Improving medical care for adults with Prader-Willi syndrome

Karlijn Pellikaan

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Improving Medical Care for Adults with Prader-Willi syndrome

Het verbeteren van de medische zorg voor volwassen met Prader-Willi syndroom

Thesis

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**General introduction
and aims of the thesis**

Prader-Willi syndrome (PWS) is a severe disorder with a high mortality. This is mainly due to an unlimited appetite that frequently leads to stomach rupture, choking, and morbid obesity. Apart from hyperphagia, features of PWS include low muscle mass, hypotonia, intellectual disability, behavioral challenges, typical facial features, and hypothalamic dysfunction. Hypothalamic dysfunction leads to hyperphagia, abnormal temperature regulation, inadequate pain registration, and pituitary hormone deficiencies (1-4).

Prader-Willi syndrome (PWS) was first described in 1956 by Andrea Prader, Alexis Labhart, and Heinrich Willi (5). It is a rare genetic disorder, affecting approximately one in 16.000-21.000 live births (6, 7). Nowadays, PWS is diagnosed by genetic testing. However, before genetic testing was available, PWS was diagnosed based only on clinical diagnostic criteria, first formulated by Holm et al. (4) and later updated by Cassidy et al. (8), see **Figure 1**.

Major criteria

1. Neonatal/infantile hypotonia and poor suck

2. Feeding problems and failure to thrive as infant

3. Weight gain at 1-6 years; obesity; hyperphagia

4. Characteristic dysmorphic facial features

5. Small genitalia; pubertal delay and insufficiency

6. Developmental delay / intellectual disability

Minor criteria

1. Decreased fetal movements and infantile lethargy

2. Typical behavioral challenges

3. Sleep apnea

4. Short stature for family by 15 years

5. Hypopigmentation compared to family

6. Small hand and feet for height

7. Narrow hands, straight ulnar border

8. Esotropia, myopia

9. Thick, viscous saliva

10. Speech articulation defects

11. Skin picking

Figure 1. Diagnostic criteria Prader-Willi syndrome
Criteria modified from Holm et al. (4) and Cassidy et al. (8). Major criteria give one point, while minor criteria give half a point. For children under 3 years old, at least four major criteria should be present with a score of five or more points in total. For patients 3 years old or older, at least five major criteria should be present, with a total score of at least eight. Picture from Zorgatlas Groeihormoon, Esculaap Media bv, 2017.

In addition to the diagnostic criteria for PWS, Holm et al. (4) also described supportive findings for PWS. Supportive findings are a high pain threshold, decreased vomiting, temperature instability in infancy or altered temperature sensitivity in older children and adults, scoliosis and/or kyphosis, early adrenarche, osteoporosis, unusual skill with jigsaw puzzles, and hypotonia with normal laboratory neuromuscular studies.

Mortality is high in PWS: 3% of children and adults die every year. In adults above 30 years old, this is even 7% (9). Half of patients with PWS die before the age of 29 (10). The

most common causes of mortality are respiratory failure (31%), cardiac disease (16%), gastro-intestinal disease (10%), infections (9%), obesity (7%), pulmonary embolism (7%), choking (6%), and accidents (6%) (10). To decrease this high mortality among adults, medical treatment for adults with PWS needs to be optimized. This thesis focuses on identifying health problems in adults with PWS and providing clinical recommendations for the optimization of treatment in these patients. With these recommendations, we aim to avoid unnecessary complications, underdiagnosis, undertreatment and early mortality.

GENETIC BACKGROUND OF PRADER-WILLI SYNDROME

PWS is caused by the lack of expression of a cluster of paternally expressed genes on chromosome 15q11.2-13, also called the PWS critical region. As a result of imprinting, this PWS critical region is methylated on the maternal allele. Therefore, the genes on the PWS critical region can only be expressed from the paternal allele. Absence of expression from this paternal allele causes PWS. This is most commonly caused by a paternal deletion of chromosome 15q11.2-13 (70-75%) or the presence of two maternal copies of chromosome 15 in absence of a paternal copy, which is called a maternal uniparental disomy 15 (mUPD, 25-30%). Less frequent causes include imprinting center defects (ICD, 1-3%) and paternal chromosomal translocations (<0.1%) (11, 12). The two most common paternal deletions are type 1 and the slightly smaller type 2 deletions. However, as genetic tests become more detailed, more different sizes of deletions are reported.

Chromosome 15q11.2-13 consists of the proximal and distal non-imprinted regions, which are expressed from the maternal and paternal chromosome; the PWS critical region, which is only expressed from the paternal allele; and the Angelman syndrome (AS) region, which is only expressed from the maternal allele. Imprinting of chromosome 15q11.2-13 is regulated by a bipartite imprinting center (IC). The location of this IC defined as the smaller regions of overlap (SRO) found in patients with ICD (13-18). It consists of the Angelman syndrome-SRO (AS-SRO) and the PWS-SRO. The AS-SRO silences paternally expressed genes on the maternal allele. The PWS-SRO activates paternally expressed genes on the paternal allele (19-21). The PWS-SRO partly overlaps with exon 1 of *SNURF-SNRPN* (22, 23). While paternal deletions and mUPD causes PWS, maternal deletions and paternal uniparental disomy of chromosome 15q11.2-13 results in Angelman syndrome, a syndrome with limited phenotypic overlap with PWS.

The PWS critical region encompasses several genes, including *MKRN3, MAGEL2, NDN, NPAP1, SNURF-SNRPN,* and numerous non-coding RNAs (ncRNAs) including small

nucleolar RNAs (snoRNAs) (12), see **Figure 2**. The exact function of most of the genes in the PWS critical region and their exact relation to the PWS phenotype remains unclear. Several case reports describe microdeletions or translocations resulting in lack of expression of *SNORD116*, which resulted in many PWS features (24-37). This suggests that *SNORD116* is a strong contributor to the PWS phenotype (38). The absence of *SNORD116* might explain part of the hypothalamic dysfunction in PWS. Although the exact mechanism is unknown, the absence of *SNORD116* may be related to deficiency of nescient helix–loop–helix 2 (NHLH2) and prohormone convertase 1 (PC1), which is associated with hypothalamic dysfunction (39-41). However, although *SNORD116* deletions seem to cause most of the major features of PWS, they may be less severe (32).
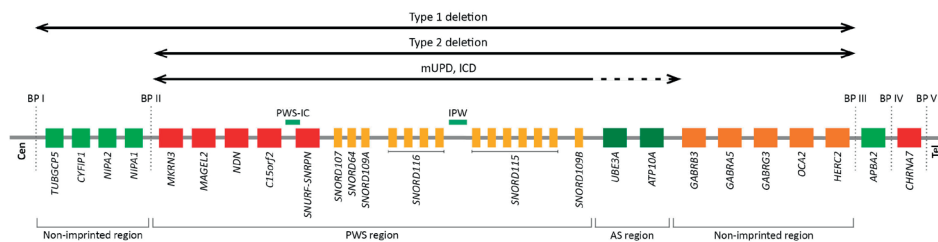
Figure 2. Overview chromosome 15q11.2-13
Abbreviations: Angelman syndrome (AS), breakpoint (BP), centromere (Cen), imprinting center (IC), Prader–Willi syndrome (PWS), telomere (Tel). Figure adapted from Cheon et al. (12) Horizontal arrows indicate regions of chromosome 15q11.2-q13 affected by the different genotypes of PWS. Solid horizontal arrows indicate diminished or loss of expression, dotted arrows indicate increased expression.

SNURF-SNRPN is another important gene on the PWS critical region. *SNURF-SNRPN* transcription is necessary for the production of downstream non-coding RNAs (ncRNAs) like *SNORD116* (42, 43). It also encodes two proteins: SNURF, a protein with unknown function, and SNRPN, which is also called SmN (44). SNRPN is predominantly expressed in neurons and is involved in mRNA splicing (45-47). In mice, SNRPN influences neurite outgrowth, neuron migration and distribution of dendritic spines (48). While other genes may also contribute to the PWS phenotype, their contribution seems less important.

PWS can be confirmed by different genetic tests. First, a methylation sensitive PCR can be used to assess methylation anomalies of the *SNRPN* gene (49). In case of hypomethylation, the diagnosis PWS is confirmed. To find out whether the underlying genetic defect is a deletion, fluorescent in situ hybridization (FISH) with a probe for *SNRPN* or multiplex ligation-dependent probe amplification (MLPA) can be performed. However, nowadays a methylation specific MLPA is often used which can assess the methylation status and the presence of a deletion of chromosome 15q11.2-13. When deletion is absent, the DNA of the parents is investigated to distinguish between an mUPD or ICD. For this purpose,

a set of highly polymorphic chromosome 15q markers can be used, but nowadays the use of SNP arrays is more common. These tests can confirm the presence of an mUPD. When an mUPD is not present, it is often assumed that the underlying genetic defect is an ICD (50).

When these genetic tests are negative in a patient with PWS features, this is called Prader-Willi-like syndrome (PWLS) (12). Many alterations on various chromosomes have been described in relation to PWLS (12, 51, 52). Cases with PWLS may provide insight into the complex genotype-phenotype relationship of PWS. This might eventually facilitate the development of new treatments in the future.

HYPERPHAGIA

Hyperphagia, one of the major features of PWS, may present as an intense and persistent sensation of hunger. However, caregivers also report food preoccupations, food-related behaviors, an extreme drive to consume food and a lack of normal satiety (53). Individuals with PWS can eat up to three times the normal caloric intake per meal. They may also hoard food and eat frozen or contaminated food or inedible items (54, 55).

The degree of hyperphagia can change over time. Neonates with PWS may display a lack of interest in food, feeding difficulties, and failure to thrive. Around five to fifteen months, these resolve and usually the infant's growth seems to normalize. Between the age of two and four years, most children with PWS start to show a significant increase in weight, which is later accompanied by an increased appetite and increased interest in food. Around the age of eight, many children with PWS display clear hyperphagia, food seeking behaviors and lack of satiety. This may lead to severe obesity in young children. Hyperphagia remains present in adults with PWS, but may decrease over time (8).

When uncontrolled, hyperphagia may lead to gastric rupture, choking, and obesity (10, 56). To avoid the short- and long-term complications of hyperphagia, constant supervision is needed to restrict access to food and food intake of individuals with PWS (55). Several methods have been used to quantify the amount of hyperphagia in individuals with PWS. The most commonly used method is the Hyperphagia Questionnaire, a 13-item questionnaire that can be filled out by parents or caregivers of individuals with PWS (57).

The pathophysiological mechanism leading to hyperphagia in individuals with PWS is not fully understood. Hyperphagia in adults with PWS is believed to result from hypo-

thalamic dysfunction, resulting in decreased satiety (8). Several functional brain studies have indicated that hypothalamic control of satiety is disrupted in patients with PWS, with dysfunction of the reward circuitry regions and impairment in inhibitory control regions (58). Besides hypothalamic dysfunction, several hormones that are involved in the regulation of food intake are also altered in patients with PWS. First, compared to obese and lean controls, patients with PWS have increased acylated ghrelin (AG) levels, while unacylated ghrelin (UAG) levels are relatively low, leading to a high AG/UAG ratio (58-60). As AG has an appetite-stimulating effect, which seems to be counteracted by UAG, this high AG/UAG ratio can result in an increased appetite in patients with PWS (61). Second, pancreatic polypeptide (PP) and peptide YY (PYY), both anorexigenic hormones, may be reduced in patients with PWS. However, contradictory results on PYY levels have been reported (58). Third, adiponectin levels are increased in patients with PWS compared to obese controls (62). Adiponectin is an anti-inflammatory agent and plays a role in the regulation of insulin sensitivity (63). Patients with PWS are less likely to develop insulin resistance compared to BMI-matched controls, which might be related to increased adiponectin levels (58, 62, 64). Lastly, leptin, an important regulator of appetite and fat storage, does not seem to be altered in PWS (compared to BMI-matched controls) (65, 66). Thus, hyperphagia in patients with PWS seems to be a complex interplay between the hypothalamus and several hormones that are involved in the regulation of satiety.

BEHAVIORAL CHALLENGES AND PSYCHIATRIC ILLNESS

Besides the food-seeking behavior, patients with PWS may have other neurobehavioral challenges, such as difficulties with social interaction, rigidity, anxiety, obsessive-compulsive behaviors, skin picking, and temper outbursts (55, 67, 68). Because of the social cognitive challenges in patients with PWS, 12-41% of patients with PWS also fulfil the criteria for autism spectrum disorder (ASD) (69, 70). ASD is more frequent in patients with an mUPD compared to patients with a deletion (69, 70). Individuals with PWS are also at risk for other psychiatric illnesses like (depressive) psychosis, bipolar disorder, and anxiety disorder (55, 71, 72). mUPD is associated with a higher risk of psychiatric illness in general, in particular psychosis and bipolar disorder (71, 73).

Due to this high prevalence of behavioral challenges and psychiatric illness, individuals with PWS are frequently prescribed psychotropic medication like antipsychotics, antidepressants, and benzodiazepines (74). Psychotropic medication can cause adverse effects and may increase the risk of other health problems, like weight gain, high blood pressure, hypothyroidism, and hyperprolactinemia (75).

HYPOTHALAMIC DYSFUNCTION AND PITUITARY HORMONE DEFICIENCIES

PWS is characterized by hypothalamic dysfunction, which may lead to hyperphagia, abnormal temperature regulation, inadequate pain registration and pituitary hormone deficiencies (1-4).

The hypothalamus is connected to the pituitary gland by the infundibular stalk. The pituitary gland is located behind the nasal bridge in the sella turcica. The hypothalamus releases stimulating and inhibiting hormones that are transported to the anterior pituitary gland through the hypothalamic-hypophyseal portal veins. In response, the anterior pituitary can produce several hormones, with different target organs, see **Figure 3**. In contrast, the posterior pituitary is an extension of hypothalamic neurons. The posterior pituitary does not produce hormones, but stores and secretes hormones that are produced in hypothalamic neurons.
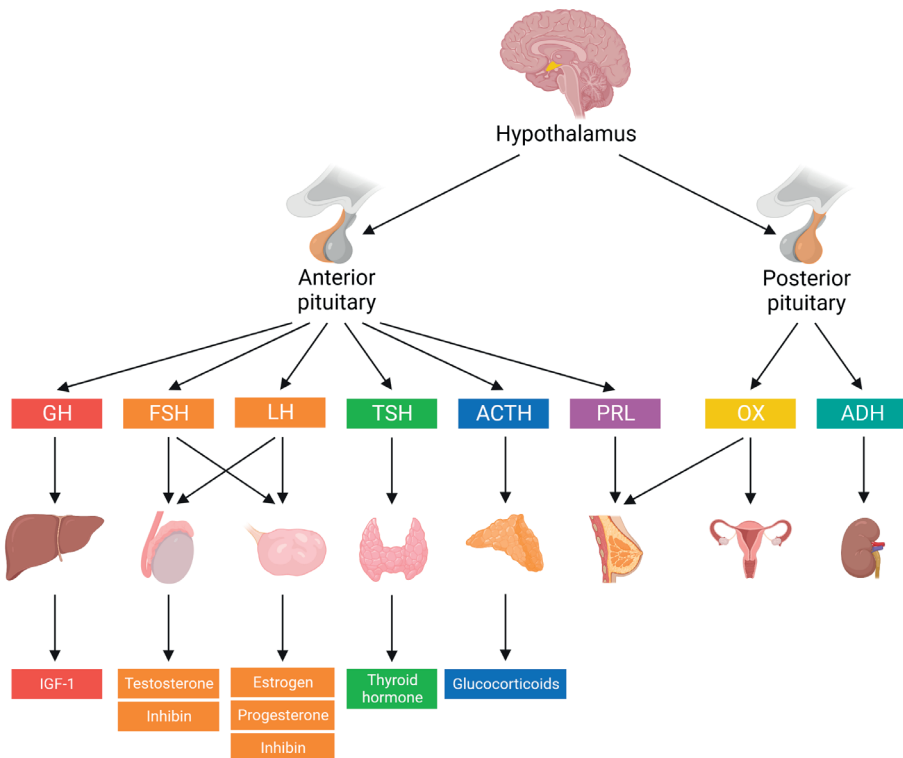

Figure 3. Pituitary hormones produced by the anterior and posterior pituitary
Abbreviations: adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), growth hormone (GH), luteinizing hormone (LH), prolactin (PRL), thyroid-stimulating hormone (THS). Figure adapted from Feldt-Rasmussen et al. (76). Created with BioRender.com.

Here we will discuss hormones secreted by the anterior and posterior pituitary gland and how these hormones may be affected in patients with PWS. For details of the different axes, see **Appendix**.

Hypothalamic-pituitary-somatotropic axis

Growth hormone (GH) is important for growth and metabolism. The effects of GH are partly mediated by insulin-like growth factor-1 (IGF-1), which is produced in the liver in response to GH. During childhood, the most important symptoms of GH deficiency are decreased height velocity and short stature (77). However, even after final height has been reached, GH is still important. GH deficiency can cause decreased lean body mass, increased fat mass, decreased bone mineral density (BMD) and an increased risk of cardiovascular disease (78).

Patients with PWS display several symptoms of GH deficiency, such as small hands and feet, short stature, low muscle strength and unfavorable body composition (79). In adults with PWS, the reported prevalence of GH deficiency ranges from 0-38% (80, 81). However, there are no adequate tests to confirm the diagnosis of GH deficiency in patients with PWS (80, 82, 83). Therefore, the prevalence of GH deficiency in patients with PWS might be underestimated. IGF-1 bioactivity and bioavailability may be decreased in patients with PWS, further complicating the diagnosis GH deficiency in these patients (84, 85). However, regardless of the presence of GH deficiency, GH treatment is beneficial for patients with PWS. During childhood, GH treatment improves cognitive functioning, psychomotor development, LDL-cholesterol values and body composition (86-94). These improvements in body composition are maintained during long-term GH treatment (95, 96). Additionally, initiation of GH treatment during adulthood also improves body composition (97).

Hypothalamic-pituitary-gonadal axis in men

In males, the hypothalamic-pituitary-gonadal axis is important for testosterone production and fertility. A deficient hypothalamic-pituitary-gonadal axis is called hypogonadism. In patients with primary hypogonadism, low testosterone levels are the result of testicular failure. Primary hypogonadism is characterized by elevated gonadotropin levels. Central hypogonadism is caused by deficient production of pituitary hormones (LH and FSH, secondary hypogonadism) or hypothalamic GnRH (tertiary hypogonadism). Hypogonadism in men can cause infertility, absence of secondary sex characteristics, fatigue, depression, increased fat mass, decreased muscle mass and strength, decreased sexual quality of life, osteoporosis (98-101) and cardiovascular disease (99, 102).

Hypogonadism is common in males with PWS, with a reported prevalence between 57 and 100% (103-115). It can be the result of hypothalamic dysfunction or primary gonadal failure (111, 112, 116), or a combination of both (111, 117, 118). Males with PWS of all ages can be affected by hypogonadism. Early in life, boys with PWS may have a small penis, hypoplastic scrotum, and cryptorchidism (119). Later, puberty is usually delayed and/or incomplete (120-122). In adulthood, men with PWS often have low levels of testosterone (103, 110, 113, 114, 123-125).

Hypothalamic-pituitary-gonadal axis in women

Impaired function of the hypothalamic-pituitary-ovarian axis (female hypogonadism) usually results in an absent or irregular menstrual cycle. Patients with central hypogonadism have inappropriately low LH and FSH levels due to a dysfunction of either the pituitary gland or the hypothalamus. Primary hypogonadism is caused by ovarian dysfunction and results in elevated gonadotropin levels. There are many causes of hypogonadism. One well-known cause is extreme obesity. Extreme obesity leads to increased estrogen production in adipose tissue, decreased SHBG levels and elevation of leptin, which causes disturbed GnRH pulsatility (126, 127). Other causes of hypogonadism include endocrinopathies like hyperprolactinemia and primary hypothyroidism (127). Female hypogonadism may have negative effects on quality of life, muscle strength, bone mineral density, and cardiovascular health (98, 102, 128-131).

In females with PWS, the prevalence of hypogonadism is 54-100% (103-110, 114, 132). While hypogonadism is most often central in origin, primary hypogonadism may also occur (107, 109, 133, 134). Signs of hypogonadism may already be present early in life (121). While breast development usually starts at a normal age, its progression is often delayed (132, 133, 135). Additionally, menarche may not occur. When menarche occurs, it is often delayed and followed by oligomenorrhea or secondary amenorrhea (108, 134).

Hypothalamic-pituitary-thyroid axis

Thyroid hormone is an important regulator of basal metabolic rate and is important for bone maintenance and brain development and function. Disturbance of the HPT axis can result in hypo- or hyperthyroidism. Symptoms of hypothyroidism include fatigue, cold intolerance, weight gain, constipation, change in voice, mood impairment, muscle weakness, and dry skin (136). Hypothyroidism also has unfavorable cardiovascular effects, leading to accelerated atherosclerosis and coronary artery disease (137). Hypothyroidism can be central, with TSH levels being inappropriately low for low fT4, or primary, with increased TSH. In subclinical hypothyroidism, TSH is increased while fT4 is within the reference range. Symptoms of hyperthyroidism include fatigue, heat intolerance,

weight loss, diarrhea, weakness, tachycardia, and emotional lability (138). In patients with subclinical hyperthyroidism, TSH is low while TH is within the reference range.

Previous research indicates that the prevalence of hypothyroidism is increased in patients with PWS and that hypothyroidism in PWS is often central in origin. However, there are contradictory results regarding the exact prevalence (119, 139). Hypothyroidism in patients with PWS might aggravate already prevalent symptoms like fatigue and exercise intolerance (1, 4, 140, 141), which eventually leads to an increase in BMI and cardiovascular risk (53, 136, 142).

Hypothalamic-pituitary-adrenal axis

Disturbance of the HPA axis leads to adrenal insufficiency, which can be primary (adrenal), secondary (pituitary) or tertiary (hypothalamic) in origin. Symptoms include fatigue, muscle weakness, loss of appetite, nausea, postural dizziness and weight loss (143). A life-threatening complication of adrenal insufficiency is an acute adrenal crisis, which can occur if patients with adrenal insufficiency are exposed to physical or psychological stress without receiving hydrocortisone stress doses. Early symptoms of adrenal crisis include fatigue, vomiting, abdominal pain and confusion. Eventually, adrenal crisis may result in hypotension, loss of consciousness, shock or coma (144). Adrenal insufficiency can be treated with hydrocortisone.

In individuals with PWS, symptoms of adrenal insufficiency may be hard to recognize. For example, weight loss can be seen as the result of lifestyle interventions, as many patients are on a diet. Moreover, fatigue and muscle weakness are prevalent in patients with PWS, regardless of adrenal function (145). A previous study in children with PWS found a high prevalence (60%) of central adrenal insufficiency (CAI) (146). In some countries, this has led to standard prescription of corticosteroid stress doses to children and adults with PWS. However, frequent use of corticosteroid stress doses can have side-effects like weight gain, hypertension, osteoporosis, myopathy and type 2 diabetes mellitus (DM2) (147). Therefore, it is crucial to prevent unnecessary prescription of hydrocortisone. As other studies found much lower prevalences (148-155), the exact prevalence of CAI in patients with PWS is unknown. Especially data on adults with PWS are scarce.

Prolactin

During pregnancy, prolactin is secreted to induce lactation. Additionally, prolactin also has metabolic and immunological effects and is important for mammary development and parental behavior. (156, 157). High prolactin levels can cause galactorrhea, gynecomastia, hypogonadism, infertility, metabolic abnormalities and increased cardiovascular risk (158-160).

Patients with PWS are at increased risk to develop hyperprolactinemia due to the frequent use of psychotropic medications. As described previously, psychotropic medication may be prescribed because of psychiatric illness or behavioral problems (74). However, no systematic assessment of hyperprolactinemia has been performed in patients with PWS.

Antidiuretic hormone

Antidiuretic hormone (ADH), also called vasopressin, is essential for osmotic balance, blood pressure regulation, sodium homeostasis and kidney function. Additionally, ADH has been associated with social functioning, as ADH levels in cerebrospinal fluid are lower in patients with autism (161, 162). Administration of intranasal ADH improves social function in children with autism (163).

Data on the occurrence of disturbances in ADH in patients with PWS is scarce. However, patients with PWS might be at increased risk for disturbances of the regulation of ADH due to hypothalamic dysfunction and frequent use of psychotropic medications that are associated with SIADH (8, 74, 164). Both studies in mouse models of PWS and patients with PWS suggest that ADH signaling is disrupted, which may contribute to the social impairments seen in patients with PWS. Administration of intranasal ADH improves social function in PWS animal models, but this has not been tested in clinical studies (165).

Oxytocin

Oxytocin is involved in lactation and uterus contractions. However, as its nickname 'the cuddle hormone' suggests, oxytocin also plays an important role in social behavior and anxiety (166, 167). Many studies have investigated the effect of the administration of oxytocin on social outcomes. As oxytocin is unable to pass the blood-brain barrier (BBB) in significant amounts, intranasal administration is often used to bypass the BBB. Although oxytocin seemed a promising new therapy for autism spectrum disorder (ASD), studies found contradictory results and meta-analyses failed to demonstrate an effect of oxytocin on anxiety, repetitive behavior or social functioning (168, 169).

Both clinical and animal studies indicate abnormal oxytocin signaling in PWS (165). Administration of intranasal oxytocin was associated with improvements in hyperphagia and obsessive-compulsive behavior in some studies, while other studies could not confirm these results (165, 170).

CARDIOVASCULAR DISEASE

In PWS, there is a complex interaction between endocrine, non-endocrine, and psychosocial problems, leading to obesity and eventually cardiovascular (CV) disease (79). Hormone deficiencies like growth hormone deficiency, hypothyroidism, and hypogonadism lead to a decreased muscle mass and function and a decreased basal metabolic rate (BMR) (79, 103, 171-182). Hypotonia and other musculoskeletal problems (183-187) can cause poor exercise tolerance. The combination of low BMR, poor exercise tolerance and hyperphagia (188-190) can results in unfavorable body composition and a high prevalence of obesity (176, 191-193). In turn, this often leads to the development of cardiovascular (CV) risk factors like hypertension, hypercholesterolemia, and DM2 (10, 16, 79, 114, 194-199). Because of this complex interplay, individuals with PWS have an increased prevalence of CV disease, already occurring early in life (123, 197, 200, 201). Apart from this indirect relation between hormone disruptions and cardiovascular problems, dysregulation of hormones might also have a more direct effect on the development of CV disease. Some of these effects are protective. For example, compared to BMI-matched controls, patients with PWS develop less insulin resistance, which might be related to increased adiponectin levels (58, 62, 64). Additionally, patients with PWS display hyperghrelinemia with a high AG/UAG ratio, which is associated with weight gain and glucose intolerance (202, 203), but also seems to have protective CV effects (204-206).

MALIGNANCIES

Given the high prevalence of obesity in PWS and the association between obesity and cancer (207), one would expect an increased risk of malignancies in patients with PWS. Previous studies on malignancies in PWS showed that the prevalence of malignancies is slightly increased, especially the prevalence of leukemia (208, 209). Additionally, multiple case reports of patients with PWS and different types of malignancies have been reported (210-219). Apart from obesity, the genes on chromosome 15q11.2-13 might influence the risk of malignancies in patients with PWS. In one patient with PWS with testicular seminoma, loss of methylation of the PWS-IC was found, which suggests involvement of genes in the PWS critical region (217). Additionally, *in vitro* studies, animal studies, and clinical studies suggest that expression of multiple genes on chromosome 15q11.2-q13 might be altered in malignant cells (220-226). However, the exact relation between the PWS genotypes and malignancies remains unclear.

MULTIDISCIPLINARY AND TRANSITIONAL CARE

Due to the complexity of the syndrome, children and adults with PWS benefit from multidisciplinary (MD) care. Ideally, the MD team should consist of at least a (pediatric) endocrinologist, a dietitian, a physiotherapist, a behavioral expert or psychologist, and a physician for intellectual disabilities (ID physician) or psychiatrist. If needed, other specialists, such as an orthopedic surgeon and a cardiologist should be consulted.

The transition from pediatric to adult care is a particularly vulnerable period (227). To avoid psychological stress and dropout, careful coordination of care is warranted during the transition phase. Ideally, transitional care should include shared visits with both the pediatric and the adult endocrinologist, followed by alternating visits at the pediatric and adult department until the final transfer to adult endocrinology. In the Netherlands, transitional care and MD care for adults with PWS has only been available since 2015. In many other countries, specialized care for adults with PWS is still unavailable.

UNDERDIAGNOSIS

In adults with PWS, undiagnosed and untreated health problems are common. This underdiagnosis and undertreatment in adults with PWS is due to several factors. First, due to intellectual disability and the PWS-specific behavioral phenotype, patients are often unable to express their complaints. Therefore, the presentation of health problems may be atypical. Second, patients with PWS tend to have a high pain threshold, a disturbed temperature regulation and a high vomiting threshold (1-4). Therefore, health problems associated with pain, fever, and nausea may be especially hard to detect. Third, the interplay between psychological and somatic problems in PWS is complex. This makes it hard for physicians to recognize the symptoms of individual health problems. Fourth, PWS is a rare disorder (228). Therefore, many physicians are unfamiliar with the syndrome and its comorbidities. Therefore, physicians are not alert to syndrome-specific problems, which may lead to doctors' delay. Fifth, many health problems associated with PWS cause symptoms that are also associated with the syndrome itself, like change in weight and leg edema (8). This can lead to underdiagnosis, as physicians may think that a symptom is 'just part of the syndrome' and therefore does not require additional diagnostics. Therefore, treatment of patients with PWS requires syndrome-specific knowledge to distinguish symptoms of the syndrome from symptoms of an underlying, treatable health problem. Combined, these factors may lead to both patients' and doctors' delay. Missing PWS-associated comorbidities often causes medical complications, leading to hospital admission, long stays on intensive care units and even death. Timely recogni-

tion can reduce the financial and personal burden of PWS-associated health problems (229). Therefore, practical guidelines for the screening and treatment of health problems in adults with PWS are urgently needed.

GAP IN KNOWLEDGE

Until a few years ago, most research into PWS had focused on children with PWS, while research on adults with PWS was extremely scarce. Due to this knowledge gap, clinical recommendations for adults with PWS were lacking. Therefore, adults with PWS were treated according to the pediatric guidelines. However, treatment regiments for children are often unsuitable for adults. In addition, problems that occur during childhood might be less prevalent among adults, leading to overdiagnostics and overtreatment (for example, standard prescription of corticosteroid stress doses). Conversely, health problems that develop during adulthood, like cardiovascular disease and other age-related health problems, often remained undiagnosed. To address this problem, Sinnema et al. (195) was the first to describe health problems in a large cohort of adults with PWS. However, the results of this study were based on interviews and not on a systematic health screening. This was done later, by Laurier et al. (198) and Coupaye et al. (114) who performed a systematic health screening in French adults with PWS. However, recommendations for the treatment of adults with PWS were still unavailable, leading to suboptimal treatment.

INTERNATIONAL COLLABORATIONS

As the incidence of PWS is one in 16.000-21.000 live births (6, 7), it is classified as a rare disease. Scientific research in patients with rare diseases is often limited by an insufficient number of patients, limiting the statistical power of clinical studies. To increase these numbers, international collaborations are essential. To that aim, an international collaboration was started between adult endocrinologists, who are experts in the field PWS: the International Network for Research, Management & Education on adults with PWS (INfoRMEd-PWS). Within this network, adult endocrinologists from seven countries work together to improve research and patient care around adults with PWS. Many of the studies described in this thesis, were carried out in close collaboration with INfoRMEd-PWS.

Figure 4. Logo of the International Network for Research, Management & Education on adults with PWS**.**

OUTLINE OF THIS THESIS

With this thesis, we fill the gap in knowledge about health problems occurring in adults with PWS. We provide clinical recommendations to avoid underdiagnosis and undertreatment. We also provide new insight into the genotype-phenotype relation in patients with PWS.

In **Chapter 2**, we give an overview of the health problems occurring in adults with PWS and we show how systematic health screening reveals yet undiagnosed comorbidities.

In **Chapter 3**, we investigate the effect of multidisciplinary pediatric care and childhood growth hormone treatment on health problems in adulthood.

In **Chapter 4**, we focus on hypogonadism in men with PWS. Based on our cohort study, a literature review and an expert panel discussion, we provide practical recommendations for the treatment of hypogonadism in this specific patient population.

In **Chapter 5**, we describe the pitfalls in the treatment of hypogonadism in women with PWS. To avoid underdiagnosis and undertreatment, we provide clinical recommendations based on our cohort study, literature review and expert panel.

In **Chapter 6**, we study thyroid disorders in adults with PWS and the relation between thyroid function and other treatments, i.e. GH treatment and psychotropic medication. We provide recommendations for the screening of thyroid disorders to avoid long-term negative consequences.

In **Chapter 7**, we investigate the prevalence of central adrenal insufficiency in a large, international cohort of adults with PWS. We provide clinical recommendations to prevent overtreatment with corticosteroids in the future.

In **Chapter 8**, we report the prevalence and causes of hyperprolactinemia in adults with PWS. We show the importance of prolactin measurement, especially in patients that use antipsychotics, to avoid long-term negative effects of undiagnosed and untreated hyperprolactinemia.

In **Chapter 9**, we analyze the occurrence and causes of hyponatremia in patients with PWS. We provide practical recommendations to avoid unnecessary morbidity and mortality due to undiagnosed and untreated hyponatremia.

In **Chapter 10**, we investigate bone health in a large international cohort of adults with PWS. We report on the high prevalence of osteoporosis, osteopenia, fractures, and scoliosis and provide clinical recommendations to avoid long-term complications.

In **Chapter 11**, we describe four adults with PWS with severe cardiovascular disease and compare these patients to our cohort of adults with PWS without cardiovascular disease. We highlight the diagnostic pitfalls regarding the prevention, detection, and treatment of cardiovascular disease in this patient population.

In **Chapter 12**, we describe the occurrence of malignancies in an international cohort of children and adults with PWS. As malignancies were significantly related to genotype, we also provide a literature review on the relation between the genes on chromosome 15q11.2-13 and malignancies.

In **Chapter 13**, we shed a new light on the genetics of PWS by presenting a patient with a unique genetic variant. Based on this patient, a literature review and functional analysis of the genetic variant, we provide an in-depth discussion of the relation between the genes on the PWS region and the clinical features of PWS.

In **Chapter 14**, we discuss the results of the studies presented and provide an overview of the clinical recommendations.

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APPENDIX TO CHAPTER 1

Regulation of pituitary hormones
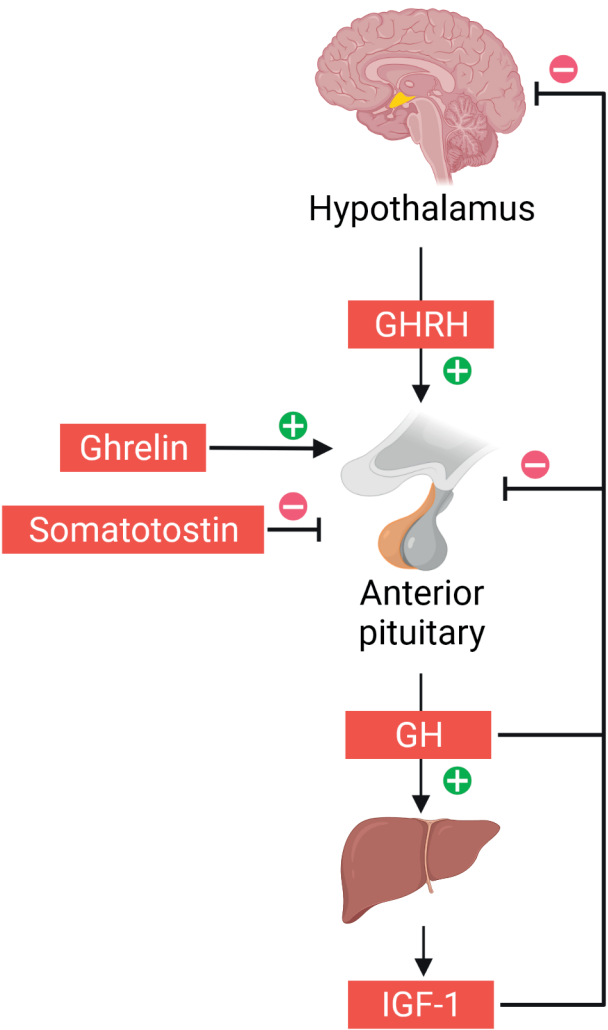
Figure 1. Hypothalamic-pituitary-somatotropic axis
Created with BioRender.com. Abbreviations: growth hormone (GH), growth hormone-releasing hormone (GHRH), insulin-like growth factor-1 (IGF-1).
The hypothalamic-pituitary-somatotropic axis is a complex axis. GHRH, produced by the hypothalamus, causes the release of GH from the pituitary gland. GH itself has many target organs, but its main action is stimulation of the liver to produce IGF-1. IGF-1 also has many target organs, including bone, adipose tissue, and muscle. IGF-1 provides negative feedback to the pituitary gland and hypothalamus. In addition to GHRH, ghrelin also stimulates the secretion of GH, while somatostatin is an important inhibitor of GH secretion (1, 2).
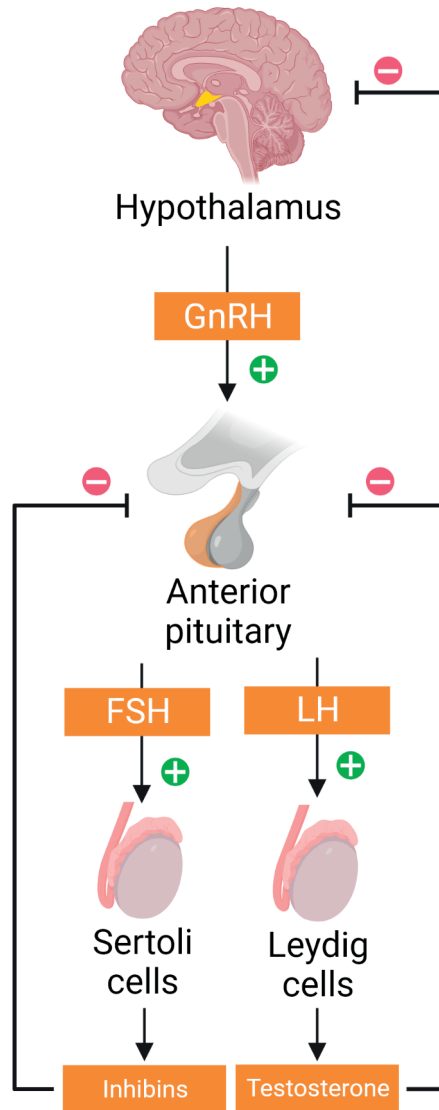
Figure 2. Hypothalamic-pituitary-gonadal axis in men

Created with BioRender.com. Abbreviations: follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH).

The hypothalamus produces GnRH in a pulsatile manner, which stimulates the production of gonadotropins, LH and FSH, in the anterior pituitary. FSH stimulates testicular Sertoli cells, playing an important role in spermatogenesis. Sertoli cells produce inhibins, which suppress FSH secretion (3, 4). LH stimulates Leydig cells to produce testosterone. Testosterone is strongly bound to sex hormone–binding globulin (SHBG), the levels of which can be altered by several conditions like obesity (5). Testosterone can also be bound by albumin. The free (unbound) testosterone, in turn, inhibits the release of GnRH and LH.
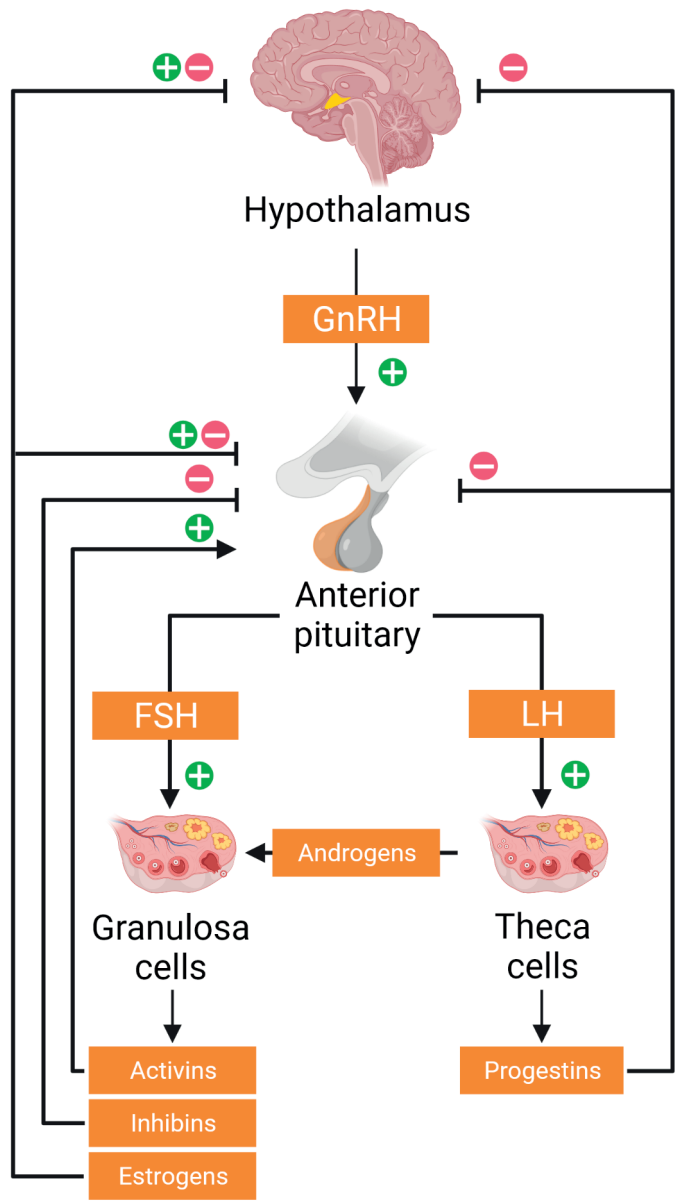
Figure 3. Hypothalamic-pituitary-gonadal axis in women
Created with BioRender.com. Abbreviations: follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH).
GnRH is released in a pulsatile manner, which stimulates the production of LH and FSH. LH stimulates the ovarian theca cells to produce androgens and progestins, while FSH stimulates ovarian granulosa cells to convert androgens to estrogens and produce inhibins and activins. Activins cause a positive feedback to the pituitary gland, while inhibins provide negative feedback (4). Low levels of estrogens and progestins provide negative feedback to the pituitary gland and the hypothalamus, while high levels of estrogens causes positive feedback, resulting in a LH surge that induces ovulation (6).
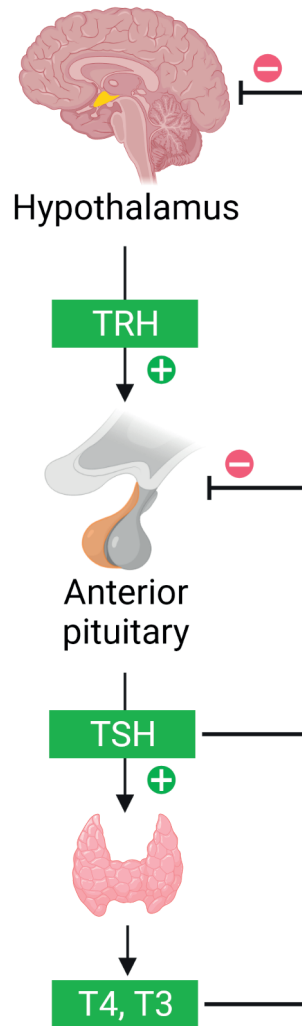
Figure 4. Hypothalamic-pituitary-thyroid axis
Created with BioRender.com. Abbreviations: thyroxine (T4), triiodothyronine (T3), thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH).
TRH is released by the hypothalamus. TRH stimulates the synthesis and release of TSH in the anterior pituitary. TSH stimulates the production of THs in the thyroid gland, which, in turn, provide negative feedback on the hypothalamus and the pituitary gland. For clinical practice, the most important thyroid hormones are T4 and T3. As most T4 and T3 is bound to plasma proteins like thyroxine binding globulin, free T4 (fT4) and free T3 (fT3) are often measured.
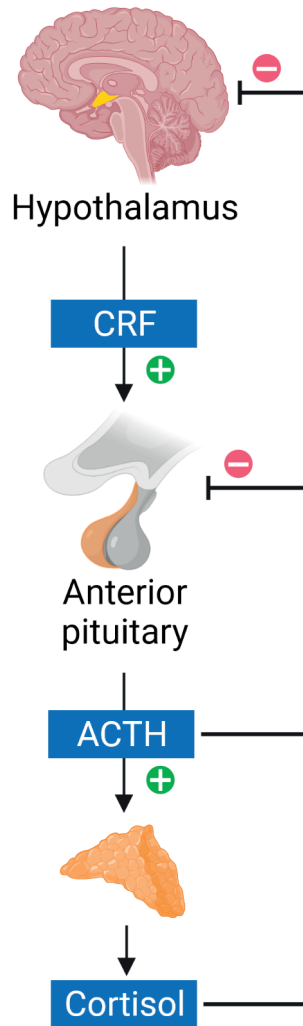
Figure 5. Hypothalamic-pituitary-adrenal axis
Created with BioRender.com. Abbreviations: adrenocorticotropic hormone (ACTH), corticotropin-releasing factor (CRF).
The hypothalamic-pituitary-adrenal axis regulates the glucocorticoid production (7).
The hypothalamus produces CRF, which is released in response to stress and stimulates the release of ACTH by the pituitary gland. ACTH stimulates the production of glucocorticoids like cortisol in the adrenal cortex, which provide a negative feedback on the hypothalamus and pituitary gland (7, 8). This axis is subject to a circadian rhythm, with cortisol levels being the highest just after waking (9).
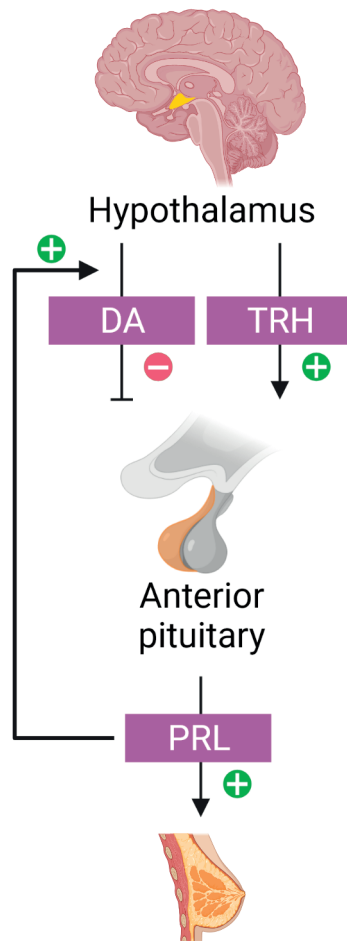
Figure 6. Prolactin
Created with BioRender.com. Abbreviations: dopamine (DA), prolactin (PRL), thyrotropin-releasing hormone (TRH).
The hypothalamus produces dopamine, which inhibit the release of prolactin by lactotroph cells in the anterior pituitary. The hypothalamus also produces TRH, which stimulates prolactin production. Prolactin stimulates the release of dopamine from the hypothalamus, thus providing negative feedback (10, 11). Disturbance in the dopamine inhibition results in hyperprolactinemia (10, 11). Several conditions and drugs influence dopamine secretion, including many antipsychotics and some antidepressants (12). Hyperprolactinemia might also be the related to stress-induced changes in dopamine and serotonin secretion (13).
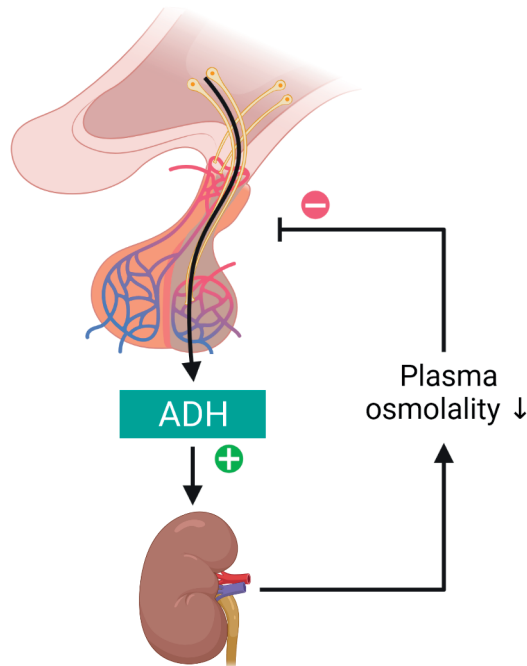
Figure 7. Antidiuretic hormone
Created with BioRender.com. Abbreviations: antidiuretic hormone (ADH).
ADH is produced in the hypothalamus and travels down the axons to the posterior pituitary gland, where it is secreted into the circulation. Osmoreceptors in the hypothalamus measure plasma osmolality. When plasma osmolality is increased, ADH is released. ADH can also be released in response to hypovolemia, as measured by baroreceptors in the left atrium, carotid artery and aortic arch. ADH increases water reabsorption in the late distal tubule and the collecting duct of the kidney. This leads to an increase in plasma volume and blood pressure. ADH also increases peripheral vascular resistance through vasoconstriction, leading to a further increase in blood pressure (14).
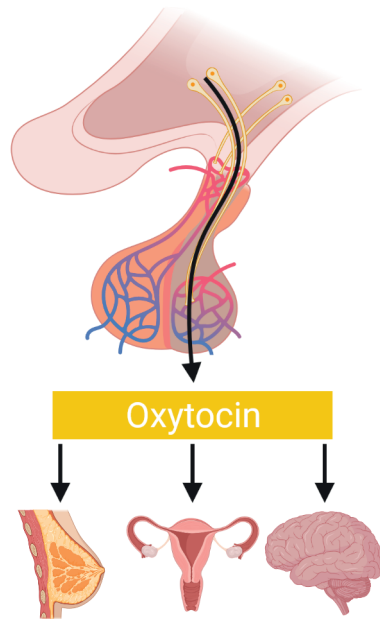
Figure 8. Oxytocin
Created with BioRender.com. Oxytocin is produced in the hypothalamus and released in the posterior pituitary. Oxytocin stimulates uterine contractions during labor and stimulates the release of milk from the mammary gland. It also has important behavioral effects. For example, oxytocin is involved in sexual arousal, prosocial behavior, empathy, and the mother-infant bond (15-17).

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2

Missed diagnoses and health problems in adults with Prader-Willi syndrome: recommendations for screening and treatment

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ABSTRACT

Context

Prader-Willi syndrome (PWS) is a complex hypothalamic disorder, combining hyper-phagia, hypotonia, intellectual disability, and pituitary hormone deficiencies. Annual mortality of patients with PWS is high (3%). In half of the patients, the cause of death is obesity related and/or of cardiopulmonary origin. Health problems leading to this increased mortality often remain undetected due to the complexity and rareness of the syndrome.

Objective

To assess the prevalence of health problems in adults with PWS retrospectively.

Patients, Design, and Setting

We systematically screened 115 PWS adults for undiagnosed health problems. All patients visited the multidisciplinary outpatient clinic for rare endocrine syndromes at the Erasmus University Medical Center, Rotterdam, Netherlands. We collected the results of medical questionnaires, interviews, physical examinations, biochemical measurements, polygraphy, polysomnography, and radiology.

Main outcome measures

Presence or absence of endocrine and nonendocrine comorbidities in relation to living situation, body mass index, genotype, and demographic factors.

Results

Seventy patients (61%) had undiagnosed health problems, while 1 in every 4 patients had multiple undiagnosed health problems simultaneously. All males and 93% of females had hypogonadism, 74% had scoliosis, 18% had hypertension, 19% had hypercholesterolemia, 17% had type 2 diabetes mellitus, and 17% had hypothyroidism. Unfavorable lifestyles were common: 22% exercised too little (according to PWS criteria) and 37% did not see a dietitian.

Conclusions

Systematic screening revealed many undiagnosed health problems in PWS adults. Based on patient characteristics, we provide an algorithm for diagnostics and treatment, with the aim to prevent early complications and reduce mortality in this vulnerable patient group.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare genetic, neuroendocrine condition caused by the absence of a normal paternal contribution to the 15q11-13 region. It is most commonly caused by a paternal deletion (65–75%) or a maternal uniparental disomy 15 (mUPD, 20–30%). In the minority of cases, PWS is caused by an imprinting center defect (ICD, 1–3%) or a paternal chromosomal translocation (0.1%) (1, 2). The syndrome is characterized by hypotonia, behavioral challenges, typical dysmorphic features, and hypothalamic dysfunction resulting in hyperphagia, pituitary hormone deficiencies, abnormal temperature regulation, and inadequate pain registration (3–6).

Annual mortality in adults with PWS is high (3%) (7, 8) compared with 1.3% annual mortality in non-PWS adults with an intellectual disability (9). More than half of these deaths are caused by cardiopulmonary pathology (8, 10) and another 7% of deaths are directly related to obesity (8). Seventy-eight percent of deaths in patients with PWS are unexpected (11).

Multiple factors contribute to the increased risk of cardiopulmonary pathology in patients with PWS. Based on our clinical experience with more than a hundred PWS adults, we hypothesize that there is a complex interaction between obesity and behavioral, endocrine, and cardiovascular (CV) risk factors that contribute to the high prevalence of cardiopulmonary disease in patients with PWS, as shown in **Figure 1**.

Obesity in patients with PWS is caused by hyperphagia (leading to a high energy intake) combined with a low energy expenditure (12, 13). This low energy expenditure is caused by low muscle mass, which is part of the syndrome. Untreated pituitary hormone deficiencies like hypogonadism, hypothyroidism, and growth hormone deficiency can affect muscle mass and function, causing a further decrease in basal metabolic rate (12, 14–21).

The total energy expenditure in adults with PWS is 20% lower than in age-matched obese adults (22). This difference in energy expenditure should be compensated by either a strict diet or by exercising for at least 1 hour a day (23). However, exercise tolerance may be low, due to hypotonia, pituitary hormone deficiencies, and (severe) vitamin D deficiency (14, 24–29). Moreover, the typical behavioral phenotype and musculoskeletal problems like scoliosis, hypotonia, and leg edema impair physical activity in adults with PWS. This results in a vicious circle of muscle weakness, exercise intolerance, and a further decrease in physical activity. The subsequent sedentary lifestyle can induce CV risk factors like hypertension, hypercholesterolemia, and type 2 diabetes mellitus (DM2) (30).
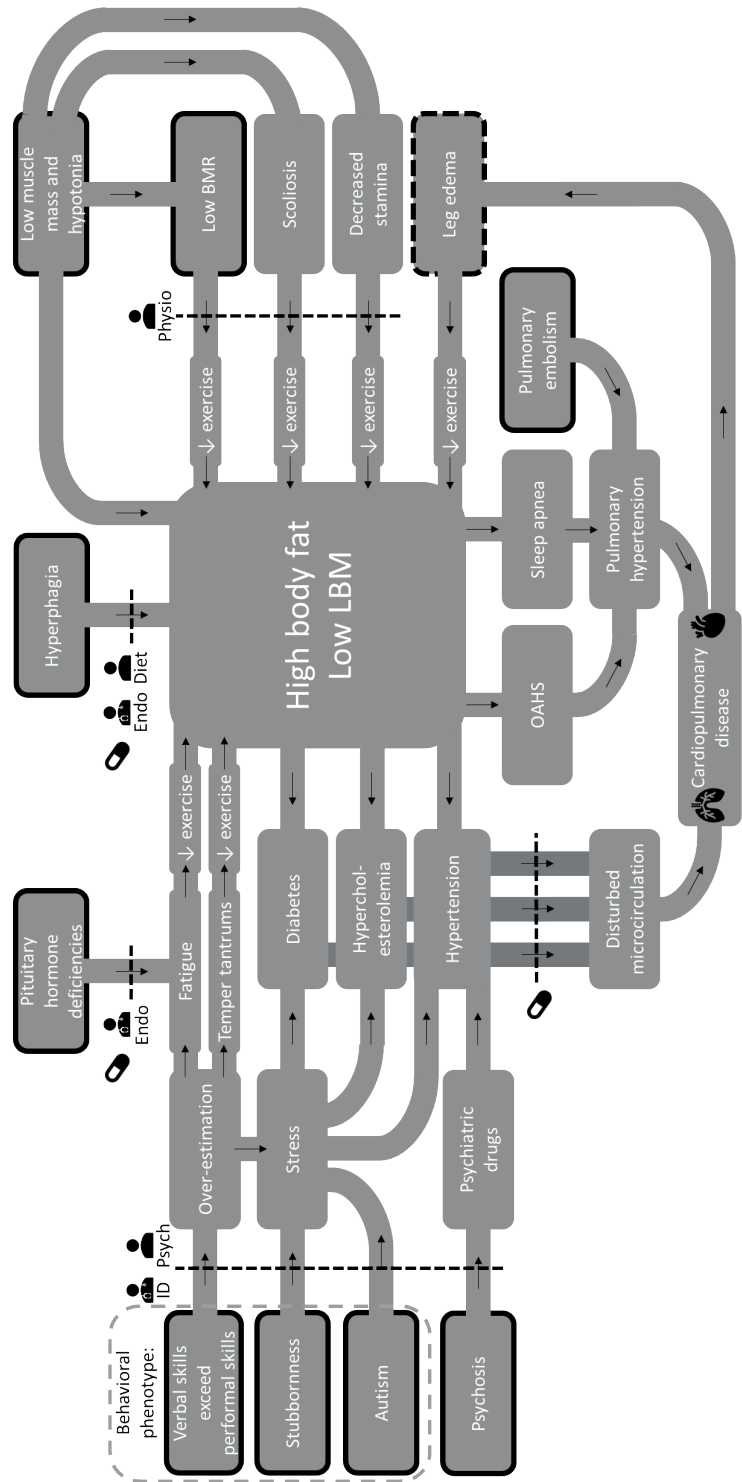
Figure 1. Factors contributing to cardiopulmonary disease in patients with PWS. Abbreviations: BMR (basal metabolic rate), diet (dietitian), ID (physician for people with intellectual disabilities), lean body mass (LBM), OAHS (obesity associated hypoventilation syndrome), physio (physiotherapist), psych (psychologist). Legend: black arrows indicate a cause-and-effect relationship; the pill stands for an intervention with medication; black borders indicate that the factor is inherent to the syndrome; dotted black border indicates that the factor is inherent to the syndrome, but can be aggravated by cardiopulmonary disease (12–39).

Other CV risk factors often present in PWS are obesity hypoventilation syndrome and sleep apnea, which can be central sleep apnea, obstructive sleep apnea, or both. Central sleep apnea, obstructive sleep apnea, and obesity hypoventilation syndrome can lead to pulmonary hypertension (31, 32), DM2 (33), and a further increase in obesity (34) and CV risk (35–38). Lastly, the cognitive phenotype of PWS (often higher verbal comprehension skills compared with performal/reasoning skills, which can easily lead to overestimation) and autism-related behavioral challenges could induce stress. Stress can induce hypertension, another important CV risk factor (39). Moreover, psychosis is prevalent in patients with PWS, often requiring psychiatric drugs. As many psychiatric drugs have CV side effects, this can lead to a further increase in CV risk (40).

The complex interplay between somatic and psychological factors requires a syndrome-specific approach to health problems. However, as PWS is a rare disorder (5), most physicians are unfamiliar with the syndrome and its associated comorbidities. Furthermore, the PWS-specific behavioral phenotype (high pain threshold and the inability to express complaints) often leads to underdiagnosis and undertreatment. Combined doctors' and patients' delay can lead to medical complications and hospital admission. Timely recognition of comorbidities can reduce medical complications and associated personal and financial burdens (41).

Previous authors have reported health problems in adults with PWS (11, 42–60). However, most of them did not perform a systematic screening and only reported health problems that had already been diagnosed. As underdiagnosis is a serious problem in this patient population, the prevalences reported in these studies are most likely underestimated. Data from systematic health screenings in adults with PWS are scarce (44, 45, 47, 48, 58) and little is known about the relation between patient characteristics (living situation, presence or absence of obesity, genotype, and demographic factors) and health problems. As a consequence, there is no consensus about periodical screening.

In our reference center, we routinely perform a systematic health screening in all adults with PWS in order to detect comorbidities at an early stage. In this article, we report the prevalence of the physical health problems detected by our screening. Based on their associations with the aforementioned patient characteristics, we provide practical advice for medical screening.

METHODS

This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center. In this retrospective study, we reviewed the medical files of all adults who visited the multidisciplinary outpatient clinic of the PWS reference center in the Erasmus University Medical Center, Rotterdam, Netherlands, between January 2015 and April 2020 and who underwent a routine systematic health screening. All patients that visited our outpatient clinic were already diagnosed with PWS, often years before visiting our outpatient clinic and/or during childhood. Before the launch of our multi-disciplinary outpatient clinic in 2015, many Dutch adults with PWS were treated by their general practitioner or physician for people with intellectual disabilities (ID physician).

Our systematic screening consists of a structured interview, a complete physical exami-nation, a medical questionnaire, a review of the medical records, biochemical measure-ments and, if indicated and feasible, additional tests such as dual energy X-ray absorp-tiometry (DEXA), polygraphy (PG), and polysomnography (PSG). We report the hidden health problems that were present but undetected and/or untreated until the moment of screening. Conditions that developed during later follow-up visits were not taken into account. Forty-two patients in the cohort that we describe were also mentioned in a previous study by Sinnema et al (43), who gave an overview of 102 adults with PWS and the health problems that had already been diagnosed (without systematic screening).

Genetic diagnosis

We performed genetic testing or collected previous genetic test results from other Dutch academic hospitals to confirm the PWS diagnosis and to determine the genetic subtype.

Medical questionnaire

As part of regular patient care, primary caregivers filled out a medical questionnaire be-fore visiting the outpatient clinic. This questionnaire included questions on the patient's medical history, medication, family history, symptoms of disease, physical complaints, behavioral challenges, and social aspects such as school, relationships, and living situa-tion. The symptoms of disease, physical complaints, and behavioral challenges are rated on a 5-point Likert scale (1 = rarely or never, 2 = not often and/or not severe, 3 = quite often and/or quite severe, 4 = often and/or severe, 5 = very often and/or very severe). A score of 3 or higher was considered clinically relevant and was further explored during the visit. Mutism is defined as the absence of speech.

Biochemical analysis

During the visit, blood samples were taken for general medical screening, including the evaluation of fat metabolism (low density lipoprotein [LDL]-cholesterol), glucose metabolism (nonfasting glucose, hemoglobin A1c), thyroid function (free T4), gonadal function (random luteinizing hormone [LH], follicle-stimulating hormone, estradiol or testosterone, sex hormone binding globulin), liver enzymes (aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, total bilirubin, lactate dehydrogenase), kidney function (urea, creatinine, estimated glomerular filtration rate [eGFR]), the hematopoietic system (hemoglobin, hematocrit, mean corpuscular volume, leukocytes, thrombocytes and, in case of microcytic anemia, ferritin), and vitamin D status (25-hydroxyvitamin D). The eGFR is estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

Cutoff levels

A hypercholesterolemia diagnosis was confirmed if the patient had a nonfasting LDL-cholesterol above 4.0 mmol/L. Type 2 diabetes mellitus was defined as a repeated fasting glucose above 6.7 mmol/L (or nonfasting glucose above 11.0 nmol/L). Hemoglobin A1c was used to assess long-term glycemic control. As hypothyroidism in PWS can be both primary and central (61), hypothyroidism was defined as a free T4 below 11 pmol/L, regardless of thyroid stimulating hormone. Hypogonadism in males was defined as a morning testosterone level below 10.0 nmol/L or a random testosterone level below 10.0 nmol/L combined with clear clinical features of hypogonadism (sparse body hair, micropenis, and the absence of spontaneous morning erections). Hypogonadism in females was defined as absent, scarce, or irregular menses. The diagnosis of hypogonadism was based on both laboratory values and clinical parameters because of the effect of adipose tissue aromatase activity on estradiol and testosterone levels (62), and the fact that hypogonadism in PWS can be both primary and central (63). Due to intellectual disability in most patients, gynecological evaluation was not routinely performed. When females used oral contraceptives or estrogen replacement therapy before screening, we asked for the presence of the menstrual cycle before the start of estrogen replacement therapy.

Severe vitamin D deficiency was defined as a 25-hydroxyvitamin D level below 20 nmol/L and a mild vitamin D deficiency was defined as a 25-hydroxyvitamin D level below 50 nmol/L. When patients used cholesterol-lowering medications, oral antidiabetics, insulin, levothyroxine, or testosterone replacement therapy before the start of the screening, we requested pretreatment laboratory values to verify the diagnosis.

Additional tests

We screened for hypertension by measuring blood pressure. If the patient's blood pressure was above 140/90 mmHg, the measurement was repeated, and if it was still elevated, a 30-minute blood pressure measurement was performed. If the patient already used antihypertensive drugs, we requested pretreatment blood pressure values.

If risk factors for osteoporosis were present (untreated hypogonadism, family history of osteoporosis, previous fractures, untreated vitamin D deficiency, and/or corticosteroid treatment), we performed a DEXA scan to screen for osteoporosis or osteopenia. Osteoporosis was defined as a T-score (comparison of a person's bone density with that of a healthy 30-year-old of the same sex) below -2.5, osteopenia was defined as a T-score between -1.0 and -2.5.

If there was a clinical suspicion of scoliosis (based on a gibbus deformity during physical examination), we performed an X-ray of the spine if (1) the patient was not previously diagnosed with scoliosis, (2) the patient suffered from back pain, or (3) the caregivers reported new or progressive postural abnormalities. Radiologically confirmed scoliosis was defined as a Cobb angle of 10° or more, as measured on a spinal X-ray.

The indication for sleep studies was based on the presence of clinical signs of sleep apnea: severe snoring, witnessed apneas, daytime sleepiness, morning headaches, hypertension, or waking up with shortness of breath, headaches, or panic. If indicated and feasible, we performed PG (ie, the continuous recording of nasal airflow, thoracic and abdominal movements, heart rate, and oxygen saturation during 1 night) or a PSG (ie, PG measurements and electroencephalography, electro-oculography, and electromyography). Also, before the start of growth hormone (GH) treatment, we performed a PSG to exclude sleep apnea, as untreated sleep apnea is an absolute contraindication for GH treatment.

Data analysis

Statistical analysis was performed using R version 3.6.3. Descriptive statistics for continuous variables are reported as median and interquartile range (IQR). For dichotomous variables, we display the number of people and the percentage of total people, n (%). We used a chi-squared test to compare the prevalence of health problems between different groups based on patient characteristics: genotype, living situation, and gender. To investigate the relationship between body mass index (BMI), age, patient characteristics, and the prevalence of health problems, we used the Wilcoxon rank sum test. For the relationship between BMI, age, and living situation, we used the Kruskal-Wallis test. A chi-squared test for trend was used to compare the number of undiagnosed health

problems between subgroups. To investigate the relationship between age and BMI, the Kendall rank correlation test was used. To investigate the effect of BMI and age on health problems and the number of undiagnosed health problems corrected for age and BMI respectively, logistic and ordinal regression models were used and a likelihood ratio test was performed.

Literature review

We reviewed the literature for studies that report physical health problems in patients with PWS. Inclusion criteria were original research articles and observational studies that reported the prevalence of physical health problems in a cohort of patients with PWS of 15 years of age or older. Exclusion criteria were clinical trials, basic or translational research, case reports, case series that included less than 10 adults with PWS, articles that were not available online, articles that were not available in English, and mixed pediatric–adult articles that did not report separate prevalences for patients with PWS of 15 years of age or older. The full search strategy used is available upon request.

RESULTS

We included 115 (56 male and 59 female) patients. Median age was 29 years (IQR 21–40) and median BMI 29 kg/m^2 (IQR 26–35). Baseline characteristics are shown in **Table 1**. The exact age at diagnosis was known for 72 patients, of which 59 patients were diagnosed during childhood. Of 115 patients, 42 underwent transition after years of medical supervision at the pediatric multidisciplinary outpatient clinic. All patients referred by the pediatrician had a personal care plan. Of the remaining 73 patients, 17 patients had been followed by an endocrinologist elsewhere during the year before the screening. A total of 46 patients had been followed by an ID physician and 14 had never visited an adult endocrinologist or ID physician before.

We refer to the repository (64) for the following supplementary data: baseline characteristics and health problems by living situation and genotype; health problems by BMI, age, and gender; information about lifestyle, behavior, and physical complaints; details of biochemical analysis (liver panel, kidney function, hematopoiesis, and electrolyte values); and data about sleep apnea, bone mineral density, and vitamin D deficiency.

Table 1. Baseline characteristics of 115 adults with Prader-Willi syndrome

	Total n = 115
Age in years, median [IQR]	29 [21 – 40]
BMI in kg/m², median [IQR]	29 [26 – 35]
Male gender, n (%)	56 (49%)
Genetic subtype	
Deletion, n (%)	64 (56%)
mUPD, n (%)[a]	41 (36%)
ICD, n (%)	3 (3%)
Unknown, n (%)	7 (6%)
Growth hormone treatment	
Only during childhood, n (%)	10 (9%)
Only during adulthood, n (%)	3 (3%)
Both, n (%)	40 (35%)
Never, n (%)	62 (54%)
Current growth hormone treatment, n (%)	41 (36%)
Use of hydrocortisone	
Daily, n (%)	4 (4%)
During physical or psychological stress, n (%)	47 (41%)
Use of estrogen replacement therapy or oral contraceptives, n (%)	34/59 females (58%)
Use of levothyroxine, n (%)	17 (15%)
Living situation	
With family, n (%)	28 (24%)
In a specialized PWS group home, n (%)	23 (20%)
In a non-specialized group home, n (%)	61 (53%)
Assisted living, n (%)	3 (3%)
Scholar level	
Secondary vocational education, n (%)	6 (5%)
Pre-vocational secondary education, n (%)	3 (3%)
Special education, n (%)	82 (71%)
No education, n (%)	4 (4%)
Unknown, n (%)	20 (17%)
Mutism, n (%)	3 (3%)
Relationship status	
In a relationship with sexual intercourse, n (%)	8 (7%)
In a relationship without sexual intercourse, n (%)	18 (16%)
Not in a relationship, n (%)	76 (66%)
Unknown, n (%)	13 (11%)

Abbreviations: body mass index (BMI), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD), Prader-Willi syndrome (PWS). [a] In 11 patients with an mUPD, the parents were not available for genetic testing. Therefore, mUPD is the most likely genotype, but an ICD could not be ruled out in these patients.

