girls, treatment is started around the age of 12 with 17β estradiol 0.25 mg/day orally or 14 µg / day transcutaneous with a patch administered twice a week. The dose is gradually increased to an adult dose of 2-4 mg/day orally or 100-200 µg/day by transcutaneous preparation. Progestin is added at a dose of 200 mg / day orally for 10th–14th day of the cycle when breakthrough bleeding occurs or after two years of isolated estrogen therapy, to prevent endometrial hyperplasia (54).



In boys, treatment begins around age 14 with testosterone enanthate 50 mg intramuscularly every four weeks, and the dose is gradually increased by 25-50 mg every six months. The adult dose is 250 mg taken every two weeks (54).

Therapy is given in agreement with the patients' family.

#### 6.5. PROMOTION OF PSICHOMOTOR DEVELOPMENT

Assessment and monitoring of psychomotor development are necessary from early childhood. Early intervention with habilitation measures, primarily continuous physical therapy and exercise that aim at improving the coordination and strength of the child and thus help achieve an appropriate developmental pattern. Patients with a speech development disorder require speech therapy that aims to improve and enhance the communication skills of children who lag behind their peers.

## 7. NOVEL THERAPIES

Hyperphagia is the major concern for individuals with PWS and their families. The inability to control food intake severely restricts the individuals' opportunities for employment, social engagement, and independent living. While strict environmental controls may help reduce caloric intake and effectively manage weight for some individuals with PWS in the home setting, the persistent hyperphagic drive and associated behavioral problems characteristic of PWS often prevent patients from achieving a high quality of life or being well-integrated into their communities (66). Growth hormone therapy is the only FDA-approved treatment, and although it provides many important benefits for the patients, it does not ameliorate PWS-associated hyperphagia (67). Although, to date, no drugs have proved effective in controlling appetite and weight gain in PWS, and still the biological mechanisms underlying hyperphagia remain poorly understood, there are reasons to be hopeful. Many anti-obesity drugs and other therapeutic modalities in clinical development have the potential to offer new therapeutic options for individuals with PWS.

## 7.1. OXYTOCIN AND CARBETOCIN THERAPY

Oxytocin (OXT) is a neuropeptide that plays an important role in modulating social interactions and mother-infant bonding (68,69). In mammals, oxytocin is critical for social recognition (70), as well as approach and avoidance behavior (71,72). Oxytocin administration to mammals has been found to restore social recognition (73), induce maternal behavior in both rats (74,75) and lambs (76,77), as well as partner preference in monogamous prairie voles (78). This has led to models proposing that oxytocin modulates attention to socially relevant cues, enhances the salience of social stimuli, and increases reward and learning in social situations (79).

Quantitative neuroanatomical studies of postmortem human hypothalamic tissue from patients with Prader–Willi syndrome have demonstrated a reduced number and volume of OXT neurons in the paraventricular nucleus in comparison with controls (80).

Much of the phenotype of PWS is consistent with a hypothalamic defect characterized by a reduction of OXT expressing neurons in the PVN, mostly represented by parvocellular OXT neurons (81). These cells project to the brainstem nuclei, including the nucleus of the solitary tract, where OXT acts as a powerful anorexic peptide (82). Normal OXT plasma levels have been detected in 17 PWS adult patients (83), but elevated cerebrospinal fluid (CSF) OXT levels have been reported in five PWS patients (84). This high level of OXT in the CSF most likely relies on OXT released from magnocellular neurons, contacting both the vasculature of the posterior pituitary and the lumen of the third ventricle (85). However, such a difference between OXT released into the blood and CSF needs further clarification, especially because of the methodological limitations for measuring OXT in biological samples (86).

An alteration in the OXT system was also described in PWS mouse models. The *Magel2* KO mouse is considered a mouse model for PWS and autism spectrum disorder (ASD) because truncated mutations in the *Magel2* gene have been reported in some patients with PWS and ASD (87). These mice showed an altered onset of suckling activity and subsequent impaired feeding leading to 50% of neonatal lethality, affecting both males and females (88). A single OXT injection before the first 5 hours of life rescued 100% of the newborn *Magel2* knock-out (KO) mice from early death by restoring normal sucking activity (89) and a daily administration of OXT in the first week of life is sufficient to

regain suckling activity at birth and to restore normal social behavior and learning abilities in adult mutant males (90). Restricted production of mature OXT despite normal prohormone production was detected specifically in the hypothalamus of the *Magel2* KO pups. Altogether, these results suggest that an alteration of the OXT system around birth has early and long-term consequences on feeding and social behaviors and cognition. Importantly, an OXT treatment of *Magel2*-deficient pups in the first postnatal week partially restores normal anatomy of the OXT system and prevents deficits in social behavior and learning in adults. This concept opened the door to a powerful pharmacological therapy in early infancy for the PWS and is considered for other pathologies such as autism spectrum disorders (91).