Health problems detected by screening

The results of our systematic health screening are shown in **Table 2** and **Figure 2**. We found undetected health problems in 61% of adults with PWS. One-fourth had more than one undetected simultaneous health problem. The most common undetected health problem was hypogonadism, which had gone unnoticed in 52% of males and

33% of females. Other undiagnosed health problems were scoliosis (20%), hypercholesterolemia (6%), DM2 (5%), hypertension (3%), and hypothyroidism (2%). Forty-five patients underwent DEXA scans as part of medical screening. This revealed 3 new cases of osteoporosis and 8 cases of osteopenia, on top of the 9 patients already known with osteoporosis and the 22 patients with osteopenia. Two males and one female (all older than 30 years of age during the screening) had osteoporosis despite previous treatment for hypogonadism. Both males had received testosterone replacement therapy for more than 15 years before screening. For the female, the exact duration of estrogen replacement therapy was unknown. Nine patients were known to have sleep apnea before the screening. Nineteen patients underwent PG or PSG, of which 11 patients were diagnosed with sleep apnea.

Table 2. Health problems in 115 adults with PWS before and after systematic screening

	Total n = 115			
	Before Screening	**Detected by screening**	**After screening**	**Missing**
Hypogonadism				
Male (n = 56)	26 (48%)	*+52%*	54 (100%)	2
Female (n = 59)	26 (60%)	*+33%*	40 (93%)	16[a]
Scoliosis	61 (54%)	*+20%*	83 (74%)[b]	3
Hypercholesterolemia	14 (13%)	*+6%*	22 (19%)	2
Type 2 diabetes mellitus	13 (12%)	*+5%*	19 (17%)	2
Hypertension	17 (15%)[c]	*+3%*	20 (18%)	3[d]
Hypothyroidism	17 (15%)	*+2%*	19 (17%)	0
Vitamin D deficiency	26 (38%)	*+40%*	54 (78%)	46[e]
Severe vitamin D deficiency			8 (13%)	55
Total undiagnosed health problems				
At least one		*70 (61%)*		
At least two		*28 (24%)*		
Three or more		*10 (9%)*		

Data are presented as n (% of total). In bold are the number and % of health problems detected by our systematic screening. Abbreviation: Prader-Willi syndrome (PWS) [a] (Caregivers of) 16 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age. [b] Twenty-eight patients had clear scoliosis at physical examination, but X-ray was not performed due to practical/behavioral issues, and 55 cases were radiologically confirmed. [c] Four patients received antihypertensive medication before screening, but the indication was unknown. [d] Blood pressure was high in 2 patients, but the measurement was not repeated due to practical/behavioral issues. [e] In 2 patients vitamin D was not measured and 44 patients used vitamin D supplementation before the screening, but it was unknown whether they had low vitamin D values before the start of vitamin D supplementation.

Comparison of health problems between groups

Living situation

Twenty-three patients lived in a specialized PWS group home (PWS home), 61 in a nonspecialized group home (non-PWS home), 28 with family, and 3 in an assisted living
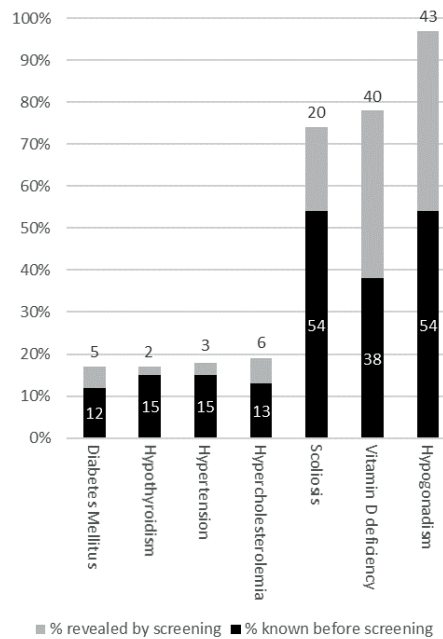
Figure 2. Health problems detected by systematic health screening in 115 adults with PWS. Abbreviation: Prader-Willi syndrome (PWS). Legend: black bars indicate the percentage of health problems already diagnosed before the screening; gray bars indicate the percentage of health problems that were revealed by screening.

facility. Patients living in non-PWS homes were significantly older (median age 36 years, IQR 28–50) than those living in PWS homes (median age 26 years, IQR 21–32) or with family (median age 19 years, IQR 19–22). Body mass index and prevalence of hypertension were significantly higher in patients living in non-PWS homes (see **Table 3**).

Patients in PWS homes exercised more than those living with family or in non-PWS homes. Patients in PWS homes all exercised at least 30 minutes a day versus 75% and 70% of those living with family or in non-PWS homes, respectively. A dietitian was involved in the care of 87% of patients living in PWS homes, 74% of those living in a non-PWS home, and 29% of those living with family.

Genotype
When comparing health problems between the 2 largest genotypic subgroups (64 patients with a deletion and 41 with mUPD), scoliosis was more frequent in patients with a deletion than in patients with an mUPD (81% vs 59%, P = 0.02). Other variables were not remarkably different between the genotypes (see **Table 3**).

Table 3. Health problems according to living situation and genotype

	Missing	PWS home[a] n = 23	Non-PWS home[b] n = 61	Family[c] n = 28	P-value	Deletion n = 64	mUPD n = 41	P-value
Age, median [IQR]	0	26 [21 – 32]	36 [28 – 50]	19 [19 – 22]	**<0.001**	28 [21 – 36]	32 [21 – 49]	0.2
BMI, median [IQR]	0	27 [22 – 30]	30 [27 – 40]	28 [26 – 36]	**0.004**	31 [26 – 38]	29 [25 – 34]	0.3
Undiagnosed health problems[d]								
At least one		9 (39%)	44 (72%)	15 (54%)	0.16	37 (58%)	25 (61%)	0.7
At least two		2 (9%)	19 (31%)	5 (18%)		14 (22%)	12 (29%)	
Three or more		2 (9%)	4 (7%)	4 (14%)		6 (9%)	3 (7%)	
Hypogonadism								
Male (n = 56)	2	9 (100%)	28 (100%)	15 (100%)	NA[e]	27 (100%)	19 (100%)	NA[e]
Female (n = 59)	16[f]	10 (100%)	20 (87%)	10 (100%)	0.2	25 (93%)	14 (93%)	0.9
Hypothyroidism	0	4 (17%)	12 (20%)	3 (11%)	0.6	11 (17%)	7 (17%)	0.99
Type 2 diabetes mellitus	2	2 (9%)	13 (22%)	3 (11%)	0.2	8 (13%)	10 (24%)	0.1
Hypertension	3	0 (0%)	17 (29%)	2 (7%)	**0.002**	9 (15%)	8 (20%)	0.5
Hypercholesterolemia	2	4 (17%)	15 (25%)	2 (7%)	0.1	11 (17%)	8 (20%)	0.7
Scoliosis	3	18 (78%)	44 (76%)	19 (68%)	0.6	51 (81%)	23 (59%)	**0.02**
Vitamin D deficiency	46[g]	14 (88%)	22 (85%)	16 (64%)	NA[h]	33 (80%)	19 (76%)	NA[h]

Data are presented as n (% of total). P-values <0.05 are bold. Abbreviations: body mass index (BMI), interquartile range (IQR), maternal uniparental disomy (mUPD), Prader-Willi syndrome (PWS). [a] Patients living in a specialized Prader-Willi syndrome group home. [b] Patients living in a non-specialized group home. [c] Patients living with family. [d] Undiagnosed health problems are: hypogonadism, hypothyroidism, type 2 diabetes mellitus, hypertension, hypercholesterolemia, scoliosis and vitamin D deficiency. [e] Not applicable as hypogonadism is present in 100%, regardless of patient characteristics. [f] (Caregivers of) 16 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age. [g] In 2 patients vitamin D was not measured and 44 patients used vitamin D supplementation before the screening, but it was unknown whether they had low vitamin D values before the start of vitamin D supplementation. [h] A P-value could not be calculated due to selective missings.

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Table 4. Health problems according to BMI, age and gender

	BMI <25 kg/m² n = 24	BMI 25-30 kg/m² n = 43	BMI >30 kg/m² n = 48	P-value[a]	Corr. P-value for age[b]	Age < 25 year n = 43	Age 25-30 year n = 21	Age > 30 year n = 51	P-value[a]	Corr. P-value for BMI[c]	Male n = 56	Female n = 59	P-value
Age, median [IQR]	24 [20 – 33]	27 [20 – 40]	32 [25 – 42]	**0.02**	NA	20 [19 – 22]	28 [26 – 29]	41 [34 – 52]	NA	NA	31 [20 – 43]	28 [22 – 38]	0.5
BMI, median [IQR]	22 [21 – 23]	27 [27 – 29]	38 [34 – 46]	NA	NA	27 [23 – 31]	29 [26 – 35]	31 [27 – 42]	**0.02**	NA	27 [25 – 32]	32 [27 – 40]	**0.02**
Undiagnosed health problems													
At least one	8 (33%)	24 (56%)	38 (79%)	**<0.001**	**<0.001**	19 (44%)	9 (43%)	42 (82%)	**0.001**	**0.02**	37 (66%)	33 (56%)	0.1
At least two	2 (8%)	9 (21%)	17 (35%)			6 (14%)	4 (19%)	18 (35%)			17 (30%)	11 (19%)	
Three or more	0 (0%)	4 (9%)	6 (13%)			3 (7%)	3 (7%)	4 (8%)			7 (13%)	3 (5%)	
Hypogonadism													
Male (n = 56)	11 (100%)	27 (100%)	16 (100%)	NA	NA	20 (100%)	7 (100%)	27 (100%)	NA	NA	54 (100%)		NA
Female (n = 59)	6 (100%)	12 (92%)	22 (92%)	0.9	0.3	18 (100%)	9 (90%)	13 (87%)	0.2	0.1		40 (93%)	NA
Hypothyroidism	5 (21%)	7 (16%)	7 (15%)	0.5	0.8	10 (23%)	5 (24%)	4 (8%)	0.2	0.4	5 (9%)	14 (24%)	**0.03**
Type 2 diabetes mellitus	2 (8%)	7 (17%)	10 (21%)	0.2	0.6	2 (5%)	2 (10%)	15 (31%)	**<0.001**	**0.001**	13 (24%)	6 (10%)	0.06
Hypertension	3 (13%)	6 (15%)	11 (23%)	0.4	0.7	3 (7%)	1 (5%)	16 (31%)	**<0.001**	**<0.001**	9 (17%)	11 (19%)	0.8
Hypercholesterolemia	4 (17%)	4 (10%)	14 (30%)	**0.01**	**0.01**	3 (7%)	2 (10%)	17 (35%)	**0.002**	**0.01**	10 (18%)	12 (21%)	0.7
Scoliosis	12 (79%)	30 (71%)	34 (74%)	0.3	0.2	30 (70%)	19 (90%)	34 (71%)	0.9	0.5	42 (76%)	41 (72%)	0.6
Vitamin D deficiency	12 (75%)	20 (77%)	22 (81%)	NA[d]	NA[d]	27 (69%)	10 (91%)	17 (89%)	NA[d]	NA[d]	25 (83%)	29 (74%)	NA[d]

P-values <0.05 are bold. Abbreviations: body mass index (BMI), interquartile range (IQR). [a] P-value calculated with BMI and age as continuous variables. [b] P-value corrected for age using regression models. [c] P-value corrected for BMI using regression models. [d] A P-value could not be calculated due to selective missings.

Body mass index

Body mass index increased with age (P = 0.02). Patients with a higher BMI had a higher total number of undiagnosed health problems (P < 0.001) and more hypercholesterolemia (P = 0.01) (see **Table 4**). This remained significant after correction for age.

Age

Older patients had a higher prevalence of DM2 (P < 0.001), hypertension (P < 0.001), and hypercholesterolemia (P < 0.002), and a higher total number of undiagnosed health problems (P = 0.001) (see **Table 4**). This remained significant after correction for BMI.

Gender

Body mass index was significantly higher in females than in males (P = 0.02). Hypothyroidism was more prevalent in females than in males (24% vs 9%, P = 0.03) (see **Table 4**).

Age and BMI

Thirteen patients had BMI < 25 kg/m^2 and age < 25 years. None of these patients had DM2, 1 patient had hypertension, and 2 had hypercholesterolemia (of which 1 case was undiagnosed before screening).

Fatigue and daytime sleepiness

Fatigue and daytime sleepiness were common. One-third of the patients (40/115) had clinically relevant daytime sleepiness (score of 3 or higher on a 5-point Likert scale). All of these 40 patients had either untreated vitamin D deficiency, untreated male hypogonadism (**Table 5**), or another treatable cause such as sleep apnea, narcolepsia, nycturia, or use of drugs that can cause sleepiness (antiepileptic drugs, antipsychotics, benzodiazepines, tricyclic antidepressants, or antihistamines). Daytime sleepiness was present in 62% of the patients with untreated vitamin D deficiency versus 36% of the patients with normal vitamin D levels (P = 0.02). It was also related to the severity of the deficieny: daytime sleepiness was present in 80% of patients with untreated severe vitamin D defiency, 57% of patients with untreated mild vitamin D defiency, and 36% of patients with normal vitamin D levels.

Biochemical analysis

Liver panel was normal in most patients. However, 19 patients had alkaline phosphatase levels above the upper limit of normal. The vast majority of them had potential underlying causes such as vitamin D deficiency (63%) and/or obesity (58%). Normocytic anemia was common in males, but not in females. There were no cases of micro- or macrocytic anemia. Of the 17 males with anemia, 13 (76%) had untreated hypogonadism. Creatinine levels were generally low: 35 males (63%) and 28 females (47%) had creatinine levels

below the lower limit of normal, and this was independent of BMI and age. Of all males, 95% had creatinine levels between 46 and 93 μmol/L and eGFR levels between 93 and 149 ml/min/1.73 m^2. Of all females, 95% had creatinine levels between 37 and 76 μmol/L and eGFR levels between 98 and 140 ml/min/1.73 m^2.

Table 5. Fatigue and daytime sleepiness and potential underlying causes

	Difficulty sleeping		P-value	Nycturia		P-value	Snoring		P-value
	-	+		-	+		-	+	
N	84	9		66	28		64	32	
Fatigue	18 (22%)	4 (44%)	0.1	12 (18%)	11 (41%)	**0.03**	11 (18%)	12 (41%)	**0.02**
Daytime sleepiness	32 (38%)	8 (89%)	**0.003**	23 (35%)	17 (61%)	**0.02**	18 (29%)	22 (71%)	**<0.001**

	Male hypogonadism		P-value	Hypothyroidism		P-value	Untreated Vit D deficiency		P-value
	T[a]	UT[b]		-	+		-	+	
N	24	31		96	19		85	28	
Fatigue	4 (19%)	7 (28%)	0.5	18 (23%)	5 (31%)	0.5	15 (22%)	8 (32%)	0.3
Daytime sleepiness	7 (33%)	17 (65%)	**0.03**	35 (44%)	6 (35%)	0.5	25 (36%)	16 (62%)	**0.02**

Abbreviations: treated (T), untreated (UT), vitamin D (Vit D). Physical complaints were scored on a 5-point Likert scale. A score of 3 or higher was seen as clinically relevant ('+'), a score below 3 was seen as not clinically relevant ('-'). [a] Treated with testosterone replacement therapy. [b] Untreated hypogonadism.

Literature review

We found 21 publications reporting one or more of the following health problems in PWS: hypogonadism, hypothyroidism, DM2, hypertension, hypercholesterolemia, scoliosis, vitamin D deficiency, sleep apnea, or osteoporosis/osteopenia. Outcomes of these studies are summarized in **Tables 6 and 7**. None of the papers reported the prevalence of vitamin D deficiency in PWS.

Algorithm for diagnostics and treatment

Based on our analysis of patients data and the literature review, we defined diagnostic and therapeutic recommendations, presented in the algorithm in **Figure 3**.

Table 6. Patient characteristics of cohorts assessed by previous studies

Article	N[a]	Country	Data-collection	Age range (years)[a]	Genotype (deletion / mUPD / ICD / translocation)	Sex	Mean BMI (kg/m^2)	Previous GH treatment (%)
Laurance et al. (1981) (51)	24	United Kingdom	NA	15-41	NA	13 M, 11 F	NA	NA
Greenswag (1987) (52)	232	United States of America	Q	16-64	NA	115 M, 117 F	34[b]	NA
Partch et al. (2000) (46)	19	Germany	MR, I, PE	18-34	NA	7 M, 12 F	46	NA
Marzullo et al. (2005) (50)	13	Italy	MR	18-NA mean ± SD: 27 ± 1	85%/15%/-/-	7 M, 6 F	46	38%
Butler et al. (2002) (53)	58	United Kingdom	I and MR	18-46	NA	32 M, 26 F	35	13%
Thomson et al. (2006) (42)	30	Australia	State health data sets	15-48	44%/10%/-/- (54% NA)[c]	23 M, 23 F[c]	NA	NA
Sinnema et al. (2011) (43)	102	The Netherlands	I and MR	18-66	54%/43%/3%/-	49 M, 53 F	32	13%
Grugni et al. (2013) (49)	108	Italy	MR and PE	18-43	68%/25%/-/2% (6% NA)	47 M, 61 F	Median in non-obese: 26 Median in obese: 45	NA
Proffitt et al. (2019) (11)	2029	United States of America	Q	0 - 84	42%/19%/2%/-[c] (37% NA)	934 M, 1000F[c]	Living: 29[c] Deceased: 52[c,d]	56%[c]

Papers without systematic screening

Table 6. Patient characterististics of cohorts assessed by previous studies (continued)

Article	Nᵃ	Country	Data-collection	Age range (years)ᵃ	Genotype (deletion / mUPD / ICD / translocation)	Sex	Mean BMI (kg/m²)	Previous GH treatment (%)
Hertz et al. (1993) (54)	15	United States of America	MR after SS	18-47	47%/-/-/- (53% NA)	7 M, 8 F	38	8%ᶜ
Richards et al. (1994) (55)	14	United Kingdom	SS	16-39	NA	9 M, 5 F	30	NA
Höybye et al. (2002) (47)	19	Sweden	SS	17–37	NA	10 M, 9 F	36	0%
Eldar-Geva et al. (2009) (56)	10	Israel	SS	15-32	50%/40%/10%/-	10 F	36	0%
Nakamura et al. (2009) (57)	34	Japan	MR after SS	16-51	79%/NA/NA/NAᶜ	NA	NA	NA
Van Nieuwpoort et al. (2011) (48) & Van Nieuwpoort et al. (2018) (58)	15	The Netherlands	SS	19-43	93%/7%/-/-	4 M, 11 F	Median: 28	27%ᵉ
Laurier et al. (2015) (44)	154	France	MR after SS	16-54	66%/16%/2%/2% (15% NA)	68 M, 86 F	42	24%
Coupaye et al. (2016) (45)	73	France	MR after SS	16-58	64%/36%/-/-	35 M, 38 F	Deletion: 41 / mUPD: 35	36%
Fintini et al. (2016) (59)	145	Italy	MR after SS	18-50	66%/32%/-/-ᶜ (2% NA)	59 M, 86 F	41	15%
Ghergan et al. (2017) (60)	60	France	SS	16-54	65%/28%/2%/- (5% NA)	26 M, 34 F	39	27%
Pellikaan et al. (2020)	115	The Netherlands	MR after SS	18-72	56%/36%/3%/- (6% NA)	56 M, 59 F	32	46%

Papers with systematic screening

Systematic screening is defined as a systematic analysis of all outcomes in which all patients are subject to I, PE, Q, laboratory analysis and/or additional testing in order to detect or exclude each diagnosis. P-values <0.05 are bold. Abbreviations: body mass index (BMI), females (F), growth hormone (GH), interviews (I), imprinting center defect (ICD), males (M), medical records (MR), maternal uniparental disomy (mUPD), paternal deletion (deletion), physical examination (PE), questionnaires (Q), systematic screening (SS). ᵃ When a subgroup analysis was performed, the N and age range for the adult group (15 years or older) is reported. ᵇ Approximation based on mean weight and height in the total population. ᶜ Percentages or values based on the whole cohort of children and adults as the values for the adult group alone are unknown. ᵈ BMI level at greatest weight. ᵉ Patients with current GH treatment were excluded.

Table 7. Health problems assessed by previous studies

Article	Hypertension (%)	Type 2 diabetes mellitus (%)	Hyper-cholesterolemia (%)	Sleep apnea (%)	Scoliosis (%)	Osteoporosis (%)	Hypogonadism (%)	Hypothyroidism (%)
Laurance et al. (1981) (51)	-	17%	-	-	58%	-	92% F (MC)	-
Greenswag (1987) (52)	17%	19%	-	-	± 50%	-	94% F (MC)	-
Partsch et al. (2000) (46)	16%	16%	37%	58%	37%	-	100% M / 100% F (MC)	-
Marzullo et al. (2005) (50)	23%	8%	-	-	-	-	100% F (MC)	-
Butler et al. (2002) (53)	13%	24%	-	-	34%	2%	-	-
Thomson et al. (2006) (42)	-	13%	-	-	37%	3%	58% F[a], 100% F (MC)	-
Sinnema et al. (2011) (43)	9%	17%	-	10%	56%	16%	91% F (MC)	9%
Grugni et al. (2013) (49)	48%	21%	-	-	-	-	-	5%
Proffitt et al. (2019) (11)	-	11%[b]	-	45%[b]	33%[b]	9%[b]	-	9%[b]
Hertz et al. (1993) (54)	-	-	-	7%	-	-	-	-
Richards et al. (1994) (55)	-	29%[c]	-	86%	-	-	-	-
Höybye et al. (2002) (47)	21%	5%	[d]	-	-	Osteoporosis: 21% Osteopenia: 58%	63% (LM)	0%
Eldar-Geva et al. (2009) (56)	-	10%[c]	-	-	-	-	40% F (LM), 100% F (MC)	-
Nakamura et al. (2009) (57)	-	-	-	-	44%	-	-	-
Van Nieuwpoort et al. (2011) (48) & Van Nieuwpoort et al. (2018) (58)	-	7%	-	-	-	Osteoporosis: 13% Osteopenia: 40%	100% M / 82% F (MC)	13%
Laurier et al. (2015) (44)	25%	25%	[e]	35%	75%	-	-	26%
Coupaye et al. (2016) (45)	16%	19%	10%	-	78%	-	96% (LM + RT)	26%
Fintini et al. (2016) (59)	-	21%	-	-	-	-	-	-
Ghergan et al. (2017) (60)	22%[c]	25%[c]	[f]	23%	-	-	-	25%[c]
Pellikaan et al. (2020)	18%	17%	19%	17% - 93%[g]	74%	Osteoporosis: 10% - 44%[g] Osteopenia: 26% - 60%[g]	100% M / 93% F (MC)	17%

Abbreviations: females (F); laboratory measurements, diagnosis of hypogonadism was based on LH, FSH and/or estrogen (LM); males (M); menstrual cycle, diagnosis of hypogonadism was based on amenorrhea or oligomenorrhea (MC); replacement therapy, diagnosis of hypogonadism was based on use of estrogen (RT). [a] 58% hypogonadism in females was reported, however the method of evaluation was not described. [b] Percentages or values based on the whole cohort of children and adults as the values for the adult group alone are unknown. [c] Reported, but to our knowledge not based on systematic screening. [d] Total cholesterol was less than 5 mmol/liter in 16 of 19 patients and 7 patients had LDL cholesterol above 3 mmol/liter, but the highest level found was 4.2 mmol/liter. [e] Hyperlipidemia in 10% (hypercholesterolemia was not described). [f] Dyslipidemia in 54% (hypercholesterolemia was not described). [g] As poly(somno)graphy and DEXA scans are not always indicated or feasible, we had many missing values for sleep apnea and osteoporosis. As the missing values were not random, we were only able to provide ranges for the prevalence of sleep apnea and osteoporosis.

New patients & yearly controls Interview Physical examination Biochemical measurements	▪ If creatinine > 93 μmol/L (males), > 76 μmol/L (females) or eGFR > 93 ml/min/1.73m^2 (males), eGFR > 98 ml/min/1.73m^2 (females): perform nephrologic analysis
Vitamin D / calcium	▪ Start vitamin D supplementation if vitamin D level < 50 nmol/L ▪ Add calcium supplementation if dairy intake < 3 units per day ▪ Start vitamin D + calcium in case of known osteopenia/osteoporosis
Start hormone replacement therapy *if*	*Females* ▪ Amenorrhea or oligomenorrhea (do not rely on estradiol levels) *Males* ▪ Sparse body hair *or* ▪ Underdeveloped genitals *or* ▪ Daytime sleepiness and testosterone < 10 nmol/L
Perform poly(somno)graphy *if*	▪ Daytime sleepiness *or* ▪ Reported apneas *or* ▪ BMI > 25 kg/m^2 *or* ▪ Hypertension *or* ▪ Plan to start GH treatment
Perform neuropsychological assessment[a] *if*	▪ High blood pressure *or* ▪ Temper tantrums *or* ▪ Fatigue / daytime sleepiness
Perform DEXA *if*	▪ Hypogonadism *or* ▪ Fractures *or* ▪ Corticosteroid use
Perform spine X ray *if*	▪ Gibbus deformity at physical examination ▪ If Cobb angle > 10 degrees and: - Asymptomatic → refer to physiotherapist to prevent progression - Symptomatic (pain, dyspnea, reduced mobility) → refer to orthopedic surgeon
Perform cardiac evaluation[a] *if*	▪ Nycturia *or* ▪ Pitting edema (do not rely on NT-proBNP if BMI > 25 kg/m^2) (65-67) *or* ▪ Shortness of breath *or* ▪ Poor exercise tolerance
Perform yearly screening Fasting glucose LDL-cholesterol Blood pressure *if*	▪ BMI > 25 kg/m^2 *or* ▪ Age > 25 year *or* ▪ GH therapy *or* ▪ Corticosteroid use
Exclude central adrenal insufficiency by ITT or MTP[b] *if*	▪ Apathy *or* ▪ Fainting *or* ▪ Observed hypotension during acute infections / stressful events *or* ▪ Other clinical signs of adrenal insufficiency

Figure 3. Algorithm for diagnostics and treatment in adults with PWS. Abbreviations: body mass index (BMI), dual energy X-ray absorptiometry (DEXA), follicle-stimulating hormone (FSH), free thyroxin (FT4), hemoglobin A1c (HbA1c), insulin tolerance test (ITT), low density lipoprotein (LDL), luteinizing hormone (LH), metyrapone test (MTP), sex hormone binding globulin (SHBG). [a] Recommendation based on expert opinion and literature review (65-67). [b] Based on data published previously (68).

DISCUSSION

We found a large number of undetected health problems among adults with PWS during our systematic health screening. To our knowledge, we are the first to translate this into an evidence-based algorithm for screening and treatment of adults with PWS. We hypothesize that the high prevalence of undiagnosed health problems is the result of most physicians' unfamiliarity with the syndrome, in combination with the complex PWS-specific behavioral phenotype.

Previous studies

Previous studies have reported prevalences of health problems in adults with PWS. However, most studies (11, 42, 43, 46, 49–53) did not perform a systematic screening. Of the 4 studies that performed a systematic health screening (44, 45, 47, 48, 58), only 2 (Laurier et al (44) and Coupaye et al (45)) included a substantial number of (over 20) patients. Six studies (54–57, 59, 60) performed a systematic screening, but focused on only 1 health problem of interest. Compared to the systematic screening described by Laurier et al (44) and Coupaye et al (45), we found a lower prevalence of DM2, scoliosis, and hypothyroidism. The difference in the prevalence of DM2 could be partly explained by the large difference in BMI, which was much lower in our cohort than in the French cohorts (**Table 7**). Moreover, the patients in our cohort had more often been treated with GH during childhood. Although GH treatment may have a short-term negative effect on glucose homeostasis due to increased insulin resistance, GH treatment also improves body composition and exercise tolerance, which has positive effects on glucose metabolism in the long term (69–71).

Prader-Willi syndrome homes and non-PWS homes

Patients in specialized PWS homes had a lower BMI and a lower frequency of hypertension than patients living in non-PWS homes. This could probably be largely explained by the age difference between the groups. Another contributing factor could be that patients in a specialized PWS home are subject to strict supervision from trained personnel. In PWS homes, food is kept out of sight and food storages are locked. According to caregivers, this greatly reduces the stress and conflicts caused by food-seeking behavior. We hypothesize it might even prevent stress-related hypertension. The fact that all patients living in PWS homes received portion-controlled meals (as determined by a dietitian) and often exercised under supervision probably explained part of the difference in the BMI between patients living in PWS homes and those living in non-PWS homes.

Fatigue and daytime sleepiness

Fatigue and daytime sleepiness were very common problems among adults with PWS. According to caregivers, these complaints often prevented them from taking part in day trips and physical activities. Daytime sleepiness is usually attributed to a lack of hypothalamic arousal and is regarded as a problem that is inherent to the syndrome. However, when we looked in more detail, all patients with clinically relevant fatigue or daytime sleepiness had treatable underlying problems such as sleep apnea, narcolepsia, nycturia, vitamin D deficiency, untreated male hypogonadism, or use of drugs that can cause sleepiness. Although we could not perform a randomized controlled trial to assess whether treating these underlying problems resolved the complaints, our clinical experience is that the majority of the patients reported less fatigue after treatment of the underlying cause. Also, caregivers reported that these patients were more actively participating in daily activities. This indicates that daytime sleepiness is not necessarily just "part of the syndrome", but could be the symptom of an underlying, treatable problem. Treating the underlying cause is important to reduce daytime sleepiness and increase physical activity.

Vitamin D deficiency and hypogonadism

Both vitamin D deficiency and hypogonadism are frequently present in adults with PWS. Low levels of vitamin D and testosterone are often attributed to obesity, as vitamin D is fat-soluble and testosterone production can be diminished by increased estradiol levels due to adipose tissue aromatase activity. However, lean male patients also had hypogonadism and vitamin D defiency. Although there is no consensus on the clinical effects of vitamin D (25, 72–74), we found a clear relation between (the severity of) vitamin D deficiency and daytime sleepiness. Although the cause of daytime sleepiness in this complex patient population is likely to be multifactorial, we believe that prescribing vitamin D may be beneficial for all PWS adults with vitamin D deficiency. The high prevalence of osteoporosis and osteopenia in adults with PWS combined with the fact that vitamin D knows little side effects (75) are additional arguments for treatment. Therefore, we recommend prescribing vitamin D supplementation in all adults with PWS with a vitamin D level below 50 ng/mL.

Creatinine levels

Creatinine levels were low in the majority of patients, regardless of sex and BMI. This indicates that normal creatinine levels in patients with PWS are lower than in healthy controls, which is explained by their low muscle mass (13). Therefore, in PWS patients, presence of high-normal creatinine levels might actually indicate impaired renal function. We recommend to adjust the reference values with −24% for males and −18% for females. In our hospital, the PWS-specific reference range for creatinine is 46 to 93

μmol/L (compared with 65–115 μmol/L for non-PWS adult males) and 37 to 76 μmol/L for females (compared with 55–90 μmol/L for non-PWS adult females). For the same reasons, we propose using PWS-specific reference values for eGFR of >98 ml/min/1.73 m^2 for PWS adult males and >93 ml/min/1.73 m^2 for PWS adult females.

Adrenal insufficiency

This is rare in adults with PWS (68). However, in cases of clinical signs of hypocortisolism, we recommend assessing the hypothalamic-pituitary-adrenal axis using the metyrapone test or, in the absence of contraindications, the insulin tolerance test (see **Figure 3**).

Strengths and limitations

Like every study, our study has strengths and limitations. The strengths of our study are the large sample size (considering the fact that PWS is a rare syndrome), its focus on adults, and the fact that we investigated health problems in relation to living situation, BMI, genotype, and demographic factors, thus making our study a powerful source of new information. Limitations may include selection bias (due to selective referral to our specialized facility) and survival bias. Moreover, we have many missing values for osteoporosis and sleep apnea. These additional tests were not always performed because they were either not indicated or impossible to perform due to behavioral issues. Therefore, these results should be interpreted with caution.

CONCLUSION

We found undetected health problems in 61% of adults with PWS. On top of this, one-third of the patients had clinically relevant fatigue or daytime sleepiness which, according to caregivers, prevented them from taking part in physical activities. Although daytime sleepiness is usually considered just "part of the syndrome", all of these patients turned out to have treatable causes such as sleep apnea, narcolepsia, nycturia, vitamin D deficiency, untreated male hypogonadism, or use of drugs that can cause sleepiness. Therefore, fatigue and daytime sleepiness should be considered not just "part of the syndrome", but the symptom of an underlying health problem. We recommend exploring and treating these underlying causes in order to optimize physical activity and prevent obesity-related cardiopulmonary problems. We provide an algorithm for diagnostics and treatment, taking into account PWS-specific pitfalls like falsely low creatinine levels and false-normal cardiac markers. Use of the algorithm will optimize the mental and physical health of adults with PWS. This will improve exercise tolerance and reduce the personal and financial burden of cardiopulmonary complications in this vulnerable patient group.

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SUPPLEMENTARY DATA

Table S1a. Baseline characteristics by living situation

	PWS home[a] n = 23	Non-PWS home[b] n = 61	Family[c] n = 28
Age in years, median [IQR]	26 [21 – 32]	36 [28 – 50]	19 [19 – 22]
BMI in kg/m², median [IQR]	27 [22 – 30]	30 [27 – 40]	28 [26 – 36]
Male gender, n (%)	9 (39%)	30 (49%)	15 (54%)
Genetic subtype			
Deletion, n (%)	16 (70%)	27 (44%)	18 (64%)
mUPD, n (%)[d]	6 (26%)	28 (46%)	7 (25%)
ICD, n (%)	0 (0%)	1 (2%)	2 (7%)
Unknown, n (%)	1 (4%)	5 (8%)	1 (4%)
Growth hormone treatment			
Only during childhood, n (%)	0 (0%)	6 (10%)	3 (11%)
Only during adulthood, n (%)	3 (13%)	0 (0%)	0 (0%)
Both, n (%)	12 (52%)	9 (15%)	19 (68%)
Never, n (%)	8 (35%)	46 (75%)	6 (22%)
Current growth hormone treatment, n (%)	14 (61%)	8 (13%)	19 (68%)
Use of hydrocortisone			
Daily, n (%)	0 (0%)	2 (3%)	2 (7%)
During physical or psychological stress, n (%)	16 (70%)	13 (21%)	17 (61%)
Scholar level			
Secondary vocational education, n (%)	0 (0%)	0 (0%)	4 (14%)
Pre-vocational secondary education, n (%)	1 (4%)	0 (0%)	2 (7%)
Special education, n (%)	16 (70%)	46 (75%)	19 (68%)
No education, n (%)	1 (4%)	3 (5%)	0 (0%)
Unknown, n (%)	5 (22%)	12 (20%)	3 (11%)
Mutism, n (%)	0 (0%)	3 (5%)	0 (0%)
Relationship status			
In a relationship with sexual intercourse, n (%)	0 (0%)	5 (8%)	2 (7%)
In a relationship without sexual intercourse, n (%)	5 (22%)	7 (12%)	5 (18%)
Not in a relationship, n (%)	14 (61%)	40 (66%)	21 (75%)
Unknown, n (%)	4 (17%)	9 (15%)	0 (0%)

Abbreviations: body mass index (BMI), interquartile range (IQR). [a] Patients living in a specialized Prader-Willi syndrome home. [b] Patients living in a non-specialized group home. [c] Patients living with family. [d] In 11 patients with an mUPD, the parents were not available for genetic testing. Therefore, an ICD could not be ruled out with total certainty in these patients.

Table S1b. Baseline characteristics by genotype

	Deletion n = 64	mUPD[a] n = 41	Other n = 10
Age in years, median [IQR]	28 [21 – 36]	32 [21 – 49]	26 [22 – 48]
BMI in kg/m^2, median [IQR]	31 [26 – 38]	29 [25 – 34]	27 [24 – 28]
Male gender, n (%)	28 (44%)	20 (49%)	8 (80%)
Growth hormone treatment			
Only during childhood, n (%)	7 (11%)	1 (2%)	2 (20%)
Only during adulthood, n (%)	3 (5%)	0 (0%)	0 (0%)
Both, n (%)	20 (31%)	16 (39%)	4 (40%)
Never, n (%)	34 (53%)	24 (59%)	4 (40%)
Current growth hormone treatment, n (%)	22 (34%)	15 (37%)	4 (40%)
Use of hydrocortisone			
Daily, n (%)	3 (5%)	1 (2%)	0 (0%)
During physical or psychological stress, n (%)	24 (38%)	18 (44%)	5 (50%)
Living situation			
With family, n (%)	18 (28%)	7 (17%)	3 (30%)
In a specialized Prader-Willi group home, n (%)	16 (25%)	6 (15%)	1 (10%)
In a non-specialized group home, n (%)	27 (42%)	28 (68%)	6 (60%)
Assisted living, n (%)	3 (5%)	0 (0%)	0 (0%)
Scholar level			
Secondary vocational education, n (%)	6 (9%)	0 (0%)	0 (0%)
Pre-vocational secondary education, n (%)	1 (2%)	1 (2%)	1 (10%)
Special education, n (%)	46 (72%)	31 (76%)	5 (50%)
No education, n (%)	0 (0%)	4 (10%)	0 (0%)
Unknown, n (%)	11 (17%)	5 (12%)	4 (40%)
Mutism, n (%)	0 (0%)	2 (5%)	1 (10%)
Relationship status			
In a relationship with sexual intercourse, n (%)	6 (9%)	2 (5%)	0 (0%)
In a relationship without sexual intercourse, n (%)	15 (23%)	2 (5%)	1 (10%)
Not in a relationship, n (%)	41 (64%)	30 (73%)	5 (50%)
Unknown, n (%)	1 (2%)	7 (17%)	4 (40%)

Abbreviations: body mass index (BMI), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD). [a] In 11 patients with an mUPD, the parents were not available for genetic testing. Therefore, an ICD could not be ruled out with total certainty in these patients.

Table S2a. Health problems before and after our systematic screening by living situation

	PWS home[a] n = 23			Non-PWS home[b] n = 61			Family[c] n = 28			P-value
	Before	After	Missing	Before	After	Missing	Before	After	Missing	
Hypogonadism										
Male (n = 54)	5 (56%)	9 (100%)	0	10 (36%)	28 (100%)	2	10 (67%)	15 (100%)	0	NA
Female (n = 58)[d]	10 (100%)	10 (100%)	4	10 (43%)	20 (87%)	8	6 (60%)	10 (100%)	3	0.2
Hypothyroidism	3 (13%)	4 (17%)	0	11 (18%)	12 (20%)	0	3 (11%)	3 (11%)	0	0.6
Type 2 diabetes mellitus	2 (9%)	2 (9%)	0	9 (15%)	13 (22%)	2	1 (4%)	3 (11%)	0	0.2
Hypertension	0 (0%)	0 (0%)	1	11 (19%)	17 (29%)	2	1 (4%)	2 (7%)	0	0.002
Hypercholesterolemia	2 (9%)	4 (17%)	0	9 (15%)	15 (25%)	2	2 (7%)	2 (7%)	0	0.1
Scoliosis	15 (65%)	18 (78%)	0	31 (53%)	44 (76%)	3	14 (50%)	19 (68%)	0	0.6
Vitamin D deficiency	11 (69%)	14 (88%)	7	7 (27%)	22 (85%)	35	8 (32%)	16 (64%)	3	0.6

Data are presented as n (%). All P-values show the difference in both groups after screening. [a] Patients living in a specialized Prader-Willi syndrome group home. [b] Patients living in a non-specialized group home. [c] Patients living with family. [d] (Caregivers of) 15 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age.

Table S2b. Health problems after our systematic screening by genotype

	Deletion n = 64	Missing	mUPD n = 41	Missing	P-value
Hypogonadism					
Male (n = 48)	27 (100%)	1	19 (100%)	1	NA
Female (n = 57)[a]	25 (93%)	9	14 (93%)	6	0.9
Hypothyroidism	11 (17%)	0	7 (17%)	0	0.99
Type 2 diabetes mellitus	8 (13%)	0	10 (24%)	2	0.1
Hypertension	9 (15%)	0	8 (20%)	2	0.5
Hypercholesterolemia	11 (17%)	1	8 (20%)	1	0.7
Scoliosis	51 (81%)	2	23 (59%)	1	0.02
Vitamin D deficiency	33 (80%)	16	19 (76%)	23	

Data are presented as n (%). Abbreviations: maternal uniparental disomy (mUPD). [a] (Caregivers of) 15 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age.

Table S2c. Health problems after our systematic screening by BMI

	BMI <25 kg/m^2 n = 24	Missing	BMI 25-30 kg/m^2 n = 43	Missing	BMI >30 kg/m^2 n = 48	Missing	P-value
Hypogonadism							
Male (n = 56)	11 (100%)	1	27 (100%)	1	16 (100%)	0	NA
Female (n = 59)[a]	6 (100%)	6	12 (92%)	2	22 (92%)	8	0.9
Hypothyroidism	5 (21%)	0	7 (16%)	0	7 (15%)	0	0.5
Type 2 diabetes mellitus	2 (8%)	0	7 (17%)	1	10 (21%)	1	0.2
Hypertension	3 (13%)	0	6 (15%)	2	11 (23%)	1	0.4
Hypercholesterolemia	4 (17%)	0	4 (10%)	1	14 (30%)	1	0.01
Scoliosis	12 (79%)	0	30 (71%)	1	34 (74%)	2	0.3
Vitamin D deficiency	12 (75%)	8	20 (77%)	17	22 (81%)	21	

Data are presented as n (%). Abbreviations: body mass index (BMI). [a] (Caregivers of) 16 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age.

Table S2d. Health problems after our systematic screening by age

	Age < 25 year n = 43	Missing	Age 25-30 year n = 21	Missing	Age > 30 year n = 51	Missing	P-value
Hypogonadism							
Male (n = 59)	20 (100%)	0	7 (100%)	1	27 (100%)	1	NA
Female (n = 56)[a]	18 (100%)	5	9 (90%)	3	13 (87%)	8	0.2
Hypothyroidism	10 (23%)	0	5 (24%)	0	4 (8%)	0	0.2
Type 2 diabetes mellitus	2 (5%)	0	2 (10%)	0	15 (31%)	2	<0.001
Hypertension	3 (7%)	1	1 (5%)	2	16 (31%)	0	<0.001
Hypercholesterolemia	3 (7%)	0	2 (10%)	0	17 (35%)	2	0.002
Scoliosis	30 (70%)	0	19 (90%)	0	34 (71%)	3	0.9
Vitamin D deficiency	27 (69%)	4	10 (91%)	10	17 (89%)	32	

Data are presented as n (%). [a] (Caregivers of) 16 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age.

Table S2e. Health problems after our systematic screening by gender

	Male n = 56	**Missing**	**Female** n = 59	**Missing**	P-value
Hypothyroidism	5 (9%)	0	14 (24%)	0	0.03
Type 2 diabetes mellitus	13 (24%)	1	6 (10%)	1	0.06
Hypertension	9 (17%)	2	11 (19%)	1	0.8
Hypercholesterolemia	10 (18%)	1	12 (21%)	1	0.7
Scoliosis	42 (76%)	1	41 (72%)	2	0.6
Vitamin D deficiency	25 (83%)	26	29 (74%)	20	

Data are presented as n (%).

Table S3. Lifestyle and behaviour

	Missing	**Total** n = 115	**PWS home**[a] n = 23	**Non-PWS home**[b] n = 61	**Family**[c] n = 28
Physical exercise <30 minutes a day	0	25 (22%)	0 (0%)	18 (30%)	7 (25%)
No dietitian	0	42 (37%)	3 (13%)	16 (26%)	20 (71%)
Increasing weight	0	44 (38%)	5 (22%)	15 (25%)	15 (54%)
Problems regarding living, work, daytime activities or care takers	24	41 (45%)	5 (22%)	25 (52%)	11 (39%)
Difficulties dealing with behavioural problems	26	42 (47%)	4 (17%)	28 (46%)	9 (32%)

Data are presented as n (%). [a] Patients living in a specialized Prader-Willi syndrome group home. [b] Patients living in a non-specialized group home. [c] Patients living with family.

Table S4. Total physical complaints

	Missing	Total n = 115
Skin picking	21	53 (56%)
Food seeking behaviour	23	42 (46%)
Daytime sleepiness	19	41 (43%)
Temper tantrums	20	40 (42%)
Leg edema	20	32 (34%)
Snoring	19	32 (33%)
Foot complaints	20	30 (32%)
Nycturia	21	28 (30%)
Fatigue	22	23 (25%)
Feeling cold	22	22 (24%)
Constipation	18	21 (22%)
Thirst	26	19 (21%)
Visual complaints	23	18 (20%)
Stomach ache	20	15 (16%)
Diarrhea	19	15 (16%)
Backache	22	15 (16%)
Pyrosis / ructus	22	13 (14%)
Pica (eating nonfood items)	23	10 (11%)
Sexual problems	22	9 (10%)
Difficulty sleeping	22	9 (10%)
Urinary incontinence	20	9 (9%)
Fecal incontinence	21	6 (6%)
Chestpain	24	4 (4%)
Bone fractures	19	3 (3%)
Orthopnea	25	3 (3%)
Vomiting	20	0 (0%)

Complaints are scored as present when the caregivers indicated a score of 3 or higher on a 5-point Likertscale. Data are presented as n (%).

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Table S5. Liver panel, kidney function, hematopoiesis and electrolyte values of 115 adults with PWS

		N	Reference range	Median [IQR]	Min-max	Patients below LLN, n (%)	Patients above ULN, n (%)
ASAT (U/L)	Male	54	<35	21 [18 – 25]	11 – 82	NA[a]	2 (4%)
	Female	55	<31	20 [17 – 25]	11 – 52		4 (7%)
ALAT (U/L)	Male	54	<45	21 [16 – 28]	10 – 149	NA[a]	5 (9%)
	Female	56	<34	20 [15 – 23]	9 – 76		5 (9%)
ALP (U/L)	Male	52	<115	86 [65 – 107]	17 – 180	NA[a]	8 (15%)
	Female	52	<98	77 [60 – 96]	25 – 211		11 (21%)
GGT (U/L)	Male	54	<55	18 [15 – 27]	9 – 165	NA[a]	2 (4%)
	Female	53	<38	19 [13 – 31]	9 – 85		9 (17%)
Total bilirubin (μmol/L)	Male	48	<17	5.0 [4.0 – 8.0]	3.0 – 25	NA[a]	2 (4%)
	Female	49	<17	4.0 [3.5 – 6.0]	3.0 – 18		1 (2%)
LDH (U/L)	Male	50	<248	200 [170 – 223]	118 – 270	NA[a]	4 (8%)
	Female	50	<247	178 [166 – 213]	132 – 299		5 (10%)
Urea (mmol/L)		107	2.5 – 7.5	4.4 [3.7 – 5.0]	1.8 – 10.6	2 (2%)	3 (3%)
Creatinine (μmol/L)	Male	56	65 – 115	61 [51 – 72]	40 – 109	35 (63%)	0 (0%)
	Female	59	55 – 90	56 [49 – 65]	31 – 89	28 (47%)	0 (0%)
Hemoglobin (mmol/L)	Male	55	8.6 – 10.5	8.9 [8.4 – 9.4]	7.3 – 10.1	17 (31%)	0 (0%)
	Female	53	7.5 – 9.5	8.2 [8.0 – 9.0]	6.8 – 9.7	1 (2%)	1 (2%)
MCV (fL)		111	80 – 100	90 [87 – 92]	78 – 101	1 (1%)	1 (1%)
Sodium (mmol/L)		111	136 – 145	140 [138 – 142]	130 – 145	8 (7%)	0 (0%)
Potassium (mmol/L)		111	3.5 – 5.1	4.3 [4.1 – 4.5]	3.4 – 5.4	1 (1%)	2 (2%)
Calcium (mmol/L)		107	2.20 – 2.65	2.4 [2.3 – 2.5]	1.2 – 4.0	3 (3%)	2 (2%)
Albumin (g/L)		105	35 – 50	45 [42 – 48]	30 – 53	3 (3%)	5 (5%)

Abbreviations: upper limit of normal (ULN), lower limit opf normal (LLN), alanine transaminase (ALAT), alkaline phosphatase (ALP), aspartate transaminase (ASAT), gamma glutamyl transpeptidase (GGT), interquartile range (IQR), lactate dehydrogenase (LDH), mean corpuscular volume (MCV). [a] Unknown, because LLN not defined.
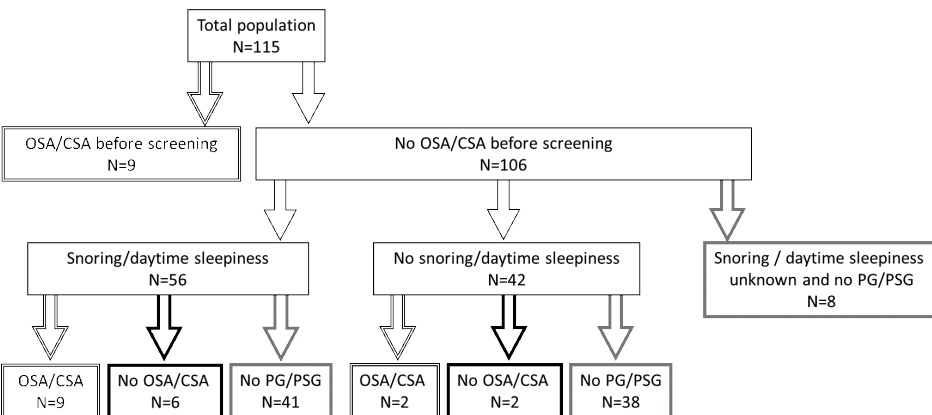

Figure S1. Sleep apnea: clinical data and poly(somno)graphy results
Abbreviations: CSA (central sleep apnea), PG (polygraphy), PSG (polysomnography), OSA (obstructive sleep apnea).
Legends: Grey arrows and squares represent patients in which polygraphy was not performed. Double lined arrows and squares represent patients that were diagnosed with sleep apnea. Bold arrows and squares represent patients in which sleep apnea was excluded.
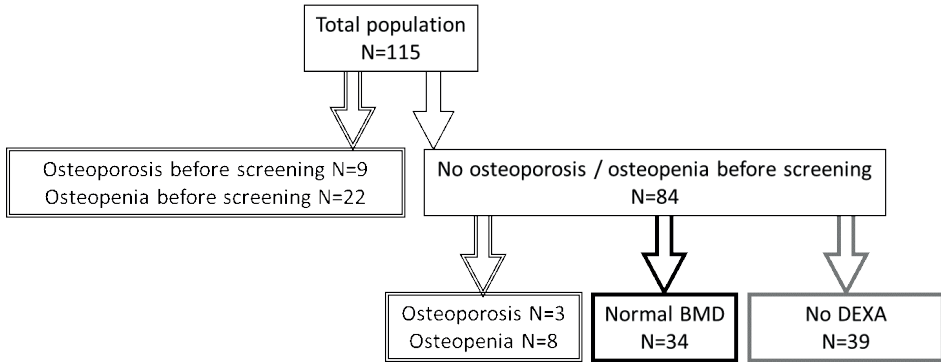
Figure S2. Osteopenia and osteoporosis
Abbreviations: BMD (bone mineral density), DEXA (dual energy X-ray absorptiometry).
Legends: The grey arrow and square represent patients in which DEXA was not performed. Double lined arrows and squares represent patients that were diagnosed with osteoporosis or osteopenia. The bold arrow and square represent patients in which osteoporosis and osteopenia were excluded.
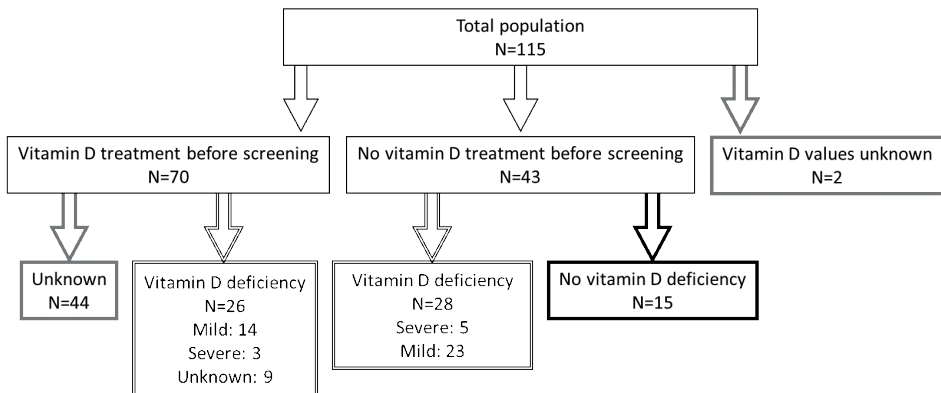

Figure S3. Vitamin D deficiency
Legends: The grey arrow and square represent patients that received vitamin D supplementation before screening for unknown reasons. Double tined arrows and squares represent patients that were diagnosed with vitamin D deficiency. The bold arrow and square represent patients in which vitamin D deficiency was excluded.

3

Effects of childhood multidisciplinary care and growth hormone treatment on health problems in adults with Prader-Willi syndrome

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ABSTRACT

Prader-Willi syndrome (PWS) is a complex hypothalamic disorder. Features of PWS include hyperphagia, hypotonia, intellectual disability and pituitary hormone deficiencies. The combination of growth hormone treatment and multidisciplinary care (GHMDc) has greatly improved the health of children with PWS. Little is known about the effects of childhood GHMDc on health outcomes in adulthood. We retrospectively collected clinical data of 109 adults with PWS. Thirty-nine had received GHMDc during childhood and adolescence (GHMDc+ group) and sixty-three had never received growth hormone treatment (GHt) nor multidisciplinary care (GHMDc- group). Our systematic screening revealed fewer undetected health problems in the GHMDc+ group (10%) than in the GHMDc- group (84%). All health problems revealed in the GHMDc+ group had developed between the last visit to the paediatric and the first visit to the adult clinic and/or did not require treatment. Mean BMI and the prevalence of diabetes mellitus type 2 were significantly lower in the GHMDc+ group compared to the GHMDc- group. As all patients who received GHt were treated in a multidisciplinary setting, it is unknown which effects are the result of GHt and which are the result of multidisciplinary care. However, our data clearly show that the combination of both has beneficial effects. Therefore, we recommend to continue GHMDc after patients with PWS have reached adult age.

INTRODUCTION

Prader-Willi syndrome (PWS) is a genetic, neuroendocrine condition caused by the loss of expression of a cluster of maternally imprinted genes on chromosome 15q11-13. This loss can be caused by a paternal deletion (65-75%), a maternal uniparental disomy 15 (mUPD, 20-30%), an imprinting centre defect (ICD, 1-3%) or a paternal chromosomal translocation (0.1%)(1-3). The prevalence of PWS is 1:10.000 – 1:30.000 (3). Newborns with PWS usually have severe hypotonia and poor suck resulting in feeding difficulties, which later in infancy switches to excessive eating. Motor and language development is usually delayed and most patients develop a complex behavioural phenotype during childhood or later in life. Moreover, children and adults with PWS have hypothalamic dysfunction resulting in hyperphagia, pituitary hormone deficiencies, abnormal temperature regulation and inadequate pain registration (3-7).

Mortality in both children and adults with PWS is high. A study of reported deaths between 1973 and 2015 showed that 25% had died before reaching the age of twenty, 50% before the age of 29, 75% before the age of 42 and 99% before the age of 60 (8). In most patients, death is the result of a complex interaction between somatic and psychosocial factors (3,9), like hyperphagia (10-12), musculoskeletal problems (13-17), low basal metabolic rate (BMR)(18-21), behavioural challenges (22,23), biochemical anomalies (3,19,24-34), and cardiovascular risk factors (obesity, hypertension, hypercholesterolemia and type 2 diabetes mellitus (DM2)) (3,8,9,35-42).

Many of these risk factors can be improved by growth hormone (GH) treatment. For many years, GH treatment in children with PWS has been approved in European countries, the USA and several other countries worldwide. GH treatment during childhood improves psychomotor development, cognitive functioning, body composition and LDL-cholesterol values (43-51), with few adverse events. The positive effects on body composition are maintained during long-term GH treatment (52,53).

GH treatment for children with PWS is often provided in a multidisciplinary (MD) setting and usually involves a paediatric endocrinologist, dietitian, physiotherapist and a behavioural expert. For adults, MD care is unavailable in many countries. In the Netherlands, adult MD care has only been available since 2015.

The Dutch Centre of reference for Prader-Willi syndrome is treating over 300 patients with PWS, of whom 140 adults. To evaluate the combined effect of GH treatment and MD care (GHMDc), we report the prevalence of physical health problems in three groups: adults with PWS who have received GHMDc from childhood to adulthood (GHMDc+

group); those who never received GHMDc (GHMDc- group) and those who have temporarily received GHMDc during childhood, but GHMDc was discontinued before adulthood (GHMDc± group).

MATERIALS AND METHODS

Ethical review and approval were waived by the Medical Ethics Committee of the Erasmus University Medical Centre. This study was performed at the Centre for Adults with Complex Rare Genetic Syndromes (CRGS) at the Erasmus University Medical Centre, Rotterdam, the Netherlands. We retrospectively reviewed the medical files of all adults who visited the MD outpatient clinic of our centre between January 2015 and January 2021 and who underwent our systematic health screening as part of their regular patient care. As described previously (see (9)), systematic screening consists of a structured interview, an extensive physical examination, a medical questionnaire, a review of the medical records, and biochemical measurements. This systematic screening was largely performed during the first visit to the outpatient clinic for CRGS. However, when parameters could not be assessed during the first visit, data from the next available date was used.

As GH treatment was part of MD childhood care, we investigated the combined effect of GH treatment and MD care and were not able to assess the independent effect of GH treatment or MD care.

The GHMDc+ group is defined as the patients who 1) were treated at our reference centre during childhood and adulthood 2) received MD care and GH treatment both during childhood and adolescence, 3) received specialized transitional care before transferring from the paediatric to the adult endocrinology department and 4) still received GH treatment and MD care at the time of this study. The GHMDc- group had never received MD care nor GH treatment before visiting our outpatient clinic for adults with CRGS, neither during childhood nor during adolescence. The patients in the GHMDc± group temporarily received both GH treatment and MD care at our reference centre during childhood but discontinued GHMDc before transition to adult care. They spontaneously visited the adult endocrinology department several years later, after which the systematic health screening was performed and MD care was resumed at the outpatient clinic for adults with CRGS. Therefore, patients in the GHMDc± group did not receive GH treatment or MD care between their last appointment at the paediatric endocrinology department and their first appointment at the adult endocrinology department of our reference centre.

MD care during childhood included treatment by a paediatric endocrinologist, a dietitian, a physiotherapist, a nurse practitioner, a physician for people with intellectual disabilities (ID physician), and, if indicated, a psychologist. Transitional care included a shared visit with both the paediatric and the adult endocrinologist, followed by alternating visits at the paediatric and adult department until the final transfer to adult endocrinology.

One patient was excluded as he had received GHMDc at our reference centre both during childhood and adolescence, but discontinued GH treatment at his own initiative when he reached adulthood. Another patient was excluded because he had received GHMDc during childhood but received GH treatment in another hospital without MD care before transferring to the adult endocrinology department of our reference centre. Eleven patients were excluded because they had received GH treatment during childhood and/or adolescence, but did not receive MD care at our reference centre during childhood.

Newly diagnosed (i.e., undetected/undiagnosed) health problems were defined as health problems that had not been diagnosed before referral to our outpatient clinic, but were diagnosed during the systematic health screening at our MD outpatient clinic for adults with complex rare genetic syndromes.

As part of regular patient care, primary caregivers were asked to fill out a medical questionnaire. In this questionnaire, subjective complaints scored on a 5-point Likert scale (1 = rarely or never, 2 = not often and/or not severe, 3 = quite often and/or quite severe, 4 = often and/or severe, 5 = very often and/or very severe). A score of 3 or higher was considered clinically relevant.

Data analysis

Statistical analysis was performed using R version 3.6.3. Descriptive statistics for continuous variables are reported as the median and interquartile range [IQR]. Dichotomous variables are displayed as count and percentage, n (%). As the GHMDc± only contained seven patients, this group was not included in the statistical analysis. We used a chi-squared test to compare living situation, the prevalence of health problems and subjective complaints between the GHMDc+ and GHMDc- group. To investigate the relationship between the GHMDc+ and the GHMDc- group and BMI and age, we used the Wilcoxon rank sum test. A chi-squared test for trend was used to compare the number of undiagnosed health problems between the GHMDc+ and the GHMDc- group. To investigate the effect of GHMDc on health problems and subjective complaints, number of newly diagnosed health problems and BMI corrected for age logistic, ordinal, and

linear regression models were used and a likelihood ratio test was performed. As this was an exploratory analysis, no correction for multiple testing was performed.

RESULTS

We included 109 (53 male / 56 female) patients who fulfilled the criteria for one of the GHMDc groups. Median age was 28 years [IQR 20 – 41] (range 18 – 72 years) and median BMI 29 kg/m^2 [IQR 26 – 36].

Thirty-nine patients had received GHMDc during childhood and adolescence and still received GHMDc at the time of the study (GHMDc+ group). Sixty-three patients had never received GHMDc (GHMDc- group). Seven had temporarily received GHMDc but did not receive GHMDc anymore at time of the study (GHMDc± group). The median age of the patients in the GHMDc+ group was 20 years [IQR 19-24], compared to 38 years [IQR 31-51] in the GHMDc- group.

Before referral to our reference centre, 15 adults in the GHMDc- and GHMDc± were treated (only) by a general practitioner, 37 were (only) treated by an ID physician, 8 (only) by an adult endocrinologist and 8 by an ID physician and an adult endocrinologist. All patients in the GHMDc+ group received MD childhood care at our reference centre before referral to the MD outpatient clinic for adults, see **Table 1**.

The prevalence of different health problems is reported in **Table 2**. In the GHMDc+ group, the BMI and the prevalence of DM2 were significantly lower than in the GHMDc- group, also after correction for age. Median BMI of the GHMDc± group was comparable to the GHMDc- group, while DM2 was rare (n = 1, 14%). Systematic screening revealed more undetected health problems in the GHMDc- group (84%) than in the GHMDc+ group (10%). Health problems that were most often newly diagnosed in the GHMDc- group were hypogonadism (for males defined as a serum testosterone concentration <10 nmol/L combined with clinical signs of hypogonadism and for females defined as an absent or irregular menstrual cycle) and vitamin D deficiency (serum vitamin D concentration < 50 nmol/L), followed by scoliosis. In the GHMDc+ group newly diagnosed health problems were hypercholesterolemia (n = 1), hypothyroidism (n = 1) and hypogonadism (n = 2). However, all newly diagnosed health problems in the GHMDc+ group had developed between the last visit to the paediatric department and the first visit to the adult outpatient clinic and/or did not require treatment.

Table 1. Baseline characteristics of 109 adults with Prader-Willi syndrome according to GHMDc group.

	GHMDc+[a] n = 39	GHMDc-[b] n = 63	GHMDc±[c] n = 7	Total n = 109
Age in years, median [IQR]	20 [19-24]	38 [31-51]	24 [22-26]	28 [20-41]
BMI in kg/m² , median [IQR]	26 [22-29]	32 [27-42]	34 [27-37]	29 [26-36]
Obesity (BMI >30 kg/m²), n (%)	6 (15%)	36 (57%)	4 (57%)	46 (42%)
Overweight (BMI 25-30 kg/m²), n (%)	17 (44%)	19 (30%)	3 (43%)	39 (36%)
Lean (BMI 19-25 kg/m²), n (%)	16 (41%)	8 (13%)	0 (0%)	24 (22%)
Male gender, n (%)	18 (46%)	33 (52%)	2 (29%)	53 (49%)
Age at diagnosis in years, median [IQR][d]	0 [0-2]	9 [3-20]	0 [0-0]	4 [0-13]
Genetic subtype				
Deletion, n (%)	20 (51%)	33 (52%)	4 (57%)	57 (52%)
mUPD, n (%)[e]	13 (33%)	25 (40%)	1 (14%)	39 (36%)
ICD, n (%)	2 (5%)	0 (0%)	1 (14%)	3 (3%)
Unknown, n (%)	4 (10%)	5 (8%)	1 (14%)	10 (9%)
Growth hormone treatment				
Only during childhood, n (%)	0 (0%)	0 (0%)	7 (100%)	7 (6%)
Only during adulthood, n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Both, n (%)	39 (100%)	0 (0%)	0 (0%)	39 (36%)
Never, n (%)	0 (0%)	63 (100%)	0 (0%)	63 (58%)
Current growth hormone treatment, n (%)	39 (100%)	0 (0%)	0 (0%)	39 (36%)
Care before referral				
Multidisciplinary childhood care, n (%)	39 (100%)	0 (0%)	0 (0%)	39 (36%)
Endocrinologist only, n (%)	0 (0%)	8 (13%)	0 (0%)	8 (7%)
ID-physician only, n (%)	0 (0%)	34 (54%)	3 (43%)	37 (34%)
Endocrinologist and ID-physician, n (%)	0 (0%)	5 (8%)	3 (43%)	8 (7%)
General practitioner only, n (%)	0 (0%)	14 (22%)	1 (14%)	15 (14%)
Unknown, n (%)	0 (0%)	2 (3%)	0 (0%)	2 (2%)
Use of hydrocortisone				
Daily, n (%)	0 (0%)	2 (3%)	0 (0%)	2 (2%)
During physical or psychological stress, n (%)	34 (87%)[f]	8 (13%)	2 (29%)	44 (40%)
Living situation				
With family, n (%)	19 (49%)	6 (10%)[g]	4 (57%)	29 (27%)
In a specialized PWS group home, n (%)	13 (33%)	8 (13%)	0 (0%)	21 (19%)
In a non-specialized facility, n (%)	7 (18%)	49 (78%)	3 (43%)	59 (54%)
Scholar level				
Secondary vocational education, n (%)	2 (5%)	2 (3%)	0 (0%)	4 (4%)
Pre-vocational secondary education, n (%)	3 (8%)	0 (0%)	0 (0%)	3 (3%)
Special education, n (%)	31 (79%)	40 (64%)	5 (71%)	76 (70%)
No education, n (%)	1 (3%)	4 (6%)	0 (0%)	5 (5%)
Unknown, n (%)	2 (5%)	17 (27%)	2 (29%)	21 (19%)
Mutism, n (%)	0 (0%)	3 (5%)	0 (0%)	3 (3%)
Relationship status				
In a relationship with sexual intercourse, n (%)	2 (5%)	5 (8%)	0 (0%)	7 (6%)
In a relationship without sexual intercourse, n (%)	5 (13%)	9 (14%)	1 (14%)	15 (14%)
Not in a relationship, n (%)	28 (72%)	40 (64%)	4 (57%)	72 (66%)
Unknown, n (%)	4 (10%)	9 (14%)	2 (28%)	15 (14%)

Abbreviations: body mass index (BMI), imprinting centre defect (ICD), physician specialized in intellectual disabilities (ID-physician), interquartile range (IQR), maternal uniparental disomy (mUPD), Prader-Willi syndrome (PWS).

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Subjective complaints according to GHMDc group are shown in **Table 3**. Skin picking, food seeking behaviour, daytime sleepiness, temper tantrums, leg edema, snoring, foot complaints, nocturia, fatigue, constipation, thirst, visual complaints, diarrhoea, backache, heartburn / belching, pica (eating non-food items), sexual problems and difficulty sleeping were more often reported by patients in the GHMDc- group. The sexual problem that was most often reported was an increased libido (often in males receiving testosterone replacement therapy), leading, for example, to masturbation in public or unwanted sexual behaviours towards other patients in the same group home. Feeling cold and stomach aches were more prevalent in the GHMDc+ group. After correction for age, only the differences in prevalence of nocturia (26% in the GHMDc+ group vs 31% in the GHMDc- group, P = 0.04) and snoring (13% in the GHMDc+ group vs 44% in the GHMDc- group, P = 0.01) were significant. When the P-value was corrected for age and BMI, snoring was no longer significant (P = 0.2).

Characteristics of the patients in the GHMDc± group are shown in **Table 4**. Six patients discontinued care at the paediatric endocrinology department because they had to be transferred to a different physician after GH treatment was discontinued, as the MD outpatient clinic for adults with PWS did not exist at the time. One patient discontinued care due to personal circumstances. Five of the seven patients showed an increase in BMI during their time without GHMDc, all of at least 5 kg/m^2. One patient developed hypothyroidism, one DM2 and one hypercholesterolemia.

Table 2. Health problems according to GHMDc group.

	Missing	GHMDc+[a] n = 39	GHMDc-[b] n = 63	P-value	P-value corr. for age[c]	GHMDc±[d] n = 7
Age in years, median [IQR]	0	20 [19-24]	38 [31-51]	<0.001	NA	24 [22-26]
BMI in kg/m^2, median [IQR]	0	26 [22-29]	32 [27-42]	<0.001	<0.001	34 [27-37]
Newly diagnosed health problems[e]						
At least one		4 (10%)[f]	53 (84%)	<0.001	<0.001	5 (71%)
At least two		0 (0%)	26 (41%)			3 (43%)
Three or more		0 (0%)	9 (14%)			0 (0%)
Hypogonadism						
Male (n = 53)	1	17 (94%)	32 (100%)	0.2	0.1	2 (100%)
Of whom treated		*14 (82%)*	*6 (19%)*			*1 (50%)*
Female (n = 56)	13[g]	15 (94%)	21 (91%)	0.8	0.5	4 (100%)
Of whom treated		*14 (93%)*	*5 (24%)*			*1 (25%)*
Hypothyroidism	0	8 (21%)	7 (11%)	0.2	0.1	1 (14%)
Of whom treated		*7 (88%)[h]*	*7 (100%)*			*0 (0%)*
Diabetes mellitus type 2	3	0 (0%)	16 (27%)	<0.001	0.005	1 (14%)
Of whom treated		*NA*	*12 (75%)*			*0 (0%)*
Hypertension	3	2 (5%)	17 (27%)	0.005	0.8	1 (20%)
Of whom treated		*1 (50%)[i]*	*13 (76%)*			*1 (100%)*
Hypercholesterolemia	2	3 (8%)	18 (30%)	0.01	0.2	1 (14%)
Of whom treated		*0 (0%)[j]*	*11 (61%)*			*0 (0%)*
Scoliosis	4	28 (72%)	42 (71%)	0.9	0.2	7 (100%)
Vitamin D deficiency	42[k]	25 (71%)	24 (92%)	NA[L]	NA[L]	5 (83%)

Abbreviations: body mass index (BMI), interquartile range (IQR). Data are presented as n (%), unless otherwise specified. "Of whom treated" refers to how many patients were treated before undergoing our systematic health screening. Only P-values for GHMDc+ vs GHMDc- are calculated. [a] The GHMDc+ group is defined as the patients who received growth hormone treatment and multidisciplinary care both during childhood and adolescence and received transitional care. [b] The GHMDc- group had not received growth hormone treatment or multidisciplinary care during childhood or adolescence. [c] P-value corrected for age using regression models. [d] The GHMDc± group had received growth hormone treatment and multidisciplinary care during childhood, but not continuously until transfer. [e] Newly diagnosed health problems are: hypogonadism, hypothyroidism, type 2 diabetes mellitus, hypertension, hypercholesterolemia, scoliosis and vitamin D deficiency. Newly diagnosed health problems were health problems that had not been diagnosed before referral to our outpatient clinic, but were diagnosed during the systematic health screening at our multidisciplinary outpatient clinic for adults with complex rare genetic syndromes. [f] One patient had newly diagnosed hypercholesterolemia, which had developed between the last visit to the paediatric endocrinologist (where LDL-cholesterol was normal) and the first visit to the adult endocrinologist. One patient had newly diagnosed hypothyroidism with a fluctuating free thyroxin level, which was not treated as discussed with the patient. Two patients had newly diagnosed hypogonadism, of whom one had developed hypogonadism between the last visit to the paediatric endocrinologist (where testosterone was normal) and the first visit to the adult endocrinologist and the other one could not be treated due to severe behavioural problems. [g] (Caregivers of) 13 female patients did not recall whether they had had a normal menstrual cycle before the start of oral contraceptives or before reaching menopausal age. [h] One patient with hypothyroidism with a fluctuating free thyroxin level, which was not treated as discussed with the patient. [i] One patient had untreated moderate hypertension, which was being monitored. [j] Treatment not indicated based on the Dutch cardiovascular risk guidelines. [k] In 2 patients vitamin D was not measured and 40 patients used vitamin D supplementation before the screening, but it was unknown whether they had low vitamin D values before the start of vitamin D supplementation. [L] A P-value could not be calculated due to selective missing values.

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Table 3. Subjective complaints.

	Observations	GHMDc+[a] n = 39	Observations	GHMDc-[b] n = 63	P-value	P-value corr. for age[c]
Skin picking	31	15 (48%)	49	32 (65%)	0.1	0.5
Food seeking behaviour	30	9 (30%)	49	27 (55%)	0.03	0.5
Daytime sleepiness	31	9 (29%)	51	29 (57%)	0.01	0.3
Temper tantrums	30	10 (33%)	51	25 (49%)	0.2	0.9
Leg edema	30	3 (10%)	51	25 (49%)	<0.001	0.2
Snoring	31	4 (13%)	52	23 (44%)	0.003	0.01[d]
Foot complaints	31	8 (26%)	50	19 (38%)	0.3	0.7
Nocturia	31	8 (26%)	49	15 (31%)	0.6	0.04
Fatigue	30	6 (20%)	49	14 (29%)	0.4	0.5
Feeling cold	29	11 (38%)	50	6 (12%)	0.007	NA[e]
Constipation	32	4 (13%)	51	13 (26%)	0.2	NA[e]
Thirst	30	6 (20%)	47	12 (26%)	0.6	NA[e]
Visual complaints	30	5 (17%)	48	10 (21%)	0.6	NA[e]
Stomach ache	32	5 (16%)	49	6 (12%)	0.7	NA[e]
Diarrhoea	32	2 (6%)	50	9 (18%)	0.1	NA[e]
Backache	30	3 (10%)	49	9 (18%)	0.3	NA[e]
Heartburn / belching	30	2 (7%)	51	11 (22%)	0.1	NA[e]
Pica (eating non-food items)	30	1 (3%)	48	7 (15%)	0.1	NA[e]
Sexual problems	31	1 (3%)	48	6 (13%)	0.2	NA[e]
Difficulty sleeping	29	1 (3%)	50	7 (14%)	0.1	NA[e]

Data are presented as n (%). Complaints are scored as present when the caregivers indicated a score of 3 or higher on a 5-point Likertscale. [a] The GHMDc+ group is defined as the patients who received growth hormone treatment and multidisciplinary care both during childhood and adolescence and received transitional care. [b] The GHMDc- group had not received growth hormone treatment or multidisciplinary care during childhood or adolescence. [c] P-value corrected for age using logistic regression models. [d] Post hoc analysis: P-value after correction for age and BMI was 0.2. [e] P-value was not calculated as there were too few patients with the outcome to fit the model.

Table 4. Characteristics GHMDc± group.

	GHMDc± group (n = 7)	
Male / female	2/5	
Total duration of growth hormone treatment, median [IQR]	4.7 [2.7-8.0]	
	Last visit paediatric endocrinologist	**First visit adult endocrinologist**
Age in years, median [IQR]	15 [14-18]	24 [22-26]
BMI in kg/m², median [IQR]	28 [27-33]	34 [27-36]

DISCUSSION

We compared health problems in PWS adults who received GH treatment and multi-disciplinary care (GHMDc+) versus those who did not (GHMDc-) and found that health outcomes differed significantly between the two groups.

In our exploratory analysis, GHMDc was associated with a lower prevalence of obesity and DM2. Whereas obesity was a major problem in the GHMDc- group (median BMI 32 kg/m^2), the GHMDc+ group had a median BMI of 26 kg/m^2. However, as the GHMDc+ group still received GH treatment and MD care to date, it is unknown whether the beneficial effects were due to the childhood GHMDc, the ongoing GHMDc, or both. As many health problems become more prevalent with age, it is important to note that the mean age of the GHMDc+ group was lower than the GHMDc- group. This can be explained by the fact that the patients in the GHMDc+ group were, by definition, referred directly by a paediatrician during adolescence or early adulthood. Another explanation is that patients were excluded from the GHMDc- group if they had received GH treatment during childhood, which is now standard care for all children with PWS, thus excluding most adolescents. After correction for age, the relationship between GHMDc and BMI and DM2 was still significant, while the relationship between GHMDc and other health problems was not. Both obesity and DM2 are important cardiovascular risk factors. As half of deaths in PWS are of cardiopulmonary origin (8,54), it is crucial to reduce obesity and DM2 in this vulnerable patient group.

Apart from obesity and DM2, the prevalence of undiagnosed health problems was also higher in the GHMDc- group (84%) and in the GHMDc± group (71%) compared to the GHMDc+ group (10%). This suggests that GHMDc prevents obesity and DM2 in patients with PWS and results in early detection of health problems that would otherwise remain undiagnosed. The fact that the results for the GHMDc± group were similar to the GHMDc- group suggests that the positive effects of GHMDc are only sustained when continued into adulthood. However, this result may be biased as patients with worse health outcomes are probably more likely to seek care from or be referred to our reference centre during adulthood. Additionally, the small size of the GHMDc± group (seven patients) prevents us from drawing any firm conclusions.

Although not significant, the prevalence of hypothyroidism found by our systematic health screening was higher in the GHMDc+ group than in the GHMDc- group. This could be the result of more frequent thyroid hormone measurements during childhood, as part of standard health watch. Additionally, GH treatment can unmask central hypothyroidism in adults with hypopituitarism (55), although this has not been shown in

children with PWS (56). In the GHMDc+ group, hypothyroidism was often mild, without clinical signs.

When we look at the GHMDc± group in more detail, we see that five of the seven patients in the GHMDc± group showed an increase in BMI of at least 5 kg/m^2 in their time without GHMDc. This resulted in more obesity (n = 4, 57%), compared to the GHMDc+ group (n = 6, 15%). Additionally, one patient developed hypothyroidism, one DM2 and one hypercholesterolemia, all accompanied by an increase in BMI. It is well known that DM2 and hypercholesterolemia are related to BMI (57-59), but also thyroid function can be affected by BMI. Obesity is associated with a higher serum thyroid stimulating hormone (TSH) concentration and a lower serum free thyroxine (free T4) concentration (60,61). On the other hand, thyroid dysfunction can increase BMI when patients are not accurately treated (61).

Our exploratory analysis for subjective complaints according to GHMDc group showed that adults in the GHMDc+ group reported fewer nocturia and snoring after correction for age. As BMI is an important cause of snoring (62,63), we performed a post hoc analysis. After adjusting the relationship between snoring and GHMDc group for age and BMI, this relationship was no longer significant. This indicates that the lower prevalence of snoring in the GHMDc+ group is mostly caused by the lower BMI. Unfortunately, we had insufficient data to report on the prevalence of sleep apnea (as assed by polysomnography) in this population. Future research is needed to investigate the relationship between GHMDc and sleep apnea. Nocturia is an important symptom of heart failure and other heart diseases (64), making this an indicator of cardiovascular health. However, more objective assessments of cardiovascular health (e.g. echocardiography) are needed before drawing any firm conclusions. It should be noted that not all patients filled in the questionnaire and that some patients skipped questions for unknown reasons, which could have influenced the results.

There are several aspects of GHMDc that could explain the differences between the GHMDc+ and GHMDc- groups.

The GHMDc+ group received GH treatment

The GHMDc+ group received GH treatment while the GHMDc- group, by definition, did not. GH status and GH treatment have been the subject of extensive research over the last decades. Individuals with PWS display signs and symptoms of GH deficiency, like short stature, small for height hands and feet, increased body fat and low muscle strength and muscle mass (3). Although the reported prevalence of GH deficiency in adults with PWS ranges from 0-38% (65,66), these percentages are only a rough estimate as there are no

adequate tests to confirm the diagnosis of GH deficiency in patients with PWS (65,67,68). The GHRH-arginine test does not detect GH deficiency of hypothalamic origin, as the underlying GHRH deficiency is reversed due to the administration of GHRH (69). The insulin tolerance test (ITT) is able to detect GH deficiency of hypothalamic origin (70), but is often contra-indicated in PWS due to the presence of epilepsy or cardiovascular disease. In addition, placing two indwelling intravenous catheters needed for the ITT is often technically impossible due to disturbed vascularisation and / or obesity (71,72). Furthermore, hypoglycaemia can be dangerous in patients with intellectual disabilities as they could be unable to accurately express their symptoms. However, recently, a more easy-to-perform test e.g. the glucagon test proved encouraging for the detection of GH deficiency in adults with PWS, although this test is also not infallible (73).

In children with PWS, GH treatment is known to improve physical health and cognition and might also improve quality of life (QoL) (45,51,52,74-78), independent of the GH status (50,68). GH treatment has become standard of care in PWS children, regardless of the presence or absence of GH deficiency (50).

In adults with PWS, GH treatment improves body composition (by increasing lean body mass and decreasing fat mass) and muscle strength, and decreases the prevalence of cardiovascular risk factors, even without proven GH deficiency. Furthermore, positive effects on endurance, several aspects of cognition, and quality of life have been reported (79-89). Despite these beneficial effects, GH treatment is often not reimbursed by healthcare insurance for adults with PWS as GH deficiency cannot be confirmed. However, in the Netherlands, adults that received GH treatment during childhood, can continue GH treatment into adulthood.

The GHMDc+ group received structured transitional care

The transition from paediatric to adult care is a vulnerable, yet important process. Structured transitional care is important to decrease drop-out (90). Paepegaey et al investigated the effect of transitional care in adults with PWS and found that the presence of structured transitional care resulted in a lower BMI (91). This is in accordance with our study. However, Paepegaey et al did not find a significant effect on type 2 diabetes mellitus (DM2). In our centre, transitional care includes a shared visit to both the paediatric and the adult endocrinologist, followed by alternating visits at the paediatric and adult department until the final transfer to adult endocrinology.

The GHMDc+ group was treated in a centre of expertise

Due to the rarity of the syndrome, care for patients with PWS should preferably be provided by dedicated physicians with PWS expertise. In our GHMDc- group, most patients

were treated by generalists, i.e. ID physicians or general practitioners. Generalists, by definition, have a broad knowledge of common disorders. Although ID physicians are specialized in syndromes, they usually lack the knowledge of internal health problems and are seldom familiar with the diagnostic and therapeutic pitfalls in the screening and treatment of internal and endocrine problems intrinsic to these rare disorders. The high number of undiagnosed and/or untreated health problems revealed by our systematic screening is probably due to referring general practitioners' unfamiliarity with the internal and endocrine health problems occurring in this syndrome.

The GHMDc+ group was treated in an MD setting during childhood

Due to the complexity of the syndrome, care for both children and adults with PWS should preferably be provided by an MD team. Ideally, the MD team consists of a (paediatric) endocrinologist to treat the pituitary hormone deficiencies, a dietitian to provide and guide a diet that compensates for low basal metabolic rate (BMR), a physiotherapist to address musculoskeletal problems and increase muscle mass to optimize BMR and an ID physician, or, if an ID physician is not available, a behavioural therapist to address behavioural issues. Ideally, a clinical neuropsychologist should also be involved, to assess cognitive, adaptive and behavioural functioning from a developmental, brain and behavioural perspective. Patients with PWS often have high verbal comprehension abilities compared to their perceptual reasoning abilities (49,92,93). Therefore, their capacities are often overestimated by caregivers. This can lead to too many responsibilities, which may cause stress, challenging behaviour and physical problems like hypertension and fatigue. Informing caregivers about the actual capacities of the PWS individual can prevent this overestimation and the associated stress-related somatic and behavioural issues.

The GHMDc+ group underwent systematic health screening

Underdiagnosis is a common problem in patients with PWS, due to the high pain threshold, PWS-specific behavioural phenotype and/or intellectual disability (7). Health problems can easily be missed when they are not actively screened for. Therefore, regular patient care should include a systematic health watch, including screening for endocrine deficiencies and cardiovascular risk factors.

Strengths and limitations

Like every study, our study has strengths and limitations. Strengths of our study are that we had a (for rare disorders) large sample size and that we provide a thorough exploratory analysis of the differences between the GHMDc+ and GHMDc- group. However, the GHMDc± group was small. As GH treatment was part of MD childhood care, we were only able to investigate the combined effect of GH treatment and MD care and could

not assess the independent effect of GH treatment or MD care. Another limitation is the limited overlap in age between the GHMDc+ and the GHMDc- group. Therefore, the results of our multivariable analysis should be interpreted with caution.

CONCLUSIONS

We demonstrated that the combination of growth hormone treatment and multidisciplinary care has beneficial effects in patients with PWS. Therefore, we recommend to continue GHMDc in patients with PWS who have reached adulthood. Unfortunately, this may not always be possible as growth hormone treatment is not available for all adults with PWS. Based on our data on the combined effect of growth hormone treatment and multidisciplinary care, supported by previously reported beneficial effects of GH treatment alone in both children and adults (43-53,68,74-89), we support the pledge by Hoybye et al for general approval of growth hormone treatment in adults with PWS (94).

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Hypogonadism in adult males with Prader-Willi syndrome - clinical recommendations based on a Dutch cohort study, review of the literature and an international expert panel discussion

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ABSTRACT

Prader-Willi syndrome (PWS) is a complex genetic syndrome characterized by hyperphagia, intellectual disability, hypotonia and hypothalamic dysfunction. Adults with PWS often have hormone deficiencies, hypogonadism being the most common. Untreated male hypogonadism can aggravate PWS-related health issues including muscle weakness, obesity, osteoporosis, and fatigue. Therefore, timely diagnosis and treatment of male hypogonadism is important. In this article, we share our experience with hypogonadism and its treatment in adult males with PWS and present a review of the literature. In order to report the prevalence and type of hypogonadism, treatment regimen and behavioral issues, we retrospectively collected data on medical interviews, physical examinations, biochemical measurements and testosterone replacement therapy (TRT) in 57 Dutch men with PWS. Fifty-six (98%) of the patients had either primary, central or combined hypogonadism. Untreated hypogonadism was associated with higher body mass index and lower hemoglobin concentrations. TRT was complicated by behavioral challenges in one third of the patients. Undertreatment was common and normal serum testosterone levels were achieved in only 30% of the patients. Based on the Dutch cohort data, review of the literature and an international expert panel discussion, we provide a practical algorithm for TRT in adult males with PWS in order to prevent undertreatment and related adverse health outcomes.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare genetic syndrome caused by the absence of expression of a cluster of paternally expressed genes on chromosome 15q11.2-q13, also called the 'PWS region'. PWS can be caused by paternal deletion of (part of) the PWS region (60-75%), maternal uniparental disomy 15 (mUPD, 20-35%), imprinting center defect (ICD, 1-4%) or paternal chromosomal translocation (0.1%) (1,2). Due to hypothalamic dysfunction, patients with PWS often have hormone deficiencies, hyperphagia, sleep disorders, abnormal temperature regulation and high pain threshold. PWS also has a characteristic neurobehavioral phenotype, including mild to moderate intellectual disability, autism-like features, obsessive compulsions, skin picking, and temper tantrums (3-7).

The most common hormone deficiency in PWS is hypogonadism. The reported prevalence of hypogonadism in adult males with PWS ranges from 57 to 100% (8-20). Although hypogonadism in PWS can be the result of hypothalamic dysfunction, recent studies show that hypogonadism in PWS can also be the result of primary gonadal failure (16,17,21), or a combination of hypothalamic and gonadal dysfunction (16,22,23).

Hypogonadism can affect males with PWS at all ages. At birth and during infancy, boys with PWS can display cryptorchidism, scrotal hypoplasia and short penile length (24). Later in life, small penile length in combination with large suprapubic fat may lead to voiding difficulties in young, obese adults with PWS (24). Puberty is usually incomplete and delayed, although precocious adrenarche and, rarely, precocious puberty can also occur (25-27). Primary testicular dysfunction is a major contributor to abnormal pubertal development in males with PWS (23). In adulthood, individuals with PWS often have low levels of sex steroids (8,15,18,19,28-30). Males with PWS are believed to be infertile and no cases of paternity have been reported in the literature (21,24).

Male hypogonadism is associated with fatigue, depression, decreased muscle strength and mass, increased fat mass, decreased sexual quality of life, and an increased risk of osteoporosis (31-33) and cardiovascular disease (32,34). As many of these factors are already prevalent in PWS (7), it is important to detect hypogonadism and start testosterone replacement therapy (TRT) at an early stage. However, TRT is a delicate matter as it may be complicated by challenging behavior (26,35).

In the current article, we share our experience with hypogonadism and its treatment in a Dutch cohort of adult males with PWS. We report the prevalence and type of hypogonadism, treatment regimen and behavioral issues encountered in adult males with PWS.

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Based on our findings, a thorough review of the literature and the clinical expertise of an international expert panel discussion, we provide a practical algorithm for the treatment of hypogonadism in adult males with PWS.

MATERIALS AND METHODS

Ethical review and approval were waived for this study by the Medical Ethics Committee of the Erasmus University Medical Center.

In this retrospective study, we included adult males who visited the multidisciplinary outpatient clinic of our PWS reference center in the Erasmus University Medical Center, Rotterdam, the Netherlands, between January 2015 and December 2020 and underwent our routine systematic health screening. As described previously (see (36)), this screening consists of a structured interview, a complete physical examination, a medical questionnaire, a review of the medical file, biochemical measurements and, if indicated and feasible, additional tests.

As part of regular patient care, primary caregivers were asked to fill out a medical questionnaire. In this questionnaire, subjective complaints (daytime sleepiness, fatigue, sexual complaints and temper tantrums) were scored on a 5-point Likert scale (1 = rarely or never, 2 = not often and/or not severe, 3 = quite often and/or quite severe, 4 = often and/or severe, 5 = very often and/or very severe). A score of 3 or higher was considered clinically relevant.

During the visit, blood samples were taken for general medical screening, including evaluation of gonadal function (luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone and sex hormone binding globulin (SHBG)) and the hematopoietic system (hemoglobin and hematocrit).

Before 1 February 2018, testosterone concentrations were measured using the PerkinElmer CHS™ MSMS Steroids Kit and an ultra-performance liquid chromatography – tandem mass spectrometer (UPLC-MS/MS) (reference range 10.0-30.0 nmol/L). After that date, testosterone concentrations were measured using an in-house assay and a UPLC-MS/MS (reference range 10.0-30.0 nmol/L). Before 1 February 2019, LH and FSH concentrations were measured using the Siemens Immulite 2000XPi (reference range 1.5-8.0 IU/L for LH and 2.0-7.0 IU/L for FSH). After that date, LH and FSH concentrations were measured using the Fujirebio Lumipulse G1200 (reference range 1.0-5.5 IU/L for LH and 0.8-5.1 IU/L for FSH). Before 15 June 2020 SHBG, concentrations were measured

using the Siemens Immulite 2000XPi (reference range 10-70 nmol/L). After that date, SHBG concentrations were measured using the IDS-ISYS (reference range 10-70 nmol/L). Hemoglobin and hematocrit were measured using the Sysmex XN1000 analyzer (reference ranges 8.6-10.5 mmol/L and 0.4-0.5 L/L, respectively). LH and FSH measurements changed methods during the study with a different calibration, testosterone and SHBG measurements also changed methods, but they were calibrated similarly, as checked by external quality assessment schemes.

The visits to our outpatient clinic are always in the afternoon. In one visit, the patients are seen by the multidisciplinary team, after which blood is collected for general health screening. Although testosterone is preferably measured in the morning (37), in our clinic this was not feasible. Hypogonadism was defined as an afternoon total testosterone value below 10.0 nmol/L (2.88 ng/mL) with normal SHBG and sparse facial hair. Only if hypogonadism was not clearly present from clinical features (prepubertal status, underdeveloped genitals and/or absent virilization), a separate morning testosterone analysis was done to confirm hypogonadism. Pubic hair Tanner stage is often relatively advanced in PWS men due to normal or increased production of adrenal androgens, however this does not represent testicular development or gonadal hormone secretion and therefore pubic hair was not considered in the diagnosis of hypogonadism (27). Due to hyperphagia, it was not feasible to obtain fasting testosterone measurements. If patients already used TRT before the first visit to our outpatient clinic, this was also considered as indicating presence of hypogonadism. Only LH, FSH and testosterone values from before the start of TRT were included.

When TRT was initiated at our outpatient clinic, a daily dose of 10 mg transdermal testosterone gel was administered, which was increased by 10 mg every 4 weeks until serum testosterone concentrations within the normal range were reached. When adverse effects occurred, the TRT dose was not further increased or was decreased, depending on the severity of the adverse effects. After TRT was started, SHBG measurements were not routinely repeated.

We defined short-acting injections as injections that have to be administered every 1-6 weeks, and long-acting injections as injections that have to be administered every 12 weeks.

Hypothalamic dysfunction of the LH/testosterone axis was defined as a low or normal LH concentration with a low testosterone concentration, while testicular dysfunction was defined as a high LH concentration with a low testosterone concentration. Hypothalamic dysfunction of the FSH/inhibin B axis was defined as a low or normal FSH concentration,

while testicular dysfunction was defined as a high FSH concentration. Inhibin B was not measured, but based on previous research, we would expect that inhibin B levels would be low in most males (16,18).

Patients that were treated by the pediatric endocrinologist at our reference center during childhood, received transitional care during transition to the multidisciplinary outpatient clinic for adults with PWS. Transitional care included a shared visit with both the pediatric and the adult endocrinologist, followed by alternating visits at the pediatric and adult department until the final transfer to adult endocrinology.

Literature search

In collaboration with the Erasmus MC Medical Library, we performed a literature search on 24 September 2020 and last updated the search on 3 June 2021. We searched the following databases: Embase, Medline (Ovid), Web of Science Core Collection and Cochrane Central Register of Controlled Trials. We reviewed the literature for articles reporting the prevalence of hypogonadism and laboratory measurements (e.g. testosterone, LH, FSH, SHBG, inhibin B) in males with PWS. Search terms included 'Prader-Willi Syndrome', 'gonadal disease', 'hypogonadism', 'puberty', and relevant laboratory measurements. For the full search strategy, see **Table S1.** We excluded conference abstracts, non-original research articles, articles that were not available in English, and articles that included less than ten adults (males and females) with PWS. When articles reported on adults and children and the prevalence of hypogonadism or laboratory values were not available for adults only, we contacted the authors to retrieve information for the adults separately. When articles reported on overlapping populations, the article with the most patients or, when the number of patients was similar, the most recent article was included. Although this search strategy resulted in articles on hypogonadism in both males and females with PWS, only the articles that provide information on hypogonadism in males are reported here.

Expert opinion

An international panel of PWS experts was asked to fill out a survey on their experience with the treatment of hypogonadism in adult males with PWS. Clinical recommendations have been made based on this survey, the results of the cohort study and the literature review. None of the experts had a financial interest in any of the modalities of TRT.

Data analysis

Statistical analysis was performed using R version 3.6.3 (https://cran.r-project.org/, accessed on 16 September 2021). Descriptive statistics for continuous variables are reported as median (interquartile range (IQR)). For dichotomous variables we display the number

and the percentage of people, n (%). For the comparison of untreated male hypogonadism compared to no hypogonadism or treated hypogonadism, we used the Wilcoxon rank sum test for continuous variables and a chi-squared test for dichotomous variables. To correct for age, we used linear and logistic regression models, respectively, with a likelihood ratio test. To compare testosterone or SHBG concentrations between genotypes and between patients who used and did not use growth hormone (GH) treatment, we used a Wilcoxon rank sum test. To correct for age, we used a linear regression model with a likelihood ratio test. To investigate the relationship between testosterone or SHBG concentrations, and body mass index (BMI) and age, the Kendall rank correlation test was used. To explore the relationship between testosterone or SHBG concentrations, and BMI corrected to age, a linear regression model and a likelihood ratio test were used. For all analysis involving FSH and LH measurements, a linear regression model with a likelihood ratio test was used and a variable indicating whether the measurement was performed before or after 01-02-2019 (when the method was changed with a different calibration) was included in the model. As this was an exploratory analysis, no correction for multiple testing was performed. P-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Baseline characteristics are shown in **Table 1**. We included 57 adult males with a median age of 29 years (IQR 20 – 40) (range 18 – 72 years). Only one patient was more than 60 years old and 21 patients were younger than 25 years old. One patient was excluded from the analysis. We did not screen for hypogonadism in this individual as TRT was unfeasible due to serious behavioral challenges that were already present before the first visit to our outpatient clinic.

Hypogonadism

Hypogonadism was present in 56 of 57 males (98%). In 28 males (49%), hypogonadism had been previously diagnosed and 24 males were already receiving TRT. Our screening revealed hypogonadism in another 28 patients (49%). Most frequent modes of testosterone administration at the first visit to our center were transdermal gel (n = 12, 50%) and intramuscular injections (n = 10, 42%). Nine males used short-acting intramuscular injections (Sustanon®) and one used long-acting intramuscular injections (testosterone undecanoate, Nebido®). For practical and / or behavioral reasons (see also discussion section), 8 patients switched from oral TRT (n = 2) or intramuscular injections (n = 6) to transdermal testosterone gel after their first visit (**Table 2**). The current and highest dose of transdermal testosterone gel of each patient is shown in **Figure 1(a,b)**.

Table 1. Baseline characteristics of 57 adult males with Prader-Willi syndrome.

	Males with PWS n = 57
Age in years, median (IQR)	29 (20 – 40)
BMI in kg/m^2, median (IQR)	27 (26 – 32)
Overweight (BMI 25 – 30 kg/m^2), n (%)	29 (51%)
Obesity (BMI > 30 kg/m^2), n (%)	17 (30%)
Genetic subtype	
Deletion	29 (51%)
mUPD [a]	20 (35%)
ICD	2 (4%)
Unknown	6 (11%)
Growth hormone treatment	
Only during childhood	4 (7%)
Only during adulthood	1 (2%)
Both	20 (35%)
Never	32 (56%)
Current growth hormone treatment	19 (33%)
Living situation	
With family	16 (28%)
In a specialized PWS group home	8 (14%)
In a non-specialized facility	32 (56%)
Other [b]	1 (2%)
Education level	
Secondary vocational education	2 (4%)
Pre-vocational secondary education	2 (4%)
Special education	43 (75%)
No education	1 (2%)
Unknown	9 (16%)
Relationship status	
In a relationship with sexual intercourse	2 (4%)
In a relationship without sexual intercourse	10 (18%)
Not in a relationship	37 (65%)
Unknown	8 (14%)
Cryptorchidism	36 (63%)
Of which underwent orchidopexy, n (% of cryptorchidism)	34 (94%)
Of which underwent orchidectomy, n (% of cryptorchidism)	2 (6%)
No cryptorchidism	5 (9%)
Cryptorchidism unknown	16 (28%)
Small penile length	
Yes	22 (39%)
No	11 (19%)
Unknown	24 (42%)

Abbreviations: body mass index (BMI), paternal deletion (deletion), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD), Prader-Willi syndrome (PWS). Data are presented as n (%), unless otherwise specified. Baseline characteristics were collected during the first visit to the multidisciplinary outpatient clinic of our PWS reference center. [a] In 9 patients with suspected mUPD, the parents were not available for genetic testing. Therefore, mUPD is the most likely genotype, but an ICD could not be ruled out in these patients. [b] One patient lived alone with ambulatory care.

Table 2. Hypogonadism in male adults with PWS.

	Males with PWS n = 57
Hypogonadism before screening, n (%)	28 (49%)
Of which treated, n (% of hypogonadal)	24 (86%)
Type of testosterone replacement therapy before screening	
Gel, n (% of treated)	12 (50%)
Injections, n (% of treated)	10 (42%)
Short-acting, n (% of treated)	9 (38%)
Long-acting, n (% of treated)	1 (4%)
Oral, n (% of treated)	2 (8%)
Newly diagnosed hypogonadism, n (%)	28 (49%)
Hypogonadism after screening, n (%)	56 (98%)
Of which currently treated, n (% of hypogonadal)	42 (75%)
Current type of testosterone replacement therapy	
Gel, n (% of treated)	37 (88%)
Injections, n (% of treated)	3 (7%)
Short-acting, n (% of treated)	3 (7%)
Long-acting, n (% of treated)	0 (0%)
Oral, n (% of treated)	2 (5%)
Decrease in testosterone dose due to challenging behavior	
Yes, n (%)	18 (32%)
Of which had increased testosterone concentrations, n (% of yes) [a]	1 (6%)
Of which had normal testosterone concentrations, n (% of yes) [a]	2 (11%)
Of which had inadequate testosterone concentrations, n (% of yes) [a]	15 (83%)
Of which reported a decrease in challenging behavior after decrease testosterone dose, n (% of yes)	11 (61%)
No, n (%)	31 (54%)
Unknown, n (%)	8 (14%)
Problems with compliance to testosterone replacement therapy	
Yes, n (%)	6 (11%)
High suspicion of non-compliance, n (%)	5 (9%)
No problems with compliance reported, n (%)	36 (63%)
Never used testosterone replacement therapy, n (%)	10 (18%)
Enlarged breasts	
Yes, n (%)	8 (14%)
Gynaecomastia, n (% of enlarged breasts)	1 (13%)
Lipomastia, n (% of enlarged breasts)	2 (25%)
Unknown, n (% of enlarged breasts)	5 (63%)
No or not assessed, n (%)	49 (86%)

Sex hormone-binding globulin (SHBG) measurements were not repeated after the start of testosterone replacement therapy, but were normal before start of testosterone replacement therapy. [a] Testosterone concentrations measured before the decrease in testosterone dose.
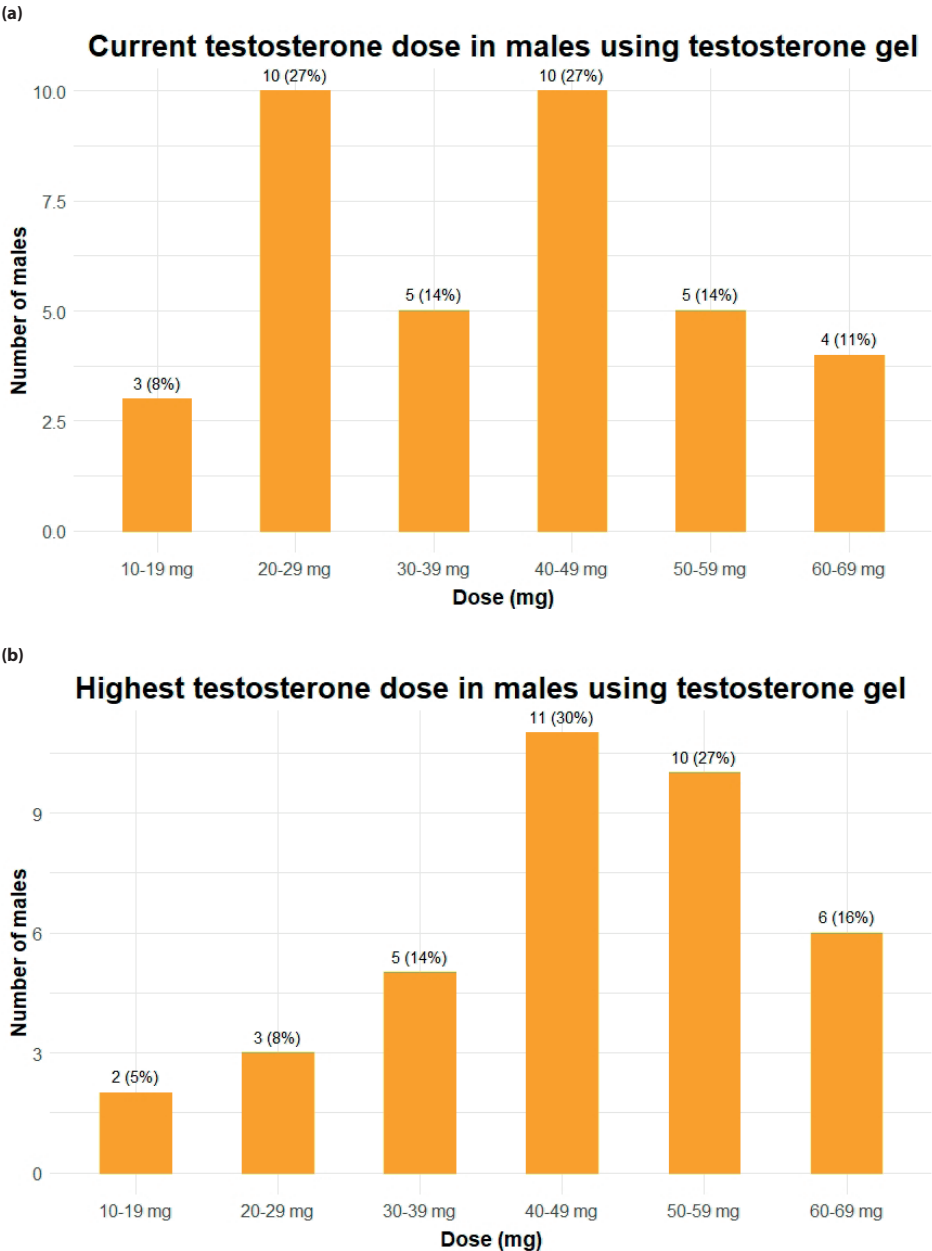
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(a)

Current testosterone dose in males using testosterone gel

(b)

Highest testosterone dose in males using testosterone gel

Figure 1. Testosterone dose in 37 males with PWS using testosterone gel. Data is given as n (%). (**a**) The testosterone doses each patient received during the last visit to the outpatient clinic. When patients died or were transferred to another hospital, the last known dose was given; (**b**) The highest dose of testosterone gel ever received while visiting our outpatient clinic for each patient. To make both graphs comparable only patients who currently still use testosterone gel are depicted in panel b (in 5 patients testosterone replacement therapy was discontinued completely).

Figure 2 shows serum testosterone concentrations according to the current testosterone dose in 22 males. This figure shows that while higher testosterone doses lead to higher serum testosterone concentrations, low testosterone concentrations can also be seen in patients using higher doses of testosterone gel. Serum testosterone levels in the normal range were reached in 17 (30%) patients. Of the 70% not reaching normal range testosterone levels, 9 patients never started TRT at all (**Figure 3(a)**), due to fear of adverse events (n = 4), increased age (n = 2), or loss to follow-up (n = 3) (**Figure 3(b)**). In 27 males, TRT dose could not be increased, either due to challenging behavior (n = 18) or for unknown reasons (n = 9). Three (5%) patients had inadequate testosterone doses because they were still gradually increasing testosterone dose at the time of publication of this manuscript.
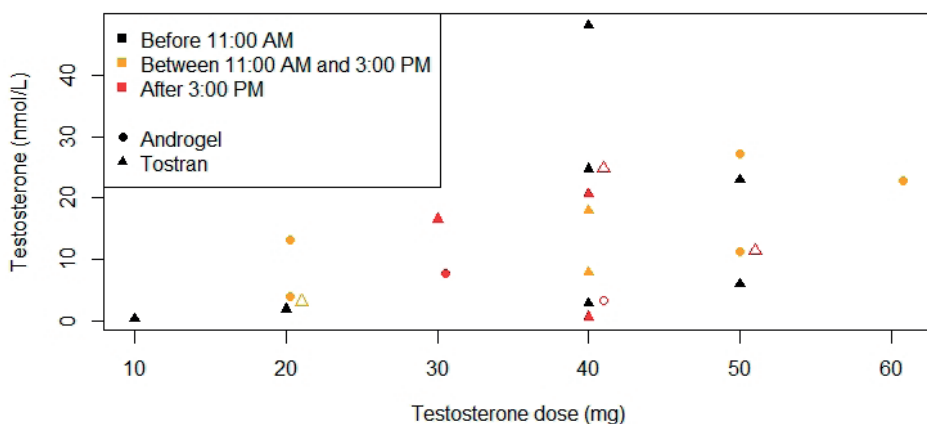

Figure 2. Serum testosterone concentrations according to testosterone dose for patients using testosterone gel. Laboratory measurements were only available for 22 males. Testosterone gel was administered by the patient in the morning. Testosterone measurements before 11:00 AM are depicted in black, between 11:00 AM and 3:00 PM in orange and after 3:00 PM in red. Only two brands were used; Androgel® is depicted with circles and Tostran® with triangles. When two points overlapped, one of the points was moved 1 mg to the right and this point was depicted an open circle or triangle instead of closed.

In 18 patients testosterone dose had to be decreased due to challenging behavior, of whom the majority (83%) had serum testosterone concentrations below the reference range. Seventeen of them used transdermal gel (10 mg daily, n = 3; 20 mg daily, n = 2; 30 mg daily, n = 2; 40 mg daily, n = 2; 50 mg daily, n = 5; 60 mg daily, n = 2; and 69 mg daily, n = 1) and one used short-acting testosterone injections (200 mg every 4 weeks). In 11 (61%) behavior improved after testosterone dose reduction.

In 5 patients TRT was stopped completely, because even 10 mg transdermal testosterone gel was followed by unacceptable behavioral challenges (n = 4) or depressive symptoms (n = 1).

(a)

- Adequate serum testosterone concentration, 17 (30%)
- Challenging behavior, 18 (32%)
- Never started TRT, 9 (16%)
- TRT dose was still being increased, 3 (5%)
- Unknown, 9 (16%)

(b)

- Fear of adverse events, 4 (44%)
- Increased age, 2 (22%)
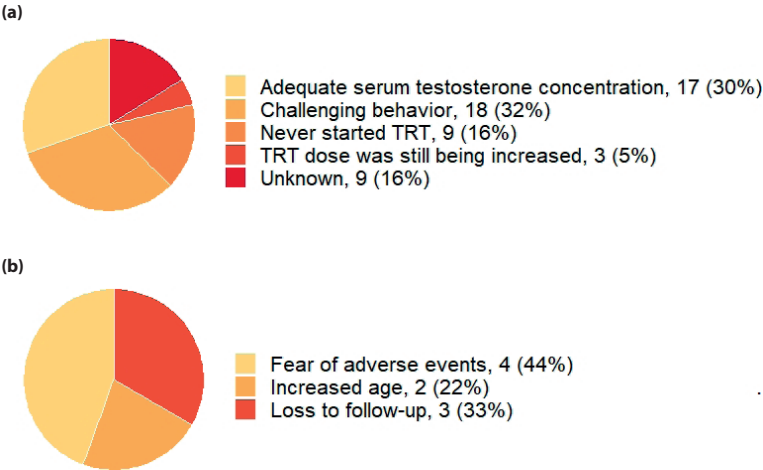- Loss to follow-up, 3 (33%)

Figure 3. Reasons for not increasing the testosterone doses and reasons for not initiating testosterone replacement therapy. Abbreviations: testosterone replacement therapy (TRT). Data is given as n (%). (**a**) The reasons for not further increasing testosterone doses in adult males with PWS and hypogonadism (n = 56). (**b**) The reasons for never initiating TRT in adult males with PWS and hypogonadism (n = 9).

Problems with compliance were fairly common, with non-compliance confirmed in 6 patients (11%) and suspected in 5 (9%).

Eight males, of whom two used short-acting testosterone injections and six with untreated hypogonadism before screening, had enlarged breasts, either due to gynaecomastia or lipomastia. In the two patients using TRT it was unknown whether breast enlargement was related to TRT. Six patients with enlarged breasts had obesity and two were overweight. In the other 49 males, the medical records did not mention gynaecomastia or lipomastia. Estradiol levels were not available.

Effect of untreated hypogonadism

We compared males with and without untreated hypogonadism at the first visit to our outpatient clinic. After correction for age, we found a significant difference in BMI (median (IQR): 29 kg/m^2 (27 – 38) in males with untreated hypogonadism and 26 kg/m^2 (23 – 37) in males with treated or no hypogonadism, P = 0.001). Three patients (12%) with treated or no hypogonadism had obesity, compared to 14 (44%) patients with untreated hypogonadism. Hemoglobin was significantly lower in males with untreated hypogonadism (median 8.2 nmol/L (IQR 8.0 – 9.0)) than in males with treated or no hypogonadism (median 9.3 nmol/L (IQR 8.6 – 9.7), P = 0.03). Although not significant, anemia was less prevalent in patients with treated or no hypogonadism (n = 4, 17%), compared to patients with untreated hypogonadism (n = 11, 34%). After correction for age, subjective complaints did not differ between the males with untreated hypogonadism and the males with treated or no hypogonadism (**Table 3**).

Table 3. Effect of untreated hypogonadism in adult males with PWS.

	Number of Observations	Untreated Male Hypogonadism n = 32	Number of Observations	Treated / No Male Hypogonadism n = 25	P-value	P-value After Correction for Age
Age, median (IQR)	32	35 (26 – 50)	25	23 (19 – 30)	0.003	NA
BMI, median (IQR)	32	29 (27 – 38)	25	26 (23 – 27)	<0.001	0.001
Overweight, n (%)		15 (47%)		14 (56%)		
Obesity, n (%)		14 (44%)		3 (12%)		
Anemia, n (%)	32	11 (34%)	24	4 (17%)	0.09	0.15
Hemoglobin in mmol/L, median (IQR)	32	8.2 (8.0 – 9.0)	24	9.3 (8.6 – 9.7)	0.009	0.03
Hemoglobin in g/dL, median (IQR)	32	13.2 (12.9 – 14.5)	24	15.0 (13.9 – 15.6)		
Hematocrit in L/L, median (IQR)	18	0.43 (0.42 – 0.45)	18	0.45 (0.43 – 0.48)	0.02	0.2
Subjective complaints						
Daytime sleepiness, n (%)	26	17 (65%)	22	7 (32%)	0.02	0.09
Fatigue, n (%)	25	7 (28%)	22	4 (18%)	0.4	0.5
Sexual complaints, n (%)	25	3 (12%)	22	2 (9%)	0.7	NA [a]
Temper tantrums, n (%)	26	13 (50%)	22	11 (50%)	1	0.7

Abbreviations: body mass index (BMI), interquartile range (IQR), not available (NA). Comparison of patients who had hypogonadism, but were not treated before screening (untreated male hypogonadism) compared to patients who either already received TRT before screening (n = 24) or who did not have hypogonadism (n = 1). Subjective complaints were scored on a 5-point Likert scale. A score of 3 or higher was seen as 'present'. Overweight is defined as a BMI between 25 and 30 kg/m² and obesity as a BMI above 30 kg/m². Reference ranges for hemoglobin and hematocrit were 8.6 - 10.5 mmol/L (13.9 – 16.9 g/dL) and 0.4 – 0.5 L/L, respectively. [a] Not enough events to fit the model to correct for age.

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We investigated the relationship between testosterone, BMI and age (**Figures 4 and 5**) because in the normal population these parameters affect serum testosterone concentrations. Testosterone concentrations measured before 11:00 AM seemed to be negatively associated with BMI and age, but this association was not significant (P = 0.4 and P = 0.3, respectively). For the relationships between laboratory values (testosterone, LH, FSH and SHBG) and genotype, GH treatment, BMI, and age, see **Tables S2 and S3.**
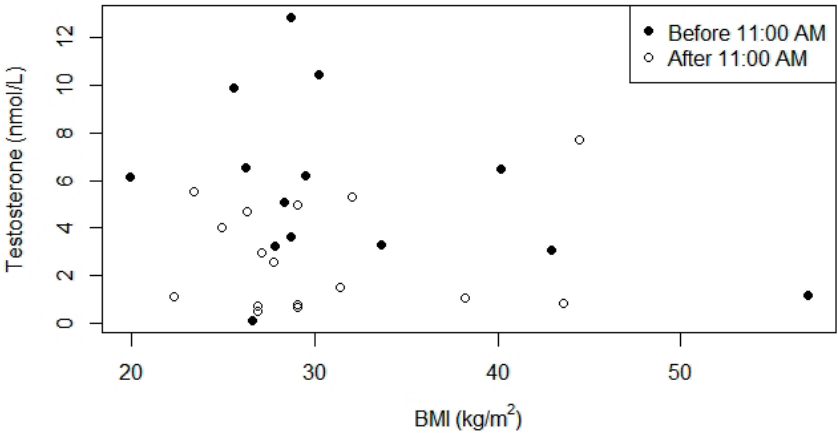

Figure 4. Relationship between serum testosterone concentrations and BMI for males who were not receiving testosterone replacement therapy. P-value for the relationship between BMI and serum testosterone concentration measured before 11:00 AM was 0.4 (0.1 after correction for age), Kendall's Tau was -0.19. P-value for the relationship between BMI and serum testosterone concentration measured after 11:00 AM was 0.9 (1.0 after correction for age), Kendall's Tau was 0.03.
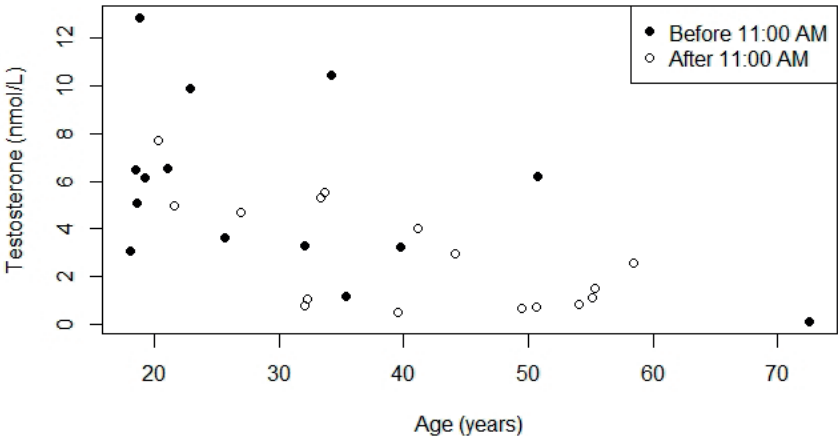

Figure 5. Relationship between serum testosterone concentrations and age for males who were not receiving testosterone replacement therapy. P-value for the relationship between age and serum testosterone concentration measured before 11:00 AM was 0.3, Kendall's Tau was -0.23. P-value for the relationship between age and serum testosterone concentration measured after 11:00 AM was 0.2, Kendall's Tau was -0.27.

Types of hypogonadism

Pre-TRT LH and FSH levels were available in 33 males. Seven patients had central hypogonadism (21%), seven had primary hypogonadism (21%), but the majority had a combination of hypothalamic and testicular dysfunction (n = 18, 55%), **Table 4**.

Table 4. LH and FSH values in males with PWS.

	FSH / Inhibin B axis		
	Hypothalamic dysfunction (low FSH)	**Hypothalamic or no dysfunction [a] (normal FSH)**	**Testicular dysfunction (high FSH)**
Hypothalamic dysfunction (low/normal LH with low T)	1 (3%)	6 (18%)	18 (55%)
No dysfunction (normal LH with normal T)	0 (0%)	0 (0%)	1 (3%)
Testicular dysfunction (high LH with low T)	0 (0%)	0 (0%)	7 (21%)

Abbreviations: follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T). Laboratory measurements of LH, FSH and testosterone were available for 33 adult males. In the other 24 males no laboratory measurements were available or only laboratory measurements during testosterone replacement therapy were available. This figure has been designed using resources from Flaticon.com (from freepik and smashicons), accessed 1 June 2021. [a] As no inhibin B measurements were available, we did not know whether patients with normal FSH had normal function of the FSH/inhibin B axis or hypothalamic FSH / inhibin B axis dysfunction with inappropriately low FSH for the low inhibin B values. Based on previous research, we assumed that inhibin B levels were low in most adult males with PWS (16,18).

Literature review

We found 13 articles that described hypogonadism in adult males with PWS and fulfilled the inclusion criteria (**Tables 5 and 6**). Most articles defined hypogonadism as a low serum testosterone concentration. The prevalence of hypogonadism ranged from 57% to 100%, with 6 of 10 articles reporting a prevalence of ≥ 90%. Central hypogonadism was the most common form of hypogonadism, while primary and mixed forms of hypogonadism were also reported. Multiple articles reported laboratory measurements and showed that testosterone and inhibin B values were below the reference range in most patients.

Table 5. Literature review hypogonadism in male adults with PWS (Part 1).

Article	n	Country	Age Range (Years)	Genotype (Deletion/ mUPD/ICD/ Translocation)	Mean BMI (kg/ m²)	Assays Used	Definition Hypogonadism
Partsch et al. (2000) (8)	7	Germany	18 – 34 [a]	All deletion or mUPD	46 [a]	commercially available immunoassays	testosterone levels below the normal range
Whittington et al. (2002) (9) [b]	31	United Kingdom	18 – 46	NA [c]	NA	NA	undescended testes at birth and / or small genitalia
Grugni et al. (2003) (10)	7	Italy	19 – 29	7/0/0/0	37	FSH, LH: immunochemiluminescent assays testosterone: chemiluminescent immunoassay	testosterone levels below the normal range
Höybye et al. (2005) (11) [b]	7	Sweden	19 – 36	0/1/0/0 (6 NA) [c]	Median 28	commercially available immunoassays	low testosterone or treatment with sex steroids
Miller et al. (2008) (12) [b]	10	Florida, USA	18 – 34	6/4/0/0	38	commercially available radioimmunoassays	Hypogonadotropic hypogonadism: delayed onset of puberty (i.e. >14 years) in addition to low gonadotropin levels for age
Brandau et al. (2008) (13) [b]	20	Missouri, USA	18 – 45	12/8/0/0	35	FSH, LH: chemiluminescence assays testosterone: radioimmunoassay	testosterone levels below the normal range
Sode-Carlsen et al. (2010) (14) [b]	21	Denmark, Norway, Sweden	18 – 39	13/1/1/0 (6 NA) [c]	Median 25	commercially available immunoassays	low testosterone or treatment with sex steroids
Van Nieuwpoort et al. (2011) (15)	4	The Netherlands	21 – 42	14/1/0/0 [a]	29	commercially available immunoassays	low testosterone or treatment with sex steroids

Table 5. Literature review hypogonadism in male adults with PWS (Part 1). (*continued*)

Article	n	Country	Age Range (Years)	Genotype (Deletion/ mUPD/ICD/ Translocation)	Mean BMI (kg/ m²)	Assays Used	Definition Hypogonadism
Radicioni et al. (2012) (16)[b]	17	Italy	18 – 42	13/4/0/0	42	FSH, LH, testosterone: chemiluminescent microparticle immunoassay. Inhibin B: enzymatically amplified two-site two-step sandwich-type immunoassay (ELISA). SHBG: immunoradiometric assay	testosterone and/or inhibin B levels below the normal range
Kido et al. (2013) (17)	16	Japan	18 – 48	15/1/0/0	33	NA	testosterone level <300 ng/dl and Tanner stage less than IV.
Hirsch et al. (2015) (18)[b]	18	Israel	18 – 36	11/7/0/0	29	LH, FSH, testosterone: immunoassays. Inhibin B, AMH: Two-site enzyme-linked immunosorbent assay (ELISA). SHBG: immunochemiluminescence	NA
Coupaye et al. (2016) (19)[b]	31	France	18 – 58[a]	42/24/0/0 [a,d]	39[a]	routine techniques	testosterone level <320 ng/dl or treatment with sex steroids
Matsuyama et al. (2019) (20)	11	Japan	18 – NA (Mean ± SD: 19.9 ± 2.3)	6/0/1/0 (4 NA)	NA	LH, FSH: two-site enzyme immune-assay. testosterone: chemiluminescent immunoassay	NA

Abbreviations: anti-müllerian hormone (AMH), body mass index (BMI), paternal deletion (deletion), follicle stimulating hormone (FSH), imprinting center defect (ICD), luteinizing hormone (LH), maternal uniparental disomy (mUPD), not available (NA), standard deviation (SD), sex hormone binding globulin (SHBG), United States of America (USA). Only articles reporting separate outcomes on adults (older than 18 years) were included. When this was not available, we contacted the authors to retrieve this information. [a] Data for all males and females included in this study. [b] Additional data was provided by the authors of this article. [c] All methylation positive. [d] Only patients with a deletion or an mUPD were included according to the inclusion criteria of this study.

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Table 6. Literature review hypogonadism in male adults with PWS (Part 2).

Article	Hypogonadism n (%)	Primary hypogonadism / central hypogonadism	FSH, mean (range)	LH, mean (range)	Testosterone, mean (range)	SHBG, mean (range)	Inhibin B, mean (range)	AMH, mean (range)
Partsch et al. (2000) (8)	7 (100%)	-[a]	-	-	-	-	-	-
Whittington et al. (2002) (9)	30 (100%) (1 NA)	-	-	-	-	-	-	-
Grugni et al. (2003) (10)	5 (71%)	-	12.4 (0.1 – 30.6) IU/L	6.0 (0.7 – 15.1) IU/L	3.0 (0.5 – 6.9) ng/mL / 10.4 (1.7 – 23.9) nmol/L	-	-	-
Höybye et al. (2005) (11)	4 (57%)	0/3 (1 NA)	4.2 (2.7 – 10) IU/L	2.8 (0.6 – 5.1) IU/L	9.7 (1.9 – 37) nmol/L	-	-	-
Miller et al. (2008) (12)	10 (100%)	0/10	-	-	-	-	-	-
Brandau et al. (2008) (13)	17 (89%) (1 NA)	-	14.8 (0.1 – 52.0) IU/L	3.1 (0.1 – 8.0) IU/L	1.3 (0.3 – 4.0) ng/ml / 4.5 (1.0 – 13.9) nmol/L	-	-	-
Sode-Carlsen et al. (2010) (14)	14 (67%)	8/2 (4 NA)	18.5 (<0.2 – 64) IU/L	3.5 (<1.0 – 13.5) IU/L	10 (1.9 – 39.5) nmol/L	-	-	-
Van Nieuwpoort et al. (2011) (15)	4 (100%)	0/2 (2 NA)	Median 1.1 IU/L	Median 0.43 IU/L	Median 3.2 nmol/L	Median 17.9 nmol/L	-	-
Radicioni et al. (2012) (16)	17 (100%)	2/9 Combined: 6	11.6 (0.05 – 46.6) IU/L	2.5 (0.04 – 7.2) IU/L	3.7 (1.4 – 13.7) nmol/L	22.9 (6.8 – 42.7) nmol/L	14.0 (3.0 – 38.3) pg/mL	-
Kido et al. (2013) (17)	-[b]	0/3 (13 NA)	18.9 (<0.5 – 43.3) IU/L	4.0 (<0.5 – 12.8) IU/L	99 (24 – 190) ng/dL / 3.4 (0.8 – 6.6) nmol/L	-	-	-
Hirsch et al. (2015) (18)	-	-	16.3 (0.1 – 55.9) IU/L	3.0 (0.1 – 10.5) IU/L	1.8 (0.2 – 4.7) nmol/L	34.2 (9.0 – 73.8) nmol/L	72.4 (0.1 – 269.0) pg/mL (n = 17)	12.13 (0.17 – 62.40) ng/mL (n = 16)
Coupaye et al. (2016) (19)	30 (97%)	-	Mean ± SD 13.2 ± 16 IU/L	Mean ± SD 3.2 ± 3.1 IU/L	1.3 (0.2 – 4.0) ng/mL / 4.5 (0.7 – 14) nmol/L	Mean ± SD 30.0 ± 20.0 nmol/L	Mean ± SD 36 ± 38 pg/mL	Mean ± SD 9.5 ± 15.3 ng/mL

Table 6. Literature review hypogonadism in male adults with PWS (Part 2). (*continued*)

Article	Hypogonadism n (%)	Primary hypogonadism / central hypogonadism	FSH, mean (range)	LH, mean (range)	Testosterone, mean (range)	SHBG, mean (range)	Inhibin B, mean (range)	AMH, mean (range)
Matsuyama et al. (2019) (20)	-	-	19.5 (7.5 – 30.8) IU/L	4.0 (1.0 – 5.3) IU/L	248 (102-509) ng/dL *8.6 (3.5 – 17.6) nmol/L*	-	-	-

Abbreviations: anti-müllerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), not available (NA), sex hormone-binding globulin (SHBG). When only laboratory measurements in non-SI units were reported, we added the converted values in *italics*. Only values for FSH, LH, and testosterone in patients who did not use sex steroid replacement therapy during blood withdrawal are included. Values that were below the measuring threshold were considered equal to the measuring threshold to calculate the mean. For example, when FSH was reported as <0.5, this was considered 0.5. [a] Gonadotropin levels were subnormal in all but one patient (of the population of 7 males and 12 females) and showed a reduced responsiveness to stimulation with exogenous gonadotropin-releasing hormone. [b] Only males with PWS with hypogonadism were included according to the in- and exclusion criteria of this study.

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Table 7. Expert panel discussion (Part 1).

	Expert 1 and expert 2 [a]	Expert 3	Expert 4
(Past) experience	Short-acting injections	Long-acting injections, transdermal gel	Long-acting injections, transdermal gel
Preferred mode of administration in PWS	Short-acting injections	Transdermal gel followed by long-acting injections	Long-acting injections, transdermal gel
Mode of administration advised against	Oral testosterone	Short-acting injections, oral testosterone	None
Preferred starting dose in testosterone naïve patients	*Short-acting injections:* 125 mg every 3-4 weeks	*Long-acting injections:* 200 mg every 12 weeks *Transdermal gel:* 10 mg daily	*Long-acting injections:* 250-500 mg for the first injection *Transdermal gel:* 10-30 mg depending on testosterone level
Preferred follow-up dose	*Short-acting injections:* After 6 months: increase to 200 mg every 3-4 weeks and then 250 mg every 3-4 weeks depending on serum testosterone concentrations, clinical signs and adverse effects	*Long-acting injections:* Every 6-9 months: increase by 200-300 mg, depending on serum testosterone and SHBG concentration *Transdermal gel:* Every 3-6 months: increase by 10 mg	*Long-acting injections:* Depending on the increase in serum testosterone concentration, injection of 500-1000 mg after 6 weeks. After 12 weeks, depending on the testosterone level obtained, injection of 500-1000 mg, which is then continued every 12 weeks aiming for a serum testosterone concentration within the normal range *Transdermal gel:* Gradual increase over 1-4 weeks to 30-50 mg daily, aiming for a serum testosterone concentration within the normal range
Biochemical follow up	Testosterone, Hb, Ht after each change of TRT dose. Once the final dose of TRT has been obtained, measurement of testosterone, Hb, Ht every year	Testosterone, Hb, Ht, SHBG, estradiol every 6 months or prior to dose increase	Testosterone, LH, FSH, Hb, Ht every 6-12 months, cholesterol every 12 months

Table 7. Expert panel discussion (Part 1). (*continued*)

	Expert 1 and expert 2 [a]	Expert 3	Expert 4
Considerations:	Only short-acting injections are reimbursed in these experts' country (France), while transdermal gel is no longer available	Start with transdermal gel as this allows gradual dose up-titration and immediate cessation if behavioral problems occur. Once established on final transdermal dose, switch to long-acting injections as this has smoother pharmacokinetics than short-acting injections and does not need to be applied daily, though depending on patient preference may continue transdermal gel	Short-acting injections not available in the expert's country (Sweden). Decision for long-acting injections or transdermal gel based on patient preference, most patients prefer injections instead of transdermal gel
Additional remarks		With gradual increases in testosterone dose behavioral problems do not appear to be an issue. Once established on long-acting injections measure testosterone concentrations ~1-2 months after injection and at trough just prior to injection as may need to increase injection frequency rather than dose	

Abbreviations: follicle stimulating hormone (FSH), hemoglobin (Hb), hematocrit (Ht), luteinizing hormone (LH), Prader-Willi syndrome (PWS), sex hormone-binding globulin (SHBG), testosterone replacement therapy (TRT). In this table the general considerations are described regarding testosterone replacement therapy for adult males with PWS who have not used testosterone replacement therapy before. However, based on patient preference, another treatment modality or dose could be prescribed. We defined short-acting injections as injections that have to be administered every 1-6 weeks, and long-acting injections as injections that have to be administered every 12 weeks. For this expert discussion we focused on the use of short-acting and long-acting injections, transdermal gel and oral testosterone only. Biochemical follow-up refers to the biochemical measurements performed during the titration of TRT dose. Physicians may perform additional measurements before the initiation of TRT (e.g. LH, FSH and / or SHBG to confirm the diagnosis hypogonadism) or during long-term follow-up (e.g. yearly measurement of prostate specific antigen in older men) and may change the frequency of biochemical measurement after reaching the final TRT dose. [a] As expert 1 and expert 2 worked closely together in the same PWS reference center and had the exact same clinical practice, they were combined into one column.

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Table 8. Expert panel discussion (Part 2).

	Expert 5	Expert 6	Expert 7	Expert 8
(Past) experience	Long-acting injections, transdermal gel, oral testosterone	Short-acting injections, transdermal gel	Short-acting injections, transdermal gel	Short-acting injections, transdermal gel
Preferred mode of administration in PWS	Transdermal gel	Short-acting injections, transdermal gel	Short-acting injections, transdermal gel	Long-acting injections
Mode of administration advised against	Short-acting injections, oral testosterone	Long-acting injections, oral testosterone	Oral testosterone	None
Preferred starting dose in testosterone naïve patients	*Transdermal gel:* 12.5 mg daily	*Short-acting injections:* 50 mg every month *Transdermal gel:* 10 mg daily	*Short-acting injections:* 100-125 mg every 4 weeks *Long-acting injections:* 250-500 mg every 3 months *Transdermal gel:* 10 mg daily	*Short-acting injections:* 100 mg every month *Transdermal gel:* 50 mg daily
Preferred follow-up dose	*Long-acting injections:* Every 3-6 months: adjust dosing interval based on trough testosterone level *Transdermal gel:* Every 3-4 months: increase dose by 12.5 mg based on serum testosterone concentration and clinical response	*Short-acting injections:* Every 3-6 months: increase by 50 mg *Transdermal gel:* Every 4 weeks: increase by 10 mg	*Short-acting and long-acting injections:* Low dose for many years *Transdermal gel:* Every 6-8 weeks: increase by 10 mg	*Short-acting injections and transdermal gel:* Check up to see if testosterone has normalized after 3-4 months and increase dose based on serum testosterone concentration and clinical response
Biochemical follow up	Testosterone, Hb, Ht every 6-12 months	LH, FSH, testosterone, Hb, Ht, liver transaminases every 6 months and prior to any dose modification	Testosterone, Hb, Ht, liver transaminases every 6-8 months	Testosterone, Hb, Ht every 3-4 months

Table 8. Expert panel discussion (Part 2). (*continued*)

	Expert 5	Expert 6	Expert 7	Expert 8
Considerations	Start with transdermal gel as this can be stopped quickly and is most suitable due to more physiological testosterone concentrations. When patient achieves normal testosterone level, either continue transdermal gel or switch to long-acting injections, based on patient preference.	Transdermal gel when the patient is compliant and the family reliable, otherwise short-acting injections.	Transdermal gel is most suitable as it results in more physiological testosterone concentrations and can be stopped instantly if behavioral problems appear, but compliance is better with injections.	Long-acting would be most suitable, but is not reimbursed in this country (Spain).

Additional remarks

Abbreviations: follicle stimulating hormone (FSH), hemoglobin (Hb), hematocrit (Ht), luteinizing hormone (LH), Prader-Willi syndrome (PWS), sex hormone-binding globulin (SHBG), testosterone replacement therapy (TRT). In this table the general considerations are described regarding testosterone replacement therapy for adult males with PWS who have not used testosterone replacement therapy before. However, based on patient preference, another treatment modality or dose could be prescribed. We defined short-acting injections as injections that have to be administered every 1-6 weeks, and long-acting injections as injections that have to be administered every 12 weeks. For this expert discussion we focused on the use of short-acting and long-acting injections, transdermal gel and oral testosterone only. Biochemical follow-up refers to the biochemical measurements performed during the titration of TRT dose. Physicians may perform additional measurements before the initiation of TRT (e.g. LH, FSH and/or SHBG to confirm the diagnosis hypogonadism) or during long-term follow-up (e.g. yearly measurement of prostate specific antigen in older men) and may change the frequency of biochemical measurement after reaching the final TRT dose.

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Table 9. Expert panel discussion (Part 3).

	Expert 9	Expert 10	Expert 11
(Past) experience	Short-acting injections, transdermal gel	Short-acting injections, long-acting injections, transdermal gel	Transdermal gel, short-acting injections, long-acting injections, oral testosterone
Preferred mode of administration in PWS	Transdermal gel	Short-acting injections, transdermal gel	Transdermal gel
Mode of administration advised against	Oral testosterone	None	Oral testosterone
Preferred starting dose in testosterone naïve patients	*Short-acting injections:* 50-100 mg, depending on serum testosterone concentration and age *Transdermal gel:* 12.5-25 mg, depending on serum testosterone concentration and age	*Short-acting injections:* 25 mg every week or 50 mg every month *Transdermal gel:* 25 mg daily	*Transdermal gel:* 10 mg daily
Preferred follow-up dose	*Short-acting injections:* Every 6 months: increase by 50 to 100 mg *Transdermal gel:* Every 6 months: increase by 12.5 to 25 mg	*Short-acting injections:* Every 3 months: increase by 25 mg *Transdermal gel:* Every 3 months: increase by 25 mg	*Transdermal gel:* Every 4 weeks: increase by 10 mg
Biochemical follow up	Testosterone, Hb, Ht every 6 months	Testosterone, Hb, Ht, inhibin B every 3 months	LH, FSH, testosterone, Hb, Ht every 3 months
Considerations	Transdermal gel is most suitable as this results in stable serum testosterone concentrations, but this requires a reliable caregiver and can cause skin irritation and skin picking.	Short-acting injections and gel are tolerated best and easy to dose adjust.	Transdermal gel is most suitable as it can be stopped immediately when behavioral challenges occur due to the short half-life time.
Additional remarks			Use gel in the morning and apply gel to shoulders, not belly (due to increased abdominal fat in PWS).

Abbreviations: follicle stimulating hormone (FSH), hemoglobin (Hb), hematocrit (Ht), luteinizing hormone (LH), Prader-Willi syndrome (PWS), sex hormone-binding globulin (SHBG), testosterone replacement therapy (TRT). In this table the general considerations are described regarding testosterone replacement therapy for adult males with PWS who have not used testosterone replacement therapy before. However, based on patient preference, another treatment modality or dose could be prescribed. We defined short-acting injections as injections that have to be administered every 1-6 weeks, and long-acting injections as injections that have to be administered every 12 weeks. For this expert discussion we focused on the use of short-acting and long-acting injections, transdermal gel and oral testosterone only. Biochemical follow-up refers to the biochemical measurements performed during the titration of TRT dose. Physicians may perform additional measurements before the initiation of TRT (e.g. LH, FSH and/or SHBG to confirm the diagnosis hypogonadism) or during long-term follow-up (e.g. yearly measurement of prostate specific antigen in older men) and may change the frequency of biochemical measurement after reaching the final TRT dose.

Table 10. Recommendations for different testosterone formulations.