Over the years, multiple clinical trials involving oxytocin were conducted in the hope of improving the lives of affected individuals. Results vary, and research is still ongoing. The first human clinical trial on OXT showed that a single intranasal administration rescue some behavioral features, such as increased trust and decreased signs of depression as well as emotional outbreaks, in patients with PWS (92). However, a double-blind randomized crossover trial of OXT nasal spray performed in 22 PWS patients (12–29 years old) did not find statistically significant effects using 18–40 IU of intranasal OXT twice daily for 8 weeks (93). The authors reported an increased number of temper tantrums in the patients receiving the high dose of OXT, and no effect was observed in patients treated with low doses. The discrepancies between these two studies may be explained by the dose used, the short washout period (15 days), the duration of treatment, and the sex ratio. Another study used body surface area to decide dosage and reported improvements for children younger than 11 years in anger, sadness, conflicts, and food-related behavior. However, the older participants reported unfavorable changes in happiness, sadness, and anger (94). A double-blind crossover trial in children aged 5–11 years, in which oxytocin or placebo was administered for five days, concluded a positive trend favoring oxytocin, though it was not significant (95). The most notable improvement was in anxiety symptoms, as reported by parents, corroborated by improvements on the self-injury subscale.

Factors associated with early intervention, placebo effects, experiment design, and dosing regimens may account for these equivocal findings.

Some of the disappointing findings may be explained by oxytocin's lack of specificity and stimulation of arginine vasopressin receptors, which are not reduced in the PWS brain. Vasopressin differs from oxytocin by just 2 amino acids and has been associated with states of negative emotional arousal and aggression (96). Thus, high doses of intranasal oxytocin may bind to the more abundant AVP receptors and worsen behavioral symptoms. In addition, oxytocin carries the risk of prolonged antidiuresis and hyponatremia due to considerable vasopressin V2 receptor activity (97,98). Therefore, an oxytocin receptor-selective compound may be preferable to avoid these vasopressin-related medical complications or potential worsening of agitated behavior. Some individuals with PWS are known to have polydipsia and low baseline serum sodium levels (99). As such, antidiuretic effects from exogenous oxytocin therapy have the potential to further increase the risk of the serious complication of hyponatremia. Knowing this, researchers decided to conduct a study using oxytocin analog carbetocin, which has an improved receptor-selectivity profile (100). Eligible patients aged 10–18 years with genetically confirmed PWS were randomized (1:1) to intranasal carbetocin or placebo 3 times daily for 14 days. This was the first randomized, placebo-controlled trial to demonstrate significant improvement in hyperphagia and related behavioral problems during treatment with the therapeutic peptide carbetocin in patients with PWS. Compared with placebo, a 14-day regimen of intranasal carbetocin significantly reduced hyperphagic symptoms, food-related behaviors and emotions, and compulsivity, while also improving overall functioning. It demonstrated a favorable safety profile, with no safety issues identified during the study.

In conclusion, larger trials with longer treatment duration are needed to further assess the efficacy of intranasal oxytocin or carbetocin on hyperphagia, compulsivity, social and emotional functioning, and determining if improvements in behavior complement weight reduction in individuals with PWS.

## 7.2. CARALLUMA FIMBRIATA EXTRACT THERAPY

Caralluma Fimbriata extract (CFE) is well known in Ayurvedic medicine. It grows wild throughout India, Pakistan, and Afghanistan. It has been ingested for centuries amongst populations as a natural appetite suppressant or as a vegetable substitute in times of famine (101,102). Genetic deletion of small nucleolar RNA (snoRNA), including the snord 116 and snord 115 contributes to the hyperphagia in PWS. One of the most important recognized disruptions is within the hypothalamic pathways, which are distributed due to disrupted SnoRNA115/HBII-52 transcription known to interact with the transcription of the serotonin 5-HT<sub>2c</sub> receptor. This receptor is involved in anorexigenic signaling within the hypothalamic appetite pathways of the central nervous system. After proving on the PWS animal model that CFE enhanced 5-HT<sub>2c</sub> receptor activity, and this activity confirmed increased satiety, a human study was conducted. It involved a female child (M), and it lasted over 12 years (103). M was diagnosed with PWS at the age of 18 months by genetic testing, which suggested maternal uniparental disomy. She had small hands and feet, dysmorphic facial features, light skin pigmentation, and severe developmental delay. At 29 months of age, the parents sourced CFE, and the daily treatment with half a capsule (250 mg) started (TABLE 2). During the earliest years of the intervention M volunteered that “she felt full” and “was not hungry”. In comparison to recorded previous appetite behaviors, this was a significant improvement. From 2–4 years, satiety eventually reduced, and there was increased asking for food. Each time this happened, the dose was increased by 250 mg/d. Decreased hunger was noted within a day of increased administration. Therefore, the dose followed a simple pattern: expressed hunger, adjustment of dose and observed satiety. This process continued over 12 years with the most recent 250 mg/d increase raising the dose to 2000 mg/d in 2018. Each time with the amount settled upon, there has been sustained freedom around food and successful appetite behavior. Other medications administered were fluoxetine (LOVAN 20 mg, 9–14 years) for anxiety and growth hormone therapy only a couple of times because M showed to be allergic. In 2010, M ceased taking the CFE for 6 days. Crying, tantrums due to food, and obvious changes to behavior were recorded. These remonstrations were far stronger than those observed by the parents when deciding to increase the dose. After 6 days the parents resumed administering CFE and continued

with a routine of a broad diet with restricted daily caloric intake to 60%. The indicators of distress or hunger ceased within days, and satiety resumed with M forgetting to eat after three days of CFE intervention. Interestingly, certain behaviors like skin picking, asking and telling, wanting to know the routine of the day, were not attenuated by CFE. These behaviors may be due to anxiety and not hyperphagia, as they occurred when no hunger was reported. Over the 12 years of administration, there have been no adverse effects. This single-case study suggests that CFE increases satiety and maintains weight overtime, within parameters of caloric restriction without adverse effects or compromising blood serum measures.

**Table 2.** Age and anthropometric measurements in single-case with Prader-Willi syndrome (PWS) ingesting *Caralluma fimbriata* extract (CFE). Anthropometric measurements behavioral indications of hyperphagia and additional intervention/exercise, in a single case of a female with PWS ingesting CFE over 12 years. The case begins as a newborn and ceases at 14 years and 4 months of age. (From Griggs J. Single-Case Study of Appetite Control in Prader-Willi Syndrome, Over 12-Years by the Indian Extract *Caralluma fimbriata*. *Genes (Basel)*. 2019;10(6):447. Published 2019 Jun 12. doi:10.3390/genes10060447).

Age Yr/Month	Weight Kg	Height Cm	Dose Mg/d	Food/Hyperphagia	Indication	Other Intervention
Birth	2.35	Unsure	nil	Failure to thrive	Unable to suck	Tube fed, phentobarbital
2.3 months	3.7	55	nil	Feeding slowly	Take out nasogastric tube	Feeding extremely slowly; adapted bottle
8 months	6.5	65	nil	Normalised appetite	Bottle fed	Feeding slowly with adapted bottle
12 months	8.2	68	nil	Normalised appetite	Bottle fed and soft food	Adapted bottle exercise program
18 months	12	72	nil	Increased hunger	Obese and ambulating after food	Diet 100% home exercise program
20 months	12.9	78	nil	Eating constantly	Obese, always hungry ambulating after food	<b>DIAGNOSIS</b>
2 years	12.5	82.5	nil	Hyperphagia	Obesity, tantrums around food and always hungry	Diet 60% home exercise program
2 years, 4 months	12	85	250	Satiated	Saying no to dinner and leaving food on plate	Diet 60% home exercise program
4 years, 11 months	19	104	500	Access to food with supervision	Saying no to dinner and minimal asking for food.	Diet 60% home exercise program
6 years, 6 months	23	112	NIL for 6 days	Ceased CFE hyperphagia returned after 1.5 days	Tantrums around food after 1.5 days. Licking empty plate	Diet 60% ballet once weekly
6 years, 6 months	23	112	500	Hunger persisted 1 day	Confirmation of satiety and interested in other activities	Diet 60% ballet once weekly
7 years, 1 month	23.5	114	750	Non-restricted with supervision	Both hunger and satiety	Diet 60% ballet once weekly
7 years, 9 months	25	119	1000	Non-restricted with supervision	Saying no to dinner and minimal hunger.	Diet 60% ballet once weekly
8 years, 8 months	29	124	1000	Non-restricted with supervision	Interested in food	Diet 60%, routine no exercise
9 years, 7 months	30	126	1250	Non-restricted with supervision	Forgetting to eat food provided	Diet 60%, routine no exercise
10 years, 1 months	34	128	1250	Non-restricted with supervision	Minimal communication of hunger.	Diet 60%, routine no exercise