TRT formulation	Examples	Starting dose	Dose increase	Advantages	Disadvantages
Transdermal gel	Testosterone (e.g. Androgel®, Testim®, Tostran®, Testogel®, Testavan®)	10 mg daily	Increase dose by 10 mg every 3-6 months until testosterone values within the normal range are achieved	- Flexible dosing - Easy application - Metered dosing with certain brands allows easy dose titration - Good skin tolerability - Less erythrocytosis compared to injections - Less fluctuation of serum testosterone concentrations than testosterone enanthate or cypionate	- Potential of transfer to a female partner or child by direct skin-to-skin contact - Testosterone concentrations may vary between applications - Skin irritation in a small proportion - Moderately high dihydrotestosterone concentrations (of unknown significance) - Some patients may have difficulties administrating the gel - Has to be administered every day, which may lead to non-compliance
Short-acting injections	Testosterone decanoate / isocaproate / phenylpropionate / propionate mixture (e.g. Sustanon®) Testosterone enanthate or cypionate (e.g. Xyosted®, Andortardyl®)	50-125 mg every month	Increase dose by 25-100 mg every 3-6 months until serum testosterone concentrations within the normal range are achieved	-Relatively inexpensive if self-administered - Flexible dosing	- Requires intramuscular injections - Risk of high peak testosterone concentrations shortly after injection - Variation in serum testosterone concentrations may be associated with fluctuations in symptoms - Coughing episode reported immediately after injection in a small number of men
Long-acting injections	Testosterone undecanoate (e.g. Nebido®, Reandron®, AVEED®)	200-500 mg every 3 months	Increase dose by 200-500 mg every 3-9 months until serum testosterone concentrations within the normal range are achieved	- Infrequent administration - Serum testosterone concentrations are maintained in the normal range in most treated men - Stable serum testosterone concentrations between injections	- Requires intramuscular injection of a large volume (3 or 4 mL) - Need to be administered by primary care nurse or physician - Coughing episode reported immediately after injection in a small number of men - Higher chance of erythrocytosis compared to gel

Recommendations for the treatment of hypogonadism in adult males with PWS who have not used testosterone replacement therapy before. Advantages and disadvantages are based on the expert panel discussion and the Endocrine Society Clinical Practice Guideline for testosterone therapy in men with hypogonadism (38).
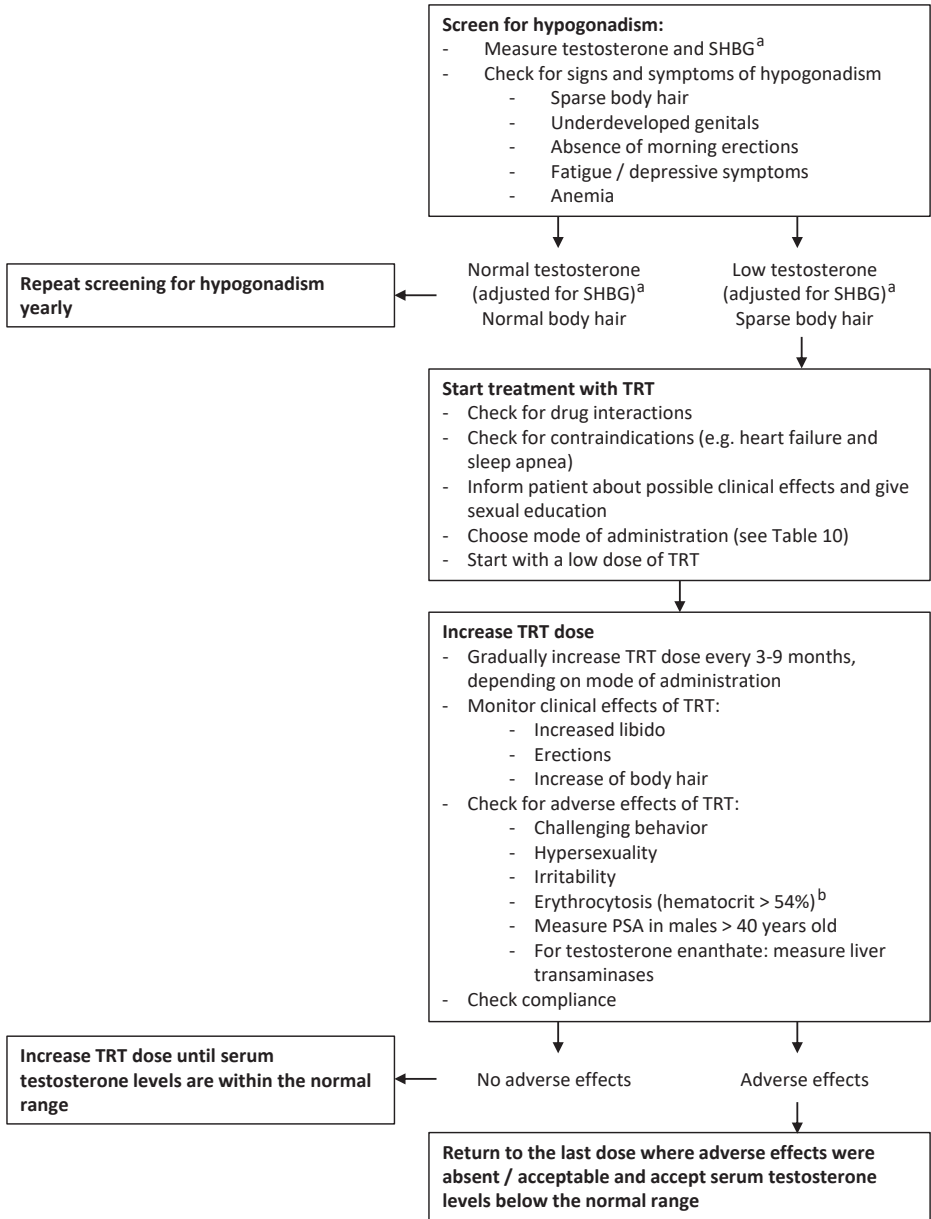
4

Screen for hypogonadism:
- Measure testosterone and SHBG[a]
- Check for signs and symptoms of hypogonadism
  - Sparse body hair
  - Underdeveloped genitals
  - Absence of morning erections
  - Fatigue / depressive symptoms
  - Anemia

Normal testosterone (adjusted for SHBG)[a]
Normal body hair

Low testosterone (adjusted for SHBG)[a]
Sparse body hair

Repeat screening for hypogonadism yearly

Start treatment with TRT
- Check for drug interactions
- Check for contraindications (e.g. heart failure and sleep apnea)
- Inform patient about possible clinical effects and give sexual education
- Choose mode of administration (see Table 10)
- Start with a low dose of TRT

Increase TRT dose
- Gradually increase TRT dose every 3-9 months, depending on mode of administration
- Monitor clinical effects of TRT:
  - Increased libido
  - Erections
  - Increase of body hair
- Check for adverse effects of TRT:
  - Challenging behavior
  - Hypersexuality
  - Irritability
  - Erythrocytosis (hematocrit > 54%)[b]
  - Measure PSA in males > 40 years old
  - For testosterone enanthate: measure liver transaminases
- Check compliance

No adverse effects

Adverse effects

Increase TRT dose until serum testosterone levels are within the normal range

Return to the last dose where adverse effects were absent / acceptable and accept serum testosterone levels below the normal range

Figure 6. Recommendations for hypogonadism in adult males with PWS. Abbreviations: sex hormone binding globulin (SHBG), testosterone replacement therapy (TRT). [a] Instead of total testosterone and SHBG, free testosterone can also be measured to diagnose hypogonadism in males with PWS. [b] Based on the Endocrine Society Clinical Practice Guideline for testosterone therapy in men with hypogonadism (38).

Expert panel and clinical recommendations

Eleven experts (C.P., M.C., A.P.G., C.H., T.P.M., G.G., An.C., As.C., H.J.H., J.L.M. and L.C.G.dG) shared their experience with the treatment of hypogonadism in adult males with PWS (**Tables 7-9**). The most frequently used types of TRT were transdermal gel and short-acting injections. Starting dose and dose increase for each modality varied between experts. Additionally, one expert stated that he routinely measured estradiol concentrations in adult males with PWS, while three experts stated that they measured estradiol only in males with gynaecomastia. The other seven experts never measured estradiol in males. The advantages and disadvantages of injections and transdermal gel reported by the experts, supplemented with advantages and disadvantages mentioned in the Endocrine Society Clinical Practice Guideline for testosterone therapy in men with hypogonadism (38), are summarized in **Table 10**. Based on this cohort study, a review of the literature and the expert panel discussion, we have made recommendations for the screening and treatment of hypogonadism in adult males with PWS (**Table 10** and **Figure 6**).

DISCUSSION

Hypogonadism is present in nearly all adult males with PWS (98%). Although untreated hypogonadism was associated with obesity and decreased serum hemoglobin concentrations, adequate treatment leading to normal serum testosterone levels was only achieved in about one third of the patients.

Type of hypogonadism

Although PWS is characterized by its hypothalamic dysfunction, hypogonadism in PWS can also be of testicular origin. *MKRN3, NDN*, and *SNORD116*, genes that are located in the PWS critical region, have been associated with GnRH secretion and hypothalamic dysfunction leading to hypogonadism (39). Testicular dysfunction in men with PWS could be related to abnormal histology of the tubules and absence of spermatogonia (20,40,41). *C15orf2*, another gene in the PWS critical region, is expressed in the testes and might play a role in spermatogenesis and therefore in the disturbance of the FSH / inhibin B axis (35,42).

The maturation of Leydig and Sertoli cells in PWS occurs independently (16). Therefore, the LH/testosterone and FSH / inhibin B axes can be affected separately, either at the central or primary level. This leads to three forms of hypogonadism in PWS: central (low/normal LH with low testosterone and low / normal FSH with low inhibin B), primary (elevated LH with low testosterone and elevated FSH with low inhibin B), and a

combination of hypothalamic and testicular dysfunction. We found a high prevalence of this mixed form of hypogonadism (55%). Central (21%) and primary hypogonadism (21%) were equally common. As previous reports show that most adult males with PWS have low inhibin B values (16,18), we assume that this is also true for our population. In that case, FSH levels in our patients might be in the normal range due to hypothalamic dysfunction of the FSH/inhibin B axis, with normal FSH levels being inadequately low for low inhibin B levels. However, as inhibin B measurements are not routinely measured as part of our standard patient care, we cannot draw any firm conclusions about the presence of hypothalamic dysfunction of the FSH / inhibin B axis.

Undertreatment

In one third of the patients, normal serum testosterone levels could not be achieved due to challenging behavior. Although this challenging behavior seemed related to the start of TRT or an increase in testosterone dose, it was not possible to exclude placebo effect or other factors that might aggravate challenging behavior. Eighteen patients required testosterone dose reduction due to the development of challenging behavior. In only 11 (61%) of these patients did a reduction in TRT dose reduce the challenging behaviors, suggesting that TRT may not necessarily be the cause of the increase in challenging behaviors.

Remarkably, low testosterone concentrations were still seen in patients with higher prescribed testosterone doses. This might be due to non-compliance or variability in biochemical measurements. Additionally, although SHBG concentrations were normal initially, they may have decreased over time.

Importance of treatment of hypogonadism

Untreated hypogonadism can aggravate PWS-related health issues including osteoporosis, decreased muscle mass and increased fat mass, fatigue, and impaired cardiovascular health.

Osteoporosis

TRT increases bone mineral density in hypogonadal males without (43) and with PWS (17,44). Therefore, it is important to treat hypogonadism to avoid osteoporosis and subsequent fractures.

Muscle and fat

Higher testosterone concentrations are associated with decreased fat mass and increased fat-free mass, muscle volume, and muscle strength (43,45). A significant decrease in body fat percentage and increase in lean body mass in males with PWS has

been demonstrated after two years of TRT (17). Patients with PWS already have a decreased muscle mass and an increased fat mass, related to impaired exercise tolerance, hyperphagia and impaired GH secretion (36,46). This abnormal body composition leads to a vicious cycle of low muscle mass, poor exercise tolerance and little physical activity, which further decreases muscle mass. Treatment of hypogonadism in males with PWS is important to increase exercise tolerance and improve muscle mass and strength to help break the vicious cycle.

After correction for age, we found that males with untreated hypogonadism had a significantly higher BMI compared to males who did not have hypogonadism or received TRT. However, patients receiving TRT are probably more likely to receive other interventions that may influence body weight, such as GH replacement, physiotherapy or dietary treatment.

Fatigue

Treatment of non-PWS male hypogonadism can have beneficial effects on vitality and quality of life, and reduce fatigue and depressive symptoms (43,47-49), although studies have reported mixed results (50).

The relation between hypogonadism and fatigue may be partly explained by anemia. In the general population, treatment of male hypogonadism increases hemoglobin levels and reduces anemia (43,45). In our population, males with untreated hypogonadism had significantly lower hemoglobin levels than those without untreated hypogonadism.

At baseline, we did not find a significant difference in subjective complaints of fatigue and daytime sleepiness between males with and without untreated hypogonadism. We did not systematically assess the psychological effects after the start of TRT. However, in our clinical experience, we did see improvements in mood and vitality in many males after the start of TRT. Further research is needed to longitudinally assess the effect of TRT on fatigue and quality of life in adult males with PWS.

Cardiovascular health

Cardiovascular (CV) risk factors, including obesity, hypertension, type 2 diabetes mellitus, sleep apnea and hypercholesterolemia are prevalent in adults with PWS (36), leading to a high risk of CV disease and CV mortality at a young age (51,52). Hypogonadism has been associated with poor CV outcomes (53,54) and TRT may improve CV health, although contradictory data have been reported and more research is needed (53,54).

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TRT warnings and precautions

TRT can cause behavioral challenges, irritability, and aggressive behavior (35). However, Kido et al. (17) found no difference in the Modifier Overt Aggression Scale (MOAS) after two years of TRT in males with PWS and did not observe challenging behaviors caused by TRT. In our population, we did see behavioral challenges during TRT. In 18 (32%) males, the testosterone dose was decreased because of behavioral challenges, leading to inadequate serum testosterone concentrations. These differences between our study and Kido et al. (17) can partly be explained by the fact that, as opposed to Kido et al., we did not exclude patients based on behavioral problems at baseline, and that Kido et al. used a different form of TRT, namely monthly intramuscular injections of 125 mg testosterone enanthate, which is half of the conventional dose. However, short-acting testosterone injection regimes (2-4 weekly) might be expected to increase the risk of behavioral problems as a result of supra-physiological testosterone concentrations shortly after injection compared to the most frequently used modality in our population, testosterone gel (38).

As TRT can induce libido and sexual activity in patients who are used to lifelong hypogonadism, it is important to inform the patients and their caregivers about these possibly confusing new feelings. A clear 'code of conduct' should be discussed with regard to sexual activity before starting TRT, in order to prevent inappropriate sexual behavior. It is important to ask about sexuality, sexual function, libido and erections to identify problems and to evaluate the effect of TRT. When discussing sexuality, it is important to use direct and very simple language.

A majority of the physicians of the expert panel discussion reported that normal testosterone values could be reached without causing behavioral problems in their population of adults with PWS. This could be related to the use of testosterone injections instead of transdermal gel or a slower increase in testosterone dose. Additionally, in the Dutch cohort a neuropsychologist was involved in the multidisciplinary care for adults with PWS, which could have led to greater identification of behavioral issues resulting from TRT. Further research is needed to accurately assess the differences in behavioral challenges between all treatment regimens and centers.

Recommendations

Based on the combined clinical experience of all co-authors, we propose clinical recommendations for the treatment of hypogonadism in adult males with PWS for TRT, see **Table 10** and **Figure 6**. We wish to highlight issues that are especially relevant when treating hypogonadism in males with PWS. For the non-PWS specific aspects of TRT, we recommend referring to the general guidelines for the treatment of hypogonadism

in men for topics not discussed here (38). As clinical practice differed greatly among experts, we provide ranges for the possible starting dose and dose increase of TRT.

Interpretation of hormone levels

Whenever possible, testosterone concentrations should be measured in the morning. When a low total testosterone concentration is found, we recommend measurement of SHBG levels before starting TRT. SHBG levels can be low due to obesity, which is often present in patients with PWS (55). During follow-up, SHBG measurement may need to be repeated if obesity or insulin resistance develop or worsen (38). Alternatively, free testosterone levels can be measured instead of total testosterone and SHBG.

Sleep apnea

Sleep apnea is common in PWS (8,56,57) and TRT can worsen symptoms of obstructive sleep apnea (57). Therefore, we recommend screening for obstructive sleep apnea before starting TRT, and if present to treat this condition. After the start of TRT, polysomnography should be performed if clinical signs of sleep apnea develop.

Drug interactions

As several drugs interact with TRT, we recommend checking for possible drug interactions before starting TRT. As use of psychotropic and anti-epileptic drugs is common in adults with PWS, it is especially important to check for interactions with drugs like selective serotonin reuptake inhibitors, anti-epileptic medication and psychostimulants like modafinil (58-63). As these drugs may influence serum testosterone concentrations, adjustment of the dose of TRT might be needed. TRT can also interact with growth hormone (GH) treatment. As TRT can increase insulin-like growth factor 1 (IGF-1) concentrations (45,64,65), it is important to evaluate and, if necessary, adjust the GH dose after initiation of TRT.

Cardiac failure

As the use of androgens might induce fluid retention (38,66) and cardiac problems are common in patients with PWS (57), we recommend excluding or appropriately managing heart failure before starting TRT. As patients with PWS are often unable to accurately express their cardiac symptoms due to intellectual disability and a high pain threshold (7), and leg edema is not a reliable marker of heart failure in patients with PWS (67), heart disease in adults with PWS can easily remain undiagnosed. Therefore, we recommend arranging an echocardiogram, checking serum N-terminal pro b-type natriuretic peptide (NT-proBNP) concentrations and/or consulting a cardiologist prior to the commencement of TRT in case of pitting edema or exercise-related shortness of breath. It should be noted that NT-proBNP can be false-negative in patients with obesity (68).

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Challenging behavior

To avoid the development or worsening of aggression, hypersexuality and temper tantrums, we recommend starting with a low dose of TRT and gradually increasing the dose every 3-6 months for testosterone gel and short-acting injections and every 3-9 months for long-acting injections. If increasing the dose is impossible due to altered (sexual) behavior, we recommend returning to the last dose where behavior was still acceptable.

Mode of administration

Among the clinicians participating in the international expert panel, many different treatment regimens were used. Due to the need for gradual increase and the possibility for rapid dose reduction in case of behavioral challenges, our general recommendation is to use transdermal gel instead of injections when initiating TRT (69,70). However, once established on a final transdermal TRT dose with satisfactory behavioral profile, it may be possible to switch to intramuscular injections. We advise against using oral testosterone preparations because of the risk of liver damage and increased intestinal conversion to dihydrotestosterone, preventing aromatisation to estrogen and thus hindering bone protection (38,71-73).

Erythrocytosis

As long-term treatment with testosterone might generate erythrocytosis, we recommend to measure hemoglobin and hematocrit regularly during TRT, similar to the recommendations for TRT in non-PWS males (38). When erythrocytosis occurs, TRT should be withheld until hematocrit has returned to the normal range. Then, TRT can be resumed at a lower dose (38).

Prostate and liver

We recommend measurement of prostate specific antigen (PSA) in men who are over 40 years old, as the long-term effects of TRT on the prostate in PWS are unknown. A urology consult should be obtained if PSA levels increase above baseline during TRT. TRT in non-PWS men does not seem to be associated with benign prostatic hyperplasia or lower urinary tract symptoms (74). Also, increased levels of liver transaminases may occur during treatment with testosterone enanthate and should be monitored (75,76).

Non-compliance

Non-compliance is frequent in adults with PWS (77), even compared to non-PWS adults with intellectual disability (78). Although many patients are grateful to receive TRT and have no problems with adhering to their TRT regime, we found that non-compliance to TRT was often seen (certain non-compliance in 11% and a high suspicion of non-compliance in 9%), especially when the patient administered his own medication. However,

as figures about non-compliance are, by definition, unreliable, actual non-compliance may be more frequent. Therefore, we recommend asking about barriers that may reduce compliance such as practical barriers (e.g. inability to administer testosterone gel, lack of caregivers who can administer the gel) and other concerns (e.g. fear of adverse events). As indicated by multiple experts during our survey, compliance might be better in patients receiving monthly or three-monthly testosterone injections, compared to testosterone gel that requires daily administration.

Role of PWS reference centers

The TRT-related challenges may cause physicians to refrain from prescribing TRT in males with PWS. However, we want to stress the importance of adequate treatment as undertreatment can have serious health consequences. PWS reference centers can be contacted for consultation or, if geographically possible, referral. If there is no PWS reference center available, we recommend the use our algorithm for treatment of hypogonadism in men with PWS (**Table 10** and **Figure 6**).

Strengths and limitations

To our knowledge, we are the first to provide a practical flowchart for the screening and treatment of hypogonadism in males with PWS (39). Another strength of our study is the relatively large cohort, given the fact that PWS is a rare syndrome. In addition we have provided a comprehensive literature review of male hypogonadism in adults with PWS. However, our study also has some limitations. First, there was limited overlap in age between the group of males with untreated hypogonadism and the group of males with treated or no hypogonadism, possibly leading to residual confounding. Second, due to the circadian rhythm of testosterone, we analyzed the testosterone levels drawn before 11:00 AM and the testosterone levels drawn after 11:00 AM separately (37). As few males had morning testosterone measurements and none had fasting testosterone measurements, we had limited power to investigate which factors influenced endogenous testosterone values. Third, physical examination reports did not always include details about lipomastia or gynaecomastia. Therefore, we cannot rule out that some men had breast enlargement that was not specifically described in their medical records. Also, we did not measure estradiol concentrations, thus we were not able to investigate the relationship between breast enlargement and estradiol. Finally, we had too few DEXA-scans available to evaluate the effect of TRT on bone mineral density, lean body mass, and fat percentage. Further research should determine the effect of TRT on these clinical effects of TRT, as they may be more important parameters to measure the effectiveness of TRT than serum testosterone measurements, although these parameters may also be influenced by GH treatment.

CONCLUSIONS

In conclusion, hypogonadism was present in nearly all males with PWS (98%) and was often a combination of hypothalamic and testicular dysfunction. Although untreated hypogonadism was associated with obesity and decreased serum hemoglobin concentrations, treatment leading to serum testosterone levels within the normal range was only achieved in one third of the patients attending our center. In order to prevent undertreatment due to behavioral challenges or other PWS-related challenges, we provide a practical algorithm for TRT in adult males with PWS.

ACKNOWLEDGMENTS

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SUPPLEMENTARY DATA

Table S2. Laboratory values in adult males with PWS (Part 1).

	Reference Range for Adult Males	n	Total n = 57	Deletion n = 29	mUPD n = 20	P-value	Current GH treatment n = 19	No current GH treatment n = 38	P-value	P-value After Correction for Age
Testosterone (nmol/L)										
Before 11:00 AM		14	5.6 [3.2 – 7.4]	6.2 [3.3 – 10.5]	4.2 [0.9 – 6.3]	0.3	6.2 [4.1 – 11.4]	3.7 [2.2 – 6.5]	0.4	NA [b]
After 11:00 AM	10.0 – 30.0	16	2.0 [0.8 – 4.9]	1.5 [0.8 – 4.7]	2.1 [0.8 – 5.2]	0.9	NA [a]	2.1 [0.8 – 4.9]	NA	NA
LH (IU/L)										
Before 01-02-2019	1.5 – 8.0	30	3.3 [1.2 – 8.1]	2.0 [0.7 – 6.1]	3.9 [1.2 – 7.8]	0.7	5.4 [2.3 – 7.7]	2.4 [0.9 – 8.4]	0.6	0.06
After 01-02-2019	1.0 – 5.5	3	1.9, 2.8, 11.0 [c]	1.9 [c]	11.0 [c]		1.9, 2.8 [c]	11.0 [c]		
FSH (IU/L)										
Before 01-02-2019	2.0 – 7.0	31	13.5 [6.4 – 34.5]	11.6 [5.4 – 30.5]	16.8 [5.8 – 46.2]	0.2	20.8 [9.8 – 44.1]	13.3 [5.0 – 34.5]	0.6	0.1
After 01-02-2019	0.8 – 5.1	3	4.8, 7.2 & 58.0 [c]	4.8 [c]	58.0 [c]		4.8, 7.2 [c]	58.0 [c]		
SHBG (nmol/L)	10.0 – 70.0	28	29.9 [20.3 – 49.4]	23.8 [19.8 – 44.2]	35.5 [23.8 – 57.8]	0.2	15.5 [8.1 – 19.4]	35.5 [23.7 – 55.5]	0.003	0.2

Abbreviations: growth hormone (GH), follicle stimulating hormone (FSH), luteinizing hormone (LH), maternal uniparental disomy (mUPD), paternal disomy (deletion), sex hormone binding globulin (SHBG), number of observations (n). Data are presented as median [IQR]. Laboratory values for patients at baseline or during the last measurement available before the start of testosterone replacement therapy for LH, FSH, and testosterone. For SHBG the measurement closest to baseline was used. Values that were below the measuring threshold were considered equal to the measuring threshold to calculate the median and IQR. For example, when FSH was below 0.5, this was considered 0.5. For LH and FSH, measurements before and after the change in measuring method on 01-02-2019 are calibrated differently, therefore these measurements are described separately and a variable indicating whether the values were measured before or after 01-02-2019 was added to the model to calculate the P-value. [a] There were no observations in this group, all patients had either a testosterone measurement before 11:00 AM, or no testosterone measurement before testosterone replacement therapy available. [b] Not enough testosterone measurements to fit the model to correct for age. [c] Individual measurements are given as there were too few observations to calculate a median and IQR.

Table S3. Laboratory values in adult males with PWS (Part 2).

	n	BMI <25 n=11	BMI 25–30 n=29	BMI >30 n=17	P-value	P-value After Correction for Age	Age <25 n=21	Age 25–30 n=9	Age >30 n=27	P-value
Testosterone (nmol/L)										
Before 11:00 AM	14	6.2 [a]	5.7 [3.4-9.0]	3.3 [2.1-8.5]	0.4	0.1	6.5 [5.1-9.9]	3.7 [a]	3.3 [0.9-7.3]	0.3
After 11:00 AM	16	5.5, 4.0, 1.1 [a]	1.7 [0.7-4.3]	1.5 [0.9-6.5]	0.9	1	5.0, 7.7 [a]	4.7 [a]	1.1 [0.8-3.5]	0.2
LH (IU/L)										
Before 01-02-2019	30	5.6 [1.8-9.0]	2.6 [1.3-8.4]	2.1 [1.0-8.0]	0.2	0.3	3.9 [1.3-5.8]	2.0 [1.7-4.7]	3.9 [0.7-9.9]	0.0003
After 01-02-2019	3	NA [b]	2.8, 11.0	1.9			1.9, 2.8 [a]	NA [b]	11.0 [a]	
FSH (IU/L)										
Before 01-02-2019	31	40.4 [13.2-55.8]	13.3 [8.6-45.4]	13.0 [3.5-27.2]	0.01	0.01	13.5 [5.0-46.9]	11.4 [7.2-24.3]	14.7 [5.5-42.7]	0.001
After 01-02-2019	3	NA [b]	7.2, 58.0 [a]	4.8 [a]			4.8, 7.2 [a]	NA [b]	58.0 [a]	
SHBG (nmol/L)	28	68.0, 58.5, 44.7 [a]	27.1 [20.0-44.7]	24.4 [17.3-49.8]	0.08	0.2	16.2 [12.6-24.8]	23.9, 19.7 [a]	42.5 [24.4-57.0]	0.0001

Abbreviations: body mass index (BMI), follicle stimulating hormone (FSH), luteinizing hormone (LH), sex hormone binding globulin (SHBG), number of observations (n). Data are presented as median [IQR]. Laboratory values for patients at baseline or during the last measurement available before the start of testosterone replacement therapy for LH, FSH, and testosterone. For SHBG the measurement closest to baseline was used. Values that were below the measuring threshold were considered equal to the measuring threshold to calculate the median and IQR. For example, when FSH was below 0.5, this was considered 0.5. For LH and FSH, measurements before and after the change in measuring method on 01-02-2019 are calibrated differently, therefore these measurements are described separately and a variable indicating whether the values were measured before or after 01-02-2019 was added to the model to calculate the P-value. P-values are calculated with age and BMI as continuous variables. [a] Individual measurements are given as there were too few observations to calculate a median and IQR. [b] There were no observations in this group.

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Hypogonadism in women with Prader-Willi syndrome – clinical recommendations based on a Dutch cohort study, review of the literature and an international expert panel discussion

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ABSTRACT

Prader-Willi syndrome (PWS) is a rare neuroendocrine genetic syndrome. Characteristics of PWS include hyperphagia, hypotonia, and intellectual disability. Pituitary hormone deficiencies, caused by hypothalamic dysfunction, are common and hypogonadism is the most prevalent. Untreated hypogonadism can cause osteoporosis, which is already an important issue in PWS. Therefore, timely detection and treatment of hypogonadism is crucial. To increase understanding and prevent undertreatment, we 1) performed a cohort study in the Dutch PWS population, 2) thoroughly reviewed the literature on female hypogonadism in PWS and 3) provide clinical recommendations on behalf of an international expert panel. For the cohort study, we retrospectively collected results of a systematic health screening in 64 female adults with PWS, which included a medical questionnaire, medical file search, medical interview, physical examination and bio-chemical measurements. Our data show that hypogonadism is frequent in females with PWS (94%), but is often undiagnosed and untreated. This could be related to unfamiliarity with the syndrome, fear of behavioral changes, hygienic concerns, or drug interactions. To prevent underdiagnosis and undertreatment, we provide practical recommendations for the screening and treatment of hypogonadism in females with PWS.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare, complex, neuroendocrine syndrome with an estimated prevalence of 1:10,000–1:30,000 (1). PWS is an imprinting disorder caused by the lack of expression of a cluster of paternally expressed genes on chromosome 15q11-13. The most common genetic abnormalities are: a paternal deletion (60–75%), a maternal uniparental disomy 15 (mUPD, 20–35%), an imprinting center defect (ICD, 1–4%) or a paternal chromosomal translocation (0.1%) (2,3). Features of adults with PWS include hypotonia, mild to moderate intellectual disability, dysmorphic features and hypothalamic dysfunction, leading to hyperphagia and early-childhood onset obesity (if uncontrolled), sleep disorders, abnormal temperature regulation, high pain threshold, and pituitary hormone deficiencies (PHD) (1,4–7). Additionally, patients with PWS may display challenging behavior, and the prevalence of psychosis is increased, especially in patients with mUPD (8–11).

The most prevalent PHD in PWS is hypogonadism. We previously reported on hypogonadism in men with PWS (12), in this study we focused on hypogonadism in women with PWS.

The reported prevalence of hypogonadism in female adults with PWS varies between 54 and 100% (13–22). Hypogonadism in patients with PWS is believed to be caused by hypothalamic dysfunction, but more recent studies indicate that primary ovarian dysfunction also occurs (17,19,23,24).

During infancy, females often present with hypoplasia of the clitoris and labia minora (25). Although there is generally a normal onset of puberty, i.e., breast development, its progression is often delayed and incomplete and spontaneous menarche usually does not occur (21,23,26). If menarche occurs spontaneously, it is often delayed (at a mean age of 20 years) and followed by irregular menstruations or secondary amenorrhea (18,24). Despite absent menstruation and impaired maturation of follicles in many female patients with PWS (26), pregnancy has been described, although rarely (27–32).

In the general population, female hypogonadism may result in a decreased quality of life, decreased muscle strength, and osteoporosis (33–36). Additionally, there are indications that estradiol has beneficial effects on the cardiovascular system (37,38). Besides hypogonadism, patients with PWS also have a high prevalence of other risk factors for osteoporosis like growth hormone (GH) deficiency and low physical activity (4,39–43). This makes early detection and treatment of hypogonadism especially important to avoid osteoporosis (44,45).

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Based on a Dutch cohort, we report the prevalence and characteristics of hypogonadism and its treatment in female adults with PWS. Additionally, we provide an overview of the current literature on the prevalence of hypogonadism and related laboratory values in women with PWS. As there are currently no PWS-specific guidelines (46), we present a practical algorithm for the screening and treatment of hypogonadism in female adults with PWS based on an international expert panel discussion.

MATERIALS AND METHODS

Ethical review and approval were waived for this study by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam, the Netherlands (MEC-2018-1389).

We retrospectively reviewed the medical files of female adults with PWS who underwent the systematic health screening at our PWS reference center (Erasmus University Medical Center, Rotterdam, the Netherlands), between January 2015 and December 2020. As previously described (see (47)), this systematic screening consisted of a medical questionnaire, a review of the medical files, a structured interview, a complete physical examination, biochemical measurements and, if indicated and feasible, additional tests.

The results of our study about hypogonadism in male adults with PWS are reported separately (12), since the characteristics of hypogonadism and its treatment differ substantially between males and females.

Terminology

We will use the term hormone-replacement therapy (HRT) for tibolone and estrogen-containing preparations that cannot be used as contraception. We will use the term hypogonadism hormone therapy (HHT) as an overarching term for HRT and estrogen-containing contraceptives.

Definition of Hypogonadism

In obese women, estradiol levels can be within the reference range despite dysfunction of the hypothalamus, pituitary or ovaries, due to increased aromatase activity in adipose tissue (48,49). Therefore, hypogonadism in women with PWS was defined as absent or irregular menstruation, regardless of serum estradiol levels. When females used HHT or progesterone-only contraceptives, including intrauterine devices (IUDs), before screening, we asked about the menstrual cycle before treatment or searched the medical files for information on the menstrual cycle before treatment or a previous diagnosis of hypogonadism. When females were over 50 years old, we asked about the menstrual cycle

before they reached menopausal age. Central hypogonadism was defined as absent or irregular menstrual cycle either 1) in the presence of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations below the reference range, or 2) combined with a low estradiol concentration in the presence of serum LH and FSH concentrations within the reference range. Primary hypogonadism was defined as an absent or irregular menstrual cycle with serum LH and FSH concentrations above the reference range. Due to intellectual disability in most patients, gynecological evaluation was not performed routinely.

Laboratory Measurements

As part of regular hospital visits, blood samples were collected for general medical screening, including measurement of LH, FSH, estradiol and sex hormone binding globulin (SHBG). When females were treated with HHT or progesterone-only contraceptives (including IUDs as these may have systemic effects (50)), only LH, FSH and estradiol values from before the start of these preparations were included. If no SHBG value from before the start of these preparations was available, an SHBG value during treatment was used. Anti-Müllerian hormone (AMH) levels were not routinely measured as this would not have any consequences for treatment in this patient population.

Estradiol concentrations were measured using the Roche Elecsys assay (reference range 55–1285 pmol/L). LH and FSH concentrations were measured using the Siemens Immulite 2000XPi (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, United States) (reference range 1.5–8.0 IU/L for LH and 2.0–7.0 IU/L for FSH) until February 2019. After that date, the Fujirebio Lumipulse G1200 (Fujirebio, Inc., Tokyo, Japan) was used (reference range 1.0–5.5 IU/L for LH and 0.8–5.1 IU/L for FSH). SHBG concentrations were measured using the Siemens Immulite 2000XPi (reference range 10–70 nmol/L) until June 2020. After that date, the IDS-ISYS (Immunodiagnostic Systems, Boldon, United Kingdom) was used (reference range 10–70 nmol/L) with a similar calibration, as confirmed by external quality assessment schemes.

Expert Panel Discussion on Diagnosis and Treatment of Hypogonadism

Eleven experts with experience with the treatment of hypogonadism in females with PWS (C.P., M.C., A.P.G., C.H., T.P.M., G.G., An.C., As.C., H.J.H., J.L.M. and L.C.G.d.G.) shared their experience regarding the diagnosis and treatment of hypogonadism in females with PWS and agreed to the clinical recommendations. They were also specifically asked what criteria they used to decide when treatment for hypogonadism shoud be started and at what age they think that this treatment should be discontinued.

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Literature Search

On 24 September 2020, we performed a literature search regarding the prevalence of hypogonadism and related laboratory measurements in adults with PWS. The search was last updated on 3 June 2021. The search strategy is provided in the **Supplementary Materials in Table S1**. We excluded manuscripts that included less than ten adults (females and males) with PWS, manuscripts that were not available in English, and conference abstracts. When the prevalence of hypogonadism or laboratory values were not available for adults only, we asked the authors to provide this information for the adults separately. When manuscripts reported on overlapping populations, the manuscript with most patients or, when the number of patients was similar, the most recent manuscript was included. This search strategy identified manuscripts on hypogonadism in both men and women with PWS, however manuscripts that did not include women were excluded in the current literature review. The results of our literature review on hypogonadism in male adults with PWS are reported separately (12).

Data Analysis

We used R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) for all statistical analyses. For dichotomous variables we display the number and the percentage of people, n (%), and continuous variables are shown as median (interquartile range (IQR)). We used a Wilcoxon rank sum test to compare estradiol or SHBG concentrations between patients with an mUPD or a paternal deletion and between patients who used or did not use recombinant human growth hormone (rhGH) treatment. We used the Kendall rank correlation test to investigate the relationship between estradiol or SHBG concentrations and age or body mass index (BMI). We used linear regression models with likelihood ratio tests to correct for age. For all analyses involving FSH and LH, a variable indicating whether the measurement was performed before or after 1 February 2019 (when the method was changed with a different calibration) was included in a linear regression model. Samples with concentrations below the assay detection limit were assigned the value of the detection limit. If there were too many ties, an exact calculation method was used. No correction for multiple testing was performed in this exploratory analysis. P-values below 0.05 were considered statistically significant.

RESULTS

Baseline Characteristics

The baseline characteristics of the Dutch adult female PWS cohort are shown in **Table 1**. We included 64 women with PWS. The median age was 28 years (IQR 22–37, range 18 –58 years). The median BMI was 32 kg/m^2 (IQR 27–40). The most frequent genetic subtype

was paternal deletion of chromosome 15q11.2-13 (n = 37, 58%). Twenty-four females (38%) received rhGH treatment and 24 females (38%) used psychotropic medication at the time of the study. Frequently used types of psychotropic medication included

Table 1. Baseline characteristics of 64 females with Prader-Willi syndrome

	Hypogonadism known n = 50	Hypogonadism unknown due to treatment [a] n = 10	Hypogonadism unknown due to age [b] n = 4	Total n = 64
Age in years, median (IQR)	27 (21–34)	25 (19–38)	56 (51–58)	28 (22–37)
Age range in years	18–52	18–49	49–58	18–58
BMI in kg/m², median (IQR)	32 (27–43)	25 (22–34)	31 (24–34)	32 (27–40)
Genetic subtype				
Deletion	30 (60%)	6 (60%)	1 (25%)	37 (58%)
mUPD [c]	16 (32%)	3 (30%)	3 (75%)	22 (34%)
ICD	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Unknown	3 (6%)	1 (10%)	0 (0%)	4 (6%)
rhGH treatment [d]				
Only during childhood	6 (12%)	2 (20%)	0 (0%)	8 (13%)
Only during adulthood	2 (4%)	0 (0%)	0 (0%)	2 (3%)
Both	18 (36%)	6 (60%)	0 (0%)	24 (38%)
Never	24 (48%)	2 (20%)	4 (100%)	30 (47%)
Current rhGH treatment	18 (36%)	6 (60%)	0 (0%)	24 (38%)
Psychotropic medication	16 (32%)	5 (50%)	3 (75%)	24 (38%)
Living situation				
With family	12 (24%)	3 (30%)	0 (0%)	15 (23%)
In a specialized PWS group home	11 (22%)	3 (30%)	1 (25%)	15 (23%)
In a non-specialized facility	27 (54%)	4 (40%)	3 (75%)	34 (53%)
Scholar level				
Secondary vocational education	2 (4%)	2 (20%)	0 (0%)	4 (6%)
Pre-vocational secondary education	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Special education	35 (70%)	6 (60%)	2 (50%)	43 (67%)
No education	2 (4%)	1 (10%)	1 (10%)	4 (6%)
Unknown	10 (20%)	1 (10%)	1 (10%)	12 (19%)
Relationship status				
In a relationship with sexual intercourse	5 (10%)	1 (10%)	0 (0%)	6 (9%)
In a relationship without sexual intercourse	8 (16%)	0 (0%)	1 (25%)	9 (14%)
Not in a relationship	33 (66%)	7 (70%)	2 (50%)	42 (66%)
Unknown	4 (8%)	2 (20%)	1 (25%)	7 (11%)

Abbreviations: body mass index (BMI), paternal deletion (deletion), recombinant human growth hormone (rhGH), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD), Prader-Willi syndrome (PWS). Data are presented as n (%), unless otherwise specified. [a] The presence of hypogonadism could not be assessed as it was unknown whether they had had a regular menstrual cycle before the start of estrogen- and/or progestogen-containing preparations. [b] The presence of hypogonadism could not be assessed as these women were already over 50 years old during their first visit to our outpatient clinic and there was no information available about the menstrual cycle before they had reached menopausal age. [c] In 6 patients with suspected mUPD, the parents were not available for genetic testing. Therefore, mUPD is the most likely genotype, but an ICD could not be ruled out in these patients. [d] Patients older than 25 years old received a starting rhGH dose of 0.3 mg/day, while in patients younger than 25 years old, the starting growth hormone dosage was 1.0 mg/m²/day, which was gradually lowered to 0.33 mg/m²/day after reaching the final height. However, this dose could be adjusted according to insulin-like growth factor-1 (IGF-1) measurements and clinical effects.

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risperidone (n = 9), benzodiazepines (n = 6), valproic acid (n = 4) and non-tricyclic anti-depressants (n = 5). Six females were in a relationship with sexual intercourse, of whom three used oral contraceptives, one used progestogen-containing contraceptive injections, one used barrier contraceptives and one was sterilized.

Hypogonadism

Of the 64 women included in this study, information about gonadal function was available for 50 patients. Of these 50 women, 47 (94%) had hypogonadism (**Table 2**), of whom 30 had been diagnosed before (but 7 of these were untreated at the first visit to our outpatient clinic) and 17 were detected by our screening. Of these 50 women, two were above 50 years old (both 52 years old), but both reported that they never had had a menstrual cycle before reaching menopausal age. The median age at the start of HHT was 20 years (IQR 16–28).

Table 2. Hypogonadism in women with Prader-Willi syndrome

	Women with PWS n = 64
Hypogonadism before screening	
Of whom untreated number of women with	30/50 (60%)
hypogonadism (%)	7/30 (23%)
Hypogonadism revealed by screening	17/50 (34%)
Hypogonadism after screening	
Of whom untreated number of women with	47/50 (94%)
hypogonadism (%)	13/47 (28%)
Age at start hypogonadism hormone therapy, median (IQR)	20 (16–28) (n = 33)
Hypogonadism after screening according to BMI group	
In females with BMI < 25 kg/m^2	7/7 (100%)
In females with BMI 25–30 kg/m^2	13/14 (93%)
In females with BMI > 30 kg/m^2	27/29 (93%)

Abbreviations: body mass index (BMI), interquartile range (IQR), Prader-Willi syndrome (PWS). Data are presented as number of patients with the outcome / number of patients for whom data was available (%), unless otherwise specified.

Four of the 14 women in whom information about gonadal function was unavailable were already aged over 50 years and there was no information about the menstrual cycle before they had reached menopausal age. For the remaining 10 patients, neither patients nor caregivers remembered whether there had been a regular menstrual cycle before the start of estrogen- and/or progestogen-containing preparations.

Supplementary Materials Tables S2 and S3 show serum estradiol, LH, FSH and SHBG concentrations according to genetic subtype, rhGH treatment, age and BMI (no significant relationships).

Figures 1 and 2 show estradiol levels according to BMI and age, both did not show a significant relationship. Three patients (aged 30–40 years) had spontaneous menstruation after significant weight loss, and one of these patients developed a regular menstrual cycle. These three women were still overweight after weight loss. All seven women with a BMI below 25 kg/m^2 had hypogonadism, indicating that obesity is not the only cause of hypogonadism.
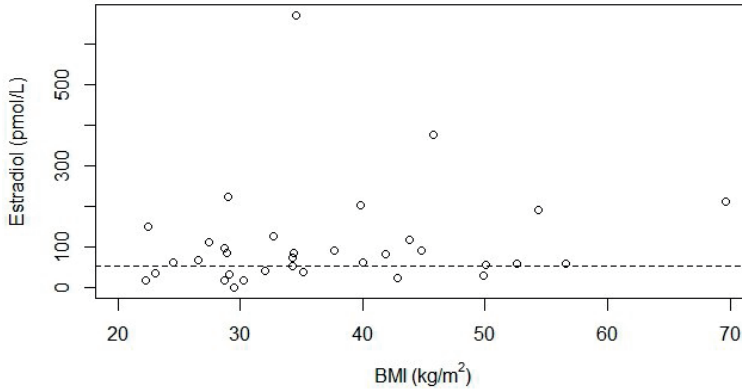

Figure 1. Relationship between serum estradiol concentrations and BMI for 34 women with Prader-Willi syndrome. Abbreviations: body mass index (BMI). The dotted line represents the lower limit of normal for estradiol of 55 pmol/L. Thirty-three women included in this figure had hypogonadism. One woman did not have hypogonadism as she had a regular menstrual cycle, she had an estradiol value of 41 pmol/L. The P-value for the relationship between estradiol and BMI was 0.13, Kendall's Tau was 0.18.

Figure 2. Relationship between serum estradiol concentrations and age for 34 women with Prader-Willi syndrome. The dotted line represents the lower limit of normal for estradiol of 55 pmol/L. Thirty-three women included in this figure had hypogonadism. One woman did not have hypogonadism as she had a regular menstrual cycle, she had an estradiol value of 41 pmol/L. The P-value for the relationship between estradiol and age was 0.27, Kendall's Tau was –0.13.
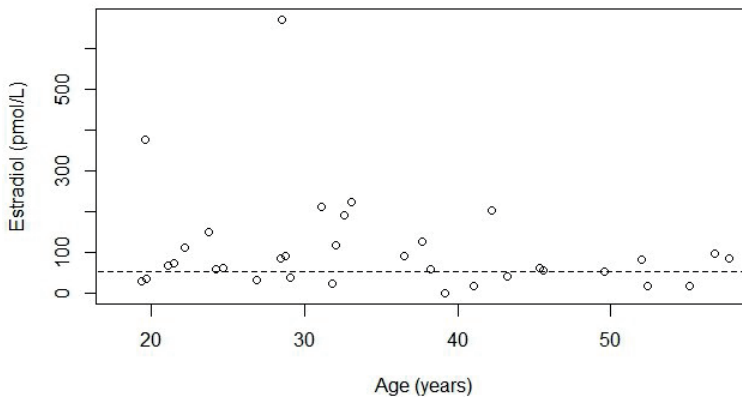
Types of Hypogonadism

LH, FSH and estradiol values were available for 27 women with hypogonadism (**Table 3**). Seven patients (26%) had central hypogonadism and one (4%) had primary hypogonadism. For the other 19 patients (70%) the type of hypogonadism could not be classified as either central or primary due to discrepant LH and FSH values. **Supplementary Materials Figure S1** shows the LH, estradiol and FSH values of the 27 individual patients.

Table 3. LH, FSH and estradiol values in 27 women with Prader-Willi syndrome and hypogonadism

Low estradiol / Normal estradiol	Low FSH	Normal FSH	High FSH
Low LH	1 (4%) / 3 (11%)	1 (4%) / 3 (11%)	1 (4%) / 1 (4%)
Normal LH	0 (0%) / 1 (4%)	2 (7%) / 8 (30%)	2 (7%) / 3 (11%)
High LH	0 (0%) / 0 (0%)	0 (0%) / 0 (0%)	0 (0%) / 1 (4%)

Abbreviations: follicle stimulating hormone (FSH), luteinizing hormone (LH). Data are presented as n (%). Laboratory measurements were available for 27 women with hypogonadism. In the other females not all three laboratory measurements were available, only laboratory measurements during the use of estrogen- and/or progestogen-containing preparations were available or the patient was over 50 years old. The type of hypogonadism is indicated with colors, where orange represents central hypogonadism and yellow represents primary hypogonadism. In the other patients, the hypogonadism could not be classified as either central or primary due to discrepant LH and FSH values. 'Low' refers to laboratory values below reference range. 'Normal' refers to laboratory values within the reference range (which may be inadequately low in case of low estradiol levels). 'High' refers to laboratory concentrations above the reference range.

Hormone Treatment

At the time of the study, 47 females used estrogen- and/or progestogen-containing preparations. Twenty-six women used oral contraceptives, 18 HRT (two of whom also used progestogen-containing contraceptive injections) and one a transdermal contraceptive patch. In addition, two women without hypogonadism used progesterone-only contraception (injections and IUD).

For oral contraceptives, spontaneously reported adverse effects were spotting (n = 3), increase in challenging behavior with psychotic symptoms (n = 2), hygienic difficulties (n = 2), headache (n = 2), stomach ache (n = 2), weight gain (n = 1), and hair loss (n = 1).

For HRT, spontaneously reported adverse effects were spotting (n = 2) and increase in challenging behavior (n = 2).

Expert Panel Discussion on Diagnosis and Treatment of Hypogonadism

Eleven experts with experience with the diagnosis and treatment of hypogonadism in females with PWS agreed to the recommendations presented in **Figure 3**.
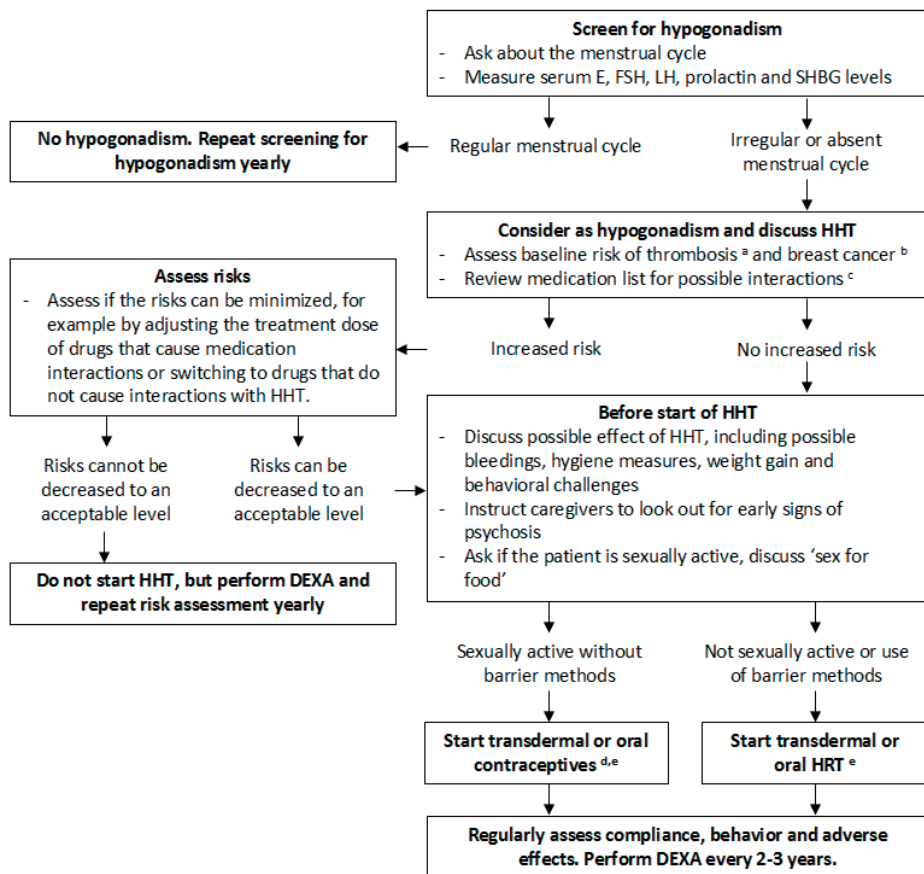
Screen for hypogonadism
- Ask about the menstrual cycle
- Measure serum E, FSH, LH, prolactin and SHBG levels

Regular menstrual cycle	Irregular or absent menstrual cycle

No hypogonadism. Repeat screening for hypogonadism yearly ← Regular menstrual cycle

Consider as hypogonadism and discuss HHT
- Assess baseline risk of thrombosis [a] and breast cancer [b]
- Review medication list for possible interactions [c]

Increased risk	No increased risk

Assess risks
- Assess if the risks can be minimized, for example by adjusting the treatment dose of drugs that cause medication interactions or switching to drugs that do not cause interactions with HHT.

Risks cannot be decreased to an acceptable level	Risks can be decreased to an acceptable level

Do not start HHT, but perform DEXA and repeat risk assessment yearly

Before start of HHT
- Discuss possible effect of HHT, including possible bleedings, hygiene measures, weight gain and behavioral challenges
- Instruct caregivers to look out for early signs of psychosis
- Ask if the patient is sexually active, discuss 'sex for food'

Sexually active without barrier methods	Not sexually active or use of barrier methods
Start transdermal or oral contraceptives [d,e]	**Start transdermal or oral HRT [e]**

Regularly assess compliance, behavior and adverse effects. Perform DEXA every 2-3 years.

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Figure 3. Recommendations for the treatment of hypogonadism in females with Prader-Willi syndrome, based on the results of this cohort study, a review of the literature and the expert opinion of an international panel of PWS-experts. Abbreviations: dual-energy X-ray absorptiometry (DEXA), estradiol (E), follicle stimulating hormone (FSH), hypogonadism hormone therapy (HHT), hormone replacement therapy (HRT), luteinizing hormone (LH), sex hormone binding globulin (SHBG). HRT includes tibolone and estrogen-containing preparations that cannot be used as contraception. HHT includes HRT and estrogen-containing contraceptives. [a] Relevant risk factors for thrombosis include: previous thrombotic events, increased age, being overweight or obese, smoking, and immobility (51). [b] Relevant risk factors for breast-cancer include: breast or ovarian cancer in first degree relatives before the age of 50, genetic mutations (e.g., BRCA), being overweight or obese and alcohol abuse. [c] For example interactions with psychotropic medication and recombinant human growth hormone treatment. [d] Start the combined oral contraceptive pill or transdermal patches containing both estrogen and progestogen. An IUD combined with oral or topical estrogens may be considered. However, general anesthesia or sedation may be necessary as insertion can be traumatic in patients with an intellectual disability and (past) sexual abuse should be excluded first. [e] Transdermal administration is preferred due to the lower risk of thrombosis, however oral preparations could be preferred in patients with skin picking.

Start Treatment for Hypogonadism

Nine experts in our expert panel discussion indicated that the decision to start HHT was not based on estradiol concentrations, but on the menstrual cycle (n = 9), bone mineral density (BMD) (n = 9), the ability of the patient to deal with possible vaginal bleeding (n = 6), sexual activity (n = 1) and/or desire of the patient to have 'normal' monthly

vaginal bleedings (n = 1). One expert based this decision on the menstrual cycle and BMD, but also considered estradiol levels, with a cut-off of 150 pmol/L. This expert did not consider the ability of the patient to deal with vaginal bleeding an issue, as she used continuous preparations that minimised vaginal bleeding in these patients. Lastly, one expert relied only on estradiol levels (below 150 pmol/L) to start HHT.

Stop Treatment for Hypogonadism

Two experts indicated that they did not have experience with HHT in women above 50 years old due to the decreased life expectancy of patients with PWS. One expert indicated that he did not have enough experience with this issue to formulate clinical recommendations. The other ten experts recommended to continue HHT until the natural age of menopause (around 50 years), similar to the clinical practice for non-PWS women. Five experts indicated that treatment could be continued longer (up until the age of 55–60) or be restarted in case of symptoms or low BMD.

Literature Review

We found ten articles that described hypogonadism or relevant laboratory values in women with PWS (**Tables 4 and 5**). Seven articles used characteristics of the menstrual cycle (i.e., absent or irregular menstrual cycle) to define hypogonadism. Seven articles reported FSH, LH and estradiol values, three SHBG values and two inhibin B and AMH values. The prevalence of hypogonadism ranged from 54% to 100%, with most articles (8 out of 10) reporting a prevalence above 80%. Hypogonadism was most often central in origin (21 reported cases), although primary hypogonadism (3 reported cases) was also reported. One article reported that most females had a combined form of primary and central hypogonadism (15 reported cases).

Table 4. Literature review of hypogonadism in women with Prader-Willi syndrome (Part 1)

Article	n	Country	Age range (years)	Genotype (deletion/ mUPD/ICD/ translocation)	Mean BMI (kg/m²)	Assays used	Definition hypogonadism
Partsch et al. (2000) (13)	9 [a]	Germany	18–34 [b]	NA/NA/0/0	46 [c]	Commercially available immunoassays	Low estradiol levels and absence of a regular MC
Whittington et al. (2002) (14) [d]	24	United Kingdom	18–47	NA [e]	NA	NA	Absence of a regular MC
Grugni et al. (2003) (15)	20	Italy	18–28	13/7/0/0	44	LH/FSH: immunochemiluminescent assays; Estradiol : chemiluminescent immunoassay	Absence of a regular MC
Höybye et al. (2005) (16) [d]	6	Sweden	19–37	NA [e]	Median 36	Commercially available immunoassays	Low estradiol, absence of a spontaneous regular MC, or treatment with sex steroids
Miller et al. (2008) (17) [d]	6	Florida, USA	18–29	4/2/0/0	32	Commercially available radioimmunoassays	Hypogonadotropic hypogonadism: delayed onset of puberty (>13 year) in addition to low gonadotropin levels for age
Brandau et al. (2008) (18) [d]	21	Missouri, USA	18–50	14/7/0/0	33	FSH, LH: chemiluminescence assays; Estradiol: radioimmunoassay	Low estradiol levels
Sode-Carlsen et al. (2010) (19) [d]	24	Denmark, Norway, Sweden	18–41	9/2/1/0 (12 NA) [e]	Median 28	Commercially available immunoassays	Low estradiol, absence of a spontaneous regular MC, or treatment with sex steroids
Van Nieuwpoort et al. (2011) (20)	11	The Netherlands	19–41	14/1/0/0 [c]	33	Commercially available immunoassays	Absence of a spontaneous regular MC
Hirsch et al. (2015) (21) [d]	19	Israel	18–47	10/8/1/0	33	LH, FSH, testosterone, estradiol: immunoassays; Inhibin B, AMH: ELISA; SHBG: immunochemiluminescence	Absence of a regular MC
Coupaye et al. (2016) (22) [d]	35	France	18–58 [c]	42/24/0/0 [c,f]	39 [c]	Routine techniques	Absence of a spontaneous regular MC, treatment with sex steroid or estradiol level < 120 ng/L at any time

Abbreviations: anti-Müllerian hormone (AMH), body mass index (BMI), paternal deletion (deletion), two-site enzyme-linked immunosorbent assays (ELISA), follicle stimulating hormone (FSH), imprinting center defect (ICD), luteinizing hormone (LH), menstrual cycle (MC), maternal uniparental disomy (mUPD), not available (NA), sex hormone-binding globulin (SHBG), United States of America (USA). Only data on adult women with PWS were included. The authors were contacted if separate data on only adults was not presented in the article. [a] Twelve women were included in this study, but in three women hypogonadism could not be investigated as they already received sex hormone replacement therapy. [b] Age range for all twelve women included in the study. [c] Data for all males and females included in this study. [d] Additional data was provided by the authors. [e] All methylation-positive. [f] Only patients a deletion or an mUPD were included.

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Table 5. Literature review of hypogonadism in women with Prader-Willi syndrome (Part 2).

Article	Hypogonadism n (%)	Primary / central hypogonadism	FSH	LH	Estradiol	SHBG	Inhibin B	AMH
Partsch et al. (2000) (13)	9 (100%)	– [a]	-	-	-	-	-	-
Whittington et al. (2002) (14)	20 (100%) (4 NA)	-	-	-	-	-	-	-
Grugni et al. (2003) (15)	17 (85%)	-	2.1 (0.1–5.1) IU/L	1.3 (0.1–5.0) IU/L	34 (15–72) pg/mL *123 (55–264) pmol/L*	-	-	-
Höybye et al. (2005) (16)	5 (83%)	0/5	4.9 (1.0–7.8) IU/L	2.1 (0.6–5.5) IU/L	104 (72–203) pmol/L	-	-	-
Miller et al. (2008) (17)	6 (100%)	1/5	-	-	-	-	-	-
Brandau et al. (2008) (18)	14 (70%) (1 NA)	-	3.8 (0.4–15.0) IU/L	1.8 (0.1–5.3) IU/L	23 (5–82) pg/mL *85 (18–301) pmol/L*	-	-	-
Sode-Carlsen et al. (2010) (19)	13 (54%)	1/5 (7 NA)	4.9 (<0.2–17.6) IU/L	2.7 (<1.0–12.9) IU/L	0.13 (0.08–0.54) nmol/L *130 (80–54) pmol/L*	-	-	-
Van Nieuwpoort et al. (2011) (20)	9 (81%)	0/4 (5 NA)	Median (IQR) 4.65 (3.49) IU/L	Median (IQR) 2.75 (2.26) IU/L	Median (IQR) 92 (257) pmol/L	Median (IQR) 25.5 (20.2) nmol/L	-	-
Hirsch et al. (2015) (21)	18 (95%)	1/2 (15 combined [b])	6.1 (0.5–18.3) IU/L	2.6 (0.1–6.8) IU/L	144 (37–733) pmol/L	47.1 (5.1–146.0) nmol/L	26.9 (10.0–73.0) pg/mL (n = 18)	1.04 (0.02–2.75) ng/mL (n = 17)
Coupaye et al. (2016) (22)	33 (94%)	-	Mean ± SD 6.4 ± 9.6 IU/L	Mean ± SD 4.2 ± 4.3 IU/L	50 (12–143) ng/L *183 (44–525) pmol/L*	Mean ± SD 36.9 ± 26.4 nmol/L	Mean ± SD 5.6 ± 6.0 pg/mL (n = 5)	Mean ± SD 0.9 ± 0.6 ng/mL (n = 5)

Abbreviations: anti-Müllerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), International System of Units (SI), interquartile range (IQR), not available (NA), standard deviation (SD), sex hormone-binding globulin (SHBG). When laboratory measurements were reported in non-SI units, the converted values are shown in *italics*. Only values for FSH, LH, and estradiol in patients that did not use estrogen- and/or progestogen-containing preparations during blood withdrawal are included. All laboratory values are presented as mean (range), unless otherwise specified. Values that were below the detection limit were considered equal to the detection limit to calculate the mean. [a] Gonadotropin levels were subnormal in all but one patient (of the total population of 7 males and 12 females) and showed a reduced responsiveness to stimulation with exogenous gonadotropin-releasing hormone. [b] Combined primary and central hypogonadism.

DISCUSSION

Hypogonadism was present in nearly all females with PWS in our cohort (94%) and was often undiagnosed (34%) and untreated. The high prevalence of hypogonadism in our cohort was in accordance with the literature.

Type of Hypogonadism

In our cohort, both central and primary hypogonadism were found, but in many patients the type of hypogonadism could not be determined due to discrepant LH and FSH values. The discrepancy between LH and FSH levels suggests that hypogonadism in females with PWS is not solely caused by hypothalamic dysfunction, but possibly also by combined ovarian and hypothalamic hypogonadism, as is seen in males with PWS (12,52,53). Previous research suggests that AMH is generally normal in girls and women with PWS (21,26), which argues against primary ovarian dysfunction. However, one study showed that AMH levels in girls with PWS were significantly lower compared to controls, while a similar assay was used in all three studies (24). Inhibin B levels are usually low or low-normal in women with PWS (21,24,26), suggesting decreased antral follicles.

Ovarian Histology

Literature on ovarian histology in females with PWS is scant. We found only two case reports (23): (i) a 32 year-old woman with PWS with delayed menarche (20 years) and an irregular menstrual cycle, who became pregnant, and in whom an ovarian biopsy (performed during caesarean section) showed normal follicles in all stages of development (29); and, (ii) a female with PWS who reported having menarche aged 11 years and a regular menstrual cycle but who died unexpectedly (age 22 years) and in whom, at autopsy, small, immature ovaries were found that showed no evidence of corpora lutea or significant follicular development (54). In order to explain the occurrence of primary ovarian dysfunction in women with PWS, more research on the ovarian histology of these females is urgently needed.

Factors influencing Hypogonadism in PWS

We did not find any significant association between laboratory measurements and genetic subtype, rhGH treatment, BMI, or age. The lack of a clear association between estradiol levels and BMI in our study might be explained by BMI's poor reflection of adiposity in PWS, due to the abnormal body composition (low muscle mass and high fat mass) (55). Many females with absent or irregular menses had estradiol levels within the normal range, possibly due to increased aromatase activity in adipose tissue, leading to normal serum estradiol levels despite hypothalamic, pituitary or ovarian dysfunction

(48,49). Supporting this hypothesis, three patients with amenorrhea developed a menstrual cycle after weight loss. The presence of hypogonadism in several females with a normal BMI also suggests obesity is not the only cause of central hypogonadism in females with PWS.

Importance of Treatment of Hypogonadism

Many beneficial effects of HHT for the treatment of hypogonadism have been described, including beneficial effects on bone health, muscle and fat, psychological outcomes and cardiovascular health. Some of these beneficial effects are especially important for patients with PWS as they already have a higher risk to develop an abnormal body composition, psychological problems and cardiovascular disease.

Bone Health

A reduced BMD is commonly seen in patients with PWS (44,45). Since estrogens play an important role in bone health (56,57), treating hypogonadism is important to optimize bone health. In the general population, low serum estradiol levels are associated with an increased risk of osteoporosis and osteoporotic fractures (58,59). Both HRT and the combined oral contraceptive pill have beneficial effects on BMD (57,60–62). In women with PWS, hypogonadism is also associated with a decreased BMD (63,64), for which for HRT is beneficial (64).

Muscle and Fat

Estrogen has important effects on muscle function (35). In women with hypopituitarism who are treated with rhGH, co-treatment with estrogen leads to an increase in lean body mass and a decrease in fat mass (65). As patients with PWS have an abnormal body composition with a high fat mass and low lean body mass, estrogen might help to improve body composition (55).

Psychological Effects

Untreated female hypogonadism can have negative consequences on mood, quality of life and energy level (33,34) and HHT has beneficial psychological effects in perimenopausal women (66–68). However, this has not been specifically investigated in women with PWS.

Cardiovascular Risk

Some studies in the general female population suggest that estradiol has beneficial effects on the cardiovascular system (37,38). In women with premature ovarian insufficiency, HRT seems to have beneficial effects on endothelial dysfunction, ischemic heart disease and cardiovascular mortality (69).

Recommendations

Based on the potential beneficial effects, we recommend treatment of hypogonadism in all adult females with PWS. For the treatment of hypogonadism in children with PWS, we refer to the recommendations for children with PWS (23).

Patients with PWS are preferably treated by a multidisciplinary team or in a PWS reference center, experienced in treating hypogonadism in females with PWS. However, as a PWS reference center is not always available, we provide clinical recommendations for the screening and treatment of hypogonadism in women with PWS (**Figure 3**). We aim to highlight topics that are especially important or difficult in females with PWS. We recommend to follow non-PWS specific hypogonadism guidelines for topics not addressed here.

Assessment of Gonadal Function
To avoid the negative consequences of long-term untreated hypogonadism, systematic screening for hypogonadism is warranted in all women with PWS. We showed that many women with PWS had estradiol values within the reference range. However, most experts in our expert panel discussion indicated that the decision to start treatment was based on the menstrual cycle and BMD rather than on serum estradiol concentrations. Therefore we recommend asking about the menstrual cycle every year and to start HHT when the menstrual cycle is absent or irregular. Additionally, a dual-energy X-ray absorptiometry (DEXA) scan should be performed as the presence of osteoporosis or osteopenia makes treatment with HHT even more urgent. Laboratory measurements could be performed to determine the type of hypogonadism and to exclude hyperprolactinemia.

Treatment Regimen
To prevent the increased risk of endometrial cancer caused by estrogen-only preparations, a progestogen must also be prescribed (70). The treatment regimen should be adjusted in case of sexual activity, as females with PWS are not always infertile and HRT does not protect against pregnancy. It is important to note that intellectual disability does not rule out the wish for sexual intercourse (71). In our cohort, six females were in a relationship with sexual intercourse. As pregnancies have been described in females with PWS (27–32), it is important to discuss contraception in patients who are sexually active. It should be noted that some types of contraception, such as progestogen-only preparations, do not aid in the treatment of hypogonadism and prevention of osteoporosis and may even lead to a decrease in BMD (72–74).

Estrogen can be administered both orally and transdermally and with same dosage as used for non-PWS women. In case of problematic skin picking, topical gels could be preferred over transdermal patches.

Drug Interactions
Frequently used medication in adults with PWS include psychotropic medication and rhGH treatment, both of which may interact with HHT.

Psychotropic Medication
Due to the increased prevalence of challenging behavior and psychosis, use of psychotropic medication is common in patients with PWS (8–11,75). There are few clinically significant interactions between HHT and psychotropic medication. However, some theoretical interactions have been proposed (76). HHT might influence the plasma concentrations of psychotropic medication. Both endogenous and exogenous estrogens induce the enzyme uridine 5'-diphospho-glucuronosyltransferase (UGT) 1A4, which is involved in the metabolism of some psychotropic medication (77–79). Therefore, HHT might lead to increased metabolism of, for example, lamotrigine (80,81), valproic acid (81), clozapine (82), olanzapine (83,84), and amitriptyline (85), for which dose adjustment might be indicated.

Moreover, estrogens (86) and progestogens (87) are metabolized by cytochrome P450 (CYP) 1A2, 2C19 and 3A4 enzymes, which could lead to competition with psychotropic medication that are metabolized via the same enzymes. This could, for example, lead to increased clozapine concentrations (77).

Some psychotropic medication may also influence the plasma concentrations of HHT. For example, lamotrigine and carbamazepine induce the production of SHBG, which could lower serum progesterone levels and decrease the effect of hormonal contraceptives (88).

Hyperprolactinemia is a common adverse effect of some antipsychotics and hyperprolactinemia might result in hypogonadism. This could be an issue in women with mild hypogonadism, and measurements of prolactin levels in women treated with antipsychotics is recommended (89). It could be considered to check the prolactin level before starting the antipsychotic therapy to avoid later unnecessary diagnostic investigations in case of mild hyperprolactinaemia during the psychiatric treatment.

Recombinant Human Growth Hormone Treatment

There is a complex interaction between GH and the gonadal system. Oral administration of estrogens inhibits hepatic insulin-like growth factor-1 (IGF-1) production, resulting in lower serum IGF-1 concentrations (65,90), while transdermal administration of estrogens may increase IGF-1 concentrations (91). Therefore, it is important to measure serum IGF-1 concentrations 3-6 months after the start or a change in dosage of HHT and to reevaluate the dose of rhGH treatment. Transdermal HHT might enable lower doses of rhGH to be administered which may have a cost benefit.

Intellectual Disability and Menstrual Hygiene

Monthly bleedings can cause practical problems for females with PWS and their caregivers (92), especially in patients with intellectual disability. Therefore, both patients and caregivers need to be informed about spotting or monthly bleedings, in order to take adequate hygiene measures. If monthly bleedings are deemed undesirable, preparations which do not lead to bleedings (tibolone, oral or transdermal preparations with continuous estrogen and progestogen administration or continuous estrogen tablets / patches in combination with progestogen-containing contraceptive injections) should be considered. However, patients and their caregivers should be warned that bleedings might occur during the first couple of weeks after starting these treatments. In patients who are sexually active, an IUD combined with oral or topical estrogens could also be considered. However, gynecological examination required to insert an IUD is often painful and stressful for women with intellectual disabilities, especially when they have a history of sexual abuse (93). Therefore, general anesthesia or sedation may be necessary during insertion of the IUD (94,95) and a possible history of sexual abuse should be excluded first (see also Section 4.5.9. Sex for food).

Challenging Behavior and Psychotic Symptoms

In our cohort, the caregivers of four females spontaneously reported an increase in challenging behaviors after start of HHT. Two of these patients even displayed psychotic symptoms. Although the behavioral challenges seemed associated with the use of HHT, it is possible that this was a coincidence as challenging behavior and psychotic symptoms are frequent in patients with PWS. Additionally, behavioral challenges were not systematically assessed and therefore it is possible that these challenges occur even more often than reported in this study. More research is needed to assess the relationship between HHT and behavior and psychotic symptoms in females with PWS. The relation between behavioral changes and HHT can be multifactorial. One possible explanation is menstrual pain or discomfort, which can cause challenging behaviors in females with an intellectual disability (96). However, the high pain threshold in most patients with PWS makes this explanation less likely. Another possible explanation is the visual exposure to

menstrual blood, which might cause feelings of fear, especially in patients with significant intellectual disabilities and the developmental age of a young child. Based on our experience, we recommend parents and caregivers are educated about early signs of psychosis and instructed to alert a physician if these symptoms occur.

Thrombosis

HHT can increase the risk of thrombosis (97). As patients with PWS already have a higher risk of thrombotic events (98), it is important to avoid any other risk factors for thrombosis, if possible. Due to lower levels of sex hormones, HRT is less thrombogenic than hormone contraception (51,99,100). Therefore, we advise to limit the use of hormone contraceptives to patients who are sexually active and unable to use non-hormonal contraception. Additionally, transdermal estrogen administration is preferred in women with PWS due to its lower risk of thrombosis compared to oral administration (101). However, it should be noted that contraceptive patches might be less reliable in obese women (102), although results are inconclusive (103). Lastly, it is important to address other risk factors for thrombosis including smoking, obesity and immobility.

Weight Gain

In our cohort, one patient spontaneously reported weight gain after starting estrogen- and progestogen-containing oral contraception. Although this might have been the result of fluid retention due to oral contraceptives (104), excessive food intake should always be considered when patients with PWS gain weight.

Breast Cancer

Two cohort studies reported an increased risk of cancer in patients with PWS (105,106), especially myeloid leukemia (106). There are no indications that the risk of breast cancer is increased in patients with PWS. However, as in non-PWS women, it is important to consider the additional risk of breast cancer resulting from HHT and to recommend participation in national breast cancer screening programs (70,107,108).

Sex for Food

Due to the combination of intellectual disabilities and hyperphagia, women with PWS are especially vulnerable to become victims of sexual abuse. Hyperphagia often leads to food-seeking behavior (109). This, especially when combined with cognitive impairment, makes them easy victims of 'sex for food' (receiving food in exchange for sexual acts) (110). Most of the patients do not disclose or understand the sexual abuse, resulting in continuation of the abuse (110). We recommend that this important topic is addressed in all women with PWS, in order to avoid negative psychological consequences and pos-

sible pregnancies. Discussing hypogonadism and HHT may provide a good opportunity to raise this sensitive topic.

Non-Compliance
Non-compliance is frequent in adults with PWS (9) and in those with intellectual disabilities (111). To improve compliance, it is important to prepare patients for monthly bleedings or spotting, and potential feelings of fear and embarrassment. Additionally, it is important to regularly ask whether the patient is still compliant and to determine reasons for non-compliance. As transdermal patches do not have to be applied daily, these treatment options may lead to better compliance.

Discontinuation of HHT
Most experts in the expert panel discussion recommend to continue HHT until the age of 50 years. Some experts also indicated that HHT could be continued longer or be restarted in case of osteoporosis / osteopenia or when symptoms occurred.

Strengths and Limitations
One strength of our study is the sample size, which is large considering the rarity of the syndrome. Moreover, we provide evidence- and expert-based practical recommendations for the screening and treatment of hypogonadism in PWS, which are urgently needed. A limitation is that many patients already used HHT before their first visit to our outpatient clinic. In these cases, we had to rely on medical files or the memory of the patients or their caregivers to determine whether the patient had hypogonadism. This also led to a large number of missing data for the analysis of laboratory values and the determination of primary or central hypogonadism. Also, we could not assess the effect of hypogonadism on BMD, as we had an insufficient number of DEXA scan results.

CONCLUSIONS

In conclusion, hypogonadism was present in nearly all females with PWS in our cohort (94%). Although adequate treatment is important for wellbeing and particularly bone health (37,38), hypogonadism is often undiagnosed (34%) and untreated. Therefore, we recommend screening for hypogonadism in all women with PWS by assessing the menstrual cycle. If the cycle is irregular or absent, treatment should be considered. To guide the screening and treatment of hypogonadism in women with PWS, we provide a practical algorithm.

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SUPPLEMENTARY DATA

Table S2. Laboratory values in women with Prader-Willi syndrome according to genotype and GH treatment

	Number of Observations	Total n = 64	Deletion n = 37	mUPD n = 24	P-value	Current GH Treatment n = 24	No current GH Treatment n = 40	P-value	P-value Corrected for Age
Estradiol (pmol/L)	34	71 (38–121)	69 (36–177)	87 (58–121)	0.7	64 (26–128)	82 (41–118)	0.4	0.3
LH (IU/L)									
Before 01-02-2019	27	2.4 (1.0–5.4)	2.9 (1.5–5.3)	1.5 (0.3–7.8)	0.9	3.6 (2.3–7.1)	1.6 (0.5–5.3)	0.4	0.2
After 01-02-2019	6	2.6 (1.2–5.4)	1.8 (0.5–9.6)	3.2 [a]		3.0 [a]	2.1 (0.8–7.7)		
FSH (IU/L)									
Before 01-02-2019	26	6.4 (3.3–8.5)	5.3 (3.0–7.5)	6.3 (3.6–9.8)	0.9	7.5 (7.0–10.0)	5.0 (2.9–8.2)	0.4	0.2
After 01-02-2019	8	5.5 (0.2–8.5)	6.7 (1.2–21.0)	0.1, 0.6, 7.5 [a]		6.4 [a]	4.6 (0.1–8.8)		
SHBG (nmol/L)	47	53 (30–73)	54 (30–81)	45 (27–60)	0.4	54 (42–68)	51 (23–75)	0.5	0.3

Abbreviations: paternal deletion (deletion), follicle stimulating hormone (FSH), growth hormone (GH), luteinizing hormone (LH), maternal uniparental disomy (mUPD), sex hormone-binding globulin (SHBG). Data are presented as median (interquartile range). Laboratory values for LH, FSH, and estradiol are given for patients at baseline or during the last measurement available before the start of estrogen- and/or progestogen-containing preparations. For SHBG the measurement closest to baseline is given. Values that were below the measuring threshold were considered equal to the measuring threshold to calculate the mean. For example, when FSH was below 0.5, this was considered 0.5. P-values are calculated with age and BMI as continuous variables.
[a] Individual measurements are given as there were too few observations to calculate a median and IQR.

Table S3. Laboratory values in women with Prader-Willi syndrome according to BMI and age

	BMI < 25 kg/m² n = 13	BMI 25–30 kg/m² n = 16	BMI > 30 kg/m² n = 35	P-value	P-value Corrected for Age	Age < 25 years n = 26	Age 25–30 years n = 13	Age > 30 years n = 25	P-value
Estradiol (pmol/L)	50 (23–129)	78 (22–108)	78 (52–144)	0.2	0.4	69 (48–131)	86 (36–381)	73 (28–126)	0.4
LH (IU/L)									
Before 01-02-2019	2.4, 4.2, 8.7 [a]	2.9 (1.4–6.4)	1.6 (0.5–5.3)	0.04	0.051	2.9 (0.1–4.6)	2.4 (1.5–5.8)	1.6 (0.8–6.4)	0.3
After 01-02-2019	3.0 [a]	NA	2.1 (0.8–7.7)			1.5, 3.0 [a]	NA	2.7 (0.6–9.9)	
FSH (IU/L)									
Before 01-02-2019	7.5, 11.1 [a]	7.5 (5.3–8.8)	4.4 (3.0–7.3)	0.08	0.06	7.5 (4.4–8.8)	7.5 (2.5–8.6)	5.4 (3.2–8.2)	0.2
After 01-02-2019	6.4 [a]	0.6 [a]	6.1 (0.1–12.9)			0.1, 4.6, 6.4 [a]	NA	7.5 (0.3–17.0)	
SHBG (nmol/L)	63 (56–84)	59 (26–89)	38 (23–59)	0.01	0.08	53 (29–85)	47 (19–77)	53 (33–68)	0.9

Abbreviations: body mass index (BMI), follicle stimulating hormone (FSH), luteinizing hormone (LH), not available (NA), sex hormone-binding globulin (SHBG). Data are presented as median (IQR). Laboratory values for LH, FSH, and estradiol are given for patients at baseline or during the last measurement available before the start of estrogen- and/or progestogen-containing preparations. For SHBG the measurement closest to baseline is given. Values that were below the measuring threshold were considered equal to the measuring threshold to calculate the mean. For example, when FSH was below 0.5, this was considered 0.5. P-values are calculated with age and BMI as continuous variables. [a] Individual measurements are given as there were too few observations to calculate a median and IQR
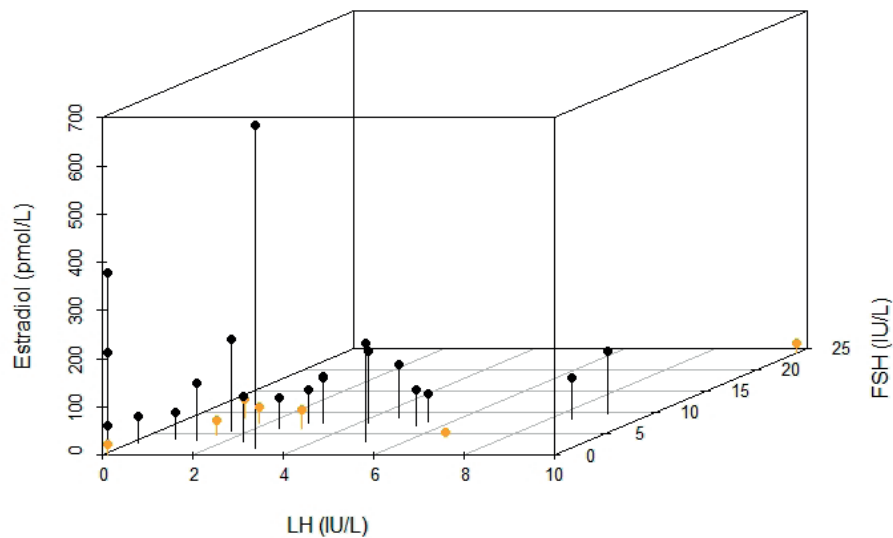
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Figure S1. LH, FSH and estradiol values for 27 women with Prader-Willi syndrome and hypogonadism.
Abbreviations: follicle stimulating hormone (FSH), luteinizing hormone (LH). In orange are women with an estradiol value below the reference range and in black the women with an estradiol value above the reference range.

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Thyroid function in adults with Prader-Willi syndrome; a cohort study and literature review

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ABSTRACT

Prader–Willi syndrome (PWS) is a complex genetic syndrome combining hypotonia, hyperphagia, a PWS-specific neurocognitive phenotype, and pituitary hormone deficiencies, including hypothyroidism. The low muscle mass associated with PWS causes a low energy expenditure due to a low basal metabolic rate. Combined with increased energy intake due to hyperphagia, this results in a high risk of obesity and associated cardiovascular disease. To reduce the high mortality in PWS (3% yearly), exercise is extremely important. As hypothyroidism can impair exercise tolerance, early detection is crucial. We performed a literature search for articles on hypothyroidism in PWS, measured thyroid hormone (TH) levels in 122 adults with PWS, and performed a medical file search for medication use. Hypothyroidism (low free thyroxin) was present in 17%, and often central in origin (80%). Triiodothyronine levels were lower in patients who used psychotropic drugs, while other TH levels were similar. One in six patients in our cohort of adults with PWS had hypothyroidism, which is more than in non-PWS adults (3%). We recommend yearly screening of free thyroxin and thyroid-stimulating hormone levels to avoid the negative effects of untreated hypothyroidism on basal metabolic rate, body mass index, and cardiovascular risk. Additionally, we recommend measuring TH concentrations 3–4 months after the start of growth hormone treatment.

INTRODUCTION

Prader–Willi syndrome (PWS) is a rare, complex, multisystem condition with an estimated prevalence of 1/10,000 to 1/30,000 (1). It is caused by loss of expression of a cluster of maternally imprinted genes on chromosome 15q11.2-q13, most commonly caused by a paternal deletion (65–75%) or a maternal uniparental disomy (mUPD, 20–30%). In rare cases, PWS is caused by imprinting center defects (ICD, 1–3%) or paternal chromosomal translocations (0.1%) (2,3). Features of PWS include hyperphagia, hypotonia, and delayed psychomotor development. In addition, hypothalamic dysfunction could result in abnormal temperature regulation, disturbed pain registration, and pituitary hormone deficiencies, including hypothyroidism. There are contradictory data regarding the prevalence of hypothyroidism in PWS (4,5).

Due to hypotonia and the low muscle mass associated with the syndrome, adults with PWS have a low basal metabolic rate (BMR) which, combined with hyperphagia, increases the risk of developing obesity. To increase energy expenditure and compensate for this low BMR, exercise is extremely important. However, if left untreated, hypothyroidism can cause fatigue and exercise intolerance (6–9). This leads to a further decrease in muscle mass and BMR, an increase in body mass index (BMI), and increased cardiovascular risk (10–12). As mortality in PWS is high (3% yearly in children and adults, and 7% yearly in adults with PWS above 30 years old) and often related to obesity and cardiovascular problems (e.g., cardiac failure and pulmonary embolism), it is of utmost importance to treat hypothyroidism and other factors affecting BMR at an early stage (6–9,13–15).

Apart from affecting BMR, hypothyroidism can also have more direct cardiovascular effects. Low thyroid hormone (TH) levels have been associated with diastolic hypertension, increased systemic vascular resistance leading to decreased cardiac output, myocardial stiffness, left ventricular diastolic dysfunction, accelerated atherosclerosis, and coronary artery disease (16). Even subclinical hypothyroidism has been associated with an increased risk of coronary heart disease and mortality (17). Treatment with levothyroxine reduces low-density lipoprotein cholesterol, total cholesterol, hypertension, and diastolic dysfunction, and delays atherosclerosis (16).

Our clinical experience is that hypothyroidism is frequently missed in adults with PWS. The PWS-specific behavioral phenotype, physicians' unawareness of the PWS-specific diagnostic pitfalls, and the lack of medical guidelines for adults with PWS can cause both patients' and doctors' delay. The intellectual disability present in most PWS adults makes it difficult for patients to express symptoms such as fatigue and constipation. In addition, physicians often falsely assume that fatigue or excessive daytime sleepiness are

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inherent to the syndrome, and do not perform further investigations. Moreover, reduced appetite and weight gain—other important symptoms of hypothyroidism—are hard to recognize, as adults with PWS have hyperphagia and are often on a diet. Therefore, hypothyroidism can easily be missed if not actively screened for.

In 122 adults with PWS, we performed TH measurements and reviewed medical files for clinical data, including use of medication. As few large studies have investigated TH measurements in adults with PWS, we provide a thorough exploratory analysis of the patient characteristics possibly associated with TH concentrations (gender, genotype, BMI, age, growth hormone (GH) treatment, and use of psychotropic drugs). Additionally, we searched the literature for the prevalence and mechanisms of hypothyroidism (central or primary hypothyroidism) in adults with PWS. Based on our findings, we present recommendations for the screening and management of hypothyroidism in adults with PWS.

MATERIALS AND METHODS

Ethical review and approval were waived for this study by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam, the Netherlands. In this retrospective study, we reviewed the medical files of adults that visited the multidisciplinary outpatient clinic of our PWS reference center in the Erasmus University Medical Center, between January 2015 and December 2020, and underwent our routine systematic health screening. This systematic screening consists of a structured interview, a complete physical examination, a medical questionnaire, a review of the medical file including medication use, biochemical measurements and, if indicated and feasible, additional tests, as described previously (see (18)).

During the visit, blood samples were taken for general medical screening, including evaluation of thyroid function (fT4, triiodothyronine (T3), TSH). The reference values in our center for TSH were 0.4–4.3 mU/L before 1 February 2019, and 0.56–4.27 mU/L after that date. The reference values for fT4 were 11–25 pmol/L (Ortho Vitros® assay, Vitros ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Rochester, MI, USA) before 12 April 2019, and 13.5–24.3 pmol/L after that date (Fuijrebio Lumipulse® assay). Reference values in our center for T3 were 1.4–2.5 nmol/L before 12 April 2019, and 0.7–2.0 nmol/L after that date. TSH, fT4 and T3 measurements changed methods during the study, but they were calibrated similarly, as checked by external quality assessment schemes.

Overt hypothyroidism was defined as an fT4 concentration below the reference range. Central overt hypothyroidism was defined as an fT4 concentration below the reference range, with a TSH concentration below or within the reference range. Primary hypothyroidism was defined as an fT4 concentration below the reference range, with a TSH concentration above the reference range. Overt hyperthyroidism was defined as an fT4 concentration above the reference range. If patients used levothyroxine before visiting our reference center, the diagnosis of overt hypothyroidism was based on referral letters and/or laboratory measurements before the start of levothyroxine; in that case, the distinction between primary or central hypothyroidism was also based on referral letters or, if available, on the laboratory measurements before the start of levothyroxine compared to the local reference values.

Subclinical hypothyroidism was defined as a normal fT4 concentration, with a TSH concentration above the reference range, based on a single measurement. It is important to note that the diagnosis of subclinical hypothyroidism is less reliable in adults with PWS. In the general population, TSH can be affected by obesity (19–22). Furthermore, hypothyroidism can be both primary and central in PWS. Taken together, this means that TSH and, therefore, the diagnosis of subclinical hypothyroidism, should be interpreted with caution.

We investigated the relationship between TH measurements and genotype, as this relationship is still largely unknown. As gender, age, BMI, and GH treatment are known to influence TH in the general population, we also investigated their effect on TH concentrations in our cohort of adults with PWS (23–30).

Literature Review

We performed a search on Embase, Medline, the Web of Science Core Collection, the Cochrane Central Register of Controlled Trials, and Google Scholar for articles that describe thyroid function and/or TH measurements in patients with PWS. The search was last updated on 22 July 2021. For the full search strategy, see **Table S1**.

Inclusion criteria were: original research articles that described the prevalence of thyroid abnormalities (including, but not limited to: central and primary hypothyroidism, hyperthyroidism, and subclinical hypothyroidism) or TH measurements (including, but not limited to: thyroxine (T4), fT4, T3, free T3 (fT3), reverse T3 (rT3), and TSH) in methylation-positive individuals with PWS. Exclusion criteria were: meeting reports, workshop summaries, conference abstracts, guidelines, articles that included 10 or fewer subjects with PWS, articles that were not available online, and articles that were not available in English. When the same population was described in multiple articles, the population

with the most laboratory values or the largest population was included. When an article described thyroid function before and after the start of GH treatment, only data at baseline were included in the table. Authors were contacted to clarify data when needed.

Statistical Analysis

Statistical analysis was performed using R version 3.6.3. Descriptive statistics for continuous variables are reported as median (interquartile range (IQR)). For dichotomous variables we display the number of patients and the percentage of the total number of patients, n (%). We used a chi-squared test to compare the prevalence of hypothyroidism between males and females, between paternal deletion and mUPD, between patients who did and did not use GH treatment, and between patients who did and did not use psychotropic drugs. To investigate the relationship between hypothyroidism, and BMI and age, we used the Wilcoxon rank sum test. We also used the Wilcoxon rank sum test to investigate the relationships between gender, genotype, and use of GH treatment and psychotropic drugs on the one hand, and laboratory measurements (fT4, T3, and TSH) on the other hand. If there were ties, an exact calculation method was used. The Kendall rank correlation test was used to assess correlations between age and BMI on the one hand, and laboratory measurements (fT4, T3, and TSH) on the other hand. As this was an exploratory analysis, no correction for multiple testing was performed.

RESULTS

Baseline

Baseline characteristics of the 122 adults with PWS participating in the study are shown in **Table 1**. The median age was 29 years (IQR 21–39), and the median BMI was 29 kg/m^2 (IQR 26–36). We included 58 males and 64 females. Paternal deletion was the most common genotype (n = 66, 54%), followed by mUPD (n = 43, 35%). A total of 63 patients (52%) had never received GH treatment, while 43 patients (35%) received GH treatment at the time of the study. Medication use before our systematic screening included use of hydrocortisone (4 adults daily and 49 adults only during physical or psychological stress), estrogen replacement therapy (34/64 females), testosterone replacement therapy (24/58 males), and thyroid hormone replacement therapy (n = 19, 16%). A total of 67 (55%) patients lived in a non-specialized facility, 24 (20%) in a specialized PWS group home, and 31 (25%) with family. Most patients had received special education (n = 87, 71%).

Table 1. Baseline characteristics of 122 adults with Prader–Willi syndrome.

	Total n = 122
Age in years, median (IQR)	29 (21–39)
BMI in kg/m², median (IQR)	29 (26–36)
Male gender, n (%)	58 (48%)
Genetic subtype	
Deletion, n (%)	66 (54%)
mUPD, n (%) [a]	43 (35%)
ICD, n (%)	3 (2%)
Unknown, n (%)	10 (8%)
Growth hormone treatment	
Only during childhood, n (%)	12 (10%)
Only during adulthood, n (%)	3 (2%)
Both, n (%)	44 (36%)
Never, n (%)	63 (52%)
Current growth hormone treatment, n (%)	43 (35%)
Use of hydrocortisone	
Daily, n (%)	4 (3%)
During physical or psychological stress, n (%)	49 (40%)
Use of estrogen replacement therapy or oral contraceptives before screening, n (%)	34/64 females (53%)
Use of testosterone replacement therapy before screening, n (%)	24/58 males (41%)
Use of thyroid hormone replacement therapy before screening, n (%)	19 (16%)
Living situation	
With family, n (%)	31 (25%)
In a specialized PWS group home, n (%)	24 (20%)
In a non-specialized facility, n (%)	67 (55%)
Education level	
Secondary vocational education, n (%)	6 (5%)
Pre-vocational secondary education, n (%)	3 (2%)
Special education, n (%)	87 (71%)
No education, n (%)	5 (4%)
Unknown, n (%)	21 (17%)

Abbreviations: body mass index (BMI), paternal deletion (deletion), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD), Prader–Willi syndrome (PWS). [a] In 14 patients with suspected mUPD, the parents were not available for genetic testing. Therefore, mUPD is the most likely genotype, but an ICD could not be ruled out in these patients.

Hypo- and Hyperthyroidism

Hypothyroidism was present in 21 patients (17%). A total of 12 patients had central hypothyroidism, 3 patients had primary hypothyroidism, and in 6 patients it was unknown whether the hypothyroidism was central or primary. In 17 patients, the diagnosis of hypothyroidism was based on referral letters. TH concentrations were provided in the referral letter in five cases. Additionally, two patients were diagnosed during childhood by the pediatric endocrinology department at our reference center, and two patients were diagnosed during our systematic health screening. The median age at diagnosis of hypothyroidism was 18 years (IQR 13–27) (age at diagnosis was unknown in two

patients). The median dose of levothyroxine in patients with hypothyroidism was 68.8 µg (IQR 50.0–100.0) daily. Additionally, one patient with very mild hypothyroidism did not receive any treatment.

Three patients (2%) had a normal fT4 concentration, with a TSH concentration above the reference range (subclinical hypothyroidism), while one patient was diagnosed with hyperthyroidism, treated with thiamazole. Although not statistically significant, hypothyroidism seemed to be more prevalent in females (23%) than in males (10%, P = 0.051). There was no relationship with genotype, age, BMI, or GH treatment (**Table 2** (**A, B, C**)).

Thyroid Hormone Levels

For the 97 patients without (subclinical) hypo- or hyperthyroidism, TH concentrations and the associations between TH concentrations and patient characteristics (gender, genotype, age, BMI, and use of GH treatment and psychotropic drugs) are shown in **Table 2** (**A, B, C**). The median fT4 concentration was 16.5 pmol/L (IQR 14.3–18.5), the median T3 concentration was 1.9 nmol/L (IQR 1.7–2.3), and the median TSH concentration was 1.6 mU/L (IQR 1.1–2.3). T3 was significantly lower in older patients and in patients without current GH treatment, while fT4 and TSH levels were similar. Gender, genotype, and BMI were not significantly related to any of the TH measurements. To visualize the exact distribution of the TH concentrations, we show the T3, fT4, and TSH concentrations according to BMI in **Figure 1** (**A, B, C**).

Psychotropic Drugs

We explored the relationship between TH concentrations and the use of psychotropic or antiepileptic drugs. Forty-nine patients used psychotropic drugs. Only two patients used antiepileptic medication, and both also used psychotropic drugs. Therefore, the relationship between the use of antiepileptic drugs and thyroid function was not further explored. Use of psychotropic drugs was not associated with hypothyroidism (**Table 2** (**C**)). However, T3 was significantly lower in patients who used psychotropic drugs (median 1.7 nmol/L (IQR 1.6–2.0)) than in those who did not (median 2.1 nmol/L (IQR 1.7–2.3), P = 0.02). The mean age of patients using psychotropic drugs was 36 years, and the mean age of patients not using psychotropic drugs was 29 years. No associations for specific types of psychotropic drugs were found (**Table S2** (**A, B**)).

Literature Review

The results of our literature review are summarized in **Table 3** (**A, B, C**). Only 5 studies reported thyroid function separately for adults, while the other 21 studies reported thyroid function in children (n = 12) or in mixed populations containing children and

Table 2(A). Prevalence of hypothyroidism and thyroid hormone levels in 122 patients with PWS.

	Missing	Total n = 122	Males n = 58	Females n = 64	P-value	Deletion n = 66	mUPD n = 43	P-value
n of males, n of females	0	58, 64	58, 0	0, 64	NA	39, 37	21, 22	NA
Hypothyroidism, n (%)	0	21 (17%)	6 (10%)	15 (23%)	0.051	12 (18%)	7 (16%)	0.8
Subclinical hypothyroidism, n (%)	0	3 (2%)	0 (0%)	3 (5%)	NA	1 (2%)	2 (5%)	NA
Hyperthyroidism, n (%)	0	1 (1%)	0 (0%)	1 (2%)	NA	0 (0%)	0 (0%)	NA
n of males, n of females with normal thyroid function (n = 97)	0	52, 45	52, 0	0, 45	NA	26, 27	19, 15	NA
fT4 (pmol/L), median (IQR) (n = 97)	2	16.5 (14.3–18.5)	16.5 (14.6–18.5)	16.5 (14.1–18.6)	0.7	16.2 (14.1–17.7)	17.1 (14.8–18.9)	0.2
T3 (nmol/L), median (IQR) (n = 97)	52	1.9 (1.7–2.3)	1.8 (1.7–2.2)	2.1 (1.7–2.3)	0.5	2.0 (1.7–2.3)	1.7 (1.5–2.3)	0.2
TSH (mU/L), median (IQR) (n = 97)	0	1.6 (1.1–2.3)	1.5 (1.0–2.1)	1.9 (1.3–2.4)	0.06	1.7 (1.4–2.3)	1.5 (1.0–2.3)	0.06

Abbreviations: paternal deletion (deletion), free thyroxine (fT4), interquartile range (IQR), maternal uniparental disomy (mUPD), not applicable (NA), triiodothyronine (T3), thyroid-stimulating hormone (TSH). Laboratory concentrations are for patients with normal thyroid function only (n = 97).

Table 2(B). Prevalence of hypothyroidism and thyroid hormone levels in 122 patients with PWS.

	Age < 25 years n = 47	Age 25–30 years n = 22	Age > 30 years n = 53	P-value	BMI < 25 kg/m² n = 25	BMI 25–30 kg/m² n = 45	BMI > 30 kg/m² n = 52	P-value
n of males, n of females	21, 26	9, 13	28, 25	NA	12, 13	29, 16	17, 35	NA
Hypothyroidism, n (%)	10 (21%)	6 (27%)	5 (9%)	0.4	5 (20%)	8 (18%)	8 (15%)	0.6
Subclinical hypothyroidism, n (%)	1 (2%)	1 (5%)	1 (2%)	NA	0 (0%)	1 (2%)	2 (4%)	NA
Hyperthyroidism, n (%)	0 (0%)	0 (0%)	1 (2%)	NA	0 (0%)	1 (2%)	0 (0%)	NA
n of males, n of females with normal thyroid function (n = 97)	18, 18	8, 7	26, 20	NA	11, 9	25, 10	16, 26	NA
fT4 (pmol/L), median (IQR) (n = 97)	16.5 (14.9–18.1)	15.4 (14.0–19.1)	16.7 (14.1–18.9)	0.2	15.9 (14.6–18.2)	16.6 (14.2–18.8)	16.7 (14.2–18.4)	1
T3 (nmol/L), median (IQR) (n = 97)	2.1 (1.8–2.2)	2.2 (1.8–2.5)	1.7 (1.6–1.9)	0.003	2.3 (1.9–2.4)	1.7 (1.6–2.3)	1.9 (1.7–2.2)	1
TSH (mU/L), median (IQR) (n = 97)	1.6 (1.2–2.2)	1.7 (1.4–2.7)	1.5 (0.9–2.3)	0.6	1.6 (1.2–2.2)	1.4 (0.9–2.3)	1.8 (1.3–2.7)	0.3

Abbreviations: body mass index (BMI), free thyroxine (fT4), interquartile range (IQR), not applicable (NA), triiodothyronine (T3), thyroid-stimulating hormone (TSH). P-values are calculated with age and BMI as continuous variables. Laboratory measurements are for patients with normal thyroid function only (n = 97).

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Table 2 (C). Prevalence of hypothyroidism and thyroid hormone levels in 122 patients with PWS.

	Current GH Treatment n = 43	No Current GH Treatment n = 79	P-value	Psychotropic Drugs n = 49	No Psychotropic Drugs n = 73	P-value
n of males, *n* of females	19, 24	39, 40	NA	25, 24	33, 40	NA
Hypothyroidism, n (%)	8 (19%)	13 (16%)	0.8	10 (20%)	11 (15%)	0.5
Subclinical hypothyroidism, n (%)	2 (5%)	1 (1%)	NA	1 (2%)	2 (3%)	NA
Hyperthyroidism, n (%)	0 (0%)	1 (1%)	NA	0 (0%)	1 (1%)	NA
n of males, *n* of females with normal thyroid function (n = 97)	17, 16	35, 29	NA	21, 17	31, 28	NA
fT4 (pmol/L), median (IQR) (n = 97)	16.0 (14.3–18.0)	16.9 (14.5–18.8)	0.3	16.6 (14.7–18.7)	16.3 (14.1–18.1)	0.8
T3 (nmol/L), median (IQR) (n = 97)	2.1 (1.8–2.4)	1.8 (1.6–2.2)	0.03	1.7 (1.6–2.0)	2.1 (1.7–2.3)	0.02
TSH (mU/L), median (IQR) (n = 97)	1.6 (1.2–2.0)	1.6 (1.0–2.3)	0.8	1.5 (0.9–2.5)	1.7 (1.2–2.2)	0.7

Abbreviations: free thyroxine (fT4), growth hormone (GH), interquartile range (IQR), not applicable (NA), triiodothyronine (T3), thyroid-stimulating hormone (TSH). Laboratory measurements are for patients with normal thyroid function only (n = 97)
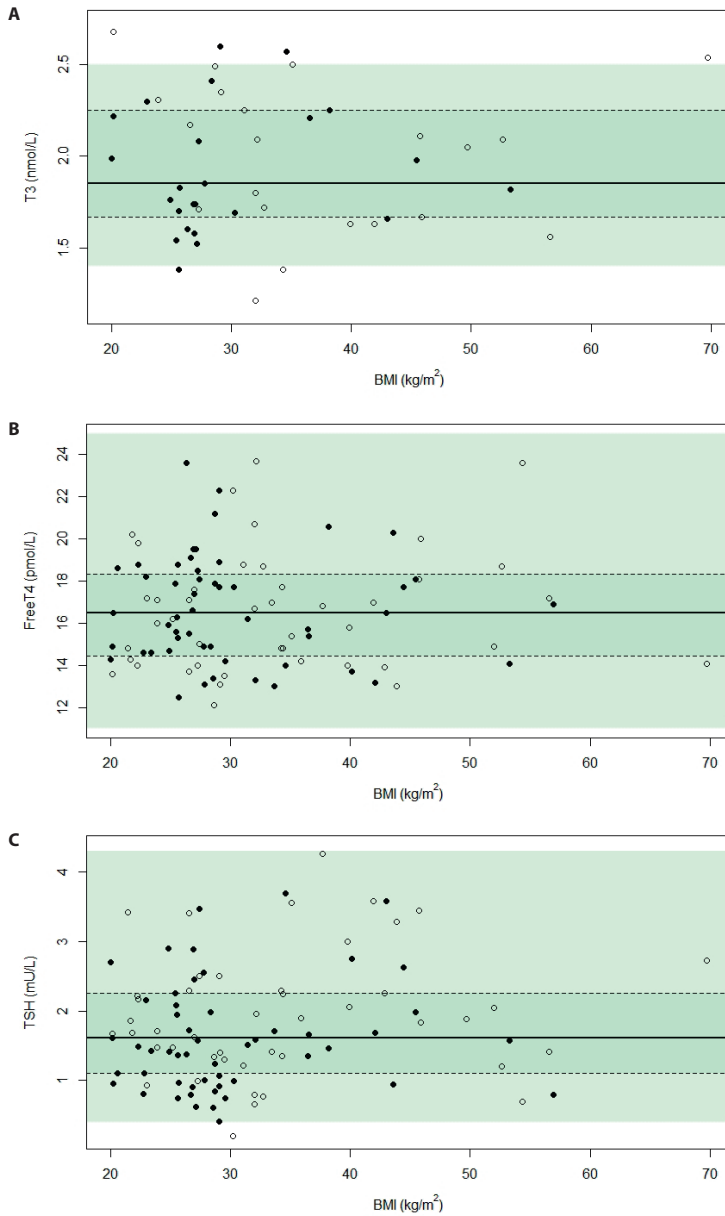
Figure 1. Scatterplots of T3, fT4, and TSH in relation to BMI.
Abbreviations: body mass index (BMI), free thyroxine (fT4), triiodothyronine (T3), thyroid-stimulating hormone (TSH). Only patients without hyperthyroidism, hypothyroidism, or subclinical hypothyroidism are depicted in this figure. Legends: males are depicted by closed dots and females by open dots. The solid line represents the median, while the dashed line with the grey rectangle represents the interquartile range. The reference range is given as a green, transparent rectangle. (**A**) T3 vs. BMI for males and females; (**B**) fT4 vs. BMI for males and females; (**C**) TSH vs. BMI for males and females. Reference values: TSH: before 1 February 2019: 0.4–4.3 mU/L (n = 69), after 1 February 2019: 0.56–4.27 mU/L (n = 28); fT4: before 12 April 2019: 11–25 pmol/L (n = 73), after 12 April 2019: 13.5–24.3 pmol/L (n = 22); T3: before 12 April 2019: 1.4–2.5 (n = 33), after 12 April 2019: 0.7–2.0 nmol/L (n = 12). Only the reference range that was valid for the most observations is shown.

adults (n = 9). Paternal deletion was the most common genotype in all studies. The prevalence of hypothyroidism differed between 0% and 33% in most studies, with one study reporting a prevalence of 72%. However, this study only included 18 children with PWS who were only up to 2 years old and, in this study, thyroid axis dysfunction was defined as serum total T4 and/or serum fT4 levels below the 2.5th percentile of a reference population. The prevalence of hypothyroidism in studies that only included adults ranged between 5% and 13%. For mixed populations of both children and adults, this prevalence was between 0% and 26%. Only two studies that included adults reported whether the hypothyroidism was central or primary in origin (31,32). Although central hypothyroidism was more prevalent (2% and 4%), primary hypothyroidism (0% and 2%) did also occur. One study reported on the prevalence of subclinical hypothyroidism in PWS, and showed a prevalence of 5% in children and 1% in adults. Additionally, 11 studies reported TSH, 5 total T4, 14 fT4, 5 total T3, 6 free T3, and 1 reverse T3 concentrations.

Clinical Recommendations

Based on the results of our cohort, the literature review, and our clinical expertise, we formulated practical clinical recommendations for the screening and treatment of hypo-
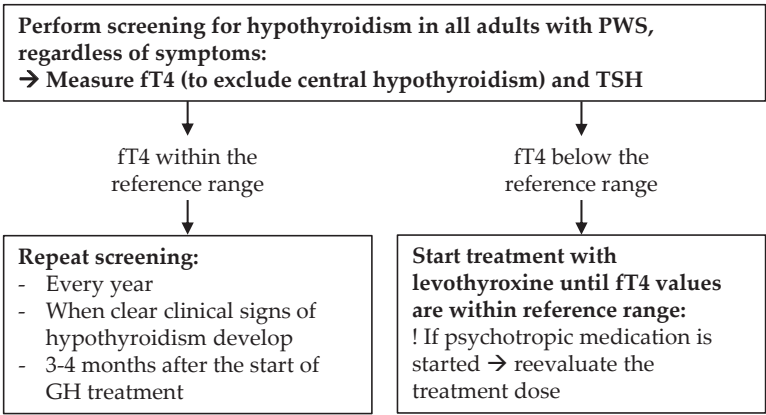

Figure 2. Recommendations for the screening and treatment of hypothyroidism in adults with Prader–Willi syndrome.

thyroidism in adults with PWS (**Figure 2**).

DISCUSSION

The prevalence of hypothyroidism detected in our cohort of 122 adults with PWS was 17%, compared to only 3% in non-PWS adults (56). The risk of hypothyroidism was

Table 3 (A). Patient characteristics of cohorts assessed by previous studies.

Article	n	Country	Age Range (years)	Genotype (Deletion, mUPD, ICD, Translocation)	Gender	Mean BMI (kg/m²)	Current GH Treatment
Tauber et al. (2000) (33)	28	France	-	36%, 14%, 0%, 0% (25% NA, 18% no genetic analysis, 7% no genetic abnormality)	12 M, 16 F	-	50%
Festen et al. (2007) (25)	75	The Netherlands	Median (IQR): 4.7 (2.7–7.6)	35%, 32%, 9%, 1% (23% NA)	39 M, 36 F	Median (IQR): 18 (16–20)	0%
Vaiani et al. (2010) (34)	18	Argentina	0 – 2	61%, 28%, 0%, 0% (11% NA)	11 M, 7 F	-	-
Wong et al. (2010) (35)	20	USA	Mean ± SD: 4.0 ± 0.8	-	12 M, 8 F	20	0%
Diene et al. (2010) (36)	127	France	0 – 18 [a]	63%, 25%, 2%, 1% (1% other, 8% NA) [a]	77 M, 65 F [a]	Median BMI Z-score +1.3 [a]	87% [a]
Sharkia et al. (2013) (37)	31 [b] TRH-ST: 21 Neonates: 23	Canada	TRH-ST: 0 – 18 Neonates: 0	TRH-ST: 62%, 29%, 0%, 0% (10% NA) Neonates: 43%, 48%, 0%, 0% (9% NA)	TRH-ST: 7 M, 14 F Neonates: 9 M, 14 F	TRH-ST: mean BMI Z-score +1.2 Neonates: NA	TRH-ST: 86% Neonates: 0%
Kim et al. (2014) (38)	14	Korea	0 – 3 [c]	93%, 7%, 0%, 0% [c]	16 M, 14 F [c]	BMI-SDS: 0.66	0%
Iughetti et al. (2019) (31)	243	Italy	0 – 18	57%, 34%, 0%, 1% (8% NA) [d]	233 M, 106 F [d]	20	27%
Oto et al. (2020) (39)	51	Japan	0 – 7	61%, 39%, 0%, 0%	29 M, 22 F	-	-
Lu et al. (2020) (40)	48	China	0 – 15	77%, 23%, 0%, 0%	32 M, 16 F	Mean BMI Z-score: 0.8	0%
Konishi et al. (2020) (41)	43	Japan	0 – 3	60%, 40%, 0%, 0%	17 M, 26 F	Median BMI-SDS: -1.47	0%
Dağdeviren Çakır et al. (2021) (42)	52	Turkey	0 – 15	69%, 12%, 2%, 0% (17% NA)	26 M, 26 F	20	40%

Children

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Table 3(A). Patient characteristics of cohorts assessed by previous studies. *(continued)*

	Article	n	Country	Age Range (years)	Genotype (Deletion, mUPD, ICD, Translocation)	Gender	Mean BMI (kg/m²)	Current GH Treatment
Children and Adults	Höybye et al. (2002) (43)	13[e]	Sweden	17 – 37	-	7 M, 6 F	35	-
	Butler et al. (2007) (32)	47	USA	10 – 44	55%, 45%, 0%, 0%	21 M, 26 F	34	0%
	Miller et al. (2008) (44)	27	USA	0 – 39	74%, 26%, 0%, 0%	17 M, 10 F	Obesity: 74%	0%
	Mogul et al. (2008) (45)	38	USA	17 – 49	-	13 M, 25 F	35	0%[f]
	Farholt et al. (2011) (46)	65	Denmark	0 – 48	65%, 20%, 3%, 0% (12% NA)	33 M, 32 F	Median BMI-SDS: 0.92	62%
	Laurier et al. (2015) (47)	154	France	16 – 54	66%, 16%, 2%, 2% (15% NA)	68 M, 86 F	42	14%
	Coupaye et al. (2016) (48)[g]	73	France	16 – 58	64%, 36%, 0%, 0%	35 M, 38 F	Deletion: 41, mUPD: 35	15%
	Proffitt et al. (2019) (49)	2029	USA	0 – 84	42%, 19%, 2%, 0% (37% NA)	934 M, 1000 F	Living: 29, deceased: 52	Living: 51%, deceased: 22%
	Pemmasani et al. (2021) (50)	480	USA	Mean ± SD: 27 ± 19	-	242 M, 238 F	Obesity: 41%	-
Adults	Van Nieuwpoort et al. (2011) (51,52)[h]	15	The Netherlands	19 – 42	93%, 7%, 0%, 0%	4 M, 11 F	Median: 28	0%
	Sinnema et al. (2011) (52)[h]	102	The Netherlands	18 – 66	54%, 43%, 3%, 0%	49 M, 53 F	32	5%
	Grugni et al. (2013) (53)[i]	108	Italy	18 – 43	68%, 25%, 0%, 2% (6% NA)	47 M, 61 F	Median in non-obese: 26, median in obese: 45	-
	Iughetti et al. (2019) (31)[i]	96	Italy	19 – 50	57%, 34%, 0%, 1% (8% NA)[d]	233 M, 106 F[d]	43	-
	Radetti et al. (2020) (54)[i]	120	Italy	18 – 59	71%, 28%, 0%, 0% (2% NA)	69 M, 51 F	37	20%

Abbreviations: body mass index (BMI), deletion (paternal deletion), females (F), growth hormone (GH), ICD (imprinting center defect), interquartile range (IQR), males (M), mUPD (maternal uniparental disomy), not available (NA or -), standard deviation (SD), standard deviation score (SDS), thyrotropin-releasing hormone stimulation test group (TRH-ST), United States of America

(USA). [a] Data for the whole cohort of 142 patients; however, hypothyroidism was only assessed in 127 patients. [b] Thirteen patients were included in both the TRH-ST group and the neonate group. [c] Data for the whole cohort of 30 patients; however, thyroid hormone values were only given for 14 patients. [d] Data for the whole cohort of children and adults. [e] Only data for methylation-positive subjects are included in the table. [f] All patients were growth hormone deficient and growth hormone treatment had not been started yet. [g] A more recent study conducted by the same research group (Paepegaey et al. 2018 (55)) with a larger study population was available. As this study population was largely the same as that of Coupaye et al. and this study did not report any laboratory values, it was not included in the table. Paepegaey et al. evaluated thyroid function in 91 adults, of whom 29 had hypothyroidism (31%). [h] Van Nieuwpoort et al. (2011) performed a systematic screening with blood measurements in patients who might also have been described in Sinnema et al. (2011), where data were collected based on questionnaires and medical histories. [i] The study population of Grugni et al. (2013) contains 30 patients who were not described in Iughetti et al. (2019) and/or Radetti et al. (2020). The study population of Radetti et al. (2020) contains 36 subjects with PWS who were not described in Iughetti et al. (2019).

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Table 3 (B). Patient characteristics of cohorts assessed by previous studies.

Article	Total Overt Hypothyroidism (Central, Primary Hypothyroidism) (%)	Subclinical Hypothyroidism (%)	TSH (mU/L)	Free T4 (pmol/L)
Tauber et al. (2000) (33)	32	-	-	Mean ± SD: 8.1 ± 1.1 pg/mL *Mean: 10 nmol/L*
Festen et al. (2007) (25)	6[a]	-	Median (IQR): 2.0 (1.6–2.7) mU/L	Median (IQR): 16.2 (14.3–17.8) pmol/L
Vaiani et al. (2010) (34)	72[b]	-	Median (range): TAD: 1.4 (0.8-5.7) mU/L NTAD: 2.9 (1.4-5.3) mU/L	Median (range): TAD: 9.1 (2.6-11.8) pmol/L NTAD: 12.9 (12.1-21.0) pmol/L
Wong et al. (2010) (35)	0	-	-	-
Diene et al. (2010) (36)	24	-	-	-
Sharkia et al. (2013) (37)	TRH-ST: 5[c] Neonates: 0[e]	-	Mean ± SD (range) TRH-ST: 1.9 ± 1.0 (0.8-4.2) mU/L[d] Neonates: 3.1 ± 2.3 (0.4-10.0) mU/L[e]	Mean ± SD (range) TRH-ST: 10.4 ± 1.1 (8.2-13.5) pmol/L[d]
Kim et al. (2014) (38)	-	-	Mean ± SD: 3.2 ± 2.1 mU/L	Mean ± SD: 1.1 ± 0.2 ng/dL *Mean: 14 pmol/L*
Iughetti et al. (2019) (31)	11 (8, 2)[f]	5	Mean ± SD (median): 2.7 ± 2.1 (2.2) mU/L	Mean ± SD (median): 10.6 ± 2.2 (10.3) pg/mL *Mean: 13.6 pmol/L, median: 13.3 pmol/L*
Oto et al. (2020) (39)	TRH-ST: 4[c]	-	Median (IQR): 2.3 (1.2–3.6) mU/L	Median (IQR): 1.18 (1.02-1.24) ng/dL *Median (IQR): 15 (13 – 16) pmol/L*
Lu et al. (2020) (40)	-	-	-	≤ 2 years old: mean ± SD: 0.7 ± 0.2 ng/dL > 2 years old: mean ± SD: 0.9 ± 0.2 ng/dL *≤ 2 years old: mean: 9 pmol/L* *> 2 years old: mean: 12 pmol/L*
Konishi et al. (2020) (41)	30	-	Median (IQR): 2.4 (1.8–3.4) mU/L	Median (IQR): 11.6 (9.9–14.0) pmol/L
Dağdeviren Çakır et al. (2021) (42)	33 (31, 2)	-	-	-

Children

Article	Total Overt Hypothyroidism (Central, Primary Hypothyroidism) (%)	Subclinical Hypothyroidism (%)	TSH (mU/L)	Free T4 (pmol/L)
Children and Adults				
Höybye et al. (2002) (43)	0	-	-	-
Butler et al. (2007) (32)	2 (2, 0)	-	Mean ± SD: 2.2 ± 1.3 mU/L	Mean ± SD: 1.1 ± 0.2 ng/dL (n = 43) *Mean: 14 pmol/L*
Miller et al. (2008) (44)	NA (19, NA) [g]	-	-	-
Mogul et al. (2008) (45)	0	-	Mean ± SD (range): 1.5 ± 0.2 (0.01-7.7) mU/L	Mean ± SD (range): 1.1 ± 0.04 (0.6-1.7) ng/dL *Mean (range): 14 (8-22) pmol/L*
Farholt et al. (2011) (46)	5	-	-	-
Laurier et al. (2015) (47)	26	-	-	-
Coupaye et al. (2016) (48)	26 [h]	-	-	Mean ± SD: Deletion: 14.0 ± 2.0 pmol/L UPD: 15.1 ± 2.7 pmol/L
Proffitt et al. (2019) (49)	9	-	-	-
Pemmasani et al. (2021) (50)	16	-	-	-
Adults				
Van Nieuwpoort et al. (2011) (51)	13 [i]	-	Median (IQR): 2.3 (1.85) mU/L	Median (IQR): 15.4 (1.9) pmol/L
Sinnema et al. (2011) (52)	9	-	-	-
Grugni et al. (2013) (53)	5	-	-	-
Iughetti et al. (2019) (31)	6 (4, 2)	1	Mean ± SD (median): 2.2 ± 1.4 (2.0) mU/L	Mean ± SD (median): 11.4 ± 2.0 (11.2) pg/mL *Mean: 15 pmol/L, median: 14 pmol/L*
Radetti et al. (2020) (54)	10	-	-	-

Abbreviations: interquartile range (IQR), not available (NA or -), non-thyroid axis dysfunction (NTAD), standard deviation (SD), thyroxine (T4), thyroid axis dysfunction (TAD), thyrotropin-releasing hormone stimulation test (TRH-ST), thyroid-stimulating hormone (TSH). When laboratory measurements were reported in non-SI units, the converted values are shown in *italics*. Total overt hypothyroidism is the sum of central, primary, and congenital hypothyroidism. [a] Five of 79 patients had free T4 levels below -2 standard deviation score; however, thyroid hormone levels of 4 of these patients are reported separately, as they already received thyroid hormone replacement therapy. [b] Percentage of thyroid axis dysfunction based on a free T4 or total T4 level below the 2.5th percentile. [c] Based on TSH response to thyrotropin-releasing hormone. [d] For patients with a normal thyrotropin-releasing hormone test only. [e] Based on blood samples collected on filter paper for the universal newborn screening for congenital hypothyroidism. [f] Congenital hypothyroidism in 2%. [g] Only central hypothyroidism was evaluated, and was present in 19%. Additionally, one patient had previously been diagnosed with autoimmune primary hypothyroidism. [h] A more recent study from the same research group (Paepegaey et al. 2018 (55)) with a larger study population was available. As this study population was largely the same as that of Coupaye et al., and this study did not report any laboratory values, it was not included in the table. Paepegaey et al. evaluated thyroid function in 91 adults, of whom 29 had hypothyroidism (31%). [i] Central in origin according to the patients. Additionally, one patient had hyperthyroidism.

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Table 3 (C). Patient characteristics of cohorts assessed by previous studies.

Article	Total T4 (nmol/L)	Free T3 (pmol/L)	Total T3 (nmol/L)	Reverse T3 (nmol/L)
Tauber et al. (2000) (33)	-	-	Mean ± SD: 118 ± 3.1 ng/dL *Mean: 1.8 pmol/L*	-
Festen et al. (2007) (25)	Median (IQR): 98.0 (85.3–113) nmol/L	-	Median (IQR): 2.6 (2.3–3.0) nmol/L	Median (IQR): 0.3 (0.3–0.4) nmol/L
Vaiani et al. (2010) (34)	Median (range): TAD: 88.8 (57.9–109) nmol/L NTAD: 112 (86.2–126) nmol/L	-	Median (range): TAD: 2.5 (1.4–3.2) nmol/L NTAD: 2.4 (2.3–3.0) nmol/L	-
Wong et al. (2010) (35)	-	-	-	-
Diene et al. (2010) (36)	-	-	-	-
Sharkia et al. (2013) (37)	-	Mean ± SD (range) TRH-ST: 6.1 ± 1.0 (4.8–8.4) pmol/L [a]	-	-
Kim et al. (2014) (38)	-	-	-	-
Iughetti et al. (2019) (31)	-	Mean ± SD (median): 3.7 ± 1.0 (3.6) pg/mL *Mean: 5.7 pmol/L, median: 5.5 pmol/L*	-	-
Oto et al. (2020) (39)	-	Median (IQR): 4.0 (3.5–4.4) pg/mL *Median (IQR): 6.2 (5.4–6.8) pmol/L*	-	-
Lu et al. (2020) (40)	≤ 2 years old: mean ± SD: 7.5 ± 1.7 µg/dL > 2 years old: mean ± SD: 9.0 ± 2.5 µg/dL *≤ 2 years old: mean: 96.5 nmol/L > 2 years old: mean: 116 nmol/L*	-	-	-
Konishi et al. (2020) (41)	-	Median (IQR): 4.8 (4.1–5.6) pmol/L	-	-
Dağdeviren Çakır et al. (2021) (42)	-	-	-	-

Children

Table 3 (C). Patient characteristics of cohorts assessed by previous studies. (*continued*)

	Article	Total T4 (nmol/L)	Free T3 (pmol/L)	Total T3 (nmol/L)	Reverse T3 (nmol/L)
Children and Adults	Höybye et al. (2002) (43)	-	-	-	-
	Butler et al. (2007) (32)	Mean ± SD: 8.1 ± 2.0 µg/dL (n = 38) *Mean: 104 nmol/L*	-	Mean ± SD: 137 ± 38 ng/dL(n = 41) *Mean: 2.1 nmol/L*	-
	Miller et al. (2008) (44)	-	-	-	-
	Mogul et al. (2008) (45)	Mean ± SD (range): 8.6 ± 0.3 (4.2-14.4) µg/dL *Mean (range): 111 (54-185) nmol/L*	-	Mean ± SD (range) : 131 ± 8 (46-251) ng/dL *Mean (range): 2.0 (0.7-3.9) nmol/L*	-
	Farholt et al. (2011) (46)	-	-	-	-
	Laurier et al. (2015) (47)	-	-	-	-
	Coupaye et al. (2016) (48)	-	-	-	-
	Proffitt et al. (2019) (49)	-	-	-	-
	Pemmasani et al. (2021) (50)	-	-	-	-
Adults	Van Nieuwpoort et al. (2011) (51)	-	Median (IQR): 5.1 (0.8) pmol/L	-	-
	Sinnema et al. (2011) (52)	-	-	-	-
	Grugni et al. (2013) (53)	-	-	-	-
	Iughetti et al. (2019) (31)	-	Mean ± SD (median): 3.1 ± 0.7 (3.0) pg/mL *Mean: 4.7 pmol/L, median: 4.6 pmol/L*	-	-
	Radetti et al. (2020) (54)	-	-	-	-

Abbreviations: interquartile range (IQR), not available (-), non-thyroid axis dysfunction (NTAD), standard deviation (SD), triiodothyronine (T3), thyrotropin-releasing hormone stimulation test (TRH-ST), thyroid axis dysfunction (TAD). When laboratory measurements were reported in non-SI units, the converted values are shown in *italics*. [a]For patients with a normal TRH-ST only.

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increased in all adults with PWS, regardless of gender, genotype, age, BMI, or use of GH treatment or psychotropic drugs.

Our prevalence of hypothyroidism was higher than that of most previous studies on hypothyroidism in adults and mixed cohorts of adults and children **Table 3** (**A, B**). However, two large French studies both showed an even higher prevalence of hypothyroidism (26%) in patients with PWS of 16 years and older (47,48). This indicates that, although the prevalence is variable, hypothyroidism is frequent in adults with PWS.

Compared to the general population, there are several aspects of PWS that increase the complexity of the diagnosis and treatment of hypothyroidism in these patients. An increased vulnerability to the effects of untreated hypothyroidism of the patients, diagnostic challenges, and altered TH metabolism make hypothyroidism a complex issue in adults with PWS.

Vulnerability of the Patients

The vulnerability of the patients makes the treatment of hypothyroidism an important topic. The common effects of hypothyroidism on the muscles and the brain can be especially harmful to adults with PWS, as they already have impaired exercise tolerance and brain function.

Exercise Intolerance and Cardiovascular Risk

Patients with PWS have a high risk of developing obesity due to hyperphagia and a low BMR inherent to the syndrome. Hypothyroidism can cause arthralgia, lethargy, exertion fatigue, shortness of breath, and muscle problems (12,57); this makes it hard to exercise, and increases the risk of obesity. Hypothyroidism is also directly associated with a decreased BMR, leading to a higher prevalence of obesity, which can further impair physical activity (58–61). Obesity results in a high cardiovascular risk. Both indirect and direct cardiovascular effects of hypothyroidism make its early detection and treatment an important topic in this already vulnerable patient population (16).

Brain Function

Thyroid function is responsible for a variety of physiological processes in the adult brain (62). Adult-onset hypothyroidism can affect both cognitive function and psychological health (63). Hypothyroidism can impair cognition, concentration, information processing speed, memory, perceptual function, and executive function (64,65). Treatment with levothyroxine can reverse these symptoms (66). Furthermore, anxiety and depressive symptoms are frequently reported in patients with hypothyroidism. These symptoms

also improve after treatment with levothyroxine, leading to an increased quality of life (67,68).

The increased vulnerability of the patients, combined with diagnostic challenges and altered thyroid hormone metabolism, make the diagnosis and treatment of hypothyroidism an important issue in adults with PWS.

Diagnostic Challenges

Diagnostic challenges include patients' delay, doctors' delay, and unreliability of TSH.

Patients' Delay

Due to the intellectual disability that is often present in PWS, patients are often unable to express their complaints. Especially when the symptoms associated with hypothyroidism are subtle (e.g., mild fatigue or muscle weakness, or a slightly changed bowel pattern), they will not be reported by the patients.

Doctors' Delay

In the general population, TH concentrations are usually measured when there is a clinical suspicion of hypothyroidism. Unexplained weight gain, reduced appetite, fatigue, and constipation are well-known clinical signs of hypothyroidism that will alert most physicians to measure TH concentrations (12). However, in patients with PWS, these symptoms are often unreliable. Unexplained weight gain will often be attributed to hyperphagia. In addition, this constant craving for food will make it easy to miss a slight reduction in appetite. Fatigue due to hypothyroidism can be easily mistaken for daytime sleepiness due to reduced hypothalamic arousal, which is often present in PWS (2,18). Lastly, constipation is already present in 40% of patients with PWS, and will not alert physicians to screen for hypothyroidism (69).

Unreliability of TSH

Apart from patients' and doctors' delay, there is another diagnostic challenge. Hypothyroidism can be both primary and central in PWS **Table 3** (**B**). In our cohort, we also found that both central hypothyroidism (n = 12) and primary hypothyroidism (n = 3) were present. Serum TSH concentrations in patients with central hypothyroidism are often normal (70–72). Furthermore, TSH can be affected by obesity (19–22). Taken together, this means that TSH is less reliable in PWS. In our clinic, we have seen several examples of patients with untreated overt central hypothyroidism, which had been missed because the physician had only measured TSH and not fT4.

As symptoms of hypothyroidism are unreliable in patients with PWS, and hypothyroidism can be both primary and central, we recommend to screen for hypothyroidism by measuring serum TSH and fT4 concentrations every year.

Altered Thyroid Hormone Metabolism

Prescription of endocrine and non-endocrine medication may disturb TH metabolism. Likewise, altered levels of "hunger hormones" may affect TH concentrations. Examples of these TH metabolism-altering factors in adults with PWS include use of psychotropic drugs, growth hormone treatment, and disturbed leptin and ghrelin levels.

Psychotropic Drugs

Psychotropic drugs can cause a disturbed synthesis and metabolism of TH (73,74). Compared to the general endocrine population, the population of adults with PWS is characterized by frequent use of psychotropic drugs (75), such as antipsychotics, anxiolytics, and antidepressants. Psychotropic drugs can influence the synthesis and metabolism of TH in a variety of ways, such as changing iodine capture or decreasing thyrotropin-releasing hormone (TRH) responsiveness. Psychotropic drugs can also cause an altered deiodination of T4 to T3 by stimulating deiodinase activity (73,74,76). The iodothyronine deiodinases D1, D2, and D3 regulate the conversion from the prohormone T4 (which is produced by the thyroid gland, and biologically inactive) to the active hormone T3. This conversion takes place mainly in peripheral tissues (77–79). In our population, 40% of the patients used psychotropic drugs. T3 was significantly lower in patients who used psychotropic drugs than in patients who did not. However, patients who used psychotropic drugs were older (mean age 36 years) compared to patients who did not use psychotropic drugs (mean age 29 years), which might have influenced the results (23,24). We did not find an association between specific types of psychotropic drugs and TH measurements. This could be related to a lack of power, as these subgroups were small.

GH Treatment

GH treatment is often prescribed to children and young adults with PWS. In our population, one-third of the patients were treated with GH. Patients who used GH treatment had generally been receiving GH treatment for several years before visiting our outpatient clinic. It has been suggested that GH treatment enhances peripheral conversion of T4 to T3, resulting in decreased fT4 and increased T3 levels (80–82). Several groups have studied the effect of GH treatment on TH levels, with contradictory results. Several studies showed that GH treatment does not induce hypothyroidism, but can unmask previously undiagnosed hypothyroidism in non-PWS individuals (83–87). In children with PWS, fT4 concentrations decreased after the start of GH treatment, but remained

within the low–normal range (25). Two randomized controlled trials in adults with PWS showed no significant effect of GH treatment on fT4 and TSH (45,88), whereas one study showed increased T3 concentrations during GH treatment (45). In our cohort, GH treatment was associated with higher T3 levels, while fT4 and TSH concentrations were similar for patients with and without GH treatment. To prevent the potential negative effects of missing "unmasked" hypothyroidism, we recommend measuring TH concentrations 3–4 months after the start of GH treatment.

Leptin and Ghrelin
High leptin levels caused by obesity in patients with PWS can lead to an increased conversion of T4 to T3 (89). This mechanism might be partially responsible for the relatively low fT4 levels found in PWS patients (25,90). Altered ghrelin levels in PWS (91,92) may impair the activity of the hypothalamic–pituitary–thyroid axis (93), which might increase the prevalence of hypothyroidism.

Strengths and Limitations
As with any study, our study has several strengths and limitations. One strength of our study is the population size, considering the rareness of the disease. Furthermore, we are the first to not only describe the prevalence of hypothyroidism and TH concentrations, but also the relationship with medication use. One of our limitations is that this was a retrospective study and, therefore, we had many missing values for T3, as this was not routinely measured in all patients. Another limitation is that thyroid peroxidase (TPO) antibodies were not measured; therefore, we were not able to distinguish autoimmune thyroid diseases. Furthermore, we did not measure thyroxine-binding globulin (TGB) and, therefore, we do not know whether free T3 levels were disturbed in our cohort. The last limitation is that laboratory measurements before the start of levothyroxine treatment were not available in 12 patients with hypothyroidism. In these cases we had to rely on referral letters that mentioned whether the patient had central or primary hypothyroidism.

CONCLUSIONS

In conclusion, one in six patients in our cohort of 122 adults with PWS had hypothyroidism, which is more frequent than in non-PWS adults. Hypothyroidism is often central in origin, and it is therefore important to measure not only TSH, but also fT4. We recommend yearly screening of fT4 and TSH to prevent the negative effects of untreated hypothyroidism on BMR, BMI, cardiovascular risk, and brain function. Additionally, we

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recommend measuring thyroid hormone concentrations 3–4 months after the start of GH treatment.

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SUPPLEMENTARY DATA

Table S2(A). Prevalence of hypothyroidism and thyroid hormone levels in relation to use of psychotropic drugs.

	Missing	Total n = 122	Psychotropic Drugs n = 49	No Psychotropic Drugs n = 73	P-value	Atypical Antipsychotics (Non Phenothiazine) n = 20	No Atypical Antipsychotics n = 102	P-value
n of males, *n* of females	0	58, 64	25, 24	33, 40	NA	10, 10	48, 54	NA
Hypothyroidism, n (%)	0	21 (17%)	10 (20%)	11 (15%)	0.5	5 (25%)	16 (16%)	0.3
Subclinical hypothyroidism, n (%)	0	3 (2%)	1 (2%)	2 (3%)	NA	0 (0%)	3 (3%)	NA
Hyperthyroidism, n (%)	0	1 (1%)	0 (0%)	1 (1%)	NA	0 (0%)	1 (1%)	NA
n of males, *n* of females with normal thyroid function	0	52, 45	21, 17	31, 28	NA	8, 7	44, 38	NA
fT4 (pmol/L), median (IQR) (n = 97)	2	16.5 (14.3-18.5)	16.6 (14.7-18.7)	16.3 (14.1-18.1)	0.8	17.2 (14.6-18.7)	16.3 (14.3-18.2)	0.5
T3 (nmol/L), median (IQR) (n = 97)	52	1.9 (1.7-2.3)	1.7 (1.6-2.0)	2.1 (1.7-2.3)	0.02	2.1 (1.8-NA)	1.8 (1.7-2.3)	0.7
TSH (mU/L), median (IQR) (n = 97)	0	1.6 (1.1-2.3)	1.5 (0.9-2.5)	1.7 (1.2-2.2)	0.7	1.5 (0.9-2.3)	1.6 (1.2-2.3)	0.3

Abbreviations: not applicable (NA), free thyroxine (fT4), triiodothyronine (T3), thyroid stimulating hormone (TSH). Laboratory measurements are for patients with normal thyroid function only (n = 97).

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Table S2(B). Prevalence of hypothyroidism and thyroid hormone levels in relation to use of psychotropic drugs.

	Nontricyclic Antidepressants n = 10	No Nontricyclic Antidepressants n = 112	P-value	Valproic Acid n = 12	No Valproic Acid n = 110	P-value	Benzodiazepines n = 14	No Benzodiazepines n = 108	P-value
n of males, *n* of females	5, 5	53, 59	NA	8, 4	50, 60	NA	8, 6	50, 58	NA
Hypothyroidism, n (%)	3 (30%)	18 (16%)	0.3	2 (17%)	19 (17%)	0.9	1 (7%)	20 (19%)	0.3
Subclinical hypothyroidism, n (%)	0 (0%)	3 (3%)	NA	0 (0%)	3 (3%)	NA	0 (0%)	3 (3%)	NA
Hyperthyroidism, n (%)	0 (0%)	1 (1%)	NA	0 (0%)	1 (1%)	NA	0 (0%)	1 (1%)	NA
n of males, *n* of females with normal thyroid function	5, 2	47, 43	NA	6, 4	46, 41	NA	7, 6	45, 39	NA
fT4 (pmol/L), median (IQR) (n = 97)	17.4 (16.5-20.0)	16.2 (14.3-18.2)	0.2	14.9 (14.2-17.8)	16.5 (14.5-18.6)	0.3	17.2 (16.2-19.4)	16.2 (14.3-18.1)	0.1
T3 (nmol/L), median (IQR) (n = 97)	1.7 (1.6-1.8)	2.0 (1.7-2.3)	0.1	2.1 (1.7-2.5)	1.9 (1.6-2.2)	0.3	1.7 (1.5-1.8)	2.0 (1.7-2.3)	0.1
TSH (mU/L), median (IQR) (n = 97)	1.8 (0.9-2.9)	1.6 (1.1-23)	0.8	2.1 (1.5-2.5)	1.6 (1.0-2.2)	0.1	1.3 (0.9-2.1)	1.7 (1.1-2.3)	0.2

Abbreviations: not applicable (NA), free thyroxine (fT4), triiodothyronine (T3), thyroid stimulating hormone (TSH). Laboratory measurements are for patients with normal thyroid function only (n = 97).
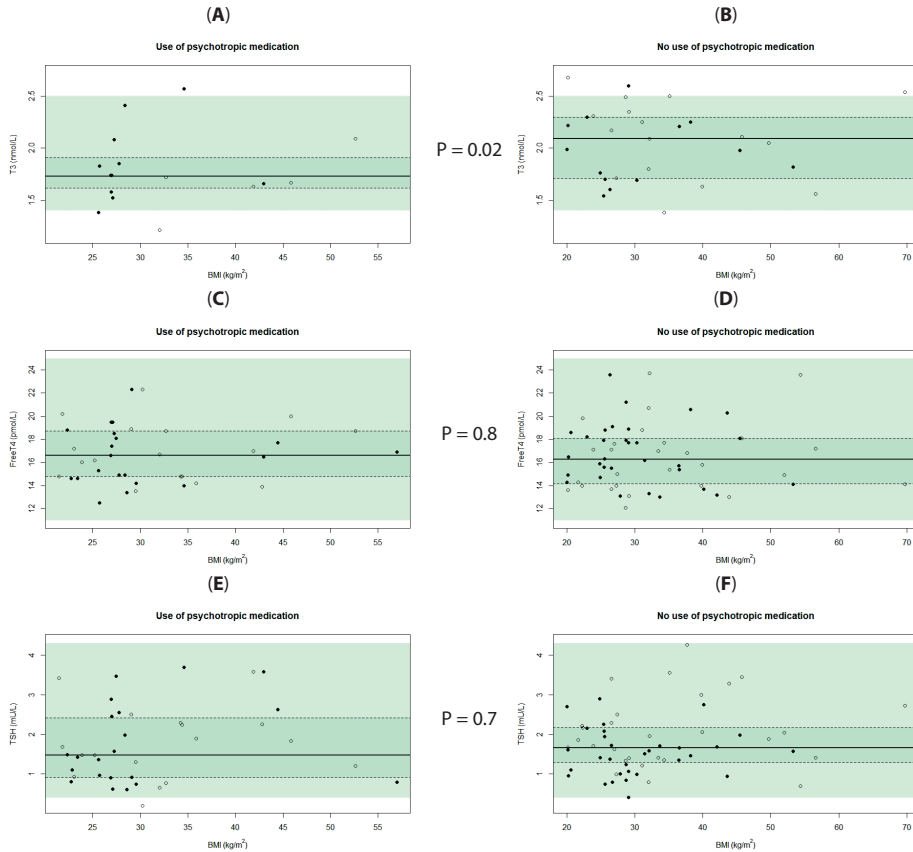
Figure S1. Scatterplot of T3, fT4, and TSH in relation to BMI for patients who use psychotropic drugs versus patients who do not.

Abbreviations: body mass index (BMI), free thyroxine (fT4), triiodothyronine (T3), thyroid stimulating hormone (TSH). Only patients without hyperthyroidism, hypothyroidism or subclinical hypothyroidism are depicted in this figure. P-values for the relationship between the use of psychotropic drugs and the thyroid hormone concentrations (T3, fT4 and TSH respectively) are shown in the middle. Legends: males are depicted by closed dots and females by open dots. The solid line is the median and the dashed line the interquartile range. The reference range is given in as a green, transparent rectangle. (**A**) T3 vs. BMI for patients who use psychotropic drugs. (**B**) T3 vs. BMI for patients who do not use psychotropic drugs. (**C**) fT4 vs. BMI for patients who use psychotropic drugs. (**D**) fT4 vs. BMI for patients who do not use psychotropic drugs. (**E**) TSH vs. BMI for patients who use psychotropic drugs. (**F**) TSH vs. BMI for patients who do not use psychotropic drugs. Reference values: TSH: before 1 February, 2019: 0.4-4.3 mU/L (n = 69), after 1 February, 2019: 0.56-4.27 mU/L (n = 28). fT4: before 12 April, 2019: 11-25 pmol/L (n = 73), after 12 April, 2019: 13.5-24.3 pmol/L (n = 22). T3: before 12 April, 2019: 1.4-2.5 (n = 33), after 12 April, 2019: 0.7-2.0 nmol/L (n = 12). Only the reference range that was valid for the most observations is shown
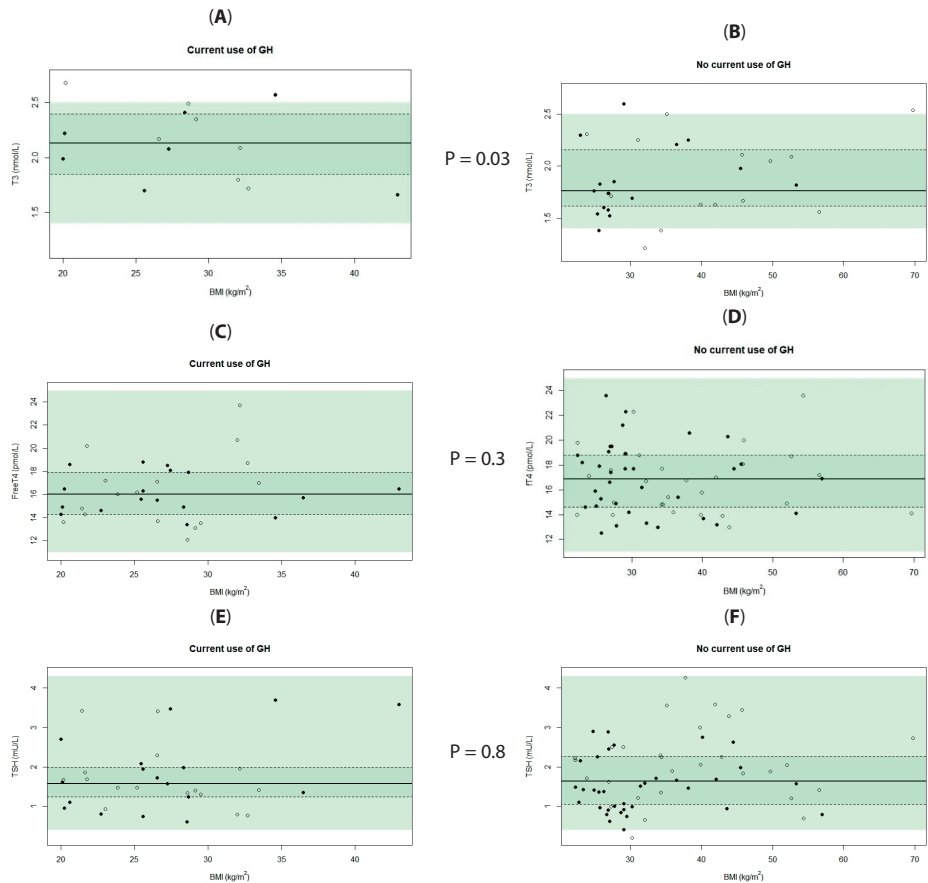
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Figure S2. Scatterplot of T3, fT4, and TSH in relation to BMI for patients who currently use growth hormone treatment versus patients who do not.