11 years, 8 months	37	129	1250	Non-restricted with supervision	Minimal communication of hunger and throws out school treats.	Diet 60%, routine ballet once weekly
12 years, 5 months	38	134	1500	Non-restricted with supervision	Minimal communication of hunger. Makes own lunch.	Diet 60%, routine swimming weekly
13 years, 1 month	39	136	1750	Non-restricted; attempting no supervision	Communication of both hunger, "I'm hungry", and satiation "I'm full".	Diet 60%, routine swimming weekly
14 years, 4 months	43	141	2000	Non-restricted environment.no supervision	Communication hunger and takes self out of difficult situations.	Diet 60%, self-managed swimming weekly

### 7.3. PITOLISANT THERAPY

Pitolisant is first-in-class histamine H3 receptor inverse agonist that enhances the activity of histaminergic neurons. It has been shown to provide effective treatment for excessive daytime sleepiness and to be well tolerated. Since individuals with PWS suffer from excessive daytime sleepiness, cataplexy-like symptoms, and cognitive impairments (104), pitolisant might not only be effective as a treatment of excessive daytime sleepiness but might also help to relieve some of the fundamental symptoms of PWS (105). A case series of three PWS patients on pitolisant therapy reported improved cognition in faster processing speed and improved mental clarity (106). In addition to improved cognition, initiation of treatment with pitolisant was associated with fewer problem behaviors. Parents also reported that children could go to longer periods without feeling an urgent need to eat. However, the data were too preliminary, the cohort too small, and the development of the children during adolescent phases too unpredictable to make any firm conclusions about the effect on hyperphagia.

#### 7.4. UPCOMING RESEARCH AND CLINICAL TRIALS

Advances in research and forthcoming clinical trials give hope for finding a cure for PWS. Notable research advances include newly developed tools, such as induced pluripotent stem (iPS) cells from patients with PWS, which provide opportunities to dissect the genetics of PWS and identify disruptions in cellular pathways. As the researchers continue to try to explain how the loss of a critical region for PWS leads to a broad phenotype that characterizes the disorder, clinical studies are progressing toward the assessment of new therapies for PWS. Several medications are in clinical trials, including beloranib (Zafgen), RM-493 (Rhythm), diazoxide (Essentialis), and AZP-531 (Alizé). Each of these agents has a different target, ranging from affecting fat metabolism to directly targeting the hypothalamic pathways of appetite and satiety. Additionally, several recently approved drugs could potentially improve satiety or help to ameliorate obesity in individuals with PWS, including exenatide (Byetta, AstraZeneca), lorcaserin (Belviq, Eisai), naltrexone/bupropion (Contrave, Takeda) and phentermine/topiramate (Qsymia, Vivus). These medications first have to be examined in long-term clinical trials for the PWS population. Some devices being tested may also improve appetite and behavior in PWS, including transcranial direct magnetic stimulation and vagal nerve stimulators<sup>lxvii</sup> (67).

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## **10. BIOGRAPHY**

I was born on April 24, 1994 in Zagreb, where I attended primary and high school. In 2013 I enrolled in Medical Studies in English, University of Zagreb. I was a demonstrator at the Department of Physiology and Immunology in 2019/2020 and an active participant at the Better Future of Healthy Ageing 2020 (BFHA 2020) conference. My interests lie mostly in psychiatry and artificial intelligence.