Abbreviations: body mass index (BMI), free thyroxine (fT4), triiodothyronine (T3), thyroid stimulating hormone (TSH). Only patients without hyperthyroidism, hypothyroidism or subclinical hypothyroidism are depicted in this figure. P-values for the relationship between the current use of growth hormone treatment and the thyroid hormone concentrations (T3, fT4 and TSH respectively) are shown in the middle. Legends: males are depicted by closed dots and females by open dots. The solid line is the median and the dashed line the interquartile range. The reference range is given in as a green, transparent rectangle. (**A**) T3 vs. BMI for patients who currently use growth hormone. (**B**) T3 vs. BMI for patients who do not currently use growth hormone. (**C**) fT4 vs. BMI for patients who currently use growth hormone. (**D**) fT4 vs. BMI for patients who do not currently use growth hormone. (**E**) TSH vs. BMI for patients who currently use growth hormone. (**F**) TSH vs. BMI for patients who do not currently use growth hormone. Reference values: TSH: before 1 February, 2019: 0.4-4.3 mU/L (n = 69), after 1 February, 2019: 0.56-4.27 mU/L (n = 28). fT4: before 12 April, 2019: 11-25 pmol/L (n = 73), after 12 April, 2019: 13.5-24.3 pmol/L (n = 22). T3: before 12 April, 2019: 1.4-2.5 (n = 33), after 12 April, 2019: 0.7-2.0 nmol/L (n = 12). Only the reference range that was valid for the most observations is shown.

7

Central adrenal insufficiency is rare in adults with Prader-Willi syndrome

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ABSTRACT

Context

Prader–Willi syndrome (PWS) is associated with several hypothalamic-pituitary hormone deficiencies. There is no agreement on the prevalence of central adrenal insufficiency (CAI) in adults with PWS. In some countries, it is general practice to prescribe stress-dose hydrocortisone during physical or psychological stress in patients with PWS. Side effects of frequent hydrocortisone use are weight gain, osteoporosis, diabetes mellitus, and hypertension—already major problems in adults with PWS. However, undertreatment of CAI can cause significant morbidity—or even mortality.

Objective

To prevent both over- and undertreatment with hydrocortisone, we assessed the prevalence of CAI in a large international cohort of adults with PWS. As the synacthen test shows variable results in PWS, we only use the metyrapone test (MTP) and insulin tolerance test (ITT).

Design

Metyrapone test or ITT in adults with PWS (n = 82) and review of medical files for symptoms of hypocortisolism related to surgery (n = 645).

Setting

Outpatient clinic.

Patients or Other Participants

Eighty-two adults with genetically confirmed PWS.

Main Outcome Measure

For MTP, 11-deoxycortisol > 230 nmol/L was considered sufficient. For ITT, cortisol > 500 nmol/L (Dutch, French, and Swedish patients) or > 450 nmol/L (British patients) was considered sufficient.

Results

Central adrenal insufficiency was excluded in 81 of 82 patients. Among the 645 patients whose medical files were reviewed, 200 had undergone surgery without perioperative hydrocortisone treatment. None of them had displayed any features of hypocortisolism.

Conclusions

Central adrenal insufficiency is rare (1.2%) in adults with PWS. Based on these results, we recommend against routinely prescribing hydrocortisone stress-doses in adults with PWS.

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INTRODUCTION

Prader–Willi Syndrome (PWS) is a rare and complex genetic disorder caused by the lack of expression of paternally inherited genes in the PWS region on chromosome 15q11-q13 (1). Apart from intellectual disability, sleep-related disorders and hypotonia, PWS is associated with hypothalamic dysfunction (1, 2), resulting in an insatiable appetite, disturbed thermoregulation, abnormal pain perception, and pituitary hormone deficiencies (2–4). In adults with PWS, growth hormone (GH) deficiency is reported in 0–38% (5, 6) and hypothyroidism in 13.6% (7) of patients. Hypogonadism is present in the majority of patients with PWS and can be either primary or central (8). There is no agreement on the prevalence of deficiencies in other pituitary hormones.

Mortality is high among patients with PWS (3% annual death rate across all ages) and death is often unexpected (9). It has been suggested that sudden death in patients with PWS might partly be explained by central adrenal insufficiency (CAI): an inadequate (increase in) cortisol production by the adrenal glands due to the insufficient secretion of adrenocorticotropic hormone (ACTH) or corticotropin-releasing hormone (CRH) by the pituitary gland or hypothalamus, respectively. If left untreated, CAI can result in an adrenal crisis, which is life-threatening. During crisis, a drop in blood pressure, organ failure, and/or mental alteration can lead to hospitalization or even treatment in the Intensive Care Unit.

Replacement with synthetic cortisol (hydrocortisone) is therefore advocated if patients have symptoms of CAI (10), which include muscle weakness, fatigue, and weight loss. However, these symptoms are unreliable in PWS. Muscle weakness and fatigue are common in PWS (11) and weight loss is not unusual, as most individuals with PWS are on a diet. In some countries, it is general practice to administer hydrocortisone during stressful situations, such as surgery, illness, or intense psychological stress (10, 12). However, stress and illness are often hard to define in individuals with PWS, as hypothalamic dysfunction reduces pain perception and the ability to mount a fever (11). Furthermore, the behavioral phenotype of PWS is characterized by frequent temper outbursts, causing psychological stress.

These uncertainties lead to frequent administration of hydrocortisone in people with PWS. Side effects of frequent overuse of hydrocortisone are weight gain, osteoporosis, diabetes mellitus, and hypertension (13), already major problems in adults with PWS (14). Ideally, hydrocortisone should only be prescribed when it is absolutely necessary.

There is no agreement on the prevalence of CAI and on the need for hydrocortisone use in adults with PWS, due to the use of different test methods and the fact that most studies involved children, not adults (10, 12, 15–21). In a previous Dutch study among 25 children with PWS, 15 (60%) were diagnosed with CAI based on ACTH levels during single-dose metyrapone tests (sMTP) using an ACTH cutoff < 33 pmol/L (12). However, the use of ACTH levels in the evaluation of the hypothalamic-pituitary-adrenal (HPA) axis has been debated, as it can lead to false-positive results (22). Studies using other test methods to diagnose CAI found much lower prevalences or even total absence of CAI in children (10, 16–21) and adults (15, 18). However, most studies used the synacthen test, which is adequate for diagnosing primary adrenal insufficiency (PAI) but less reliable for diagnosing CAI (23).

As both untreated CAI and overtreatment with hydrocortisone can have severe adverse consequences for the patient, it is important to know the true prevalence of CAI in adults with PWS. National PWS experts from 7 countries have collaborated to define the prevalence of CAI in 82 adults with PWS, which is a large group for such a rare disorder. As the use of less sensitive diagnostic tests causes uncertainty, we only report the results from the 2 most robust tests for diagnosis of CAI: the insulin tolerance test (ITT) and multiple-dose metyrapone test (MTP). Apart from collecting MTP and ITT data, we reviewed the medical records of 645 adults with PWS to define the true prevalence of CAI in adults with PWS.

METHODS

All participating centers obtained approval from ethics committees and/or individual patients to retrospectively collect data on the ITT and MTP performed in adults with PWS.

Part A: diagnosis of CAI

The HPA axis was tested in 56 Dutch, 10 French, 10 British, and 6 Swedish adults with PWS as part of regular patient care. Part of the data on the 6 Swedish patients has been published previously (18). Eight adults with PWS did not undergo ITT/MTP, because they used daily hydrocortisone based on synacthen test failure or extremely low baseline cortisol. Although diagnosis of CAI was not based on MTP or ITT, the patients were not retested by MTP or ITT due to behavioral issues or for other patient-related reasons.

Metyrapone test procedure

Patients were hospitalized for 2 consecutive days. On day one, after a 10- to 12-hour overnight fast, blood samples for ACTH and cortisol were taken at 7:45 am, and metyrapone (750 mg, Laboratoire HRA Pharma, Paris, France) was administered orally at 8:00 am, 12:00 pm, 4:00 pm, 8:00 pm, 12:00 am, and 4:00 am. Patients were fed at 6:00 pm. On day 2, blood samples for cortisol and 11-deoxycortisol were taken at 7:45 am after a 10- to 12-hour overnight fast. Blood samples were taken through a peripheral intravenous catheter. Patients were recumbent from 7:00 am until the blood collection was completed. To ensure appropriate cortisol suppression, we used a day 2 morning cortisol cutoff of 200 nmol/L.

To assess the clinical value of ACTH during MTP in the diagnosis of CAI, ACTH was also measured. Delta ACTH was defined as the difference between ACTH at the start of the test (baseline ACTH) and the peak ACTH level.

Insulin tolerance test procedure

Patients were hospitalized for 1 day. After a 10- to 12-hour overnight fast, short-acting insulin (Insuman Rapid®, Actrapid®, 0.15U/kg) was administered at t = 0 in order to achieve hypoglycaemia (blood glucose ≤ 2.2 mmol/L). Blood samples for cortisol and glucose were taken at t = 0, 30, 60, and 90 minutes through a peripheral intravenous catheter. In Dutch and British patients, ACTH was also measured. Additional insulin (dose based on the actual glucose level and weight of the patient) was administered if the glucose level was ≥ 2.2 mmol/L at t = 60, unless patients showed severe clinical signs of hypoglycaemia, and blood samples were taken at t = 80, 90, 100, 120, and 150 minutes. The patients were recumbent from the start of the study until the final blood sample was collected. If women were taking oral estrogens, these were stopped at least 6 weeks before the ITT to avoid artefactual elevations of measured cortisol due to increased levels of cortisol-binding globulin. Corticosteroids were ceased at least 1 week before testing, both for MTP and ITT.

Assays

Adrenocorticotropic hormone and cortisol levels were measured with Siemens Immulite 2000XPi (Dutch patients; British patients for all ACTH measurements and for cortisol before August 2010), chemiluminescence immunoassay Abbott Architect i2000 (British patients for cortisol measurements after August 2010, to which all British cortisol results were aligned based on a field comparison study), immunochimiluminescence Roche Cobas (French patients), or electrochemiluminescence immunoassay Elecsys, Roche (Swedish patients). Blood glucose was measured with Roche Cobas C (Dutch, French, and Swedish patients) and Abbott Architect i2000 (British patients). 11-deoxy-

cortisol was measured with UPLC-MSMS (Waters TQS, Etten-Leur, the Netherlands) in all patients. For MTP, 11-deoxycortisol > 200 nmol/L (>230 nmol/L or 7.9 µg/dL in the Dutch center due to harmonization) was considered sufficient (24). For Dutch, French, and Swedish patients who underwent ITT, cortisol > 500 nmol/L (18.1 µg/dL) was considered sufficient, whereas for British patients cortisol > 450 nmol/L (16.3 µg/dL) was considered sufficient (after alignment of the previous > 500 nmol/L [18.1 µg/dL] cutoff from Siemens Immulite 2000 assay to the Abbott Architect i2000 assay). The reference range for baseline cortisol was 200–700 nmol/L.

Part B: patient file review

We reviewed the medical files of all 645 adult patients with PWS who visited the centers participating in the International Network for Research, Management & Education on Adults with PWS: Italy (240), France (110), the Netherlands (110), Australia (60), Spain (45), Sweden (38), and the UK (42). We collected clinical data to determine rates and means of diagnosis of CAI, the number of patients on continuous hydrocortisone treatment, and the number of patients that underwent surgery with and without stress doses of hydrocortisone.

Part C: literature review

We performed a PubMed search and reviewed the medical literature for studies that have assessed adrenal function in > 1 patient by dynamic testing of the HPA axis. We used the following search strategy: "Prader–Willi Syndrome" [Mesh] AND "adrenal" [All Fields].

Data analysis

Data were analyzed with R version 3.6.0. Continuous data are presented as median (range) and categorical data are presented as count. We calculated Spearman's rho for the analysis of correlations. P-values of < 0.05 were considered significant.

Role of the funding source

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Table 1. Characteristics of the study population

Patients with PWS	ITT (n = 36)			MTP (n = 46)			Total (n = 82)		
	Male	**Female**	**All**	**Male**	**Female**	**All**	**Male**	**Female**	**All**
n	19	17	36	27	19	46	46	36	82
Nationality									
British	5	5	10	0	0	0	5	5	10
Dutch	6	4	10	27	19	46	33	23	56
French	3	7	10	0	0	0	3	7	10
Swedish	5	1	6	0	0	0	5	1	6
Age (years)									
Median	25.0	24.0	24.9	28.0	22.5	25.3	25.9	23.5	25.1
Range	18.0 – 36.0	18.0 – 55.3	18.0 – 55.3	18.1 – 55.5	18.2 – 39.0	18.1 – 55.5	18.0 – 55.5	18.0 – 55.3	18.0 – 55.5
BMI (kg/m²)[d]									
Median	28.3	32.0	30.3	27.4	31.5	28.4	28.2	31.7	29.1
Range	21.2 – 62.0	20.3 – 58.2	20.3 – 62.0	20.0 – 57.0	21.2 – 49.7	20.0 – 57.0	20.0 – 62.0	20.3 – 58.2	20.0 – 62.0
Genotype									
mUPD[e]	2	5	7	10	8	18	12	13	25
DEL[f]	9	9	18	16	10	26	25	19	44
ICD[g]	1	0	1	0	0	0	1	0	1
mUPD or ICD	1	2	3	0	0	0	1	2	3
mDEL[h]	1	0	1	0	0	0	1	0	1
Methylation positive	5	1	6	1	1	2	6	2	8
GH[i] treatment during childhood	4	6	10	11	13	24	15	19	34
Current GH treatment	3	1	4	8	11	19	11	12	23

Abbreviations: body mass index (BMI), paternal deletion (DEL), growth hormone (GH), imprinting center defect (ICD), insulin tolerance test (ITT), *SNORD116* microdeletion (mDEL), multiple-dose metyrapone test (MTP), uniparental maternal disomy (mUPD), Prader–Willi syndrome (PWS).

RESULTS

Eighty-two patients (46 males, 36 females) were tested for CAI. Forty-six patients underwent MTP and 36 patients underwent ITT. None of the patients underwent both tests. Patient characteristics are shown in **Table 1**.

Multiple-dose metyrapone test

The results of the MTP are shown in **Table 2** and **Figure 1**. All patients' 11-deoxycortisol levels were above 230 nmol/L (median 440.1, range 247.8–694.0 nmol/L). In 2 patients, the day 2 morning cortisol was above the cutoff of 200 nmol/L, namely 213 and 211 nmol/L. Although this was suggestive of inadequate inhibition of 11-β hydroxylase, it still provoked an adequate increase of 11-deoxycortisol (298.2 and 425.3 nmol/L at day 2), confirming that the function of the HPA axis was normal.

Table 2. Results of the multiple-dose metyrapone test

	Before[a]		After[b]			Delta[c]	CAI Cut-off[d]
	ACTH (pmol/L)	Cortisol (nmol/L)	ACTH (pmol/L)	Cortisol (nmol/L)	11-Deoxycortisol (nmol/L)	ACTH (pmol/L)	11-Deoxycortisol (nmol/L)
Median	3.5	325.5	37.7	70.0	440.1	33.4	<230
Range	1.3 – 16.2	126.0 – 764.0	2.8 – 132.0	28.0 – 213.0[e]	247.8 – 694.0	-1.4 – 118.9	

Abbreviation: central adrenal insufficiency (CAI). [a] Before metyrapone administration. [b] After metyrapone administration. [c] Increase in ACTH after metyrapone administration. [d] 11-deoxycortisol cutoff for diagnosis of CAI. [e] In 2 patients, the day 2 morning cortisol was above the cutoff of 200 nmol/L, namely 213 and 211 nmol/L. Although this was suggestive of inadequate inhibition of 11-β hydroxylase, it still provoked an adequate increase of 11-deoxycortisol (298.2 nmol/L and 425.3 at day 2), showing function of the HPA axis was normal.

There was no significant relation between baseline cortisol and 11-deoxycortisol after metyrapone administration (ρ = 0.16; P = 0.28), as shown in **Figure 1A**. All patients with a baseline cortisol below the lower reference limit of 200 nmol/L (lowest: 126.0 nmol/L) had a sufficient 11-deoxycortisol response.

The median (range) ACTH level after metyrapone administration was 37.7 (2.8–132.0) pmol/L. The ACTH level during MTP correlated poorly with 11-deoxycortisol level (ρ = 0.35; P = 0.02; **Figure 1B**), as did delta ACTH (ρ = 0.38; P = 0.01).

Insulin tolerance test

The results of the ITT are shown in **Table 3**. Only 2 patients did not reach the target hypoglycaemia of ≤ 2.2 mmol/L as at near-target glucose levels (2.6 mmol/L in 1 patient and 2.4 mmol/L in the other); they already had clinical signs of severe hypoglycaemia (somnolence, reduced arousal, and increased perspiration) such that it was considered unethical to administer more insulin.
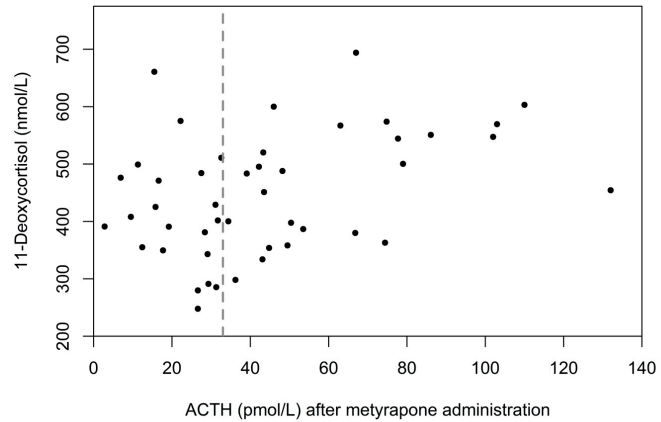
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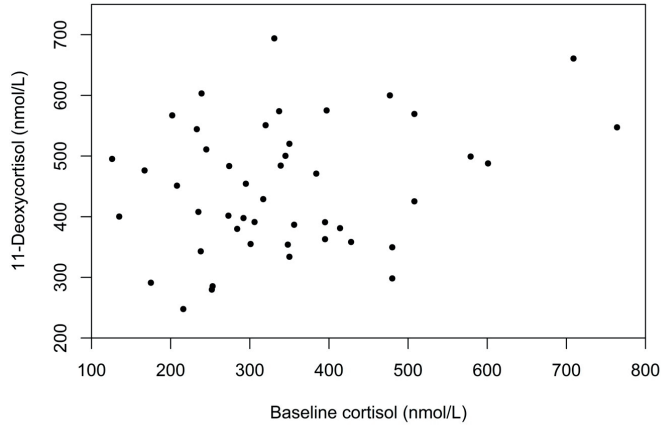
B

Figure 1. Results of the multiple-dose MTP in patients with Prader–Willi syndrome. n = 46. **A**: Relation between base-line cortisol (nmol/L) and 11-deoxycortisol (nmol/L). Spearman's rho was 0.16 (P = 0.28). Even patients with low baseline cortisol had normal 11-deoxycortisol levels. **B**: Relation between ACTH (pmol/L) after metyrapone administration and 11-deoxycortisol (nmol/L). Spearman's rho was 0.35 (P = 0.02). The dotted line represents the cutoff of 33 pmol/L used by the Dutch pediatric study (12), which would falsely classify 21 patients with sufficient increase in 11-deoxycortisol levels as "adrenal insufficient."

During the ITT, 35 of 36 patients (including the 2 who did not reach hypoglycaemia of ≤ 2.2 mmol/L) had peak cortisol levels above the cutoff of 500 nmol/L. Only 1 French patient, who had no physical signs of CAI but was tested because of the transition from pediatric to adult care, had a suboptimal peak cortisol level of 494 nmol/L. He was pre-scribed hydrocortisone for use during physical stress. Since it was very difficult to obtain intravenous access in this patient, the ITT was not repeated.

Table 3. Results of the insulin tolerance test

	Baseline Cortisol (nmol/L)	Peak Cortisol (nmol/L)	Glucose (mmol/L) [a]	Baseline ACTH (pmol/L)	Peak ACTH (pmol/L)	Delta ACTH (pmol/L)[b]	CAI Cut-off (nmol/L) [c]
France (n = 10)							
Median	229.0	735.5	1.6	N/A	N/A	N/A	<500
Range	102.0 – 384.0	494.0 – 1021.0	0.6 – 2.2	N/A	N/A	N/A	
The Netherlands (n = 10)							
Median	233.0	702.0	1.9	3.3	61.2	57.1	<500
Range	119.0 – 502.0	530.0 – 883.0	1.4 – 2.4	1.1 – 6.2	23.8 – 93.5	21.2 – 90.5	
Sweden (n = 6)							
Median	185.5	722.5	1.7	N/A	N/A	N/A	<500
Range	175.0 – 265.0	502.0 – 822.0	1.2 – 2.6	N/A	N/A	N/A	
United Kingdom (n = 10)							
Median	172.5	522.5	1.5	3.7	N/A	N/A	<450
Range	93.0 – 545.0	455.0 – 971.0[d]	1.0 – 2.1	1.7 – 6.4	N/A	N/A	

Abbreviations: central adrenal insufficiency (CAI), not available (N/A). [a] Two patients had glucose levels of 2.4 mmol/L and 2.6 mmol/L, respectively. All other patients had glucose levels ≤ 2.2 mmol/L. [b] Increase in ACTH after insulin administration. [c] Peak cortisol cutoff for diagnosis of CAI. One French patient had peak cortisol < 500 nmol/L. [d] In the UK, the cutoff for CAI is 450 nmol/L (see also: methods).

The peak cortisol correlated poorly with peak ACTH (ρ = -0.04; P = 0.91) and delta ACTH during ITT (ρ = 0.05; P = 0.88).

Reviewing medical files

We reviewed the medical files of 645 adult patients with PWS. Six French, 1 Australian, and 1 British patient used daily hydrocortisone based on a previous low morning cortisol, low-dose short synacthen test or high-dose short synacthen test. Two Dutch patients did allow to be retested, although they used daily hydrocortisone (1 based on a CRH test and the other after an event during surgery, which at that time was misinterpreted as an adrenal crisis). In these patients, hydrocortisone was successfully tapered and CAI was excluded by MTP.

As Dutch guidelines recommend the use of hydrocortisone during physical or psychological stress, even in patients without proven CAI, 30 of the 110 Dutch patients whose medical files were reviewed, received hydrocortisone during surgery (without performing an HPA function test first). Eighteen of these 30 subjects were subsequently formally tested for CAI (15 MTP; 3 ITT) and all of them were found to have sufficient HPA function, ie, no indication for perioperative hydrocortisone. Fifty-three Dutch patients had surgery without hydrocortisone, as they were operated before the guidelines were published. None of them had any complications during or after surgery. Twenty-six of

these 53 subjects were subsequently tested for CAI (20 MTP; 6 ITT) and all were found to be sufficient. None of the 535 non-Dutch patients received hydrocortisone stress-dose during illness or surgery without undergoing an HPA function test (**Table 4**).

Table 4. Review of medical files of adult patients with Prader-Willi syndrome

Country	Patient files reviewed (n)	Surgery with HC (n)	Surgery without HC (n)	Adrenal crisis during surgery (n)
Italy	240	0	97	0
UK	42	0	13	0
Sweden	38	0	8	0
Spain	45	0	7	0
France	110	0	9	0
Australia	60	1[a]	13	0
The Netherlands	110	30[b]	53[c]	0
Total	645	31	200	0

Abbreviation: hydrocortisone stress dose (HC). [a] The patient had been using daily hydrocortisone after an insufficient low-dose synacthen test. [b] 2 patients had been using daily hydrocortisone but were later tested sufficient; 28 had been using hydrocortisone during operation (16 of them were later tested sufficient). [c] 26 patients later tested sufficient (20 multiple-dose metyrapone test, 6 insulin tolerance test).

In total, of the 645 patients whose files were reviewed, 200 underwent surgery without the administration of stress doses of hydrocortisone. None of them displayed any features of hypocortisolism or adrenal crisis.

Based on ITT and MTP, the prevalence of CAI in the 82 adults with PWS was 1.2%. Findings from our study and those from other groups (10, 12, 15–21) are detailed in **Table 5**.

DISCUSSION

We tested the HPA axis in 82 adult patients with PWS and conclude that CAI is very rare (1.2%) in adults with PWS. This low prevalence of CAI is in line with the majority of studies investigating CAI in people with PWS (10, 15–21) (**Table 5**) but is in sharp contradiction with the Dutch pediatric study by De Lind van Wijngaarden et al (12), who diagnosed CAI in 60% of Dutch children with PWS.

A likely explanation for the discrepancy between the Dutch pediatric study and the other studies investigating CAI in people with PWS is the difference in the type of provocative test used. The different types of provocative tests used for diagnosing CAI are described in the supplementary data (**Table S2**), which are located in a digital data repository (25).

Table 5. Summary of studies investigating the prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome

Study	n	Median age, years (range)	GH treatment (%)	Testing method	Prevalence (%)
Lind van Wijngaarden, et al. (2008)[12]	25	9.7 (3.7 – 18.6)	100	sMTP	60
Connell, et al. (2010)[17]	4 6 15	7.16 (0.43 – 16.27)	N/A	LDSST HDSST ITT	4
Nyunt, et al. (2010)[16]	41	7.68 (±5.23)[a]	46	LDSST	0
Farholt, et al. (2011)[18]	58 8	22 (0.42 – 48)	62	HDSST ITT	0 0
Corrias, et al. (2012)[10]	84 9[b]	7.7 (±5.0)[a]	63	LDSST HDSST	14.2 4.8
Grugni, et al. (2013)[15]	53 6[b]	27.9 (18.0 – 45.2)	30	LDSST HDSST	15 7.5
Beauloye, et al. (2015)[21]	14 7[c]	4.55 (0.8 – 14.7) 5.6 (3.5 – 14.4)	25	GT ITT	5
Obrynba, et al. (2018)[19]	21[d]	13.9 (±10.9)[a]	76	LDSST sMTP	29 0
Oto, et al. (2018)[20]	36	2.0 (0.6 – 12)	0	ITT	0
This study (2019)	46 36	25.3 (18.1 – 55.5) 24.9 (18.0 – 55.3)	28	MTP ITT	0 2.8

Abbreviations: glucose tolerance test (GT), high-dose synacthen test (HDSST), insulin tolerance test (ITT), low-dose synacthen test (LDSST), multiple-dose metyrapone test (MTP), not available (N/A), single-dose metyrapone test (sMTP). [a] Age expressed as mean ± SD. [b] Number of subjects who failed the LDSST and underwent HDSST confirmation test. [c] 1 subject was tested by GT and ITT. [d] All subjects were tested by LDSST and sMTP.

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In the Dutch pediatric study (12), the sMTP was used to assess the prevalence of CAI. Patients were considered as having CAI when postmetyrapone ACTH levels were < 33 pmol/L (150 pg/mL) (26). However, a Dutch reference range study (24) showed that ACTH levels during sMTP in healthy adult volunteers ranged from 9.2 to 211.0 pmol/L (42–960 pg/mL), which suggests that the cutoff used in the Dutch pediatric study (<33 pmol/L) is too high, giving substantial false-positive results. Other studies have also debated the use of ACTH levels in the evaluation of the HPA axis function, as it can lead to false-positive results, and recommended that the assessment of CAI should be based on 11-deoxycortisol (19, 27). Our study also confirmed the inferiority of ACTH cutoff of 33 pmol/L in the interpretation of the MTP: 21 of 46 patients who tested sufficient based on 11-deoxycortisol had peak ACTH levels < 33 pmol/L (**Figure 1b**). This implies that 45.7% of our patients would have tested false-positive and would be given hydrocortisone treatment based on the ACTH cutoff used in the Dutch pediatric study (12). Some patients showed only minimal ACTH increase during MTP, whereas their 11-deoxycortisol levels strongly increased (**Table 2** and **Figure 1**). In 1 patient with a sufficient 11-deoxycortisol response, the ACTH level even decreased during MTP. Also,

Delta ACTH correlated poorly with the 11-deoxycortisol level. These results confirm that the ACTH level during the MTP is not a reliable parameter to diagnose CAI.

An alternative explanation for the difference in test results could be that the MTP suppresses the HPA for 24 hours, whereas the sMTP, used in the Dutch pediatric study, suppresses the HPA only briefly. The administration of multiple metyrapone doses might give the patient more time to produce adequate 11-deoxycortisol levels, leading to higher 11-deoxycortisol levels. The sMTP, in which metyrapone is administered once at midnight and blood samples are collected between 8:00 am and 9:00 am, might better mimic the real-life situation in which an acute event (infection, surgery) requires a fast response of the HPA. However, this explanation seems unlikely, as CAI prevalences found by our "slow," multiple-dose MTP are equally low as those found during ITT (in which there is an acute, short stimulation of the HPA). Furthermore, Obrynba et al, who used the sMTP (requiring a fast response), also found a low prevalence of CAI (0%) (19).

Another hypothetical explanation for the low rates of CAI in adults compared to children is that all children with CAI may have died before reaching adulthood. However, based on the incidence of PWS of around 1:16.000 live births (28) and the overall death rate in PWS of approximately 3% per year (9), this is very unlikely. Yearly, 170.000 children are born in the Netherlands (Central Bureau for Statistics, 2019) of whom 10 would be expected to have PWS. Thus, in the last 55 years, approximately 550 people with PWS are likely to have been born. If 60% of the children with PWS had CAI and all died before reaching adulthood (apart from the regular PWS mortality of 3% per year), we would expect only 220 (40% of 550) patients would be alive, of which 152 would be adults. However, in the Dutch national center of reference, over 110 adults with PWS were registered at the moment of submission of this manuscript, and we know this is far from the total Dutch adult PWS population. Therefore, the assertion that the lower rates of CAI in adults with PWS are explained solely by excess mortality due to CAI is highly unlikely.

A last theoretical explanation could be the difference in GH treatment between the Dutch pediatric study and our study. In the Dutch pediatric study all patients received GH treatment as part of a clinical trial, compared to only 28% in our study. This difference might be relevant as untreated GH deficiency may mask CAI. Low insulin-like growth factor I (IGF-I) levels result in increased expression and activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the enzyme that converts cortisone to cortisol (29). Therefore, untreated GH deficiency may result in increased, thus falsely normal, cortisol levels. However, none of our patients had untreated GH deficiency, as all patients were tested for GH deficiency as part of regular care. Besides, we saw no differences in the peak cortisol levels between GH-treated patients and non-GH treated patients. Further-

more, in a study by Obrynba et al (19), 76% of the patients received GH treatment and none of them were diagnosed with CAI. This suggests that the low CAI prevalence that we found is not explained by untreated GH deficiency.

The review of the medical files of 645 adults with PWS attending PWS centers worldwide revealed that none of the 200 patients who underwent surgery without using hydrocortisone displayed any symptoms of hypocortisolism or adrenal crisis. This finding is in line with the results of the MTP and ITT, demonstrating that CAI is virtually absent in adults with PWS.

Only 1 patient was diagnosed with CAI, based on a peak cortisol level of 494 nmol/L during the ITT, which is just under the cutoff of 500 nmol/L. We calculated the 95% confidence interval (CI) and intra-assay coefficient of variation (VC) of the cortisol assay over 1 year to better understand the significance of this borderline-low value. The VC percentage was 6.9 and the 95% CI was 496 to 504 nmol/L; therefore, in the statistical analysis, this single patient was scored as having CAI.

To prevent further overtreatment of adults with PWS, our results will be implemented in a new guideline on the clinical management of adults with PWS. The lack of reliability of ACTH in the diagnosis of CAI will also be emphasized in the new guidelines.

In conclusion, CAI is very rare (1.2%) in adults with PWS. In order to prevent overtreatment with hydrocortisone, we advise against routine hydrocortisone administration during psychological stress, illness, or surgery in adults with PWS. In patients in whom there is a significant clinical suspicion of hypocortisolism (such as apathy, fainting, or observed hypotension during acute infections or other stressful events), we recommend testing to exclude CAI and only administer hydrocortisone if CAI is confirmed by ITT or (s)MTP.

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Hyperprolactinemia in adults with Prader-Willi syndrome

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ABSTRACT

Prader-Willi syndrome (PWS) is a rare neurodevelopmental genetic disorder typically characterized by body composition abnormalities, hyperphagia, behavioural challenges, cognitive dysfunction, and hypogonadism. Psychotic illness is common, particularly in patients with maternal uniparental disomy (mUPD), and antipsychotic medications can result in hyperprolactinemia. Information about hyperprolactinemia and its potential clinical consequences in PWS is sparse. Here, we present data from an international, observational study of 45 adults with PWS and hyperprolactinemia. Estimated prevalence of hyperprolactinemia in a subset of centres with available data was 22%, with 66% of those related to medication and 55% due to antipsychotics. Thirty-three patients were men, 12 women. Median age was 29 years, median BMI 29.8 kg/m^2, 13 had mUPD. Median prolactin was 680 mIU/L (range 329–5702). Prolactin levels were higher in women and patients with mUPD, with only 3 patients having severe hyperprolactinemia. Thyroid function tests were normal, 24 were treated with growth hormone, 29 with sex steroids, and 20 with antipsychotic medications. One patient had kidney insufficiency, and one a microprolactinoma. In conclusion, severe hyperprolactinemia was rare, and the most common aetiology of hyperprolactinemia was treatment with antipsychotic medications. Although significant clinical consequences could not be determined, potential negative long-term effects of moderate or severe hyperprolactinemia cannot be excluded. Our results suggest including measurements of prolactin in the follow-up of adults with PWS, especially in those on treatment with antipsychotics.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare and complex neurodevelopmental disorder, characterized by hypothalamic dysfunction. PWS is caused by a lack of expression of paternally inherited genes in the PWS region of chromosome 15q11–13 (1). Approximately 65–70% of the patients have a paternal deletion, 30% a maternal uniparental disomy (mUPD), 2–5% an imprinting defect, and 0.1% chromosomal translocation (1,2). In adults, PWS is clinically characterized by endocrine deficiencies, hyperphagia, obesity and its associated comorbidities, intellectual disability, and a characteristic neuropsychological and behavioural profile (1,2). The incidence of psychosis in adults with PWS is high (10–20%), particularly in those with the mUPD genotype (1,2). Hypogonadism with various degrees of genital hypoplasia, delayed or incomplete pubertal development, and infertility is seen in about 90% and involves both hypothalamic and primary gonadal abnormalities (1-6). PWS is associated with hypogonadism irrespective of prolactin levels.

Prolactin is produced by the lactotroph cells in the anterior gland of the pituitary (7,8). Dopamine from the hypothalamus exerts a tonic inhibition on pituitary prolactin production and secretion (7,8). Any condition, hypothalamic disorder or medication interfering with dopamine secretion or action (e.g., antipsychotics, some antidepressants) might lead to an increase in prolactin levels, i.e., hyperprolactinemia (7,8,9). Prolactin can also be released from the hypothalamic paraventricular nucleus and medial pre-optic area, in response to physiological stimuli, including stress (7,8). The main physiological effects of prolactin are enlargement of breasts during pregnancy, milk production, amenorrhea during breast feeding, and stimulation of immune system-growth factors (7,8).

Hyperprolactinemia inhibits the pulsatile secretion of gonadotropin releasing hormone (GnRH), and thereby the production of gonadotropins (FSH and LH), which results in a reduction of sex hormones and development of hypogonadism (7,8,10,11). Hypogonadism can cause decreased libido, oligo- or amenorrhea and infertility in women, and decreased libido, impotence, infertility, and gynecomastia in men (7,8,10,11). Galactorrhoea might be present in both genders, but is more prevalent in women. Osteoporosis is common among patients with hyperprolactinemia and is considered to be the result of hyperprolactinemia-induced hypogonadism (11). Furthermore, severe hyperprolactinemia can lead to unfavourable metabolic effects, including increased blood glucose, LDL-cholesterol and triglycerides levels and has been shown to increase cardiovascular mortality in non-PWS males (7,8,12,13). Treatment with dopamine agonists such as cabergoline can reverse these effects as well as decrease body mass index (BMI) and total body fat in patients with hyperprolactinemia (13).

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Among the adverse effects of hyperprolactinemia, it is of particular importance in PWS to consider the potential unfavourable metabolic effects due to the hyperphagia, a decreased basal metabolic rate and high risk of developing morbid obesity (2,14). Several causes of hyperprolactinemia might be present in PWS. For example, the patients are easily stressed when routines are changed, and many patients have psychotic illnesses, depression, or mood instability treated with antipsychotics (dopamine antagonists) or antidepressants (tricyclics or selective serotonin reuptake inhibitors, SSRIs) that can cause hyperprolactinaemia (9,14). To date, high levels of prolactin in PWS adults have been reported anecdotally in both sexes, especially during therapy with psychotropic medications, and a thorough assessment of hyperprolactinemia in PWS is lacking. The knowledge about prolactin levels in PWS is sparse, and the aim of this study was to characterize adults with PWS and hyperprolactinemia.

PATIENTS AND METHODS

Data was collected from adults with PWS from reference centres for PWS in Rotterdam (Netherlands), Rome and Piancavallo (Italy), London (UK) and Stockholm (Sweden). Demographic data, BMI, co-morbidities, medication, and results of blood tests were collated from the patients' medical records.

All clinic visits and assessments were part of routine clinical practice. BMI was calculated as weight divided by the square of height in meters, kg/m^2. BMI from 18.5–25 kg/m^2 was defined as normal, between 25–30 kg/m^2 as overweight and above 30 kg/m^2 as obese, according to the WHO criteria. Results for analyses of prolactin, sex hormones, gonadotropins, TSH, free thyroxine (fT4), free triiodothyronine (fT3), kidney, and liver function were retrieved from the patients' medical records. Analyses were performed according to local assays. The same methodology for analysis was used in the participating sites, but with different equipment, see **appendix Table A1**. All prolactin concentrations and reference values were converted to mIU/L. The overall reference range for prolactin in men was 0–500 mIU/L between centres (lower reference limit between 0 and 100; upper reference limit between 300 and 500) and for women 85–700 mIU/L (lower reference limit between 85 and 102; upper reference limit between 490 and 700).

In the individual centres, it was also noted if prolactin concentrations had been measured on just a single occasion, if macroprolactin (an immunological artefact of no known clinical significance that raises measured prolactin concentrations (15)) was analysed, and if performed, the results of MRI or CT imaging of the pituitary to exclude a visible pituitary adenoma.

This study was approved by the ethical committee of participating centres and/or informed consent was collected from individual patients in accordance with national laws and regulations.

Statistics

Data are presented as median (range). Statistical analysis was performed using SPSS (version 26.0 for Mac, IBM Corp., Armonk, NY, USA). Differences between groups were calculated by Mann–Whitney U-test. A two-way ANCOVA was conducted to examine differences in prolactin levels depending on gender and the two most common genetic factors in the study; paternal deletion and mUPD. In this analysis, age was entered as a covariate. Statistical significance was set at $P < 0.05$.

RESULTS

A total of 441 adults with PWS were seen in the participating clinics. Among them 45 patients (33 men and 12 women) (15 from the Netherlands, seven from Italy, ten from United Kingdom and 13 from Sweden) had hyperprolactinemia. The hyperprolactinemia was diagnosed both without clinical suspicion, and by routine measurements. Repeated prolactin measurements (more than once) were performed in 37 patients, in whom prolactin remained elevated, while in eight patients the prolactin was measured only on one occasion.

It was not possible to determine the true prevalence of hyperprolactinemia in the whole cohort, since not all patients had routine measurements of prolactin or had data available on the number of patients with a non-elevated prolactin. However, in those centres where all prolactin results were available, and measurement was part of a routine assessment, the prevalence of hyperprolactinemia was as follows: Netherlands, 15 out of 89 (16.9%) with 73% of those related to medication (62% of total with hyperprolactinemia on antipsychotics), UK, 10 out of 50 (20.0%), with 50% of those related to medication (40% antipsychotics), and Sweden, 13 out of 33 (39.4%) with 69% of those related to medication (62% antipsychotics), giving an average prevalence of 38 out of 172 (22.1%), with 66% of those related to medication (55% antipsychotics).

The patients with hyperprolactinemia had a median age of 29 years (range 19–58) and median BMI was 29.8 kg/m^2 (range 20.5–45.5) (**Table 1**). Twenty-four patients had a paternal deletion (53.3%), 13 mUPD (31.1%) and three an imprinting defect or chromosomal translocation (6.7%). Four were *SNRPN* methylation positive for PWS, but further genetic characterization was not available (8.9%). The distribution of patients from each site were similar regarding gender and genotypes.

Table 1. Characteristics of 45 adults with Prader-Willi syndrome and hyperprolactinemia.

Demographics	n = 45
Men/women (n/n)	33/12
Age (years)	29 (19–58)
BMI (kg/m^2)	29.8 (20.5–45.5)
Treatment with sex hormones (n)	29
Untreated hypogonadism (n)	16
Treatment with growth hormone (n)	24
Treatment with antipsychotics (n)	20
Treatment with serotonin re-uptake inhibitors (SSRI)	9
Pituitary microadenoma	1
Hormone values	
TSH (mU/L)	1.6 (0.3-3.8)
Free T4 (pmol/L)	15 (8.2-22.2)
Free T3 (pmol/L)	4.4 (2.9-5.4)
Prolactin (mIU/L)	680 (329-5950)

Results are shown as median and range, unless otherwise specified.

Median prolactin was 680 mIU/L (range 329–5950), 615 mIU/L in men and 1061 mIU/L in women (**Figure 1**), and 625 mIU/L (range 360–1190) in patients with paternal deletion and 695 mIU/L (range 337–1421) in patients with mUPD (**Figure 2**). One man and two women had prolactin levels >2200 mIU/L. Using two-way ANCOVA to assess the patients with paternal deletion and mUPD, we found higher prolactin levels in patients with mUPD compared to those with paternal deletion (P = 0.005), and higher prolactin in women compared to men (P = 0.003). For the two-way ANCOVA analysis, two outliers with paternal deletion were excluded; one with a known microprolactinoma and one being the only patient from a site and suspected to be due to a different laboratory methodology.

Macroprolactin was not found in any of the 17 patients in whom it was looked for (nine out of 10 patients in UK, seven out of seven patients in Italy and one patient out of 15 patients in The Netherlands).

Median serum TSH was 1.6 mU/L (range 0.3–3.8), fT4 15 pmol/L (range 8.2–22.2) and fT3 4.4 pmol/L (range 2.9–5.4). Several different assay methods were used, but thyroid function was determined as normal in all patients; in particular, none of the patients had primary hypothyroidism with TSH above the local reference value. Two patients (4.4%) were treated with levothyroxine. Twenty-four patients (53.3%), 17 men and seven women, were treated with growth hormones. One patient had renal insufficiency (2.2%), while none of the patients had liver insufficiency. Four patients had type 2 diabetes

mellitus (8.9%). Twenty-two men and seven women were treated with sex steroids for hypogonadism. Sixteen other patients had untreated hypogonadism.
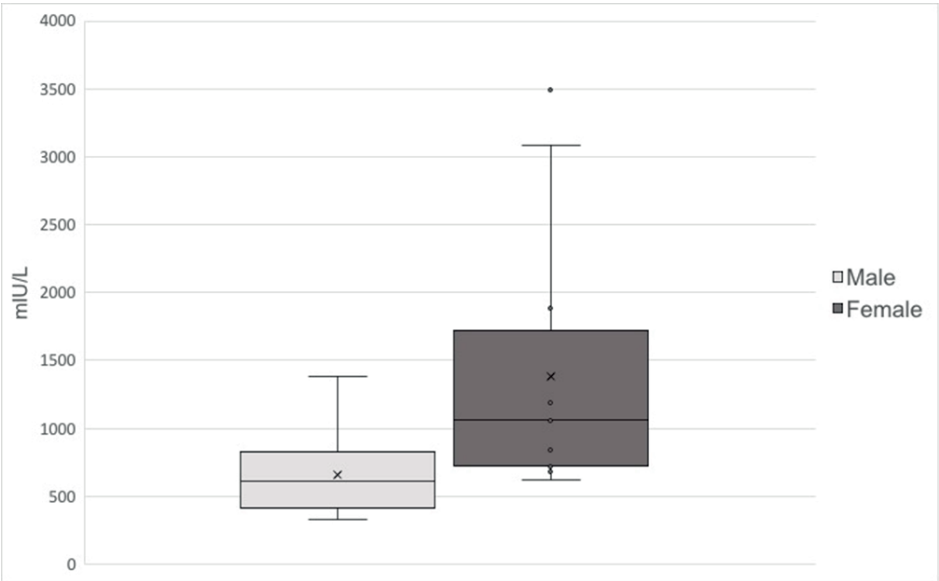

Figure 1. Prolactin levels in 33 men and 12 women with PWS and laboratory confirmed hyperprolactinemia.
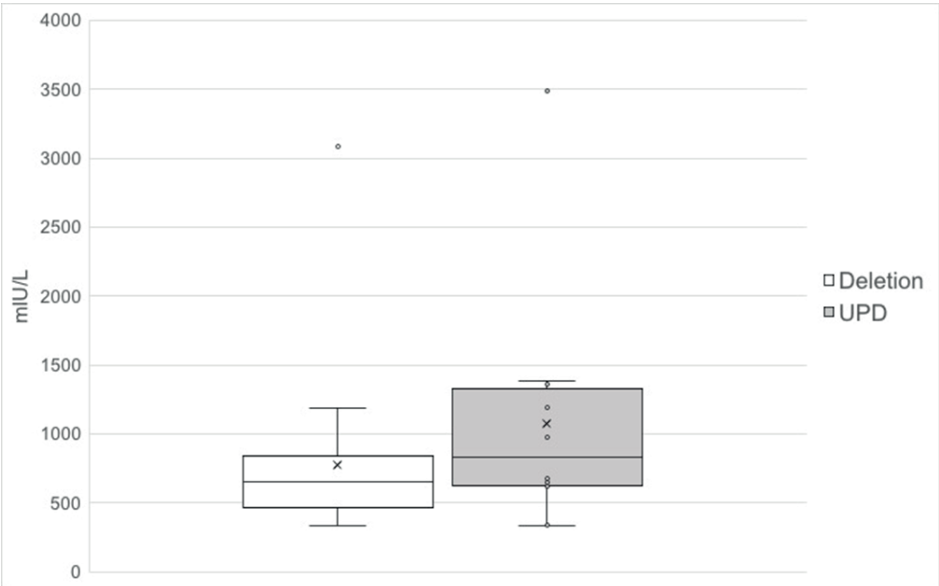

Figure 2. Prolactin levels in 24 adults with paternal chromosome 15q11–13 deletion and 14 adults with chromosome 15 maternal uniparental disomy.

For most of the patients, behavioural or psychiatric problems were reported. In total, 28/45 (62%) were on a medication known to cause hyperprolactinemia. Among them, 14 men and four women were treated with risperidone, two men with methoprazine, three men with quetiapine, one man with piamperone, one man with olanzapine, and one woman with levopromazin, totalling 25) on an antipsychotic medication (56% of all patients with raised prolactin), while 9 (20%) were on an SSRI (sertraline, fluoxetine or citalopram), of whom three were also on an antipsychotic medication.

Only twelve patients (26.7%) underwent radiological examinations (11 MRI, one CT) of the pituitary or brain. Amongst this one man had a cystic microprolactinoma with a prolactin of 5702 mIU/L at diagnosis. The hyperprolactinemia normalised on treatment with cabergoline. This patient and a woman with hyperprolactinemia were the only patients who received dopamine agonist treatment. In the UK cohort, 8 out of 10 of the patients with hyperprolactinaemia had a dedicated MRI pituitary scan with gadolinium contrast of which all were normal. In these UK patients, median (range) prolactin was 617 (386–3488) mIU/L, and 62.5% were on psychotropic medication(s) that may raise prolactin (antipsychotic or antidepressant). In the Swedish cohort, one patient underwent a CT scan of the brain and one patient MRI of the brain, but these examinations were not dedicated for the pituitary.

The patient with renal insufficiency underwent kidney transplantation the year before this study. The patient was not treated with antipsychotic medication, but was on a stable dosage of testosterone replacement therapy. Over time prolactin levels gradually increased as the kidney function decreased. Two years before the kidney transplantation eGFR was 11 mL/min (reference value > 80) and prolactin 787 mIU/L. After the transplantation eGFR increased to 58 mL/min and prolactin decreased to 175 mIU/L.

No clear clinical effects of hyperprolactinemia were identified in our cohort. Galactorrhoea or gynecomastia were not reported in any patient.

DISCUSSION

In this study of 45 adults with PWS and hyperprolactinemia the majority were men, and the most frequent aetiology was treatment with antipsychotic medication in 56%. The prolactin levels were generally only mildly increased and were higher in women and in patients with mUPD. Clear clinical consequences of the hyperprolactinemia were not observed.

Hyperprolactinemia is defined as sustained levels of prolactin above the laboratory upper limit of normal (7,8). A grading of hyperprolactinemia in psychotic patients according to its clinical severity has been suggested (16). Accordingly, hyperprolactinemia can be mild (<1100 mIU/L or <50 ng/mL), moderate (1100–1600 mIU/L or 51–75 ng/mL), or severe (>2200 mIU/L or >100 ng/mL). Prolactin levels between 1600 mIU/L or 75 ng/L and 2200 mIU/L or 100 ng/L were not included in the grading, but in the present study they were considered as moderately increased. Using these definitions, the hyperprolactinemia in the present study was generally mild (80.0%), with only six patients (13.3%) having moderate and three (6.7%) severe hyperprolactinemia. Hyperprolactinemia can cause hypogonadism, reduced bone mineralization and an increased cardiovascular risk (7,8,11-14,17,18). In PWS, these risks are already high and mainly related to hypogonadism, GH deficiency and obesity, independent of hyperprolactinemia. In eugonadal patients or patients with mild hypogonadism, hyperprolactinemia might further lower sex hormone levels further increasing the risks. Clinical consequences of hyperprolactinemia were not observed in the present study, probably because prolactin was only mildly increased.

Several factors can affect prolactin measurements, and to minimize the confounding effect of them, blood samples should be taken in an un-stressed condition (19,20). In patients with PWS, blood sampling is often stressful and difficult due to small, thin veins. Presuming the stress elicited by taking blood samples decreases when the procedure is repeated, this was not the case in 37 patients in whom hyperprolactinemia continued to be elevated in repeated measurements, indicating that the stress did not decrease, or the hyperprolactinemia had another aetiology. Other than medications as discussed below, this could be related to hypothalamic defects in PWS interrupting the dopaminergic inhibition of pituitary prolactin secretion.

Moreover, the release of prolactin is pulsatile with the highest levels in the early morning and measurement of prolactin is recommended 2–3 h after awakening (19,20). The presence of macroprolactin might falsely increase the prolactin level (19-22). Most circulating prolactin is monomeric (23 kDa), but prolactin also circulates in larger isoforms called macroprolactin. These are complexes between prolactin and IgG antibodies causing hyperprolactinemia through reduced clearance and are not considered to have any clinical effect (15,19-22). In the present study, the presence of macroprolactin was not controlled for and excluded in all laboratories' prolactin analyses. However, in our view, the presence of macroprolactin was unlikely to have complicated our results as in the 37 patients in whom it was measured with negative macroprolactin.

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The aetiology of hyperprolactinemia can be physiological like in pregnancy and breast feeding, stress, exercise, or food intake (7,8). It can also be pharmacological, induced by several drugs (antipsychotics, antidepressants, opioids, phenytoin, verapamil, antiemetic, oestrogen, metoclopramide, cimetidine, omeprazole, and several other medications) (7-9). Other aetiologies are hypothalamic tumours, prolactinomas or other pituitary tumours and after radiotherapy of the area (7,8). Furthermore, primary hypothyroidism, chronic renal insufficiency, liver cirrhosis and polycystic ovarian syndrome (PCOS) can lead to hyperprolactinemia and finally hyperprolactinemia can be idiopathic (7,8). It is, therefore, important to consider secondary causes through a careful medical history including use of drugs, clinical examination, and evaluation of kidney, liver and thyroid gland function and exclusion of pregnancy in fertile women (7,8). In our study, the most common aetiology of hyperprolactinemia was the treatment with antipsychotic medication. Psychosis is more common in patients with mUPD (2,23) and approximately 30% of our cohort had mUPD. In our cohort, prolactin levels were higher in patients with mUPD compared to patients with paternal deletion. The reason for this could be that patients with mUPD were more frequently treated with antipsychotics. Only one patient had a microprolactinoma. One patient had previously suffered from severe renal insufficiency, and during that time, prolactin increased. After kidney transplantation and improvement in kidney function, prolactin normalized.

Hyperprolactinemia is a common consequence of treatment with some antipsychotics, mainly risperidone, paliperidone, and amisulpride, but less frequently related to olanzapine (24). Dopamine binds to D2 receptors on the lactotroph cells in the pituitary, which inhibits both the synthesis and secretion of prolactin (24). Administration of dopamine antagonists, such as antipsychotics, might therefore lead to hyperprolactinemia. The highest rates of hyperprolactinemia are consistently reported for conventional antipsychotic drugs, but hyperprolactinemia also occurs with the atypical antipsychotics, but with aripiprazole and quetiapine having the most favourable profile (24). There are large variations in the individual increase in prolactin caused by antipsychotic drugs and transient elevations can also be seen (24). In a recent study of 170 patients treated with antipsychotics, female gender was associated with an increase in serum prolactin levels (25). In the present study, risperidone was the most frequently used antipsychotic medication. Too few patients were on other antipsychotics for a difference in prolactin levels to be assessed.

It was difficult to determine the overall prevalence of hyperprolactinemia in all centres, but in individual centres where sufficient data was available, the prevalence varied from 20–39%, averaging 22% with 66% of those related to medication (55% antipsychotics). Since this proportion of these patients who were on medications elevating prolactin

was similar to that in the whole cohort, it is likely that this prevalence is a reasonable estimate of true prevalence in the patients seen in all these centres. However, it should be noted that there may be a referral bias for patients with behavioural issues, and therefore on psychotropic medication to these referral centres. In comparison, cross-sectional studies of patients with schizophrenia and bipolar diseases have reported a prevalence of hyperprolactinemia of 44–75% in women and 23–72% in men (26). Considering, that 80% of our PWS patients with hyperprolactinemia had only a mild hyperprolactinemia and that some antipsychotics (such as risperidone, paliperidone and amisulpride) might raise the serum prolactin level at relatively low doses (24), we have no indications that patients with PWS have a greater susceptibility for developing hyperprolactinemia during anti-psychotic treatment than other patient groups.

Studies of non-PWS adults with psychotic illnesses treated with antipsychotics have reported a frequency of galactorrhoea of 10–90% in women, whereas it is rarely reported in men (14,15,27). It has also been shown that gynecomastia occurs in 1–11% of men (27). However, galactorrhoea and gynecomastia were not reported in any of the patients in the present study. Menstrual irregularities in non-PWS women with schizophrenia receiving long term treatment with conventional antipsychotics or risperidone were reported with a prevalence ranging between 25–78% (17,23). Due to hypogonadism in PWS and the use of sex hormone replacement therapy, it was not possible to evaluate this issue in the current study. Long-term treatment with antipsychotic medications and hyperprolactinemia have been observed to decrease bone mineral density in 32–65% of patients on antipsychotics, but confounders like poor diet, low physical activity, smoking, low exposure to sun light were considered to affect the results (17,23). Bone mineral density measurements were not available for the present cohort and the effect on bone mineral density is therefore unknown.

An interesting aspect is whether hyperprolactinemia due to treatment with antipsychotic medications might induce a prolactin producing pituitary adenoma. Long-term treatment with antipsychotics in mice has led to the development of prolactinomas (28), but from currently available pharmacovigilance data it is difficult to conclude whether antipsychotics are implicated in the development or progression of prolactinomas (28,29,30). Only one patient in the present cohort was diagnosed with a microprolactinoma and this patient did not receive treatment with antipsychotics. A microprolactinoma has been previously reported in only one patient with PWS (31). However, only 12 patients in our cohort underwent radiological examination of the pituitary or brain. Given the lack of pituitary macroadenomas and rarity of visible microprolactinomas in our cohort, it remains to be determined under which criteria a dedicated MRI or CT scan of the pituitary gland should be considered if an adult with PWS is found to have a

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raised prolactin. If a clear reason for the hyperprolactinemia is lacking perhaps hyperprolactinemia in the severe range (>2200 mIU/L or >100 ng/mL) might be an appropriate cut-off to warrant dedicated pituitary imaging (6.7% of our cohort had a prolactin level in the severe range, including one patient with a microprolactinoma). Given the possibility of causing a pituitary adenoma, a dedicated MRI or CT of the pituitary should also be considered in patients with moderately increased prolactin levels for long-term.

When indicated hyperprolactinemia is usually treated with dopamine agonists. The most common side effects of dopamine agonists are nausea, vomiting, dizziness, postural hypotension, headache, nasal congestion, and constipation. In addition to these side effects, dopamine agonist treatment can trigger or worsen an impulse control disorder, such as hypersexuality and gambling addiction, or psychotic illness. Furthermore, long-term treatment with ergot-derived dopamine agonists (including carbergoline) in patients with Parkinson's disease has shown an increased incidence of heart valve insufficiencies, though at much higher doses than usually used to treat hyperprolactinaemia from pituitary adenomas (32,33). Concerning treatment with cabergoline in patients with prolactinomas, no increased risk of clinically significant valve insufficiency has been found to date (32,33). Only two patients were treated with cabergoline and treatment was successful and without side effects. However, attention should always be paid to potential mental side effects of dopamine agonists in a vulnerable group of patients such as patients with PWS.

The strength of the study is the careful evaluation of all patients with hyperprolactinemia and the use of the same method for analysis of prolactin in all countries. Limitations to the study are the retrospective character and that equipment from different manufacturers were used for the analysis of prolactin in each country. However, after conversion of all prolactin levels to mIU/L the differences in upper and lower reference values was not considered of significance for the results of this study, in contrast to the clinical settings where an exact value is of importance. Furthermore, not all countries had formally excluded macroprolactin, and in some of the patients the diagnosis of hyperprolactinemia was based on randomly measured prolactin as not all countries measured prolactin concentrations regularly in every patient. For this reason, it was not possible to determine the true prevalence of hyperprolactinemia in PWS.

CONCLUSIONS

The present cohort of 45 adults with PWS and hyperprolactinemia consisted of more men than women. Prolactin levels were higher in women and in patients with mUPD.

Estimated prevalence was 22%, with two thirds of those related to psychotropic medication with the most common aetiology antipsychotic medication in 55%. In most cases, the hyperprolactinemia was mild and without clear clinical consequences. However, potential negative long-term effects cannot be excluded. Due to cognitive impairment, behavioural problems, and hypogonadism, the clinical effects of hyperprolactinemia might not be noticed. Therefore, it is important to routinely measure serum prolactin concentrations, especially during treatment with antipsychotics, to repeat measurements if an initial level is raised, to exclude macroprolactin artefact, and to consider a dedicated MRI or CT pituitary scan if there is moderate or severe hyperprolactinemia.

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Hyponatremia in children and adults with Prader-Willi syndrome: a survey involving seven countries

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ABSTRACT

In Prader–Willi syndrome (PWS), conditions that are associated with hyponatremia are common, such as excessive fluid intake (EFI), desmopressin use and syndrome of inappropriate antidiuretic hormone (SIADH) caused by psychotropic medication. However, the prevalence of hyponatremia in PWS has rarely been reported. Our aim was to describe the prevalence and severity of hyponatremia in PWS. In October 2020, we performed a retrospective study based on the medical records of a large cohort of children and adults with PWS from seven countries. Among 1326 patients (68% adults), 34 (2.6%) had at least one episode of mild or moderate hyponatremia ($125 \leq Na < 135$ mmol/L). The causes of non-severe hyponatremia were often multi-factorial, including psychotropic medication in 32%, EFI in 24% and hyperglycemia in 12%. No obvious cause was found in 29%. Seven (0.5%) adults experienced severe hyponatremia ($Na < 125$ mmol/L). Among these, five recovered completely, but two died. The causes of severe hyponatremia were desmopressin treatment for nocturnal enuresis (n = 2), EFI (n = 2), adrenal insufficiency (n = 1), diuretic treatment (n = 1) and unknown (n = 1). In conclusion, severe hyponatremia was very rare but potentially fatal in PWS. Desmopressin treatment for nocturnal enuresis should be avoided. Enquiring about EFI and monitoring serum sodium should be included in the routine follow-ups of patients with PWS.

INTRODUCTION

Hyponatremia, defined as a serum sodium concentration below 135 mmol/L, is the most common electrolyte disorder encountered in clinical practice and has affected up to 30% of hospitalized patients across numerous studies throughout the world over the last several decades (1,2). Severe hyponatremia (<125 mmol/L) can lead to life-threatening neurological symptoms (e.g., confusion, seizures, coma), especially if it occurs rapidly (3). Several factors that might contribute to hospital-acquired hyponatremia have been identified, including increasing age (1), diuretics, surgery, hypotonic intravenous fluids and several drugs and diseases that promote the release of antidiuretic hormone (4). Indeed, the syndrome of inappropriate antidiuretic hormone (SIADH) is the most common cause of hyponatremia and occurs when there is persistent secretion of antidiuretic hormone (ADH, also called vasopressin) despite hyponatremia (5,6).

Prader–Willi syndrome (PWS) is a rare genetic neuro-endocrine developmental disorder and the most common form of syndromic obesity with an incidence of approximately 1 in 21,000 newborns (7). This genetic syndrome is caused by a loss of the expression of paternally inherited imprinted alleles on chromosome 15q11-q13 that can occur via three mechanisms. The main genetic mechanism is a paternal deletion in about 60%, followed by maternal uniparental disomy (mUPD) in 36% and imprinting defects in 4% of the overall PWS population (8). Impaired hypothalamic development and function are the causes of many of the phenotypes comprising the developmental trajectory of PWS: from anorexia at birth to excessive weight gain preceding hyperphagia and early-onset severe obesity with combined hormonal deficiencies, behavioral problems and dysautonomia (9). Among the hormonal deficits, hypogonadism is the most frequent in PWS, with expression in both sexes and at all ages (9) and sex hormone substitution could present a greater risk for hyponatremia in women (10). In addition, central adrenal insufficiency is rare, about 1% in adults with PWS (11), but is known to induce hyponatremia.

Severe hyponatremia in patients with PWS was only reported in three studies involving adults (12,13) and an infant (14), despite universal hypothalamic–pituitary axis dysfunction in PWS, as well as an increased risk of water intoxication by excessive fluid intake (EFI), at least in patients with mUPD (12). In addition, patients with PWS are frequently exposed to psychotropic medications that are known to cause SIADH (i.e., carbamazepine, tricyclic and selective serotonin reuptake inhibitor (SSRI) antidepressants, phenothiazines, haloperidol) (6). Moreover, some patients may receive desmopressin treatment (synthetic analog of ADH) to manage nocturnal enuresis, which is observed in PWS (15). Finally, the possibility of SIADH due to dysfunction of the hypothalamic nuclei

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engaged in ADH production in patients with PWS was suggested in one study (12) but has not been confirmed clinically.

Recently, four French adults with PWS experienced severe hyponatremia, leading to death in one case, prompting a national survey. However, no other case of severe hyponatremia was reported in adults or children. This led to performing a retrospective cohort study in nine reference centers for PWS of the International Network for Research, Management and Education on adults with PWS (INfoRMEd-PWS) to collect all the cases of hyponatremia. The aim of this study was to describe cases of hyponatremia in patients with PWS that were followed in seven countries and provide clinical recommendations to prevent severe hyponatremia.

METHODS

Study Rationale and Design

The first part was a case series of four severe hyponatremia patients who were investigated using a descriptive clinical study. Then, we conducted a retrospective cohort study based on medical records of nine reference centers for PWS in seven countries constituting the INfoRMEd-PWS. All patients with genetically confirmed PWS had at least one systematic health screening every year, consisting of a medical interview, a complete physical examination and routine biochemical measurements. In October 2020, each center's investigator reviewed medical data from the last decade of their reference center for PWS to report all cases of patients with hyponatremia (Na < 135 mmol/L). Then, the investigator completed the characteristics of patients with hyponatremia (demography, comorbidities), the possible causes and the management of hyponatremia.

All participating centers (nine reference centers for PWS in seven countries) obtained approval from ethics committees and patients or caregivers were informed about the retrospective analysis of the data.

Statistical Analysis

Data are expressed as mean ± SD (range) or numbers (%). We used descriptive statistics to report the demographic information and medical data of the patients with hyponatremia.

RESULTS

The survey was based on 1326 patients (430 children and 896 adults) with PWS that were currently or had been (patients lost to follow-up or deceased) under the care of nine reference centers for PWS in seven countries in Europe and Australia: France (265 children and 315 adults), Italy (135 children and 252 adults), the Netherlands (122 adults), Spain (30 children and 50 adults), United Kingdom (46 adults), Sweden (41 adults) and Australia (70 adults).

Among these 1326 patients with PWS, 34 (2.6%) had at least one episode of mild or moderate hyponatremia (125 ≤ Na < 135 mmol/L), including two children (0.02%), and 7 (0.5%) had a severe episode of hyponatremia (Na < 125 mmol/L), all being adults.

Individual Clinical Presentation of the Four Most Recent Cases with Severe Hyponatremia

We first detailed the four most recent and severe French cases from the reference center of Pitié Salpêtrière hospital in Paris.

The first case was a 41-year-old male with mUPD. His routine treatment comprised 12.5 mg of the antipsychotic medication loxapine at bedtime, glucose-lowering medications (gliclazide, metformin, sitagliptin), antihypertensive medication (irbesartan) and allopurinol. He had nocturnal enuresis for several years, for which a urologist initially prescribed 120 μg/day of sublingual desmopressin (Minirin Melt®), but since this was ineffective, the dose was doubled to 240 μg/day. Three days later, the patient developed generalized tonic-clonic seizures and was admitted to the intensive care unit (ICU). His serum sodium on admission was 115 mmol/L. His brain computed tomography (CT) scan was normal. Neither urine electrolyte, urine osmolality analysis, measurement of serum cortisol, nor thyroid function tests were performed, as the etiology of the hyponatremia was considered to be undoubtedly related to the desmopressin. The serum sodium returned to normal on day 3 after fluid restriction and administration of oral NaCl. Desmopressin was stopped and there has been no recurrence of hyponatremia.

The second case was a 27-year-old male with mUPD with a history of EFI for 5 years after beginning psychotropic drug therapy (12.5 mg per day of loxapine and 200 mg per day of topiramate). In December 2019, he had an episode of diarrhea for 3 days and suddenly developed generalized tonic-clonic seizures. On admission to the ICU, his serum sodium was 119 mmol/L, urinary osmolality was 169 mOsmol/kg and urinary sodium was 25 mmol/L. There was no thyroid nor cortisol insufficiency. He recovered in 5 days with fluid restriction and the hyponatremia has not recurred to date with fluid restriction (1.5 L/d).

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The third case was a 23-year-old female with mUPD. Her only known medication was a growth hormone treatment. She had a history of nocturnal enuresis and was treated with oral desmopressin in childhood (Minirin® tablets). The dose was decreased from 200 to 100 μg daily when she was transferred to the adult nutrition department because her serum sodium concentration was 131 mmol/L. Desmopressin was stopped in January 2020 because of persistent mild hyponatremia and a lack of efficacy. In June 2020, she started to have vertigo, then became comatose and was admitted to ICU. Her serum sodium was 115 mmol/L and her potassium was also low at 3.2 mmol/L (3.5–5.1). There was no thyroid nor cortisol insufficiency. Her mother reported 3 days of EFI before the coma, but the urinary osmolality was high at 482 mOsmol/kg. Urinary sodium was also elevated at 75 mmol/L, consistent with SIADH. After the serum sodium returned to normal with fluid restriction, she admitted that she secretly took desmopressin tablets. She no longer has access to any desmopressin and there has been no recurrence of hyponatremia to date.

The fourth case was a 31-year-old man with mUPD who was admitted to the psychiatry unit in July 2020 because of psychiatric decompensation, which was related to severe anxiety, in part due to the COVID-19 pandemic. After increasing his psychiatric medication (diazepam, clopixol, loxapine, valproic acid), he displayed confusion, for which he required short-term hospitalization. His serum sodium was 122 mmol/L at presentation and he recovered within 48 h with fluid restriction, resulting in the normalization of his serum sodium (139 mmol/L). In November 2020, he slipped and fractured his ankle, for which he had surgery on 11 November 2020. When the cast was removed on 1 December 2020, the scar was noted to be infected and admission for debridement was recommended, but this was delayed and finally scheduled for 21 December 2020. Serum sodium was 135 mmol/L on 18 December 2020. On the morning of 21 December 2020, he was found unconscious in the bathroom and was transferred to ICU. His serum sodium was 112 mmol/L, potassium 4.0 mmol/L (3.5–5.1) and urea 2.3 mmol/L (2.5–7.4). The brain CT scan found a global erasure of cortical furrows without bleeding or signs of ischemia. Despite the correction of hyponatremia, he developed bilateral areactive mydriasis. A second brain CT scan found an increase in diffuse edema with effacement of the basal cisterns and an absence of vascularization in the arterial and venous phase. He died on 24 December 2020. After questioning his mother, it is very likely that he drank copious amounts of water from the bathroom tap in response to anxiety about the forthcoming surgery, which was postponed twice.

Severe Cases of Hyponatremia in PWS

Table 1 shows the seven cases of severe hyponatremia (Na < 125 mmol/L) in adults with PWS from three countries (France, the Netherlands and the United Kingdom), including

the four French cases described above. The mean age was 37.2 ± 11.7 years (23-55), the sex ratio was close to 1 (4 males and 3 females) and mUPD was the predominant genetic diagnosis (71%). In two patients, the hyponatremia was due to the desmopressin that was used to control nocturnal enuresis. In four patients, there were several possible contributory factors, and in the remaining patient, there was no apparent cause. All patients with severe hyponatremia were treated in emergency care and then admitted to ICU. Five patients recovered completely but two patients died (one from cerebral edema and one from probable inhalation of gastric content).

Mild or Moderate Cases of Hyponatremia in PWS

Thirty-four cases of moderate or mild hyponatremia were reported from seven countries (**Table 2**). All patients were asymptomatic (incidental finding), except one who presented with confusion (serum sodium 127 mmol/L), the mean age was 36 years, the sex ratio was 1 and 32% had an mUPD genotype. Two Italian patients were aged under 18 years (14.3 and 17.7 years), no other pediatric cases were reported. Obesity was present in 47%, type 2 diabetes mellitus in 35% and hypertension in 29% of patients, all being adults. One-third of patients took psychotropic medication that is known to cause SIADH (carbamazepine, SSRI antidepressant), one-quarter had EFI and, in 12% of cases, the hyponatremia was likely due to hyperglycemia. No cause of hyponatremia was found in 29%, including the two children (neither thyroid nor cortisol insufficiency), but SIADH was not excluded (no urine electrolyte or osmolality measurements in 30 out of 34 patients).

There was no specific management in 38% of these patients. Fluid restriction was introduced in 24%, medication (psychotropics or antihypertensive treatments) was changed in 18% and improvement of glycemic control was sought in 18% of patients (**Table 2**).

DISCUSSION

We reported the largest cohort of patients with PWS with a history of hyponatremia to identify the possible causes of hyponatremia and interventions to prevent the development of severe hyponatremia. In our study with data from seven countries, severe hyponatremia was rare (0.5%), but moderate or mild cases were more frequent, occurring in 2.6% of patients with PWS. While this prevalence is low, it is relevant in this vulnerable population. The variability of the prevalence of non-severe hyponatremia among countries can probably be explained by the variability of the frequency of routine serum sodium assessment in patients with PWS among countries.

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Table 1. Description of severe cases of hyponatremia in adults with Prader–Willi syndrome from three countries, possible causes and evolution.

	Country	Gender, Age (years), Genetic Diagnosis	Clinical Symptoms	Serum Sodium (mmol/l)	Urinary Osmolarity (mOsmol/kg)	Urinary Sodium (mmol/L)	Possible causes	Evolution
Case 1	France	Male, 41, UPD	Coma (seizures)	115	-	-	Desmopressin for nocturnal enuresia (Minirin Melt ® 240 µg/d for 3 days)	Recovery; no recidivism after stop ping desmopressin intake
Case 2	France	Male, 27, UPD	Coma (seizures)	119	169	25	Excessive fluid intake and diarrhea for 3 days	Recovery; no recidivism with fluid restriction (1.5 L/d)
Case 3	France	Female, 23, UPD	Coma (seizures)	115	482	75	Hidden intake of desmopressin (treatment stopped for several months)	Recovery; no recidivism
Case 4	France	Male, 31, UPD	Coma	112			Possible excessive fluid intake in few hours during hospitalization in orthopedics	Death (cerebral edema due to severe hyponatremia)
Case 5	The Netherlands	Male, 55, UPD	Seizures	121			Unknown	Death (probably due to inhalation of gastric content)
Case 6	The Netherlands	Female, 29, Del	Seizures	Unknown [a]			Treatment with furosemide and salt restriction for cardiac failure	Recovery (stopped furosemide)
Case 7	United Kingdom	Female, 39, Del	Confusion	122	149	37	Central adrenal insufficiency and possible SIADH due to sertraline (but no regression after stopping sertraline)	Recovery; fluid restriction necessary (2 L/d) to maintain normal serum sodium

Maternal uniparental disomy (mUPD), deletion (Del), syndrome of inappropriate antidiuretic hormone (SIADH). [a] Serum sodium was not available for this case but hyponatremia was mentioned in the medical report.

Table 2 Cases of mild or moderate hyponatremia in patients with Prader–Willi syndrome from seven countries.

Countries	France: 1; Spain: 1; Australia: 3; United Kingdom: 3; Sweden: 4; The Netherlands: 10; Italy: 12
Serum sodium (mmol/L)	131.4 ± 2.7 (124–134)
Absence of symptoms of hyponatremia (%)	33 (97)
Age (years)	35.8 ± 10.2 (14.3–55)
Gender (%)	Female: 17 (50); male: 17 (50)
Genetic subtype (%)	Deletion: 18 (53); mUPD: 11 (32); ICD: 1 (3); unknown: 4
Body mass index (kg/m^2)	32.2 ± 8.7
Obesity [a] (%)	16 (47)
Type 2 diabetes (%)	12 (35)
Type 1 diabetes (%)	1 (3)
Hypertension (%)	10 (29)
Possible causes of hyponatremia (%)	
Excess fluid intake (EFI)	8 (24)
Desmopressin treatment	2 (6)
Psychotropic treatment	11 (32)
-Carbamazepine	*6 (18)*
-SSRI antidepressant (Fluoxetine, Citalopram)	*5 (15)*
Diuretics	3 (9)
-Bumetanide	*1 (3)*
-Hydrochlorothiazide	*2 (6)*
Hyperglycemia	4 (12)
Unknown cause	10 (29)
Management (%)	
Fluid restriction	8 (24)
Reduce or stop treatment causing SIADH	4 (12)
Change of antihypertensive treatment	2 (6)
Improve glycemic control	6 (18)
No specific management	13 (38)

Characteristics of patients with mild or moderate hyponatremia (n = 34). Results are expressed as mean ± SD (range) for continuous variables and as number (percentage) for categorical variables. mUPD: maternal uniparental disomy. ICD: imprinting center defect. BMI: body mass index. SSRI: selective serotonin reuptake inhibitor. SIADH: syndrome of inappropriate antidiuretic hormone. [a] Obesity was defined as BMI ≥ 30 kg/m^2 in adults and a BMI Z-score ≥ 3 in children.

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In our cohort, the prevalence of severe hyponatremia was higher in patients with mUPD (5 out of 7 patients), probably due to an increase in EFI and the presence of psychotropic medication that may promote SIADH. Indeed, behavioral problems and psychiatric diagnoses, such as psychosis, are more common in patients with the mUPD genotype (16). In agreement with our findings, a previous study reported severe hyponatremia in two adults with PWS due to mUPD, in whom the precipitating factors were EFI and treatment with psychotropic medication known to induce SIADH (12). In another report of severe hyponatremia in an adult with PWS, water intoxication due to desmopressin treatment for nocturnal enuresis was implicated (13). In our study, apart from the direct

effect of desmopressin treatment in two adults, the etiology of the severe hyponatremia in the other adults with PWS was less clear and likely based on several factors, including EFI and treatment with psychotropic medications known to be associated with SIADH.

Similarly, the etiology of mild–moderate hyponatremia in our cohort was often multi-factorial. Type 2 diabetes mellitus was almost twice as common in this cohort (35% of patients) than the rate of 20% reported in European adults with PWS (17-19). In 12% of those with mild–moderate hyponatremia, hyperglycemia was present and these patients may be more prone to glucose-induced hyponatremia than the general population. While we did not find the cause of non-severe hyponatremia in 29% of patients, whether they displayed SIADH is not known as urine electrolytes and osmolarity were not measured.

These unexplained cases of hyponatremia could suggest a heightened sensitivity of the hypothalamic nuclei to the over-secretion of ADH in PWS. Given the numerous central endocrine abnormalities in this syndrome (9), a dysregulation of hypothalamic nuclei, both spontaneously and by drugs, is probable and could be contributing to the increased prevalence of hyponatremia in PWS. In the postmortem study of Swaab et al., the number of vasopressin neurons in the hypothalamic paraventricular nucleus of the five cases with PWS was not significantly different from 27 controls, whereas the number of oxytocin neurons was lower in the PWS cases compared to the controls (20). Although the number of vasopressin cells is not apparently increased in PWS, the density of vasopressin immunostaining was not examined and there may still be over-secretion of vasopressin. Alternatively, increased renal sensitivity to vasopressin in PWS could be another explanation, though this is difficult to confirm in clinical practice.

Finally, EFI, alone or combined with other causes, was the most frequent etiology of hyponatremia in our cohort, where it was present in up to 15% of adults with PWS in a previous Swedish study (12). While the majority of infants with PWS drink an unusually small amount, as they age, episodes of consumption of excessive amounts of water can occur and water intoxication was a frequent cause for hospitalization in Dutch adults with PWS in one study (21). EFI is likely to be exacerbated by psychiatric medications, many of which result in mouth dryness, which is compounded by the sticky saliva of people with PWS (22). It is thus important that individuals with PWS, their parents and their caregivers are well informed about this health hazard; the PWS Association USA has published a "water intoxication alert" on their website (23). Fluid intake in individuals with PWS should always be monitored because of the risk of it becoming excessive with the subsequent development of hyponatremia.

The other unambiguous cause of severe or non-severe hyponatremia is desmopressin treatment in adults with nocturnal enuresis. If this treatment is instituted, fluid intake should be restricted for at least one hour before and eight hours after taking the treatment (24). However, as water intake is difficult to control in most adults with PWS, desmopressin treatment should be avoided in adults with PWS. In cases of enuresis, patients should be comprehensively reviewed to find the exact mechanism(s) of enuresis and an alternative treatment to improve it (25).

Our study has limitations. The first is the retrospective design of the study. The second is the heterogeneity of the cases between countries. The last one is the absence of the comparison of data due to the absence of accurate retrospective data on the group of patients with PWS without a history of hyponatremia.

CONCLUSIONS

Severe hyponatremia is rare in PWS (0.5%) but is potentially life threatening, with two deaths in our cohort. Even if there are often multiple causes, the prevalence of severe hyponatremia increases in individuals with mUPD, in whom EFI appears to be more common, and is compounded by the use of psychotropic treatments that can induce SIADH, as well as the use of desmopressin treatment. In our opinion, desmopressin should not be used to treat nocturnal enuresis in adults with PWS because EFI is frequent in these patients and not easily prevented. In addition, treatments that are known to promote SIADH should be used carefully in patients with PWS, especially carbamazepine and SSRI antidepressants. Finally, we recommend that serum sodium should be measured regularly in individuals with PWS, especially in those who demonstrate EFI or take psychotropic drugs that may promote SIADH.

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Bone health in adults with Prader-Willi syndrome: clinical recommendations based on a multicenter cohort study

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ABSTRACT

Context

Prader–Willi syndrome (PWS) is a rare complex genetic syndrome, characterized by delayed psychomotor development, hypotonia, and hyperphagia. Hormone deficiencies such as hypogonadism, hypothyroidism, and growth hormone deficiency are common. The combination of hypotonia, low physical activity, and hypogonadism might lead to a decrease in bone mass and increase in fracture risk. Moreover, one would expect an increased risk of scoliosis due to hypotonia and low physical activity.

Objective

To study the prevalence and risk factors for skeletal problems (reduced bone mineral density, fractures, and scoliosis) in adults with PWS.

Methods

We retrospectively collected patient characteristics, medical history, medication, biochemical measurements, dual-energy X-ray absorptiometry scans, and spinal X-rays and reviewed the current literature.

Results

We included 354 adults with PWS (median age 31 years; 43% males), of whom 51 (14%) had osteoporosis (T-score below −2.5) and 143 (54%) had osteopenia (T-score −1 to −2.5). The most prevalent modifiable risk factors for osteoporosis were hypogonadism, insufficient dairy intake, sedentary lifestyle, and corticosteroid use. Male sex was associated with osteoporosis (P = 0.005). Growth hormone treatment was not associated with osteoporosis. A history of vertebral fractures was present in 10 (3%) and nonvertebral fractures in 59 (17%). Scoliosis was present in 263 (80%), but no modifiable risk factors were identified.

Conclusion

Besides scoliosis, osteoporosis is common in adults with PWS. Based on the literature and the risk factors for osteoporosis found in our cohort, we provide practical clinical recommendations to avoid skeletal complications in these vulnerable patients.

INTRODUCTION

Prader–Willi syndrome (PWS) is a rare, complex genetic syndrome with an estimated prevalence of 1:10.000 to 30.000 (1). It arises from a loss of expression of paternally expressed genes in the PWS region on chromosome 15q11.2-13, most often caused by a de novo paternal deletion (65-75%), a maternal uniparental disomy (mUPD, 20-30%), an imprinting center defect (ICD, 1-3%), or a paternal chromosomal translocation (0.1%) (2, 3). Clinical features of PWS include infantile hypotonia, obesity due to hyperphagia (an insatiable appetite), sleep disorders, disturbed temperature regulation, disturbed pain perception, challenging behavior, and intellectual disability (4–6). Furthermore, hypothalamic dysfunction in PWS can lead to multiple pituitary hormone deficiencies (1, 4–7).

In patients with PWS, 29% to 44% have a history of bone fractures, which might be related to the high recorded prevalence of osteoporosis of up to 21% (8–12). Several risk factors contribute to this high prevalence. Firstly, there is a high prevalence of hypogonadism in adults with PWS (up to 100% in both males and females) (13–16), leading to increased bone turnover (17, 18). Secondly, adults with PWS are considered (functionally) growth hormone (GH) deficient, and GH deficiency has been linked to osteoporosis (19–24). Other risk factors for osteoporosis that are prevalent in adults with PWS are reduced physical activity (25–28) and low levels of fat-soluble vitamin D (29). A prevalent risk factors for fractures without a low bone mineral density (BMD) is type 2 diabetes mellitus (30, 31), which is present in 11% to 24% of adults with PWS compared with 5% to 7% in the general population (11, 32). The combination of hypotonia, hypogonadism, and decreased physical activity can lead to decreased bone mass and an increased fracture risk, as has previously been reported in Down syndrome (33).

Another frequent bone-related health problem in both children and adults with PWS is scoliosis. The reported prevalence of scoliosis ranges between 38% and 86% (8, 10, 34–40). Scoliosis is likely related to hypotonia of (paravertebral) muscles, low physical activity, and obesity (35, 38, 41). Additionally, late menarche and low estrogen levels might be linked to scoliosis in females with PWS (42–45), although results are inconclusive (40).

Therefore, we studied the prevalence of osteoporosis, osteopenia, fractures, and scoliosis and their risk factors in a large cohort of adults with PWS. In addition, we performed a literature review. Based on both clinical and literature data, we provide clinical recommendations for prevention and treatment of skeletal problems in adults with PWS.

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MATERIALS AND METHODS

This study is a multicenter, retrospective cohort study. Ethical approval and/or individual informed consent was obtained by the participating centers according to local rules and regulations. We included patients with genetically confirmed PWS, aged 18 years or older with available dual-energy X-ray absorptiometry (DXA) scans who had been treated at one of the participating PWS reference centers. A total of 354 patients were included from six countries: Australia (n = 51), The Netherlands (n = 78), France (n = 64), Italy (n = 130), Sweden (n = 4), and Spain (n = 27).

Data on osteoporosis, risk factors for osteoporosis, growth hormone treatment (GHt), sex hormone replacement therapy (SHRT), scoliosis, previous fractures, medication, and genotype were collected from medical records. We studied demographic (eg, ethnicity, sex), life style–related (eg, physical inactivity, smoking, alcohol intake, body mass index [BMI], and vitamin D and calcium intake), and comorbidity-related risk factors (eg, previous fractures, type 2 diabetes mellitus, hypogonadism, hyperparathyroidism, hyperthyroidism, chronic corticosteroid use, and malabsorption) (22, 46–49).

A BMI between 18.5 and 25 kg/m^2 was considered lean, 25 to 30 kg/m^2 overweight, and more than 30 kg/m^2 obesity, according to the 1997 World Health Organization criteria (50). Exercise of less than 30 minutes per day was considered insufficient for adults with PWS.

Scoliosis was diagnosed when a gibbus deformity was observed during physical examination and/or if a Cobb angle of ≥10 degrees was present on X-ray, according to the Scoliosis Research Society criteria (51). If a patient had more than one spinal curvature, the largest Cobb angle was used. If a patient had received surgery for scoliosis, the Cobb angle before surgery was used.

The most recent 25-hydroxyvitamin D (25(OH) vitamin D) levels were collected in patients who did not receive vitamin D supplementation. Vitamin D deficiency was defined as a 25(OH) vitamin D level <50 nmol/L (52), and severe vitamin D deficiency was defined as a 25(OH) vitamin D level of <20 nmol/L.

Bone mineral density was measured using DXA scans. Different DXA machines were used (Hologic DEXA systems® or GE Healthcare Lunar), depending on availability in participating centers. In the absence of a spine phantom, we calculated the standardized BMD (sBMD) in order to compare the results from the different machines. sBMD in g/cm^2 was calculated using the following formulas (53, 54):

sBMDspineLunar=0.9683×(BMDspine-1.100)+1.0436

sBMDspineHologic=1.0550×(BMDspine-0.972)+1.0436

sBMDfemurneckLunar=0.939×BMDfemur-0.023

sBMDfemurneckHologic=1.087×BMDfemur+0.019

Osteopenia was defined as a T-score between −1 and −2.5 SD and osteoporosis was defined as a T-score below or equal to −2.5 SD, according to the World Health Organization criteria (55–58). T-scores are calculated based on a reference population, according to sex, ethnicity, skeletal site, and the bone densitometer used. When only Z-scores were available and not T-scores, osteopenia and osteoporosis were considered missing.

When information on the day and month of biochemical and/or imaging data were missing while the year was known, we assigned the date of July 1 to calculate the age and other time intervals.

Literature Review

In collaboration with the Medical Library of the Erasmus University Medical Center, a literature search was performed in March 2021 and last updated on 21 June 2022. D.v.A. and K.P. reviewed the medical literature on osteoporosis, fractures, scoliosis, and bone-related factors in adults with PWS in several databases (Embase, Medline All, Web of Science Core Collection, Cochrane database, and Google Scholar). Search terms included "Prader-Willi Syndrome", "osteoporosis", "osteopenia", "fracture", "scoliosis", "DEXA", "DXA", "bone health", and "bone mineral density". The full search strategy is available elsewhere (**Table S1** (59)). Additionally, the references of relevant articles were screened. We included articles reporting on osteoporosis, scoliosis, or bone-related factors in adults with genetically confirmed PWS (≥16 years old). Nonoriginal research articles, conference abstracts, articles describing fewer than 10 patients, nonhuman research, non-English articles, and articles without full-text availability were excluded. Articles about both children and adults were only included if a subgroup analysis for the age group of 16 years old or older was available. When articles reported on the same population, both articles were combined.

Data Analysis

Data were analyzed using IBM SPSS version 25.0. Continuous variables were displayed as median (interquartile range [IQR]), dichotomous variables as number and percentage of patients, n (%). To investigate the relationship between the determinants and osteoporosis

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or scoliosis, a chi-squared test was used for dichotomous variables, a Mann–Whitney U test for continuous variables when we compared 2 groups, and a Kruskal–Wallis H test for continuous variables to compare 3 or more groups. Ordinal and logistic regression models were used to correct for GHt, age, height, weight (and thereby indirectly for BMI), and/or sex. BMI was considered a possible confounder as high BMI is associated with an increased BMD (60–62). Correction for GHt was performed because GHt improves body composition (63–65) and could therefore influence BMD. P < 0.05 was considered to be statistically significant.

RESULTS

Baseline Characteristics

We included 354 adults (152 males, 202 females) with PWS. The median age was 31 years (IQR 25-40 years) and 43% were male. Median BMI was 33 kg/m^2 (IQR 27-41 kg/m^2); 62% had obesity. Paternal deletion was the most common genotype (61%), followed by mUPD (30%). ICD (2%) was less common. One percent of subjects were methylation positive, but the underlying genetic defect was unknown. In the remaining 6%, the genotype was either mUPD or ICD (not specified) or a rare genetic defect. Sixty-four percent (223 of 351) had ever received GHt. The median (IQR) duration of GHt during childhood was 6.0 (3.3-9.5) years. Baseline characteristics are displayed in **Table 1**.

Osteoporosis

Fifty-one of 354 patients (14%) had osteoporosis based on a T-score below or equal to −2.5 SD at the time of data collection ("current osteoporosis"); 143 of 263 (54%) had current osteopenia based on a T-score between −1 and −2.5 SD. Seventy-five patients (21%) had ever been diagnosed with osteoporosis, either at the time of data-collection or at some point in their medical history. The median T-scores and sBMD values of the most recent DXA scans are shown elsewhere (**Table S2** (59)). The median age of patients currently diagnosed with osteoporosis was 45 years (IQR 32-52 years) compared with 31 years (IQR 25-39 years) for patients with a normal BMD and 31 (26-40) in osteopenia (P < 0.001 after adjusting for sex, height, and weight) (**Table 2**). There was a male predominance in the osteoporosis group, but not in the normal BMD group (61% vs 31% P < 0.001); males had a significantly lower BMI than females (31 [IQR 27-38] kg/m^2 vs 35 [IQR 27-42] kg/m^2, P = 0.013). After adjusting for age, height, and weight, sex remained significantly associated with current osteoporosis (P < 0.001). Both height and weight were significantly associated with osteopenia or osteoporosis (adjusted P = 0.045 and P < 0.001 respectively). We corrected for BMI indirectly by correcting for height and weight. Genotype (deletion vs mUPD) was not significantly related to osteoporosis or osteopenia (adjusted P = 0.86).

Table 1. Baseline characteristics of 354 adults with PWS

	Total n = 354
Age at baseline in years, median [IQR][a]	31 [25-40]
Age at first DXA (T-scores), median [IQR]	24 [21-33], n = 332
Male sex	152 (43)
Height in m, median [IQR]	1.56 [1.49-1.64]
Weight in kg, median [IQR]	81.3 [67.2-98.1]
BMI in kg/m², median [IQR]	33.3 [26.8-41.4]
Obesity	219 (62)
Ever received GHt	223 (64), n = 351
Genetic subtype	
Deletion	217 (61)
Deletion type 1	24
Deletion type 2	43
Deletion, unspecified	141
Atypical deletion	9
mUPD	105 (30)
ICD	7 (2)
Other[b]	25 (7)
Country	
Australia	51 (14)
France	64 (18)
Italy	130 (37)
Sweden	4 (1)
Spain	27 (8)
The Netherlands	78 (22)
Ethnicity	
White/Caucasian	313 (88)
Black/African American	5 (1)
Hispanic	2 (1)
Asian	6 (2)
Arabic	6 (2)
African American	5 (1)
Hispanic	2 (1)
Eurasian	1 (0)
Unknown	21 (6)
Osteoporosis	
Currently diagnosed	51 (14)
Ever diagnosed	75 (21), n = 340
Current osteopenia[c]	143 (54), n = 263
History of vertebral fracture(s)	10 (3), n = 336
History of non-vertebral fracture(s)	59 (17), n = 326
Scoliosis	263 (80), n = 329
Largest cobb angle in degrees, median [IQR]	23.0 [13.0-41.5]

Data are presented as n (%), unless otherwise specified. Abbreviations: Body Mass Index (BMI), Dual-energy X-ray Absorptiometry (DXA), growth hormone therapy (GHt), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD). [a] Current age or age of death for deceased patient. [b] Other genetic subtypes included non-specified mUPD or ICD, non-specified methylation positive, and rare genetic subtypes such as translocations. [c] In patients not currently diagnosed with osteoporosis.

Table 2. Characteristics and risk factors of PWS patients with normal BMD, osteopenia and osteoporosis

	Number of observations	Normal BMD (n = 120)	Number of observations	Osteopenia (n = 143)	Number of observations	Osteoporosis (n = 51)	P-value	Adjusted P-value[j]
Age in years, median [IQR][a]	120	31.4 [24.8-38.5]	143	31.4 [26.3-39.8]	51	44.8 [32.4-52.2]	**<0.001**	**<0.001**
Male sex	120	41 (31)	143	60 (46)	51	31 (61)	**0.005**	**<0.001**
Height in m, median [IQR]	120	1.56 [1.48-1.65]	143	1.56 [1.49-1.63]	51	1.54 [1.44-1.61]	0.34	**0.045**
Weight in kg, median [IQR]	120	87.4 [68.0-102.6]	143	80.6 [66.7-95.1]	51	74.0 [63.1-92.4]	**0.010**	**<0.001**
BMI in kg/m², median [IQR]	120	34.9 [27.2-42.9]	143	32.9 [27.0-40.6]	51	30.3 [26.5-38.9]	0.12	0.58
Genotype	120		143		51		0.13[h]	0.86[h]
Deletion		73 (61)		87 (61)		36 (71)		
mUPD		38 (32)		43 (29)		8 (16)		
ICD		2 (2)		3 (2)		1 (2)		
Other		7 (6)		11 (8)		6 (12)		
Scoliosis	111	82 (74)	135	115 (85)	44	38 (86)	**0.049**	0.061
Cobb angle, median [IQR]	48	19.5 [10.4-44.8]	63	24.0 [15.0-43.0]	18	25.0 [16.5-39.0]	0.66	0.75
Fractures								
Vertebral	117	0	137	4 (3)	42	6 (14)	**<0.001**	**0.025**
Non-vertebral	116	15 (13)	133	24 (18)	41	16 (39)	**0.001**	**0.010**
Bisphosphonate treatment	120	0 (0)	142	5 (4)	47	19 (40)	**<0.001**	**<0.001**
Alcohol usage	112	6 (5)	135	10 (7)	47	5 (10)	0.49	0.68
Units/week median [IQR]	6	2.5 [1.0-6.5]	10	1.5 [1.0-3.3]	5	3.0 [0.5-4.0]	0.76	0.72
Smoking	112	9 (8)	133	12 (9)	48	6 (13)	0.67	0.69
Cigarettes/week median [IQR]	9	50 [15-85]	12	57 [26-70]	6	70 [70-98]	0.14	0.20
GHt								
Ever	120	78 (65)	140	93 (66)	51	23 (45)	**0.020**	0.88
During childhood[b]	109	67 (62)	117	70 (60)	38	10 (26)	**<0.001**	0.45
During adulthood[c]	91	49 (54)	95	48 (51)	42	14 (33)	0.080	0.73
Current	119	40 (34)	136	40 (29)	47	11 (23)	0.42	0.65

Table 2. Characteristics and risk factors of PWS patients with normal BMD, osteopenia and osteoporosis (continued)

	Number of observations	Normal BMD (n = 120)	Number of observations	Osteopenia (n = 143)	Number of observations	Osteoporosis (n = 51)	P-value	Adjusted P-value[j]
Hypogonadism males	41	37 (90)	59	57 (97)	30	28 (93)	0.42	0.13
Ever received SHRT	40	30 (75)	60	41 (68)	30	22 (73)	0.75	0.96
Untreated hypogonadism	37	7 (19)	57	17 (30)	28	6 (21)	0.44	0.61
Hypogonadism females	78	66 (85)	82	71 (87)	20	16 (80)	0.76	0.45
Ever received SHRT	75	55 (73)	79	56 (71)	19	15 (79)	0.77	0.43
Untreated hypogonadism	66	13 (20)	71	16 (23)	16	2 (13)	0.66	0.70
Hyperthyroidism	120	1 (0.8)	143	1 (0.7)	51	2 (4)	0.071	0.088
Hyperparathyroidism	120	0	143	1 (0.7)	50	2 (4)	NA[i]	NA[i]
Diabetes Mellitus	120		143		48			
Type 1		0		0		0	NA[i]	NA[i]
Type 2		27 (23)		37 (26)		10 (21)	0.69	0.96
Not specified		1 (0.8)		2 (1)		0	NA[i]	NA[i]
IGT		10 (8)		6 (4)		1 (2)	NA[i]	NA[i]
Current corticosteroid use	119	16 (13)	140	11 (8)	47	3 (6)	0.22	0.14
Exercise <30min/day	109	38 (35)	131	55 (42)	40	16 (40)	0.52	0.19
Dairy intake <3 units/day	82	69 (84)	105	95 (91)	37	29 (78)	0.15	0.74
Calcium supplements	116	10 (9)	136	35 (26)	44	24 (55)	**<0.001**	**<0.001**
Vit D supplements	116	83 (72)	138	112 (81)	46	40 (87)	0.055	0.25
Vit D levels in nmol/L without supplements, median [IQR]	30	71.4 [58.4-85.5]	25	61.9 [40.4-86.4]	5	77.4 [50.5-129.1]	0.39	0.93
Vit D deficiency[d]	30	5 (17)	25	8 (32)	5	1 (20)	0.40	0.18
Severe vit D deficiency[e]	30	1 (3)	25	2 (8)	5	0	NA[i]	NA[i]
Malabsorption								
Gastro-intestinal surgery[f]	116	9 (8)	138	8 (6)	48	7 (15)	0.15	0.23
Gastro-intestinal comorbidities[g]	117	4 (3)	137	4 (6)	47	6 (13)	0.74	0.16

Data are presented as n (%), unless otherwise specified. P-values are calculated using a Chi-squared test for dichotomous variables and a Kruskal Wallis H test for continuous variables. The adjusted P-value was calculated using an ordinal regression analysis. Forty patients were excluded from this analysis, as they were not diagnosed with osteoporosis but it was unknown whether they had osteopenia or a normal BMD.

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Abbreviations: Body Mass Index (BMI), bone mineral density (BMD), deletion (del), growth hormone treatment (GHt), impaired glucose tolerance (IGT), interquartile range (IQR), maternal uniparental disomy (mUPD), not applicable (NA), sex hormone replacement therapy (SHRT), vitamin (vit). As not all variables were available for all included patients, we display the number of observations, representing the amount of patient for whom that variable was known. [a] Current age or age of death for deceased patients. [b] During childhood includes all patients who received GHt at some point during childhood, independent of whether the patient received GHt during adulthood, compared to patients who never received GHt. [c] During adulthood includes all patients who received GHt at some point during adulthood, independent of GHt during childhood, compared to patients who never received GHt. [d] Vitamin D deficiency was defined as a vitamin D level <50 nmol/L without the use of vitamin D supplements. [e] Severe vitamin D deficiency was defined as a vitamin D level <20 nmol/L without the use of vitamin D supplements. [f] In the normal BMD group operations included sleeve gastrectomy and/or gastric bypass (n = 4), cholecystectomy (n = 3), appendectomy (n = 1), and bioenteric intragastric balloon (n = 1). In the osteopenia group, operations included sleeve gastrectomy, gastric bypasss or biliopancreatic diversion (n = 6), and cholecystectomy (n = 3). In the osteoporosis group, operations performed were biliopancreatic diversion (n = 4), and cholecystectomy (n = 2), which was combined with ileal resection for ileus in one patient (n = 1). [g] In the normal BMD group, gastro-intestinal comorbidities included malabsorption after previous sleeve gastrectomy (n = 4), dysbiosis (n = 1), acute alithiasic cholecystitis (n = 1). In patients with osteopenia, the most common causes of malabsorption were post-operative (n = 3), inflammatory bowel disease (n = 2), and hepatic disease (n = 2). In the patients with osteoporosis, three patients had malabsorption due to biliopancreatic diversion. [h] P-value calculated for deletion vs mUPD only, other genotypes were excluded from this analysis. [i] P-value not calculated because of small numbers. [j] P-values were adjusted for age, sex, height, and weight except for hypogonadism. For hypogonadism the P-value was adjusted for age, height and weight only.

Risk Factors for Osteoporosis

Use of alcohol (7%) and tobacco (9%) was not significantly related to osteoporosis or osteopenia (**Table 2**). Hypogonadism, irrespective of treatment with SHRT, was the most prevalent risk factor (with a prevalence of 93% in males and 80% in females), followed by insufficient physical exercise (present in 40%). No significant association was found between (un)treated hypogonadism and normal BMD, osteopenia, or osteoporosis. Use of GHt at some point in life (ie, either current use or use in the past) was lower in patients with osteoporosis than in patients with normal bone density (45% vs 65%, P = 0.020). However, after adjusting for age, height, weight, and sex, this difference was no longer statistically significant (P = 0.88). GHt during childhood was not significantly related to osteoporosis after correction for age, sex, weight, and height either (P = 0.45). Only 4 patients had hyperthyroidism and 3 had hyperparathyroidism. Scoliosis was not significantly more prevalent in adults with osteopenia (85%) or osteoporosis (86%) than in those with normal bone density (74%, adjusted P = 0.061). Eleven percent of the cohort used corticosteroids, either daily or only during physical or psychological stress. Corticosteroid use was not significantly related to osteoporosis or osteopenia (adjusted P = 0.14). Osteoporosis showed no correlation with gastrointestinal comorbidities or surgery either (adjusted P = 0.16 and P = 0.23 respectively).

Fractures

Ten of 326 (3%) patients had previously suffered at least one vertebral fracture. In six patients, the fractures were spontaneous, in other words, not caused by any (observed or reported) mechanical trauma. Fifty-nine (17%) of all patients had suffered from a nonvertebral fracture at any point in life. In eight patients (14%), this was a spontaneous fracture without previous (observed or reported) adequate trauma. Forty-five patients had had a single fracture; the remaining 14 patients suffered from multiple fractures, either at different time points or simultaneously. The maximum number of fractures in one patient was six. This patient had osteoporosis and received SHRT for hypogonadism. Four patients, all previously diagnosed with osteoporosis, had suffered from both verte-bral and at least one nonvertebral fracture. Fractures (both vertebral and nonvertebral) were more frequent in patients with a current diagnosis of osteoporosis. However, after correcting for age, sex, weight, and GHt, only nonvertebral fractures remained signifi-cantly associated with osteoporosis (P = 0.003). Patients with (vertebral or nonvertebral) fractures were significantly older than adults without fractures (P < 0.001 and P = 0.001 respectively, **Table 3**). GHt was not associated with either vertebral or nonvertebral frac-tures after correcting for age, sex, height, and weight. We did not find any association between (vertebral or nonvertebral) fractures and hypogonadism or SHRT in either of the sexes.

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Table 3. Comparison of adults with PWS with and without fractures

	Number of observations	No fracture	Number of observations	Vertebral fracture	P-value	Number of observations	Non-vertebral fracture	P-value
Age, median [IQR][a]	326	31.3 [25.2-40.0]	10	54.5 [45.2-56.3]	**<0.001**	59	36.3 [30.5-45.3]	**0.001**
Male sex	326	134 (41)	10	7 (70)	0.068	59	34 (58)	**0.005**
Height in m, median [IQR]	326	1.56 [1.49-1.64]	10	1.56 [1.50-1.65]	0.92	59	1.59 [1.49-1.68]	0.13
Weight in kg, median [IQR]	326	82.0 [67.1-98.5]	10	71.4 [55.3-80.4]	0.069	59	82.1 [72.2-104.1]	0.14
BMI in kg/m², median [IQR]	326	33.4 [26.8-41.6]	10	28.4 [24.4-31.4]	0.056	59	34.3 [26.9-40.5]	0.48
Current Osteoporosis	153	36 (24)	6	6 (100)	**<0.001[b]**	31	16 (52)	**<0.001[c]**
Current osteopenia	250	133 (53)	4	4 (100)	0.062	39	24 (62)	0.27
Scoliosis	313	251 (80)	8	5 (63)	0.22	53	42 (79)	0.77
Cobb angle, median [IQR]	144	22.5 [13.0-41.0]	1	51.0	0.23	20	23.5 [13.5-37.75]	0.95
GHt								
Ever	326	217 (67)	10	2 (20)	**0.002[d]**	58	35 (60)	0.36
During childhood	278	169 (61)	10	2 (20)	**0.010[e]**	50	27 (54)	0.33
During adulthood	237	128 (54)	8	0 (0)	**0.003[f]**	41	18 (44)	0.22
Hypogonadism males	133	123 (93)	6	6 (100)	0.49	33	32 (97)	0.26
Ever received SHRT	131	93 (71)	7	5 (71)	0.98	33	24 (73)	0.59
Hypogonadism females	188	162 (86)	3	2 (67)	0.34	24	22 (92)	0.40
Ever received SHRT	184	133 (72)	2	2 (100)	0.38	24	18 (75)	0.77

Data are presented as n (%), unless otherwise specified. P-values are calculated using a Chi-squared test for dichotomous variables and a Mann-Whitney U test for continuous variables. P-values are calculated for patients with vertebral fractures versus no fractures and for patients with non-vertebral fractures versus no fractures. Abbreviations: Body Mass Index (BMI), growth hormone treatment (GHt), interquartile range (IQR), sex hormone replacement therapy (SHRT). [a] Current age or age of death for deceased patients. [b] P = 0.996 after adjusting for age, sex, weight and GHt (ever received) for patients with osteoporosis compared to normal BMD. [c] P = 0.003 after adjusting for age, sex, weight and GHt (ever received) for patients with osteoporosis compared to normal BMD. [d] P = 0.867 after adjusting for age, sex, height and weight. [e] P = 0.995 after adjusting for age, sex, height and weight. [f] P = 0.117 after adjusting for age, sex, height and weight.

Scoliosis

Scoliosis was present in the majority of patients (80%, 263 out of 329 patients) with a median Cobb angle of 23.0 (IQR 13.0-41.5) degrees. **Table 4** shows the clinical characteristics of adults with PWS with and without scoliosis. No significant difference was found for age, sex, BMI, genotype (deletion vs mUPD), GHt at any point in life, GHt during childhood, hypogonadism, or SHRT between patients with and without scoliosis. Scoliosis was not related to osteoporosis or fractures.

Table 4. Comparison of individuals with PWS with and without scoliosis

	Number of observations	No scoliosis (n = 66)	Number of observations	Scoliosis (n = 263)	P-value
Age in years, median [IQR][a]	66	30.1 [24.8-36.8]	263	31.4 [25.4-40.2]	0.33
Male sex	66	34 (52)	263	106 (40)	0.10
Height in m, median [IQR]	66	1.58 [1.51-1.69]	263	1.56 [1.48-1.64]	**0.007[b]**
		85.2 [71.4-104.8]		79.7 [66.2-95.3]	**0.006[c]**
Weight in kg, median [IQR]	66	33.7	263	32.6	0.21
BMI in kg/m², median [IQR]	66	[28.6-42.5]	263	[26.5-40.1]	
Deletion vs mUPD	59	39 (66) del / 20 (34) mUPD	243	166 (68) del/ 77 (32) mUPD	0.74
GHt					
Ever	66	42 (64)	263	179 (68)	0.49
During childhood[d]	57	33 (58)	222	138 (62)	0.56
During adulthood[e]	52	28 (54)	183	99 (54)	0.97
Current	66	23 (35)	252	82 (33)	0.72
Hypogonadism males	34	31 (91)	104	98 (94)	0.53
Ever received SHRT	32	25 (78)	105	72 (69)	0.30
Hypogonadism females	30	23 (77)	155	136 (88)	0.11
Ever received SHRT	29	22 (76)	151	108 (72)	0.63

Data are presented as n (%), unless otherwise specified. P-values are calculated using a Chi-squared test for dichotomous variables and a Mann-Whitney U test for continuous variables. Abbreviations: Body Mass Index (BMI), deletion (del), growth hormone treatment (GHt), interquartile range (IQR), maternal uniparental disomy (mUPD), not applicable (NA), sex hormonal replacement therapy (SHRT). As not all variables were available for all included patients, we display the number of observations, representing the amount of patient for whom that variable was known. [a] Current age or age of death for deceased patients. [b] P <0.001 after adjusting for GHt (ever received). [c] P = 0.004 after adjusting for GHt (ever received). [d] During childhood includes all patients who had received GHt at some point during childhood, independent of whether the patient received GHt as an adult, compared to patients who never received GHt. [e] During adulthood includes all patients who had received GHt at some point when they were 18 years old, or older, independent of GHt during childhood, compared to patients who never received GHt.

Results of the Literature Review

Our search resulted in 1464 articles. A total of 1289 articles were excluded based on title and abstract. The remaining 175 articles underwent full-text screening. One article was excluded because the full-text was unavailable. Another 149 articles were excluded when, after reading the full-text, it turned out they did not meet the inclusion criteria. Twenty-six articles were included in the review.

Osteoporosis and Bone Mineral Density

Table 5 shows the results of the literature review of articles reporting on osteoporosis and BMD in adults with PWS. Osteoporosis was present in 2% to 26% of adults with PWS. Sinnema et al. (10) reported that osteoporosis was more prevalent in adults with PWS with a deletion. However, Faienza et al. (66) did not find a significant association between genotype and osteoporosis. Jørgensen et al. (67) reported that Z-scores of men were significantly lower than those in women with PWS and that a BMI > 30 kg/m^2 was associated with a higher BMD. Van Nieuwpoort et al. (68) reported that male sex was associated with lower lumbar spine T-scores. In this study, 87% of patients (both male and female) had hypogonadism, of whom 54% were treated with SHRT. Information on (the treatment of) hypogonadism was not given for males and females separately.

A prospective cohort study by Kido et al. (69) found that testosterone replacement therapy increased BMD after 2 years. Donze et al. (71) found a significant increase in SDS of total body BMD after 2 years of SHRT. However, Longhi et al. (70) did not find an association between SHRT and BMD.

Growth Hormone Treatment in Relation to Bone Health

Table 6 shows the studies reporting on the effect of GHt on skeletal problems (osteoporosis and/or scoliosis). Previous literature remains controversial regarding the effects of GHt. Several studies (65, 71–76) did not find any significant effect of GHt on BMD. Other studies showed conflicting results (67, 77).

Vitamin D Levels and Bone Markers and Bone-related Factors

Table 7 summarizes the results of the studies that investigated 25(OH) vitamin D and the bone related factors irisin, N-terminal propeptide of type I procollagen (P1NP), osteocalcin, Receptor activator of nuclear factor-κB Ligand (RANKL), osteoprotegerin (OPG), and N-oleoyl serine in patients with PWS (66, 67, 73, 78).

Longhi et al. (70) and Brunetti et al. (78) found lower levels of vitamin D in adults with PWS than in controls. However, Purtell et al. (29) did not find any association. According to Faienza et al. (66) vitamin D levels were not related to genotype.

Scoliosis

According to our literature review, the prevalence of scoliosis in adults with PWS is 5% to 100% **Table 8**. The large variation in this prevalence could be explained by underestimation of the prevalence due to the use of interviews and/or questionnaires (without physical examination or X-ray) to diagnose scoliosis in some studies (10, 11, 81–83). Studies that systematically screened for scoliosis by physical examination and/or spinal

X-ray reported a prevalence between 47% and 100%. Some studies reported a significantly higher prevalence of scoliosis in females (10, 11, 82) and patients with an mUPD (8). However, not all studies replicated these findings (10, 37, 39, 82, 84). Sode-Carlsen et al. (64) found that 16% of patients receiving GHt had progression of scoliosis (ie, increase in the Cobb angle) and 8% had a decrease in the Cobb angle after 2 years of GHt.

DISCUSSION

In this study, we showed that osteoporosis, osteopenia, and scoliosis are common skeletal problems in adults with PWS. Modifiable risk factors for osteoporosis such as hypogonadism, insufficient dairy intake, sedentary lifestyle, and corticosteroid use were often present, but we did not find modifiable risk factors for scoliosis.

The prevalence of osteoporosis found in this study is in line with previous studies in adults with PWS that showed a prevalence of 2% to 26% (8, 10–12, 75). However, not all studies performed a systematic screening for osteoporosis using DXA scans, likely leading to an underestimation of the true prevalence in these studies. Osteoporosis was more prevalent in males than in females, and this remained significant even after correction for age, height, weight, and genotype. In a previous study, males and females differed with regard to the use of psychotropic medication, which was slightly increased in males (85). As the use of some types of psychotropic medication is related to decreased BMD (86, 87), this might partly explain this difference. Fractures were more prevalent in males. Behavioral challenges are comparable between males and females, which suggests that the increased risk of fractures in males is not due to temper outburst (88, 89).

GHt may increase BMD and decrease the risk of osteoporosis in patients with PWS (77), although results are inconclusive (65, 67, 70, 76, 82). In our study, GHt was not associated with a decreased risk of osteoporosis. However, Longhi et al. showed that adults with PWS have unfavorable bone geometry and reduced bone strength, leading to an increased risk of fractures independent of BMD. GHt improves bone geometry, possibly reducing fracture risk without increasing BMD (70).

Risk factors for osteoporosis were prevalent. However, in our cohort many well-known risk factors for osteoporosis were not significantly related to osteoporosis or osteopenia. This is could be caused by lack of statistical power due to early treatment of risk factors such as hypogonadism or hyperthyroidism, leading to small numbers of untreated patients. Additionally, some risk factors were rare, also resulting in low statistical power.

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Table 5. Results of studies reporting on osteoporosis and bone mineral density in adults with Prader-Willi syndrome

Author (year)	Country	Patient characteristics	Methods
Butler et al. (2002)[11]	UK	N: 58 Age range: 18-46 years Sex: 32M, 26F Mean BMI: 35 kg/m^2 Genotype: NA GHt: NA SHRT: 13%	Cross-sectional study. Semi-structured interviews with family or caregivers.
Sinnema et al. (2011)[10]a	NL	N: 102 Mean age: 36 years (range 18-66) Sex: 49M, 53F Mean BMI: 32 kg/m^2 (range 17-52 kg/m^2) Genotype: 55 del, 44 mUPD, 3 ICD GHt: 5%, past 8% SHRT: males: current 16%, past 14% females: current 17%, past 9%	Cross-sectional study. Semi-structured interviews with caregivers and review of medical files.
Butler et al. (2013)[65]	USA	N: 11 Mean age: 32 years (range 23-50) Sex: 5M, 6F BMI: 34.5 kg/m^2 Genotype: 9 del, 1 mUPD, 1 ICD GHt: no prior GHt SHRT: NA, but evidence of hypogonadism and low sex steroid levels were present	Cohort study. 12 months GHt followed by 12 months no GHt. BMD assessment by DXA at baseline and 12 and 24 months.
Jørgensen et al. (2013)[67]a	Scandinavia	N: 42 Mean age: 28.5 years Sex: 21M, 21F Mean BMI: 28.1 kg/m^2 Genotype: NA GHt: no GHt at least 12 months before study, none during adulthood SHRT: not started at least 12 months before study 43%M, 29%F	Double blind RCT for GHt vs placebo for 12 months, open label, GHt for additional 24 months. Controls were 15 healthy age-, weight- and sex matched Norwegian subjects. Bone density assessment by DXA at baseline, 12, 24 and 36 months.

Outcome	Influencing factors	Remarks
Osteoporosis: 1/58 (2%). History of any fractures: 25/58 (43%). History of >1 fractures: 13/58 (22%).	-	Not all cases with PWS were genetically confirmed. No systematic BMD assessment.
Osteoporosis: 16/102 (16%).	Osteoporosis more prevalent in del (13/55, 24%) compared to mUPD (2/44, 5%, P = 0.02). No significant different was found in the prevalence of osteoporosis for different age groups or sex.	No systematic BMD assessment.
Osteoporosis: NA. Total body BMD (mean ± SE) at baseline: 1.14 ± 0.05 g/cm^2.	-	-
Osteoporosis: NA. BMD lumbar spine: 1.038 ± 0.138 g/cm^2. BMD total hip: 0.864 ± 0.120 g/cm^2. Z-score lumbar spine, mean (95%CI): -1.4 (-1.8 to -1.0). Z-score total hip, mean (95%CI): -1.5 (-1.8 to -1.3). Z-score total body, mean (95%CI): -1.0 (-1.4 to -0.6). Compared to healthy controls, BMD of lumbar spine and BMD z-score were significantly lower in adults with PWS However, BMD was not significantly different after correction for height.	Z-scores of men at lumbar spine and total body were significantly lower than Z-scores of women (lumbar spine, mean (95%CI): -1.9 (-2.5 to -1.3) vs -1.0 (-1.5 to -0.5), P <0.05, total body, mean (95%CI): -1.5 (-2.0 to -1.0) vs -0.5 (-0.9 to 0.0), P <0.01). No significant difference was found for total hip. BMD Z-score of eugonadal women at baseline were normal and higher than hypogonadal women (mean (95%CI): 0.0 (-0.6 to 0.6) vs -1.0 (-1.7 to -0.4), P <0.05). A BMI > 30 kg/m^2 was associated with higher baseline BMD of the lumbar spine (P <0.01), total hip (P <0.01) and total body (P <0.05). Regression analysis showed baseline BMD as a predictor of total hip BMD (P = 0.028). Lumbar spine BMD predictors were BMI (P = 0.010) and sex (P = 0.026).	-

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Table 5. Results of studies reporting on osteoporosis and bone mineral density in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Kido et al. (2013)[69]	Japan	N: 22 Age range: 16-48 years Sex: 22M Mean BMI: 33.6 kg/m^2 Genotype: 21 del, 1 mUPD GHt: 9% Hypogonadism: 100% SHRT: 0% at baseline, then SHRT was initiated in 100%	Observational cohort study. BMD assessment by DXA.
Longhi et al. (2015)[70]a	Italy	N: 41 Mean age: 29.4 years Sex: 17M, 24F Mean BMI: males 41.6 kg/m^2, females 41.2 kg/m^2 Genotype: 33 del, 8 mUPD GHt: current 34%, 22% past SHRT: 6%M, 54%F	Cross-sectional study. Controls matched for age and sex. BMD assessment by DXA scan.
Donze et al. (2018)[71]a	NL	N: 27 Mean age: 17.2 years Sex: 8M, 19F Mean BMI SDS: 0.9 Genotype: 9 del, 15 mUPD, 2 ICD, 1 translocation GHt: 100% at time of inclusion Hypogonadism: 88%M and 84%F SHRT: 38%M, 42%F	Baseline characteristics of a crossover GHt RCT. BMD assessment by DXA scan. Compared to age- and sex matched references.
Van Nieuwpoort et al. (2018)[68]	NL	N: 15 Median age: 22.0 years (range 19.2-42.9) Sex: 4M, 11F Median BMI: 27.5 kg/m^2 Genotype: 93.3% del, 6.7% mUPD GHt: current 0%, past 27% Hypogonadism: 87% SHRT: 54%	Cross-sectional study. BMD assessment by DXA scan.

Outcome	Influencing factors	Remarks
Osteoporosis: NA. At baseline (before start of SHRT): Lumbar spine BMD: 0.8505 ± 0.0426 g/cm^2 (n = 18) Lumbar spine T-score: -1.547 ± 1.871 Lumbar spine Z-score: -1.510 ± 1.871.	Two years of monthly intramuscular testosterone injections of 125 mg significantly increased Lumbar spine BMD (to 0.9035 ± 0.0465 g/cm^2, P = 0.036), lumbar spine T-scores (to -1.092 ± 1.333, P = 0.036) and lumbar spine Z-scores (to -0.934 ± 1.333, P = 0.036).	-
Osteoporosis: NA. No significant difference between PWS and controls In total body BMD (1.13 ± 0.01 vs 1.07 ± 0.16 g/cm^2), L2-L4 (1.04 ± 0.14 vs 1.21 ± 0.13 g/cm^2) or femur neck (0.89 ± 0.14 vs 1.02 ± 0.17 g/cm^2) after correcting for height.	No significant differences found between BMD total body, L2-L4 or femur neck for patients with and without SHRT.	Control group had a lower BMI (mean BMI 24.5 kg/m^2 in males and 21.1 kg/m^2 in females) than PWS patients.
Osteoporosis: NA. At adult height, total body BMD SD (Z-score) was -0.7 ± 1.1, which was significantly lower compared to healthy peers (P <0.01). 4 out 27 (15%) had total body BMD SD of <-2.0.	There was no significant difference in total body BMD SDS between hypogonadal adults with and without SHRT (P = 0.49 and P = 0.39 respectively).	All patients had received GHt during childhood. Not clear if all patients were aged >15 years.
Osteoporosis: Lumbar spine: 2 (13%). Total hip: 2 (13%). Osteopenia: Lumbar spine: 6 (40%). Total hip: 10 (67%). Median lumbar spine BMD: 0.91 (IQR 0.17) g/m^2. Median T-score lumbar spine: -1.4 (IQR 2.0). Median Z-score lumbar spine: -1.7 (IQR 1.4). Median total hip BMD: 0.76 (IQR 0.10) g/m^2. Median T-score total hip: -1.6 (IQR 0.9). Median Z-score total hip: -1.5 (IQR 1.1). Median total body BMD: 0.99 (IQR 0.13) g/cm^2.	Lumbar spine and total hip T-scores were significantly lower in men (median T-scores -2.6 and -2.0 respectively) compared to women (median T-scores -0.9 and -1.2 respectively, P <0.05). BMD for lumbar spine, hip and total body and Z-scores were not significantly different.	Criteria for osteoporosis not specified. DXA-scans only performed in 11 patients.

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Table 5. Results of studies reporting on osteoporosis and bone mineral density in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Viardot et al. (2018)[72]	Australia	N: 11 Mean age: 27.6 years Sex: 7M, 4F Mean BMI: 37.4 kg/m^2 Genotype: NA GHt: 0% SHRT: 71%M, 25%F	Cross-sectional study. BMD assessment by DXA scan. 2 control groups: one obese (n = 12) and one lean (n = 10) group.
Baraghithy et al. (2019)[73]	Israel	N: 30 Mean age: 29.9 years Sex: 60% M Mean BMI: 28.4 kg/m^2 Genotype: 16 del, 13 mUPD, 1 ICD GHt: NA SHRT: NA	Cross-sectional study. BMD assessment by DXA scan. Compared with 28 age- and BMI-matched controls.
Damen et al. (2021)[74]a	NL	N: 43 Mean age (range): 19.5 years (18.7 – 20.7) for males and 18.4 years (15.8 – 23.8) for females Sex: 18M, 25F Mean BMI: 24.5 kg/m^2 Genotype: 18 del, 20 mUPD, 4 ICD, 1 translocation GHt: 100% Hypogonadism: 93% SHRT: 83% Hypogonadal without SHRT: 16%	Baseline characteristics of a prospective cohort study on GHt. BMD assessment with DXA scans, compared to age- and sex matched references.

Outcome	Influencing factors	Remarks
Osteoporosis: NA. Total body BMD (mean ± SE): PWS: 1.21 ± 0.07 g/cm². Obese controls: 1.25 ± 0.07 g/cm². Lean controls: 1.13 ± 0.06 g/cm². No significant differences between PWS and the control groups in total body BMD.	Multiple linear regression in all (PWS and non-PWS) included individuals showed that total lean mass was a significant predictor of BMD (coefficient 0.005, P = 0.024), while having obesity, having PWS, fat mass, height and age were not.	No DXA results for lumbar spine or hip. SHRT no included in multiple regression model. Matching was performed by recruiting control groups with similar baseline characteristics to the PWS group rather than case to case matching. Unknown how patients with similar characteristics were selected.
Osteoporosis: NA. Z-scores of PWS patients significantly lower than controls (femoral neck: -1.6 ± 1.0 vs 0.2 ± 1.2, P <0.001, total hip -1.3 ± 1.4 vs 0.4 ± 1.1, P <0.001, lumbar spine -1.6 ± 1.3 vs -0.4 ± 1.3, P = 0.001 and forearm -2.4 ± 1.2 vs -0.4 ± 0.7 P <0.001).	-	-
Osteoporosis: NA. Total body BMD (median (IQR)) was 1.15 (1.08 to 1.19) g/cm², total body SDS -0.78 (-1.31 to 0.11), which was significantly lower than in the reference group (P <0.01). 9 out of 43 (21%) patients total body SD <-2.0. Lumbar spine BMD[c] (median (IQR)): 1.19 (1.09 to 1.26) g/cm², lumbar spine SDS -0.62 (-1.16 to -0.09) One patient (2%) had a fracture during the study.	-	All patients had received GHt for at least 5 years during childhood. No baseline characteristics of controls.

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Table 5. Results of studies reporting on osteoporosis and bone mineral density in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Faienza et al. (2021)[66)a]	Italy	N: 52 Mean age: 30.6 years (SD 10.7 years) Sex: 22M, 30F Median BMI: 35.3 kg/m^2 Genotype: 32 del, 20 mUPD GHt: 6 SHRT: 5%M, 33%F Vitamin D: 50% supplementation	Cross-sectional study. Biochemical measurement from blood samples and DXA scans to asses BMD. Compared with 54 normal weight adult controls.
Noh et al. (2022)[75]	Korea	N: 68 Age range: 19-34 years Sex: 39M, 29F Mean BMI: 35 kg/m^2 Genotype: 44 del, 24 other GHt: 48 previous, 10 current SHRT: NA	Cross-sectional study. Data collection from patient records. Compared with 204 age, sex and BMI-matched controls. BMD assessment by DXA scan.

Data are presented as mean ± SD, unless otherwise specified. When articles reported subgroup analysis for adults and children or patients > 16 years old and patients < 16 years old, only information for the adults or patients >16 years old are reported here whenever possible. Abbreviations: Body Mass Index (BMI), Bone Mineral Density (BMD), deletion (del), Dual-energy X-ray Absorptiometry (DXA), females (F), growth hormone treatment (GHt), imprinting center defect (ICD), interquartile range (IQR), males (M), maternal uniparental disomy (mUPD), not available (NA), number of patients (N), randomized controlled trial (RCT), sex hormone replacement therapy with estrogen or testosterone (SHRT), standard deviation (SD), standard deviation score (SDS), standard error (SE), the Netherlands (NL), United Kingdom (UK), United States of America (USA), World Health Organization (WHO). [a] Partly overlapping study population as current study. [b] Data for the entire cohort of 56 patients, which also included patients <15 years old. [c] Data on lumbar spine BMD and SDS for n = 34 due to scoliosis surgery.

Outcome	Influencing factors	Remarks
Osteoporosis: NA. T-score BMD of lumbar spine (median (IQR)): -1.10 (0.20). Lumbar spine BMD: 1.05 (0.06) g/cm^2. Total body less head BMD: 1.18 (0.08) g/cm^2 No significant difference between del and mUPD genotype. Five (10%) adults had a history of post-traumatic fractures.	No significant difference between del and mUPD.	Controls are normal weight adults, while irisin is also released from adipose tissue. Exclusion criteria: use of mineral or vitamin supplements (except for vitamin D), presence of chronic diseases with possible impact on bone metabolism, use of medication affecting bone turnover and fractures in the 6 months preceding the study.
Decreased bone density: 18/68 (26%) in PWS adults, compared to 2/204 (1%) in controls (P <0.001).	Decreased bone density according to GHt: Without GHt: 2/10 (20%) Previous GHt: 14/48 (29%) Current GHt: 2/10: (20%) *p*>0.05 for all comparisons	-

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Table 6. Results of studies reporting on growth hormone treatment in relation to bone mineral density in adults with Prader-Willi syndrome

Author (year)	Country	Patient characteristics	Methods
Butler et al. (2013)[65]	USA	N: 11 Mean age: 32 years (range 23-50) Sex: 5M, 6F BMI: 34.5 kg/m^2 Genotype: 9 del, 1 mUPD, 1 ICD GHt: no prior GHt SHRT: NA, but evidence of hypogonadism and low sex steroid levels were present	Cohort study. 12 monts GHt, followed by 12 months without GHt. BMD assessment by DXA at baseline and 12 and 24 months.
Jørgensen et al. (2013)[67)a]	Scandinavia	N: 42 Mean age: 28.5 years Sex: 21M, 21F BMI: 28.1 kg/m^2 Genotype: GHt: no GHt at least 12 months before study, none during adulthood SHRT: 43% M, 29%F, not started at least 12 months before study	Double blind RCT for GHt vs placebo for 12 months, open label GHt for additional 24 months. Controls were 15 healthy age-, weight- and sex matched Norwegian subjects. Bone density assessment by DXA at baseline, 12, 24 and 36 months.
Khare et al. (2014)[77]	USA	N: 18 Mean age: >15 years old Sex: NA BMI: NA Genotype: 9 del, 8 mUPD, 1 unknown GHt: 0% prior to study SHRT: NA	Cross-sectional study. 7 subjects received GHt and 11 subjects had never received GHt. Assessment of BMD by DXA scan.
Longhi et al. (2015)[70)a]	Italy	N: 41 Mean age 29.4 years Sex: 17M, 24F BMI: males 41.6 kg/m^2, females 41.2 kg/m^2 Genotype: 33 del, 8 mUPD GHt: current 34%, 22% past SHRT: 6%M, 54%F	Cross-sectional study comparing previous or current GHt (n = 23) vs no GHt (n = 18). BMD assessment by DXA scan.
Donze et al. (2018)[71)a]	NL	N: 27 Mean age: 17.2 years Sex: 8M, 19F Mean BMI SDS: 0.9 Genotype: 9 del, 15 mUPD, 2 ICD, 1 translocation GHt: 100% at time of inclusion Hypogonadism: 88%M and 84%F SHRT: 38%M, 42%F	Double blind RCT 1 year GHt vs placebo followed by cross-over to the alternative treatment for 1 year. BMD assessment by DXA scan at baseline, 6, 12, 18 and 24 months.

Outcome	Influencing factors	Remarks
Total BMD (mean ± SE) At baseline: 1.14 ± 0.05 g/cm^2. At 12 months: 1.12 ± 0.05 g/cm^2. At 24 months: 1.15 ± 0.05 g/cm^2. No significant change in BMD was found during follow-up.	-	All low plasma IGF-1 level at baseline.
12 months of GHt significantly decreased Z-score of lumbar spine (-2.1%±3.4%) compared to placebo (+1.9%±3.4%, P <0.01). No other changes in BMD at other sides or any significant changes after 24 months of GHt were found.	After 24 months of GHt, Z-score of men remained significantly lower than women (lumbar spine, mean (95%CI): -2.2 (-2.8 to -1.6) vs -1.1 (-1.6 to -0.5), P <0.05. Total body, mean (95%CI): -1.7 (-2.2 to -1.1) vs -0.5 (-0.9 to 0.0), P <0.01).	Patients lost-to-follow-up were excluded from analysis.
BMD Z-score of the spine was significantly higher in the GHt group compared to the no GHt group (P = 0.021).	There was no statistically significant difference in BMD Z-score of the spine between patients with a del or mUPD.	-
No significant difference between GHt and no GHt PWS patients in BMD (g/cm^2) of total body (1.11 ± 0.09 vs 1.15 ± 0.12), lumbar spine (1.02 ± 0.12 vs 1.05 ± 0.16) or femur neck (0.86 ± 0.13 vs 0.91 ± 0.16).	-	No distinction between current and past GHt.
GHt did not affect BMD measurements. No significant difference was found in total body and lumbar spine BMD SDS after 12 months of GHt compared to placebo (P = 0.51 and P = 0.37 respectively). After two years, BMD of total body SDS did not change significantly (P = 0.20), but BMD SDS of the lumbar spine corrected for bone size declined significantly, independent of GHt (P <0.01). There was no significant difference in change in BMD SDS for total body and BMD SWS for lumbar spine corrected for bone size between GH and placebo. There were no bone fractures during the study period.	SHRT did influence BMD measurements. Independent of GHt or placebo, total body BMD SDS did not change significantly in hypogonadal patients without SHRT (-0.8 to -0.9, P = 0.11), while there was a significant increase in total body BMD SDS from -1.1 to -0.7 (P <0.01) in patients with SHRT after two years. SDS of the BMD of the lumbar spine corrected for bone size decreased from -0.2 to -0.6 in hypogonadal patients without SHRT (P = 0.01), while it remained similar in patients with SHRT (-0.5 at baseline and after 2 years, P = 0.79).	All patients had received GHt during childhood. Not clear if all patients were aged >15 years.

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Table 6. Results of studies reporting on growth hormone treatment in relation to bone mineral density in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Damen et al. (2021)[74]a	NL	N: 43 Mean age (range): 19.5 years (18.7 – 20.7) for males and 18.4 years (15.8 – 23.8) for females Sex: 18M, 25F Mean BMI: 24.5 kg/m^2 Genotype: 18 del, 20 mUPD, 4 ICD, 1 translocation Hypogonadism: 93% SHRT: 83% Hypogonadal without SHRT: 16%	Open label prospective cohort study, patients received GHt during 3 years. BMD assessment with DXA scans.

Data are presented as mean ± SD, unless otherwise specified. Abbreviations: Body Mass Index (BMI), Bone Mineral Density (BMD), deletion (del), Dual-energy X-ray Absorptiometry (DXA), imprinting center defect (ICD), females (F), growth hormone treatment (GHt), males (M), maternal uniparental disomy (mUPD), not available (NA), number of patients (N), sex hormone replacement therapy (SHRT), standard deviation (SD), standard deviation score (SDS), standard error (SE), the Netherlands (NL), United States of America (USA), 95% confidence interval (95% CI). [a] Partly overlapping study population as current study.

Outcome	Influencing factors	Remarks
No significant difference were found for BMD of total body at start and after three years (SDS -0.76 (-1.11 to -0.41) vs -0.90 (-1.27 to -0.54), P = 0.11).	In men, a significant decrease in SDS BMD of total body after three years was found (-1.10 (-1.70 to -0.49) vs -1.46 (-1.94 to -0.98), P = 0.008). In women, no significant different was found in total body BMD SDS (P = 0.78). No significant different was found in total body BMD of 33 male and female subjects with SHRT after three years (P = 0.37). In men receiving SHRT, a significant decrease in total body BMD was found during three years from -1.33 (-1.96 to -0.69) to -1.59 (-2.15 to -1.01), P = 0.014. In women, no difference was found (P = 0.72). Regression analysis showed and association between female sex and higher BMD total body at baseline (β=1.956, P <0.001) and after three years (β=2.100, P <0.001). No associated was found between age and genetic subtypes.	All patients had received GHt for at least 5 years during childhood. No baseline characteristics of controls.

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Table 7. Results of studies reporting on vitamin D and bone related factors in adults with Prader-Willi syndrome

Author (year)	Country	Patient characteristics	Methods
Jørgensen et al. (2013)[67]a	Scandinavia	N: 42 Mean age: 28.5 years Sex: 21M, 21F BMI: 28.1 kg/m^2 Genotype: NA GHt: no GHt at least 12 months before study, none during adulthood SHRT: 43%M, 29%F, not started at least 12 months before study.	Randomized controlled double blind for GHt vs placebo for 12 months, open label GHt for additional 24 months. Blood samples were collected for biochemical essay.
Longhi et al. (2015)[70]a	Italy	N: 41 Mean age: 29.4 years Sex: 17M, 24F BMI: males 41.6 kg/m^2, females 41.2 kg/m^2 Genotype: 33 del, 8 mUPD GHt: 34% current, 22% past SHRT: 6%M, 54%F	Cross-sectional study. Controls matched for age and sex. Blood samples were taken for 25(OH) vitamin D, intact PTH and BAP measurements.
Purtell et al. (2016)[29]	Australia	N: 10 Mean age: 27.9 years Sex: NA Mean BMI: 37.0 kg/m^2 Genotype: NA GHt: 0%	Cross-sectional study. Blood samples were taken for analysis. 2 control groups: one obese (n = 12) and one lean (n = 10) group.
Brunetti et al. (2018)[78]	Italy	N: 14 Mean age: 29.5 years (SD7.2 years) Sex: 5M, 9F Mean BMI: 44.6 kg/m^2 Genotype: 12 del, 2 mUPD GHt: 43% SHRT: 0%M, 43%F	Cross-sectional study. Venous blood samples were collected to assess blood values. BMD assessed by DXA scan. 15 normal weight controls matched for sex and age.
Baraghithy et al. (2019)[73]	Israel	N: 30 Mean age: 29.9 years Sex: 18M, 12F Mean BMI: 28.4 kg/m^2 Genotype: 16 del, 13 mUPD, 1 ICD GHt: NA SHRT: NA	Cross-sectional study. Measurement of *N*-oleoyl serine, 25(OH) vitamin D, calcium, alkaline phosphatase and phosphatase in venous blood. Assessment of Z-scores by DXA scan.

	Outcome	Influencing factors	Remarks
	At baseline: P1NP was at the high end of normal (73.8±36.6 μg/L), osteocalcin was low (4.1±2.8 μg/L), cross-linked N-telopeptides of type I collagen was in the upper range of expected (20.7±6.2 nM).	GHt for 12 months increased P1NP levels (P <0.001) and normalized osteocalcin levels (P <0.05). Cross-linked N-telopeptides of type I collagen did not change significantly.	Patients lost-to-follow-up were excluded from analysis.
	Vitamin D levels of PWS patients were significantly lower than controls (19.8 ± 9.7 vs 36.2 ± 16.7 μg/L, P <0.01). PWS patients showed significantly higher value of BAP (15.1 ± 7.7 vs. 12.2 ± 3.8 μg/L, P < 0.05) compared to controls.	-	Control group had a lower BMI (mean BMI 24.5 kg/m^2 in males and 21.1 kg/m^2 in females) than PWS patients. Use of vitamin D supplements unknown.
	No significant differences between 25(OH) vitamin D levels between the lean (23.4 ± 4.4 ng/mL), obese (18.6 ± 3.1 ng/mL) and PWS group (12.7 ± 1.5 ng/mL). The mean vitamin D level in PWS was in the range of mild to moderate vitamin D deficiency.	-	Unknown if any individuals used vitamin D supplements.
	25(OH) vitamin D (ng/mL), osteocalcin (ng/mL), OPG (pg/mL) and sclerostin (pg/ml) levels significantly lower in PWS adults compared to controls (25.17 ± 11.83 vs 35.2 ± 5.8, P <0.001, 7.91 ± 7.94 vs 21.3 ± 3.21, P <0.01, 317.2 ± 77.5 vs 443.5 ± 116, P <0.006 and 1298 ± 318 vs 1906 ± 698, P <0.01 respectively). RANKL (pg/mL) significantly higher in PWS adults than controls (77.5 ± 42.2 vs 51.8 ± 20.9, P <0.004). However, this was no longer significant after correction for GHt and SHRT. CTX and DKK-1 were not significantly different between PWS and controls.	Multivariate analysis showed no correlation between RANKL and T-score of lumbar spine BMD (β=-0.033, P = 0.134) but a positive correlation between OPG and T-score of lumbar spine BMD (β=1.521, P = 0.0001) and sclerostin and T-score of lumbar spine BMD (β=0.331, P = 0.0001).	Patients and controls that used vitamin D or mineral supplements, had chronic diseases impacting bone metabolism, used medications affecting bone turnover or had a fracture in the 6 months preceding the study were excluded.
	Vitamin D levels in PWS were 74.5 ± 79.3 ng/mL. N-oleoyl serine was significantly lower in PWS compared to controls (1.4 ± 0.7 pmol/mL vs 2.4 ± 0.95 pmol/mL, P <0.001). No significant differences in 25(OH) vitamin D, calcium, alkaline phosphatase and phophatasae between patients with and without PWS.	N-oleoyl serine was positively associated with Z-scores of femoral neck (r=0.405, P = 0.0018), total hip (r=0.439, P = 0.0007), lumbar spine (r=0.296, P = 0.0251) and forearm (r=0.349, P = 0.0186).	Use of vitamin D supplements unknown.

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Table 7. Results of studies reporting on vitamin D and bone related factors in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Barrea et al. (2020)[79]	Italy	N: 15 Mean age: 28 years (range 19-41 years) Sex: 6M, 9F Mean BMI: 44 kg/m^2 Genotype: NA GHt: none current, 100% past	Cross-sectional study. Data collected by interview, physical examination and biochemical essays. Compared with 15 age, sex and BMI-matched controls.
Damen et al. (2021)[74)a]	NL	N: 43 Mean age (range): 19.5 years (18.7 – 20.7) for males and 18.4 years (15.8 – 23.8) for females Sex: 18M, 25F Mean BMI: 24.5 kg/m^2 Genotype: 18 del, 20 mUPD, 4 ICD, 1 translocation Hypogonadism: 93% SHRT: 83% Hypogonadal without SHRT: 16%	Open label, prospective cohort study, patients received GHt during 3 years. Biochemical measurement from blood samples.
Faienza et al. (2021)[66)a]	Italy	N: 52 Mean age: 30.6 years (SD 10.7 years) Sex: 22M, 30F Median BMI: 35.3 kg/m^2 Genotype: 32 del, 20 mUPD GHt: 6 SHRT: 5%M, 33%F Vitamin D: 50% supplementation	Cross-sectional study. Biochemical measurement from blood samples and DXA scans to asses BMD. Compared with 54 normal weight adult controls.

Outcome	Influencing factors	Remarks
Vitamin D deficiency: 15/15 (100%). Dietary vitamin D intake was significantly lower in adults with PWS than controls (4 ± 1 ug/1.000 kcal vs 5 ± 1 ug/1.000 kcal, P = 0.01). 25(OH) vitamin D in PWS adults significantly lower than controls (22 ± 7 vs 35 ± 10, P = 0.001), regardless of BMI or fat mass category.	25(OH) vitamin D levels were significantly associated with BMI (r=-0.52, P = 0.04), waist circumference (r=-0.56, P = 0.03), fat mass (r=-0.52, P = 0.04) and dietary vitamin D intake (r=0.91, P <0.001).	Many exclusion criteria, including current therapy with calcium, osteoporosis therapies and medications that may affect vitamin absorption of metabolism like SHRT. Inclusion period October to March.
25(OH) vitamin D levels at baseline (mean (range)): 66.0 (55.3 to 88.8) nmol/L.	No significant difference was found in 25(OH) vitamin D levels after three years of GHt.	All patients had received GHt for at least 5 years during childhood.
25(OH) vitamin D Median (IQR) vitamin D level in adults with PWS was 28.8 (9.2) ng/mL. *Irisin* No significant difference between PWS adults and controls (6.65 ± 4.49 µg/ml vs 7.24 ± 5.20 µg/ml).	After adjusting for age, irisin in adults with PWS the best predictors for irisin levels were the genetic background (β=-0.365, P = 0.0001), 25(OH) vitamin D levels (β=0.346, P = 0.0001), GHt (β=-0.139, P = 0.0001), age at start (β=-0.317, P = 0.0001) and duration of GHt (β=-0.139, P = 0.0001 age at start of SHRT (β=-0.324, P = 0.0001), IQ (β=0.910, P = 0.0001) and total body BMD adjusted for height (β=0.412, P = 0.0001). *25(OH) vitamin D* No significant difference between 25(OH) vitamin D levels of adults with deletion and mUPD genotype (31.6±9.2 vs 28.7±10.5 ng/mL). *Irisin* Significantly reduced in del compared to controls (P <0.04), but not in mUPD compared to controls. PWS patients without vitamin D supplementation had a significant reduction in irisin compared to controls (P <0.001) and patients with supplementation (P <0.02), for both the del (P <0.004) and mUPD (P <0.001) genotype.	Controls are normal weight adults, while irisin is also released from adipose tissue. Exclusion criteria: use of mineral or vitamin supplements (except for vitamin D), presence of chronic diseases with possible impact on bone metabolism, use of medication affecting bone turnover and fractures in the 6 months preceding the study. Study population might overlap with Brunetti et al. (76).

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Table 7. Results of studies reporting on vitamin D and bone related factors in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Casamitjana et al. (2022)[80]	Spain	N: 27 Median age: 26 years (all >18 years) Sex: 12M, 15F Median BMI: 34.5 kg/m^2 Genotype: 18 del, 6 mUPD, 3 ICD GHt: 0% at baseline SHRT: 8M, 7F	Cohort study with GHt for 12 months in the PWS adults. Fasting blood samples were collected for biochemical essay. Control group: 22 volunteers from hospital staff or acquaintances, median age 27.5 years, 13 women.

Data are presented as mean ± SD, unless otherwise specified. When articles reported subgroup analysis for adults and children or patients > 16 years old and patients < 16 years old, only information for the adults or patients >16 years old are reported here whenever possible. Abbreviations: Body Mass Index (BMI), bone alkaline phosphatase (BAP), Bone Mineral Density (BMD), C-terminal telopeptide of type I collagen (CTX-1), deletion (del), Dickkopf-1 (DKK-1), Dual-energy X-ray Absorptiometry (DXA), females (F), growth hormone treatment (GHt), males (M), maternal uniparental disomy (mUPD), not available (NA), N-terminal propeptide of type I procollagen (P1NP), number of patients (N), osteoprotegerin (OPG), receptor activator of nuclear factor-κB ligand (RANKL), sex hormone replacement therapy (SHRT), 25-hydroxy vitamin D (25(OH) vitamin D). [a] Partly overlapping study population as current study.

Outcome	Influencing factors	Remarks
At baseline: Median (IQR): Irisin was 982.3 (519.4-1789.6) ng/mL in PWS adults compared to 89.8 (41.8-219.4) ng/mL in controls (P <0.0001). Myostatin and IL-6 did not differ significantly between PWS adults and controls (*p*>0.05).	After 12 months of GHt the median (IQR) irisin level was 906.8 (583.5-1770.4) ng/mL (P = 0.76 compared to baseline). No significant change was observed for myostatin and IL-6 (*p*>0.05 for both)	-

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Table 8. Results of studies reporting on scoliosis and other orthopedic conditions in adults with Prader-Willi syndrome

Author (year)	Country	Patient characteristics	Methods
Holm et al. (1981)[35]	USA	N: 10 Mean age: 28 years (range 20-41 years) Sex: 6M, 4F BMI: NA Genotype: NA GHt: NA	Cross-sectional study. Cobb angle of ≥ 10° on spinal X-ray.
Partsch et al. (2000)[81]	Germany	N: 19 Mean age: 23 years (range 18-34 years) Sex: 7M, 12F Mean BMI 46 kg/m^2, range 31-74 kg/m^2 Genotype: All deletion or mUPD GHt: 0%	Cross-sectional study. Data collection from patient records.
Butler et al. (2002)[11]	UK	N: 58 (information about scoliosis known for 56) Age range: 18-46 years Sex: 32M, 26F Mean BMI: 35 kg/m^2 Genotype: NA GHt: NA	Cross-sectional study. Semi-structured interview with family or carers.
Nakamura et al. (2009)[39]	Japan	N: 34 Age range: 16-50 years Sex: 67M, 34F [b] BMI: NA Genotype: 80 del, 21 no del [b] GHt: 57% [b]	Retrospective cohort study. Cobb angle of ≥ 10° on spinal X-ray.
Sode-Carlsen et al. (2011)[64]a	Scandinavia	N: 43 Mean age: 29.5 years (range 16-42) Sex: 19M, 24F Mean (SD) BMI at baseline 28.9 (19.4-44.8) kg/m^2 Genotype: NA SHRT: 47%M, 25%F	Multicenter international RCT. GHt vs placebo for 1 year, followed by open label GHt for all patients for 1 year (GHt group) or 2 years (placebo group), until all patients had received GHt for 2 years in total. Scoliosis evaluated by spinal X-ray and defined as Cobb angle >10° and progression as a change of ≥ +5° change in Cobb angle.

	Prevalence scoliosis and orthopedic conditions	Influencing factors	Remarks
	Scoliosis: 10/10 (100%). Average Cobb angle: 22.5º. Kyphosis: 5/10 (50%) Mean curve kyphosis: 61º.	-	Diagnosis PWS based on clinical criteria instead of genetic tests.
	Scoliosis: 7/19 (37%). Kyphosis: 19/19 (100%). Gonarthrosis: 1/19 (5%).	-	Unknown whether spinal X-ray was performed.
	Suspected scoliosis/kyphosis: 23/56 (41%). Scoliosis observed by a professional: 19/56 (34%). Serious scoliosis or intervention for scoliosis: 7/56 (13%).	Scoliosis more prevalent in females compared to males (1.23:1 and 2.3:1 for severe deformity). BMI not significantly associated with scoliosis.	Not all cases with PWS were genetically confirmed. No spinal X-ray.
	Scoliosis: 16/34 (47%). Mean Cobb angle: 27º. Severe scoliosis: 3/34 (9%). Surgery for scoliosis: 1/34 (3%).	Genotype and GHt were not significantly associated (analysis in entire group of children and adults).	-
	Scoliosis: 23 / 38 (61%) Median Cobb angle: 13º. Operation for scoliosis: 2/38 (5%).	After two years of GHt, 6 patients (16%) showed progression of scoliosis and 3 (8%) showed a decrease of Cobb angle >5º.	Unknown GHt prior to study, but none was treated for at least one year preceding the study. 4 patients did not complete the study.

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Table 8. Results of studies reporting on scoliosis and other orthopedic conditions in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Sinnema et al. (2011)[10] and Sinnema et al. (2013)[82]a	NL	N: 102 Mean age: 36 years (range 18-66 years) Sex: 49M, 53F Mean BMI: 32 kg/m^2 (range 17-52 kg/m^2) Genotype: 55 del, 44 mUPD, 3 ICD GHt: 5% current, 8% past	Cross-sectional study. Semi-structured interviews with caregivers and review of medical files.
Laurier et al. (2015)[37]a	France	N: 154 Mean age: 28 years (range 16-54 years) Sex: 68M, 86F Mean BMI ± SD: 42 ± 11 kg/m^2 Genotype: 101 del, 24 mUPD, 3 ICD, 3 translocation, 18 AMP non del, 5 AMP GHt 14% current, 24% past	Cross-sectional study. Spinal X-ray.
Coupaye et al. (2016)[84]a	France	N: 73 Mean age: 25 years (range 16-58 years) Sex: 35M, 38F Mean BMI ± SD: del-group: 41 ± 11 kg/m^2 mUPD-group: 35 ± 10 kg/m^2 Genotype: 47 del, 26 mUPD GHt: 15% current, 36% past	Cross-sectional study. Systematic examination at outpatient clinic.
Woods et al. (2018)[83]	USA	N: 19 Mean age: 34.5 (range 18-62 years) Sex: 11M, 8F Mean BMI: 27 kg/m^2 (range 19.5 – 35.0 kg/m^2) Genotype: NA GHt: NA	Cross-sectional study. Questionnaires filled in by guardians or caregivers.
Pellikaan et al. (2020)[8]a	NL	N: 115 Median age: 29 years (range 18-72 years) Sex: 56M, 59F Median BMI: 29 kg/m^2 [IQR 26-35 kg/m^2] Genotype: 64 del, 41 mUPD, 3 ICD, 7 unknown GHt: 36% current	Cross-sectional study. Spinal X-ray if gibbus deformity present during physical examination.

	Prevalence scoliosis and orthopedic conditions	Influencing factors	Remarks
	Scoliosis: 57/102 (56%). Foot problems: 81/102 (79%). Knee problems: 6/102 (6%). Hip problems: 9/102 (9%). Surgery for musculo-skeletal conditons: 28/102 (27%). Of which: Surgery for scoliosis: 11/102 (11%). Hip surgery: 4/102 (4%). Arthroscopy: 4/102 (4%). Knee surgery: 3/102 (3%). Osteosynthesis: 3/102 (3%). Foot surgery: 3/102 (3%).	Patients with a deletion had more knee problems compared to other genotypes (11% vs 0%, $P = 0.02$), there was no difference in scoliosis, foot problems or hip problems. BMI and age were not associated to any orthopedic conditions.	Unknown if scoliosis was confirmed by spinal X-ray in all cases.
	Scoliosis: 95/126 (75%).	No association with genotype (del vs mUPD) or age.	-
	Scoliosis: 57/73 (78%). Severe/operated scoliosis: 16/73 (22%).	No association between scoliosis and genotype, but severe/operated scoliosis was more prevalent in mUPD (9/26, 35%) compared to del (35/47, 15%, $P = 0.047$).	Unknown if spinal X-rays were performed.
	Scoliosis: 1/19 (5%).	-	Unknown if PWS was genetically confirmed.
	Scoliosis after systematic screening: 83/112 [c] (74%). Scoliosis was undiagnosed before systematic health screening in 22 (20%) patients.	Scoliosis was more frequent in patients with an mUPD (59%) compared to with a deletion (81%, $P = 0.02$). Scoliosis was not associated with BMI, age, sex or living situation.	-

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Table 8. Results of studies reporting on scoliosis and other orthopedic conditions in adults with Prader-Willi syndrome (*continued*)

Author (year)	Country	Patient characteristics	Methods
Crinò et al. (2022)[40]		N: 74 Age range: 18-50 years Sex: 34M, 40F Mean BMI ± SD: 36 ± 9 kg/m^2 Genotype: 46 del, 28 mUPD GHt: 53 current or past	Cross-sectional study. Observation of the standing and sitting posture, Adam's forward bend test, and spinal X-ray.
Noh et al. (2022)[75]	Korea	N: 68 Age range: 19-34 years Sex: 39M, 29F Mean BMI: 35 kg/m^2 Genotype: 44 del, 24 other GHt: 48 previous, 10 current	Cross-sectional study. Data collection from patient records.

Data are presented as mean ± SD, unless otherwise specified. When articles reported subgroup analysis for adults and children or patients > 16 years old and patients < 16 years old, only information for the adults or patients >16 years old are reported here whenever possible. Abbreviations: abnormal methylation profile (AMP), Body Mass Index (BMI), deletion (del), females (F), growth hormone treatment (GHt), interquartile range (IQR), males (M), maternal uniparental disomy (mUPD), number of patients (N), not available (NA), United Kingdom (UK), United States of America (USA). [a] Study population partly overlapping with current study. [b] Data for the entire cohort of 101 patients, which also included patients <16 years old. [c] Scoliosis was missing for three patients.

Prevalence scoliosis and orthopedic conditions	Influencing factors	Remarks
Scoliosis: 87.8%	-	-
Scoliosis: 41/68 (60%).	-	Unknown whether spinal X-ray was performed.

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In our cohort, vertebral fractures were found in 3%, which is similar to the prevalence found by Waterloo et al. (90) for vertebral fractures in the general population before the age of 60. However, the patients in our cohort were relatively young with a median age of only 31 years (IQR 25-40), with only a few patients who were above 50 years old. Therefore, we were not able to investigate the prevalence of fractures in older adults. Moreover, data on vertebral fracture assessments were not available for all patients, possibly leading to an underestimation.

The prevalence of scoliosis found in this study was 80%. Previous research reported a prevalence of scoliosis between 5% and 100% (**Table 8**). Scoliosis is thought to be related to obesity and hypotonia of (paravertebral) muscles (35, 38). However, in our cohort, scoliosis was not significantly related to BMI and hypotonia was not assessed. According to Burwell et al, childhood GHt could be related to an increased risk of scoliosis due to increased growth velocity (91). Sode-Carlson et al. found a progression of the Cobb angle of >5° in 16% of patients with PWS after 2 years of GHt, although no control group was available (64). However, in our study, childhood GHt was not associated with scoliosis. This is in line with previous pediatric studies that reported no effect of GHt on onset of scoliosis, curve progression or need for surgery in patients with PWS (38, 92, 93).

Although the median age of our cohort was only 31 years, we already found a high prevalence of osteoporosis (14%) and osteopenia (54%). Due to improved health care, life expectancy of patients with PWS has drastically increased (94). Therefore, early prevention and detection is crucial to prevent complications later in life.

Clinical Recommendations

Due to the complexity of the syndrome, patients with PWS are preferably treated in a PWS reference center. However, reference centers are not always available. Therefore, we have defined practical clinical recommendations for the optimization of skeletal health in adults with PWS (**Figures 1 and and 2**) that can be used in any clinical setting.

We recommend screening for osteoporosis and scoliosis in all adults with PWS and assessing risk factors for osteoporosis. The screening should consist of a DXA scan (if possible, with vertebral fracture assessment) every 5 years in patients with normal BMD on the previous DXA scan and every 2 years in patients with osteopenia or osteoporosis. For scoliosis, yearly evaluation should be performed. We want to stress the fact that osteoporotic fractures can be easily missed due to the high pain threshold and intellectual disability often present in patients with PWS. Preventive measures to avoid the development of osteoporosis in adults with PWS include (1) optimizing calcium and vitamin D intake, (2) optimizing physical activity, (3) avoidance of unnecessary use of
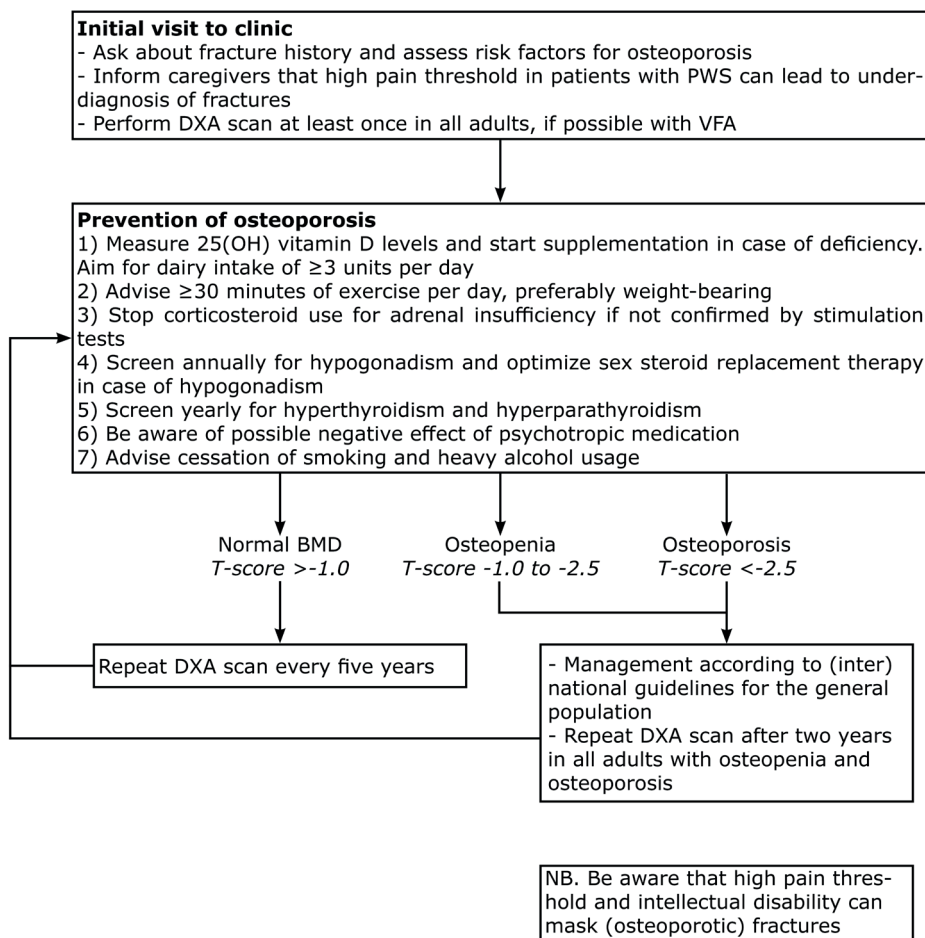
Initial visit to clinic
- Ask about fracture history and assess risk factors for osteoporosis
- Inform caregivers that high pain threshold in patients with PWS can lead to under-diagnosis of fractures
- Perform DXA scan at least once in all adults, if possible with VFA

Prevention of osteoporosis
1) Measure 25(OH) vitamin D levels and start supplementation in case of deficiency. Aim for dairy intake of ≥3 units per day
2) Advise ≥30 minutes of exercise per day, preferably weight-bearing
3) Stop corticosteroid use for adrenal insufficiency if not confirmed by stimulation tests
4) Screen annually for hypogonadism and optimize sex steroid replacement therapy in case of hypogonadism
5) Screen yearly for hyperthyroidism and hyperparathyroidism
6) Be aware of possible negative effect of psychotropic medication
7) Advise cessation of smoking and heavy alcohol usage

Normal BMD
T-score >-1.0

Osteopenia
T-score -1.0 to -2.5

Osteoporosis
T-score <-2.5

Repeat DXA scan every five years

- Management according to (inter)national guidelines for the general population
- Repeat DXA scan after two years in all adults with osteopenia and osteoporosis

NB. Be aware that high pain threshold and intellectual disability can mask (osteoporotic) fractures

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Figure 1. Recommendations for the prevention, detection and treatment of osteoporosis in adults with PWS (49, 95, 96) Abbreviations: Dual-energy X-ray Absorptiometry (DXA), Prader-Willi Syndrome (PWS), Vertebral Fracture Assessment (VFA).

corticosteroids, (4) yearly screening for (and treatment of) hypogonadism, (5) yearly screening for hyperthyroidism and hyperparathyroidism, (6) extra caution in patients using psychotropic medication, and (7) cessation of smoking and alcohol use.

Recommendation 1: Optimizing Calcium and Vitamin D Intake

In our study, we showed that 78% of participants used vitamin D supplements and 14% had a dairy intake of ≥3 units/day. Vitamin D has a direct effect on osteocytes, osteoblasts, and osteoclasts and regulates calcium and phosphate metabolism (100, 101). In the general population, vitamin D deficiency increases the risk of osteoporosis and studies with vitamin D supplementation show a reduced fracture risk and increased BMD (102,
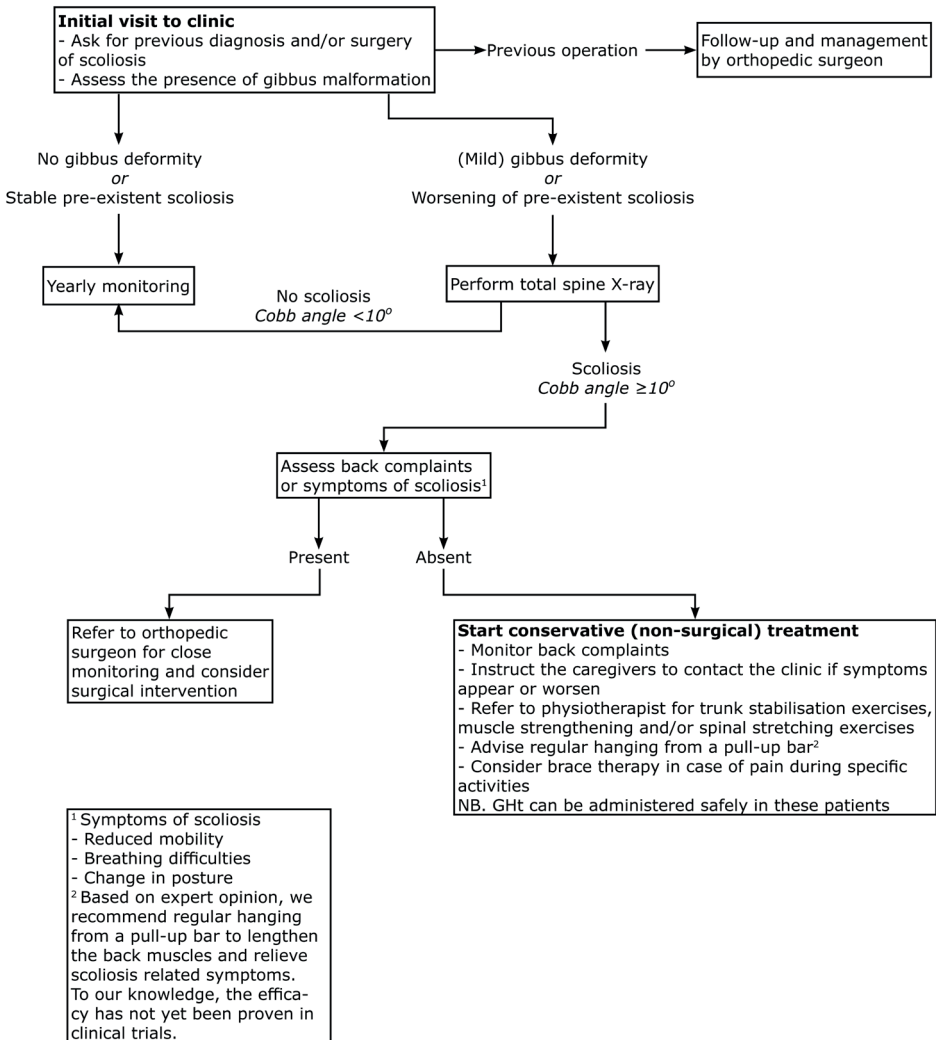
```
┌─────────────────────────────────────────┐
│ Initial visit to clinic                 │
│ - Ask for previous diagnosis and/or     │        ┌──────────────────────────┐
│ surgery of scoliosis          Previous  │───────▶│ Follow-up and management │
│ - Assess the presence of      operation │        │ by orthopedic surgeon    │
│ gibbus malformation                     │        └──────────────────────────┘
└─────────────────────────────────────────┘
```

No gibbus deformity	(Mild) gibbus deformity
or	*or*
Stable pre-existent scoliosis	Worsening of pre-existent scoliosis

```
┌──────────────────┐     No scoliosis        ┌────────────────────────┐
│ Yearly monitoring│   Cobb angle <10°       │ Perform total spine X-ray│
└──────────────────┘                         └────────────────────────┘
```

Scoliosis
Cobb angle ≥10°

```
┌──────────────────────────┐
│ Assess back complaints   │
│ or symptoms of scoliosis[1]│
└──────────────────────────┘
```

Present   Absent

┌────────────────────────┐   ┌──┐
│ Refer to orthopedic   │   │ **Start conservative (non-surgical) treatment**   │
│ surgeon for close   │   │ - Monitor back complaints   │
│ monitoring and consider│   │ - Instruct the caregivers to contact the clinic if │
│ surgical intervention │   │ symptoms appear or worsen   │
└────────────────────────┘   │ - Refer to physiotherapist for trunk stabilisation │
                                 │ exercises, muscle strengthening and/or spinal   │
                                 │ stretching exercises   │
                                 │ - Advise regular hanging from a pull-up bar[2]   │
                                 │ - Consider brace therapy in case of pain during   │
                                 │ specific activities   │
                                 │ NB. GHt can be administered safely in these patients│
                                 └──┘

┌────────────────────────────┐
│ [1] Symptoms of scoliosis   │
│ - Reduced mobility   │
│ - Breathing difficulties   │
│ - Change in posture   │
│ [2] Based on expert opinion,│
│ we recommend regular hanging│
│ from a pull-up bar to   │
│ lengthen the back muscles   │
│ and relieve scoliosis   │
│ related symptoms.   │
│ To our knowledge, the   │
│ efficacy has not yet been   │
│ proven in clinical trials. │
└────────────────────────────┘

Figure 2. Recommendations for the detection, monitoring of progression and treatment of scoliosis in adults with PWS (97-99)
Abbreviations: Growth hormone therapy (GHt).

103). We did not find a difference in 25(OH) vitamin D levels or vitamin D supplementation between patients with and without osteoporosis, probably due to lack of power as few patients had low vitamin D levels and many patients already received vitamin D supplementation. However, previous studies reported reduced vitamin D levels in adults with PWS, especially in those with obesity (70, 78, 79). The reduced 25(OH) vitamin D levels could be related to reduced exposure to sunshine and an increased volume of distribution in adipose patients as vitamin D is fat soluble (104). We recommend yearly measurement of 25(OH) vitamin D levels in all adults with PWS not receiving vitamin D

supplementation. We recommend starting vitamin D supplementation in patients with PWS with a vitamin D level below the reference, irrespective of DXA scan results. Additionally, we recommend a dairy intake of ≥3 units a day or calcium supplementation to ensure adequate calcium intake.

Recommendation 2: Optimizing Physical Activity

Many patients (39%) in our cohort exercised less than 30 minutes a day. In nonsyndromic pre- and postmenopausal women with osteoporosis, physical activity is known to improve bone mineral content and BMD, provided enough nutrients, calcium, and vitamin D are available (105). In children with PWS, Duran et al. (26, 106) showed a positive association between moderate weight-bearing physical activity and hip BMD. The effect of physical activity on BMD in adults with PWS has, to our knowledge, not yet been studied in clinical trials. However, beside the (expected) possible positive effects on BMD, regular exercise is also important in patients with PWS to decrease body fat mass, increase lean body mass, and improve coordination to decrease fall risk (25). Therefore, we recommend regular physical activity of at least 30 minutes, but ideally at least 1 hour daily, preferably weight-bearing in order to maintain and possibly improve BMD and body composition. We also recommend aerobic and muscle strengthening exercises, but we realize this might be difficult due to hypotonia and challenging behavior.

Recommendation 3: Avoidance of Unnecessary Corticosteroid use in Central Adrenal Insufficiency

One in 10 adults in our cohort used corticosteroid replacement, mostly only during physical or psychological stress. Few patients had proven central adrenal insufficiency; most patients received corticosteroids as part of local guidelines for the treatment of PWS.

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Corticosteroid-induced osteoporosis is the most common cause of secondary osteoporosis in the general population (107, 108). Glucocorticoid use increases the risk of fractures (109, 110), which can occur as early as 3 months after start of corticosteroids and even with low doses of steroids (eg, 2.5-7.5 mg of prednisone daily) (111). In our clinical experience, when adults with PWS are prescribed corticosteroid stress doses, these stress doses are sometimes frequently administered due to recurrent episodes of psychological stress. As we previously showed that central adrenal insufficiency is rare in adults with PWS (1.2%) (112), we strongly recommend refraining from routine corticosteroid "stress-doses" in adults with PWS. In order to prevent secondary osteoporosis due to corticosteroid use, we advise only prescribing corticosteroids when central adrenal insufficiency is proven.

Recommendation 4: Yearly Screening for (and Treatment of) Hypogonadism

Hypogonadism was prevalent in both males (93%) and females (86%). Hypogonadism is a well-known risk factor for osteoporosis and timely treatment with hormone replacement therapy can reduce this risk (113–115). In this study we did not find an association between hypogonadism or SHRT and osteoporosis. However, as all patients were treated in a PWS reference center, untreated hypogonadism was rare, resulting in low statistical power. Previous research has shown a significant improvement in BMD in men (69) and women (71) with PWS after the start of SHRT. Therefore, we recommend yearly screening for hypogonadism. If hypogonadism is present, adequate SHRT should be started as soon as possible to avoid the negative effects of hypogonadism on BMD (69, 70). As starting and maintaining SHRT can be challenging, we have previously defined practical recommendations for hormone replacement therapy in males (15) and females (16).

Recommendation 5: Yearly Screening for Hyperthyroidism and Primary Hyperparathyroidism

The prevalence of hyperthyroidism in this study was 1.1%, which is similar to the prevalence in the general population in Europe (<1%) and the United States (1.3%) (116, 117). The prevalence of primary hyperparathyroidism found in the current study was 0.8%, which is in line with prevalence in the general adult population (0.1-0.7%) (118–121). Patients with PWS are already at risk of developing osteoporosis and they might be unable to express the subtle complaints of hyperthyroidism and hyperparathyroidism due to intellectual disability. Therefore, we recommend yearly screening for hyperthyroidism and hyperparathyroidism by measuring thyroid-stimulating hormone, free thyroxine, and serum calcium levels. In case of hypercalcemia, parathyroid hormone (PTH) should also be measured. Depending on local hospital policy and the costs of PTH measurement, yearly (simultaneous) screening for both calcium and PTH could also be performed.

Recommendation 6: Extra Caution in Patients Using Psychotropic Medication

Psychiatric disorders, such as manic or depressive episodes with psychotic features are common in PWS, with an estimated prevalence of 16% to 28% (122–125). Psychotic features are most common in patients with an mUPD (122–125). Most psychotropic medications, such as selective serotonin reuptake inhibitors, benzodiazepines, tricyclic antidepressants, and conventional antipsychotics (eg, haloperidol) have been associated with an increased (osteoporotic) fracture risk in the general population (126–131). Furthermore, selective serotonin reuptake inhibitors, benzodiazepines, and atypical antipsychotics (eg, risperidone, clozapine) have been associated with a higher osteoporosis risk, though tricyclic antidepressants might reduce the risk for osteoporosis (86). It has been suggested that use of conventional antipsychotics may cause higher prolactin levels, possibly leading to hypogonadism and osteoporosis (131). There is conflicting

evidence regarding the effect on BMD of antipsychotics that do not raise prolactin levels (132). The relationship between osteoporosis and the use of psychotropic medication in adults with PWS is still unknown. As current evidence is scarce, we suggest performing a DXA scan every 5 years in adults with PWS who are taking psychotropic medication, just like in adults who do not use psychotropic medication. We advise continuing psychotropic treatment as long as it is indicated, regardless of the BMD.

Recommendation 7: Cessation of Smoking and Alcohol use

In our cohort, there was no relation between alcohol usage and osteoporosis. However, adults with osteoporosis smoked significantly more cigarettes per week than those without osteoporosis. In the general population, smoking has been associated with low BMD (133–135) and increased fracture risk (133, 136). Cessation of smoking has been shown to increase BMD (137–139). Furthermore, chronic heavy alcohol usage has been associated with decreased BMD (140, 141). In contrast, light to moderate alcohol consumption might increase BMD in females (141, 142). We recommend that smoking and heavy alcohol usage is discouraged in all adults with PWS.

Awareness of High Pain Threshold and Intellectual Disability

Patients with PWS seldomly report pain. This can be due to their high pain threshold and intellectual disability (6, 143), which may impair their ability to express physical complaints. This may lead to delay in the diagnosis of (osteoporotic) fractures. In our cohort, several fractures had remained unnoticed for weeks, as the only symptoms had been change of behavior or walking pattern (personal communications). Therefore, it is important to keep in mind that patients with PWS may have an atypical presentation of fractures. Thorough physical examination should be performed in case of unexplained behavioral changes or refusal of physical activities to exclude underlying physical problems such as undiagnosed fractures. Additionally, when a DXA scan is performed, preferably a vertebral fracture assessment should also be performed to exclude undiagnosed vertebral fractures.

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Yearly Screening for Scoliosis and Other Orthopedic Conditions

We recommend clinical assessment of scoliosis in all patients with PWS during yearly physical examination. Furthermore, we recommend that a standing full spine posterior-anterior X-ray is performed in case of doubt, progression, or when spine deformity surgery is considered (see **Figure 2**). When scoliosis and low BMD are both present, complications of surgery such as iatrogenic instability and postoperative fractures are more prevalent (144, 145). Therefore, surgical correction of scoliosis is more challenging in patients with low BMD. Besides scoliosis, patients with PWS also have an increased risk of other orthopedic conditions, such as hip dysplasia, genu valgum, and kyphosis (146).

In case of suspect kyphosis, a lateral view total spine X-ray should be performed. These conditions were not systematically documented in the medical records and could not be investigated in this retrospective study.

Strengths and Limitations

The current study is, to our knowledge, the first multicenter study on osteoporosis including more than 300 adults with PWS. We were able to assess not only the prevalence of scoliosis and osteoporosis, but also the risk factors for osteoporosis in this rare genetic syndrome. Additionally, we performed an extensive literature review on both osteoporosis and scoliosis in adults with PWS. However, our study also has some limitations. First, as data were collected retrospectively from patient records in different centers, screening and treatment protocols varied between centers. In particular, the assessment of vertebral fractures varied between centers, possibly leading to under-diagnosis of asymptomatic vertebral fractures in some centers. In some centers, DXA scans were only performed when risk factors for osteoporosis were present. As we only included patients for whom DXA scans results were available, the prevalence of osteoporosis and osteopenia we report might be an overestimation. Second, scoliosis was not systematically assessed using standing full spine X-rays in all patients, possibly leading to an underestimation of scoliosis prevalence. Third, as this was an international study, different DXA machines were used. To compare the results of different machines, the sBMD was calculated for all DXA scan results. However, earlier research has shown that a small bias might remain (147). Furthermore, the results could have been influenced by obesity, which might lead to an overestimation of BMD (12, 148). Moreover, previous research has shown that the true BMD might be underestimated in subjects with short stature (12, 149). As the median height in our cohort was 1.56 (IQR 1.49-1.64) meters, our reported prevalence of osteoporosis and osteopenia might be an overestimation. Uniform prospective studies are needed to overcome these limitations and to prospectively assess the fracture incidence.

CONCLUSION

In conclusion, osteoporosis, fractures, and scoliosis are common skeletal problems in adults with PWS. Male sex was associated with a higher prevalence of osteoporosis. In our cohort, the most prevalent modifiable risk factors for osteoporosis were (male and female) hypogonadism, insufficient dairy intake, sedentary lifestyle, and corticosteroid treatment. We did not identify any risk factors for scoliosis. In particular, GHt was not associated with scoliosis. Based on the cohort study and literature review, we provide

practical clinical recommendations to prevent complications in this vulnerable patient population.

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SUPPLEMENTARY DATA

Supplementary table S2. Most recent T-scores and standardized bone mineral density of adults with PWS

	T-score (SD)			sBMD (g/cm^2)	
	Lumbar spine	Hip	Total body	Lumbar spine	Hip
Normal BMD	0.0 [-0.5 to 0.8], n = 110	-0.1 [-0.5 to 0.5], n = 99	0.6 [-0.3 to 1.3], n = 73	1.150 [1.083 to 1.246], n = 102	1.028 [0.933 to 1.129], n = 70
Osteopenia	-1.3 [-1.7 to -0.8]*, n = 143	-1.4 [-1.8 to -1.0]*, n = 141	-0.8 [-1.5 to -0.2]*, n = 90	0.999 [0.954 to 1.067]*, n = 137	0.826 [0.751 to 0.917]*, n = 109
Osteoporosis	-2.7 [-3.2 to -2.1]*, n = 44	-2.8 [-3.2 to -2.3]*, n = 45	-2.2 [-2.5 to -1.3]*, n = 30	0.868 [0.779 to 0.925]*, n = 42	0.653 [0.592 to 0.758]*, n = 30
All	-0.9 [-1.7 to -0.1]*, n = 298	-1.1 [-1.8 to -0.3]*, n = 286	-0.5 [-1.3 to -0.6]^a, n = 194	1.045 [0.961 to 1.153]*, n = 294	0.875 [0.765 to 1.008]*, n = 222

Data are presented as median [IQR]. Abbreviations: interquartile range (IQR) Standard deviation (SD), standardized bone mineral density (sBMD). ^{a} P <0.001 (Kruskal Wallis H test for three groups, Mann-Whitney U test for osteopenia vs normal BMD and osteoporosis vs normal BMD), compared with adults with Prader-Willi syndrome a normal BMD.

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What endocrinologists can do to prevent cardiovascular complications in adults with Prader-Willi syndrome: lessons from a case series

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ABSTRACT

Context

Prader-Willi syndrome (PWS) is a complex rare genetic syndrome. Mortality in patients with PWS is 3% per year. In nearly half of the patients, the cause of death is of cardio-pulmonary origin. Prevention, diagnosis and treatment of cardiovascular (CV) disease in PWS adults is complicated by the behavioral phenotype, reduced ability to express physical complaints, high pain threshold and obesity.

Objective

To describe the challenges in prevention, diagnosis and treatment of CV disease in PWS adults, in order to increase awareness and improve medical care.

Methods

Retrospective study of medical records of adults visiting the Dutch PWS reference center.

Results

We describe the challenges encountered during diagnosis and treatment of four PWS adults with heart failure. All had pre-existent peripheral edema. CV risk factors in these patients were obesity (n = 4), type 2 diabetes mellitus (n = 2), hypertension (n = 2), hypo-gonadism (n = 3) and sleep apnea (n = 2). Remarkably, all patients were younger than 40 years during their first cardiac decompensation. All patients presented with progressive shortness of breath and/or orthopnea and progressive pitting edema. In 117 controls with PWS without CV problems, 31% had leg edema.

Conclusion

Diagnosing CV problems in PWS adults is challenging. Peripheral edema is common in PWS adults without CV morbidity, which makes edema in general a poor marker for heart failure. However, when edema is of the pitting kind and progressive, this is a strong predictor of cardiac decompensation. We provide practical recommendations for diag-nosing and treating CV problems in this vulnerable patient population.

INTRODUCTION

Prader-Willi syndrome (PWS) is a complex genetic disorder caused by the loss of func-tion of a cluster of paternally expressed genes on chromosome 15q11.2-q13. It occurs in 1:15.000 – 25.000 live births (1). PWS can result from a paternal deletion (65-75%), a maternal uniparental disomy 15 (mUPD, 20-30%), an imprinting center defect (ICD, 1-3%) or a paternal chromosomal translocation (0.1%) (2, 3). During infancy, patients with PWS often have muscular hypotonia, low muscle mass, feeding difficulties, failure to thrive and delayed development. During childhood, most patients develop an insatiable appetite, often leading to obesity. Patients with PWS have an abnormal body composi-ton with a high fat mass and low muscle mass (4, 5). Additionally, patients with PWS have hypothalamic dysfunction resulting in pituitary hormone deficiencies, abnormal temperature regulation and inadequate pain registration (6–9).

Mortality in patients with PWS is as high as 3% per year (10, 11). In nearly half of the patients, the cause of death is of cardiopulmonary origin and three-quarters of deaths are unexpected (10, 12). Cardiovascular (CV) abnormalities can occur already early in life (13) and patients with PWS have an increased risk to develop CV disease at a young age (10, 14–16). As previously described (17), this increased CV risk is caused by a complex interplay between somatic and behavioral aspects of the syndrome. Musculoskeletal problems associated with the syndrome (i.e. scoliosis and foot problems), hypotonia and pituitary hormone deficiencies can lead to poor exercise tolerance, which can be further aggravated by behavioral challenges. This exercise intolerance combined with hyperphagia, can lead to an increase in body fat and decrease in lean body mass. This leads to a low basal rest metabolism, which further deteriorates body composition. Increased body fat eventually leads to an increased prevalence of CV risk factors like type 2 diabetes mellitus (DM2), hypertension, hypercholesterolemia and sleep apnea (10, 18–24).

Besides abnormal body composition, appetite regulating hormones like ghrelin and leptin can also affect the CV system. PWS is associated with high ghrelin levels and an elevated acylated ghrelin/unacylated ghrelin (AG/UAG) ratio (25). While high levels of both AG and UAG seem to have protective CV effects (26–28), high ghrelin levels could cause weight gain and glucose intolerance (29, 30). Another key player in appetite regulation, leptin, has been associated with the presence, severity, extent and lesion complexity of coronary atherosclerosis (31). Leptin levels in non-obese PWS males are nearly five times higher than in non-obese control males (32). However, the interplay between leptin levels, obesity and CV pathology in PWS has not been investigated.

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Apart from the complex etiology, the diagnostic trajectory of CV disease in adults with PWS is also complex. Physicians usually rely on common symptoms of heart disease, that are reliable indicators of cardiac problems in the general population, such as chest pain, orthopnoea, shortness of breath, decreased exercise tolerance, palpitations, fatigue and peripheral edema (33, 34). However, in PWS, some of these symptoms are less reliable. Chest pain can be easily missed due to the high pain threshold in PWS and many PWS adults lack the verbal skills to express complex physical complaints like orthopnoea and palpitations. Physical signs like decreased exercise tolerance and peripheral edema are already common in adults with PWS without CV disease (17, 22, 23) and are therefore unreliable parameters. This combination of factors can easily lead to diagnostic delay (3, 6, 7). The diagnostic process can be further hindered by obesity, which complicates the interpretation of transthoracic cardiac ultrasound (35) and can lead to false-normal NT-proBNP values (36).

In the current case series, we describe the challenges that occurred during the diagnostic trajectory and treatment of four adults with PWS and CV problems. Moreover, we compare clinical characteristics between patients with and without CV events. Based on this comparison and literature data, we provide clinical recommendations for the diagnosis and treatment of CV disease in adults with PWS.

METHODS

This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam, the Netherlands. In this retrospective study, we reviewed medical files of adults with PWS who underwent our routine systematic health screening at the multidisciplinary outpatient clinic of the Center for Adults with rare genetic syndromes at the Erasmus University Medical Center, Rotterdam, the Netherlands between 2015 and 2022. This systematic screening consists of a structured interview, a complete physical examination, a medical questionnaire, a review of the medical file and biochemical measurements, as described previously (17). Biochemical measurements that were routinely measured were: low density lipoprotein (LDL)-cholesterol, nonfasting glucose, hemoglobin A1c, thyroid-stimulating hormone, free thyroxine, luteinizing hormone, follicle-stimulating hormone, estradiol or testosterone, sex hormone binding globulin, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transpeptidase, total bilirubin, lactate dehydrogenase, urea, creatinine, estimated glomerular filtration rate, hemoglobin, hematocrit, mean corpuscular volume, leukocytes, thrombocytes and 25-hydroxyvitamin D.

We systematically assessed symptoms of CV disease like orthopnea, dyspnea, nocturia, swollen legs and chest pain. During physical examination the presence of edema was objectified and cardiac auscultation was performed. Additional cardiac diagnostics, like NT-proBNP, chest X-ray, cardiac ultrasound or electrocardiogram (ECG) were not performed routinely, but only if CV problems were suspected.

We describe four adults with PWS who had suffered a CV event in their medical history or who developed CV problems while under treatment at the outpatient clinic of our center. We compare these patients to 117 control patients with PWS without known CV events.

Data analysis

Descriptive statistics for continuous variables are reported as median [interquartile range (IQR)]. For dichotomous variables we display the number and the percentage of people, n (%).

RESULTS

We describe four patients with CV events, all with manifestations of heart failure, of whom detailed clinical information was available.

Patient 1

A 39-year-old male was hospitalized with severe dyspnea and weight gain. Physical examination revealed orthopnea, bronchospasm, severe pitting edema up to his chest and generalized rhonchi. He had severe tachypnea with up to 40 breaths per minute. NT-proBNP was normal (20 pmol/L). Based on the acute clinical presentation, pulmonary congestion was suspected. The ECG showed no abnormalities. Chest X-ray was normal (i.e. no signs of pulmonary congestion), besides a slightly blurred left heart margin. Intravenous diuretics were started to reduce edema and beta-agonist inhalers were started to reduce bronchospasm. Within 48 hours, he had lost three kilograms and his respiration frequency had returned to normal (16 breaths per minute). Afterwards, cardiac ultrasound showed normal systolic left ventricular function and no signs of significant diastolic dysfunction. Right ventricular pressure was increased. Although computed tomography angiography (CTA) of the coronary arteries did not reveal any significant obstructions, the calcium score was 146 (> 90th percentile), which indicated the presence of coronary sclerosis. He was prescribed atorvastatine and reduced his cigarette use from 75 to 21 cigarettes per week.

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CV risk factors included morbid obesity (body mass index (BMI) 45 kg/m^2), heavy smoking, hypertension and dyslipidemia. Another risk factor was hypogonadism (37), which was untreated as testosterone therapy had caused behavioral challenges. Detailed analysis of additional CV risk factors revealed frequent hypoglycemias (38). The patient used insulin, which he administered himself. It turned out that he injected himself with too much insulin, in order to induce hypoglycemia and receive extra food. Finally, sleep apnea (apnea-hypopnea index (AHI) of 15) was also present. To reduce pulmonary resistance and optimize cardiac function, continuous positive airway pressure (CPAP) was started.

Patient 2

A 37-year-old female was hospitalized for analysis and treatment of generalized edema, with clinical suspicion of heart failure. Nephrogenic causes of edema, like nephrotic syndrome, had been excluded. CV risk factors included a family history with CV disease, DM2, morbid obesity and hyperlipidemia. ECG showed negative T waves in V1-3, indicating right ventricular hypertrophy. Cardiac ultrasound showed tricuspid valve insufficiency with signs of elevated right ventricular pressure and a dilated vena cava inferior, but transthoracic ultrasound quality was poor due to obesity. It was hypothesized that she had pulmonary hypertension caused by morbid obesity (BMI 44 kg/m^2) and severe sleep apnea (AHI of 59). CPAP was initiated, which led to recompensation. Three months later, she was hospitalized again. An upper respiratory tract infection had caused an increase of her pre-existent pulmonary hypertension, which caused decompensated right heart failure. After a short stay in the hospital, she could be dismissed, but returned half a year later. Then, she admitted that she had discontinued CPAP. Insufficient surveillance in her residential facility had led to nonadherence to CPAP. She had also gained a lot of weight, due to insufficient external food control. She had access to her own debit card, which she used to buy food.

Patient 3

A 29-year-old female with hypogonadism and central hypothyroidism was hospitalized because of orthopnea, progressive exercise-related shortness of breath, hyponatremia (134 mmol/L) and progressive pitting edema. NT-proBNP was increased (99 pmol/L). Chest X-ray showed an enlarged heart. During her stay at the hospital, she developed severe epileptic seizures which eventually required intubation and transfer to the intensive care unit (ICU). Magnetic resonance imaging (MRI) and electroencephalography (EEG) did not show any abnormalities. A year earlier, she had undergone cardiac evaluation because of peripheral edema. At that time, ECG did not show any signs of ischemia and cardiac ultrasound came back normal. However, retrospectively, interpretation at that time was probably already hindered by her morbid obesity. A new cardiac ultrasound,

performed at the ICU, showed a normal systolic left ventricle function (LVEF 55%), but a dilated right ventricle and tricuspid valve insufficiency. This was probably the result of right heart failure due to pulmonary hypertension, resulting from restrictive lung function caused by scoliosis and morbid obesity (BMI 48 kg/m^2). The patient was treated with intravenous diuretics and fluid and salt restriction, after which she recompensated. After 3 weeks she was sent home with oxygen therapy.

Patient 4

A 32-year-old male presented for the first time at our outpatient clinic. He had progressive exercise-related shortness of breath, extreme peripheral edema with blisters and weight gain of 20 kilograms in one month. He had morbid obesity (BMI 53 kg/m^2). At physical examination, his oxygen saturation was 80%. Initially, he refused physical examination and venipuncture. ECG showed left axis cardiac deviation and a right bundle branch block, but no signs of acute ischemia. Chest X-ray showed an enlarged heart and hilar enlargement. He was admitted to the hospital for treatment of his congestive heart failure, but refused medication, oxygen and other medical interventions. As he refused medical treatment without being able to understand the consequences, he was considered to be a danger to himself. The medical staff tried to arrange the legal documents needed for involuntary commitment. However, his aggressive behavior made it impossible to keep him on the ward and the patient left the hospital. As the stress of forced hospitalization and forced treatment was expected to further aggravate his cardiac problems, it was decided to try to treat him in the residential home where he lived. He was treated with oral diuretics (bumetanide) and a salt and water restriction. Eventually, he took the prescribed medication for two days and agreed to undergo venipuncture, which showed an increased NT-proBNP (230 pmol/L). The physician for intellectual disabilities (ID physician) reported that, after an initial weight loss of 3 kilograms, he had gradually increased in weight again. The patient adhered less and less to his salt and water restriction. His hyperphagia, aggressive behavior and the complex psychosocial situation made it impossible for his physician and caregivers to improve adherence to treatment. Eventually, he ate a large amount of salty food and spent two nights drinking water in his shower. He was readmitted to the hospital due to shortness of breath, where he died shortly after arrival. Autopsy confirmed that his death was the result of congestive heart failure. There was severe peripheral edema, the heart was enlarged and the right ventricle was dilated. Apart from congestion of the abdominal organs and the brain, autopsy revealed ascites and pericardial effusion which supported the clinical diagnosis fluid retention due to congestive heart failure.

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Comparison to controls

In **Tables 1 and 2**, we compare the four cases with CV events to a control population of 117 adults with PWS without CV events. Compared to the cases, controls had a lower BMI and more often had used growth hormone treatment during childhood. CV risk factors were also frequent in control patients. Peripheral edema was present in 31% of the controls.

Based on the described cases, the literature and our clinical expertise, we formulated recommendations for prevention, diagnosis, and treatment of CV events in adults with PWS, see **Figure 1**.

Prevention		Diagnosis	
Lifestyle intervention for all adults with PWS Treatment by a dietitian if BMI > 25 kg/m² Exercise at least 60 min/day Arrange 24/7 supervision in case of severe hyperphagia Educate caregivers about alarm symptoms of CV events		**Perform cardiac evaluation** Transthoracic echocardiography, NT-proBNP, ECG, chest X-ray *if*	▪ Increase in peripheral edema *or* ▪ Shortness of breath *or* ▪ Poor exercise tolerance *or* ▪ Nocturia *or* ▪ Unexplained weight gain
Perform yearly screening Fasting glucose LDL-cholesterol Blood pressure *if*	▪ BMI > 25 kg/m² *or* ▪ Age > 25 year *or* ▪ GH therapy *or* ▪ Corticosteroid use	! **Patients with PWS have a high pain treshold** → be alert if any symptoms of CV problems occur, regardless of the presence of thoracic pain	
Perform poly(somno)graphy *if*	▪ BMI > 25 kg/m² *or* ▪ Daytime sleepiness *or* ▪ Reported apneas *or* ▪ Hypertension	! **NT-proBNP can be false-normal in case of obesity** → consider performing cardiac imaging or start treatment for heart failure if clinical suspicion is high	
		! **Interpretation of cardiac ultrasound may be difficult in patients with high thoracic fat** → consider alternative cardiac imaging (MRI, CT) or start treatment for heart failure if clinical suspicion is high.	
Treatment			
! **without competent supervision, non-adherence to treatment regimens and lifestyle interventions is high** → increase awareness of caregivers of necessity of strict adherence to treatment and lifestyle interventions.			

Figure 1 Recommendations for prevention, diagnosis and treatment of cardiovascular events in adults with Prader-Willi syndrome. Abbreviations: 24 hours a day, 7 days a week (24/7), body mass index (BMI), computed tomography (CT), cardiovascular (CV), electrocardiogram (ECG), growth hormone (GH), low-density lipoprotein (LDL), minutes (min), magnetic resonance imaging (MRI), Prader-Willi syndrome (PWS). These recommendations only consider factors related to cardiovascular disease. For the full screening protocol for prevention, see Pellikaan et al. (17)

DISCUSSION

We describe the syndrome-specific challenges encountered during the diagnosis and treatment of severe CV problems, i.e. heart failure, of four adults with PWS. Diagnosis was complicated by obesity (BMI between 44 and 53 kg/m²) and pre-existent peripheral edema.

Pulmonary hypertension played a key role in the pathogenesis of CV disease in all patients described. Dilated right ventricle and dilated vena cava inferior, both signs of increased right heart pressure, were present in most patients. Left ventricle function was usually normal.

Table 1. Patient characteristics, cardiovascular risk factors and childhood GH status of four patients with PWS with cardiovascular events, compared to 117 PWS adults without cardiovascular events.

	Age (years)[a]	Genotype	BMI (kg/m²)	Type 2 diabetes mellitus	Hypertension	Hypercholesterolemia	Sleep apnea	GH during childhood
Patient 1	39	Deletion	45	Yes	Yes	Yes	Yes	No
Patient 2	37	Deletion	44	Yes	No	Yes	Yes	No
Patient 3	29	Deletion	48	No	No	No	No	No
Patient 4	32	Unknown	53	NA	Yes	No	NA	No
Control (n = 117)	28 [20 – 38]	Deletion: 61 (52%) mUPD: 42 (36%) ICD: 3 (3%) Unknown: 11 (9%)	29 [26 – 35]	13 (11%)	17 (15%)	20 (17%)	17 (15%)	58 (50%)

Data presented as yes (present), no (absent) or NA (not available) for individual patients and as n (%) for control PWS adults. For controls the age and BMI are given as median [IQR]. Abbreviations: body mass index (BMI), growth hormone (GH), imprinting center defect (ICD), maternal parental disomy (mUPD). [a] Age at first event for cases and age at data collection for controls.

Table 2. Physical complaints and signs of cardiac decompensation of four patients with PWS with cardiovascular events, compared to 117 PWS adults without cardiovascular events.

	Chest pain	Peripheral edema	Orthopnea	Progression of edema	Increased NT-proBNP	Difficulty interpreting cardiac ultrasound	Signs of pulmonary hypertension[a]
Patient 1	No	Yes	Yes	Yes	No	Yes	No
Patient 2	Yes	Yes	NA	Yes	NA	Yes	Yes
Patient 3	No	Yes	Yes	Yes	Yes	Yes	Yes
Patient 4	No	Yes	Yes	Yes	Yes	NA	Yes
Control[b]	2/91 (2%)	29/95 (31%)	1/90 (1%)	NA	NA	NA	NA

Data presented as yes (present), no (absent), NA (not available) for individual patients and as n (%) for the group of control patients. [a] Signs of pulmonary hypertension include: right axis deviation on ECG, signs of increased systolic RV-pressure on echocardiogram (in absence of pulmonary valve stenosis): increased TR-velocity, enlarged vena cava and/or right ventricle dilatation or hypertrophy. [b] Number of controls differs as a result of missing values.

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Factors contributing to CV disease were obesity (n = 4), DM2 (n = 2), hypertension (n = 2), hypogonadism (n = 3) and sleep apnea (n = 2) (37). Remarkably, all patients had their first cardiac decompensation before the age of 40, the youngest patient being 29 years old. This is in contrast with the low prevalence of heart failure of 0.1-0.5% found in non-PWS adults in this age category (with and without overweight) (39).

CV disease in adults with PWS is caused by a complex interplay of several syndrome-specific characteristics, which eventually leads to obesity (17). Obesity is associated with systemic low-grade inflammation and oxidative stress, hypertension, hypercho-lesterolemia, insulin resistance and DM2, all associated with increased CV risk (40–43). Furthermore, obesity can lead to obesity-associated hypoventilation syndrome (OAHS) (44), with subsequent pulmonary hypertension and right ventricular failure, if left un-treated. In addition to the obesity-related increase in CV risk, patients with PWS have an additional risk due to decreased microvascular function that is associated with the syndrome (45), as endothelium and microvessels may play an important role in the pathogenesis of heart failure (46, 47). Lastly, scoliosis is often present in patients with PWS (23). If severe, scoliosis can cause restrictive pulmonary dysfunction, pulmonary hypertension and eventually CV decompensation (48, 49).

Due to the cumulative effect of the above-mentioned mechanisms, the patients we described developed cardiac decompensation at an exceptionally young age. To prevent the development of CV disease, it is important to identify and treat CV risk factors early in life. Prevention and treatment of obesity may be complicated due to intellectual disability and hyperphagia. Therefore a multidisciplinary approach is needed. A (pediatric) endocrinologist, specialized dietitian, physiotherapist and, if needed, a behavioral expert or psychologist should work together to avoid or treat obesity. Additionally, it is essential to have adequate supervision at home to control food intake. Specialized PWS homes can be beneficial to ensure this supervision. If PWS homes are unavailable, caregivers should receive clear instructions about restricting the patient's food intake and increasing physical activity. Additionally, the endocrinologist should screen yearly for CV risk factors like hypertension, DM2, and hypercholesterolemia. For this screening, our previously described algorithm may be helpful (17). These measures might prevent long-term debilitating CV complications as seen in the four patients we described.

If, despite prevention, heart failure develops, the diagnosis of heart failure can be challenging in patients with PWS, especially when obesity is present. Peripheral non-pitting edema (lipedema and lymphedema) is common in PWS adults without CV morbidity (31%), which makes edema, in general, a poor marker for CV deterioration. However, in all four patients who eventually developed cardiac decompensation, edema was of the

pitting kind and progressive. Therefore, an increase in peripheral pitting edema should be considered an alarm symptom and should trigger further investigation.

Apart from progressive pitting edema, all cases showed orthopnea and/or progressive shortness of breath. Although not systematically assessed, none of the patients complained of nocturia. When symptoms of heart failure like dyspnea and/or orthopnea are present, additional testing is needed to rule out cardiac problems. It should be emphasized that, in obese subjects, false negative NT-proBNP values can put physicians on the wrong track. Likewise, cardiac ultrasound can be hard to interpret due to poor imaging quality resulting from obesity (35).

Besides diagnostic challenges, it can also be challenging to initiate and maintain adequate cardiac treatment. In general, diuretics and fluid and salt restriction are essential for treatment of congestive heart failure. However, due to hyperphagia and intellectual disability, nonadherence to fluid and salt restriction is common in adults with PWS. Adherence can only be guaranteed by competent supervision, which means that 24/7 supervision is often crucial in heart failure treatment in PWS. Non-adherence to CPAP may also occur. As patients with OAHS benefit from CPAP, this is a useful intervention. However, the CPAP mask can cause anxiety, especially in case of intellectual disability. In that case, stepwise introduction of the mask (starting with a few minutes per day) and gradual increase in usage is crucial to prevent non-compliance. Also, for the acceptance of CPAP, it may be useful to involve a psychologist or behavioral expert.

Strengths and limitations

One of the strengths of the current study is the detailed description of four cases of PWS adults with CV events. To our knowledge, we are the first to describe cases of PWS adults with CV events and to provide practical recommendations based on the similarities between these cases. A limitation of this study is that this study was retrospective and therefore some details were unknown. Moreover, the diagnostic tests were performed in different hospitals, which may have caused some variation in results of imaging and/or biochemical tests.

CONCLUSION

In conclusion, the diagnostic trajectory and treatment of CV disease in adults with PWS can be extremely challenging. Peripheral edema, a reliable marker of right-sided heart failure in the general population, is frequently present in the general PWS population and is therefore not a good indicator of heart failure. Diagnosis of heart failure is further

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hindered by the decreased reliability of NT-proBNP levels and increased technical challenges in transthoracic echocardiography in case of obesity. To prevent doctors' delay, it is important to inform general practitioners, ID physicians, internists and cardiologists about these diagnostic pitfalls. To prevent patient delay, it is important to inform caregivers about early signs of cardiac failure, like exercise-related shortness of breath, progression of peripheral edema and unexplained weight gain. Diagnosis and treatment can be complicated by PWS-specific behavior, non-compliance to salt and water restriction, fear of CPAP and refusal of medication. Therefore, preventive measures, diagnostics and treatment of CV disease should preferably be guided by a multidisciplinary team.

ACKNOWLEDGMENTS

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Malignancies in Prader-Willi syndrome: results from a large international cohort and literature review

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ABSTRACT

Context

Prader-Willi syndrome (PWS) is a complex disorder combining hypothalamic dysfunction, neurodevelopmental delay, hypotonia, and hyperphagia with risk of obesity and its complications. PWS is caused by the loss of expression of the PWS critical region, a cluster of paternally expressed genes on chromosome 15q11.2-q13. As life expectancy of patients with PWS increases, age-related diseases like malignancies might pose a new threat to health.

Objective

To investigate the prevalence and risk factors of malignancies in patients with PWS and to provide clinical recommendations for cancer screening.

Methods

We included 706 patients with PWS (160 children, 546 adults). We retrospectively collected data from medical records on past or current malignancies, the type of malignancy and risk factors for malignancy. Additionally, we searched the literature for information about the relationship between genes on chromosome 15q11.2-q13 and malignancies.

Results

Seven adults (age range 18-55 years old) had been diagnosed with a malignancy (acute lymphoblastic leukemia, intracranial hemangiopericytoma, melanoma, stomach adenocarcinoma, biliary cancer, parotid adenocarcinoma and colon cancer). All patients with a malignancy had a paternal 15q11-13 deletion. The literature review showed that several genes on chromosome 15q11.2-q13 are related to malignancies.

Conclusion

Malignancies are rare in patients with PWS. Therefore, screening for malignancies is only indicated when clinically relevant symptoms are present such as unexplained weight loss, loss of appetite, symptoms suggestive of paraneoplastic syndrome, or localizing symptoms. Given the increased cancer risk associated with obesity, which is common in PWS, participation in national screening programs should be encouraged.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare genetic, multisystem disorder characterized by hypothalamic dysfunction, developmental delay, hypotonia, increased pain threshold and typical dysmorphic features. Hypothalamic dysfunction may lead to several clinical features, including hyperphagia and pituitary hormone deficiencies (1-3). Hyperphagia in combination with a decreased basal metabolic rate and reduced physical activity results in a high prevalence of obesity (1,4,5).

PWS is caused by the absence of expression of a cluster of paternally expressed, maternally imprinted genes on chromosome 15q11.2-q13, also called the "PWS critical region". In 65-75% of the patients, the underlying genotype is a type I (40%) or type II (60%) paternal deletion. Maternal uniparental disomy 15 (mUPD) occurs in 20-30% and 1-3% have an imprinting center defect (ICD). Balanced translocations (0.1%) and individual gene mutations (<0.1%) are rare (6).

As a result of earlier diagnosis, multidisciplinary care and better weight management, the life expectancy of patients with PWS has substantially increased (7,8). As patients with PWS become older, the development of age-related diseases is becoming increasingly relevant. Additionally, adults with PWS have shorter leukocyte telomere lengths, premature symptoms of aging, an early functional decline and higher brain age, all suggesting accelerated aging (9,10). This highlights the importance of knowledge about the occurrence of age-related diseases in adults with PWS, such as malignancies.

Previous studies investigating malignancies in PWS are limited by low numbers, lack of older patients, and results that were based on questionnaires only. Questionnaire studies could underestimate the occurrence of malignancies, as underdiagnosis of diseases in general is a common problem in patients with PWS (11). Underdiagnosis is common for several reasons, including their high pain threshold, specific behavioral phenotype and the high prevalence of intellectual disability (1,12).

In vitro studies, animal studies, and studies in non-PWS participants suggest that multiple genes in the 15q11.2-q13 chromosomal region may be involved in the development of malignancies (13-19). However, the relationship between genetic subtype and the development of malignancies has, to our knowledge, never been investigated.

To investigate the need to screen for malignancies in patients with PWS, we assessed the prevalence of malignancies in a large international cohort of adults and children with PWS. To understand the pathogenesis of malignancies in patients with PWS, we

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provide a literature overview of the relationship between the genes on chromosome 15q11.2-q13 and different types of malignancies.

METHOD

All participating centers obtained approval from ethics committees and/or individual patients to retrospectively collect data on patients with PWS.

We collected data from patient records of 706 individuals (160 children and 546 adults) with PWS that were visiting or had previously been under the care of one of the centers participating in the INfoRMEd-PWS network in: Netherlands (115), United Kingdom (45), France (92), Spain (94), Italy (290) or Australia (70). The local investigators collected data from patients on: 1) past or current malignancies, and if applicable, which type; 2) GH treatment during childhood and adulthood; 3) treatment with testosterone or estrogen replacement therapy; 4) for males, history of cryptorchidism and 5) measurements of prostate-specific antigen (PSA); 6) type 2 diabetes mellitus; 7) family history of malignancy; 8) alcohol use; 9) smoking; 10) other substance abuse and 11) baseline characteristics, including anthropometric measurements, current age, gender, genotype and whether patients were still alive at the time of data collection. Data on height and weight was used to calculate body mass index (BMI). As measurements of fat mass were not available for all patients, obesity was defined as a BMI >30 kg/m^2 for adults and a BMI > +2 standard deviation score (SDS) for children.

Literature review

In collaboration with the Medical Library of the Erasmus University Medical Center, we performed a literature search on Embase, Medline, the Web of Science Core Collection, Cochrane Central Register of Controlled Trials and Google Scholar. The search was last updated in September 2022. We reviewed studies that reported on the relationship between the expression of genes on chromosome 15q11.2-q13 and malignancies. Inclusion criteria were: clinical trials, basic or translational research, and case reports or case series that researched the expression or methylation of one or more genes on chromosome 15q11.2-q13 in malignancies compared to normal cells/tissue. Exclusion criteria were: meeting reports, workshop summaries, reviews, conference abstracts, guidelines, articles that were not available online and articles that were not available in English. Articles that only reported on the relationship between gene expression and the prognosis or survival of patients with malignancies were also excluded. The full search strategy is included in **Table S1**. As most genes were associated with both up- and downregulation, we concluded that a gene was mainly upregulated, when it was

upregulated in ≥ 80% of studies and mainly downregulated, when it was downregulated in ≥ 80% of studies.

Data analysis

Descriptive statistics for continuous variables are reported as median [interquartile range (IQR)]. For dichotomous variables the number and the percentage of people, n (%), are displayed. To investigate the relationship between malignancies and nominal variables the Fisher's exact test was used. To investigate the relationship between malignancies and genotype, genotype was dichotomized into deletion or no deletion. For the relationship between malignancies and continuous variables the Wilcoxon rank sum test was used. The relationship between malignancies and anthropometric measurements (height, weight and BMI) was investigated in adults only.

RESULTS

Baseline characteristics are shown in **Table 1.** We included 160 children and 546 adults. The median age was 25 years [IQR 18-33 years]. Thirty-seven patients were 50 years old or older. Of the patients included, 326 (46%) were males. Obesity was prevalent (53%), with a median BMI of 32 kg/m^2 [IQR 25-42 kg/m^2]. Deletion was the most common genotype (58%). Patients from six countries were included in this study. Most patients had received GH treatment at some point in their life (65%) and 227 (32%) received GH treatment at the time of data collection.

Of 706 patients, seven adults (four males and three females), had been diagnosed with a malignancy, see **Table 2**. Patients with a malignancy were significantly older with a median age of 39 years [IQR 22-46 years] compared to 24 years (IQR 18-34 years) in the control group. All patients with a malignancy had a paternal deletion, compared with 58% in patients without a malignancy P = 0.045). There was no relation between malignancies and gender, country, GH treatment, anthropometric measurements, use of alcohol or tobacco, sex hormone replacement, cryptorchidism, family history or T2DM.

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Table 1. Baseline characteristics of 706 children and adults participating in this study

	Number of observations	Total n = 706	Children n = 160	Adults n = 546
Age[a]				
Median [IQR]	706	25 [18 - 33]	9 [5 - 14]	28 [22 - 38]
Range		0.4-73	0.4-18	18-73
Male gender	706	326 (46%)	75 (47%)	251 (46%)
Anthropometric measurements				
Height, cm, median [IQR]	690	153 [144 - 163]	135 [105 - 151]	156 [149 - 164]
Height, SDS, median [IQR]	120		-1.0 [-1.9; 0.16]	
Weight, kg, median [IQR]	690	78 [60 - 98]	38 [18 - 59]	83 [68 - 102]
Weight, SDS, median [IQR]	28		-0.5 [-1.3 - 1.6]	
BMI, kg/m^2, median [IQR]	690	32 [25 - 42]	21 [17 - 27]	34 [27 - 44]
BMI, SDS, median [IQR]	96		1.2 [0.01 - 1.9]	
BMI, range	690	13-80	13-80	17-73
Obesity	642	376 (53%)	22 (23%)	354 (65%)
Genotype				
Deletion		410 (58%)	78 (49%)	332 (61%)
mUPD		236 (33%)	74 (46%)	162 (30%)
ICD	706	13 (2%)	4 (3%)	9 (2%)
mUPD or ICD		20 (3%)	0 (0%)	20 (4%)
Translocation		1 (0%)	1 (1%)	0 (0%)
Other		8 (1%)	0 (0%)	8 (2%)
Unknown		18 (3%)	3 (2%)	15 (3%)
Country				
Netherlands		115 (16%)	0 (0%)	115 (21%)
United Kingdom		45 (6%)	1 (1%)	44 (8%)
France	706	92 (13%)	4 (3%)	88 (16%)
Spain		94 (13%)	54 (34%)	40 (7%)
Italy		290 (41%)	96 (60%)	194 (36%)
Australia		70 (10%)	5 (3%)	65 (12%)
GH treatment				
During childhood	706	420 (60%)	145 (91%)	275 (50%)
During adulthood	704	156 (22%)	NA	156 (29%)
Childhood and/or adulthood	706	462 (65%)	145 (91%)	317 (58%)
Current	693	227 (32%)	110 (69%)	117 (21%)
Duration, median [IQR]	396	8 [4 - 12]	7 [3 - 10]	8 [4 - 13]

Data are displayed as n (%). Abbreviations: imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD), standard deviation score (SDS). [a] Current age or, for deceased patients, age of death.

Four patients with malignancies had died, of whom three had died as a result of their malignancy and one from an infection two years after being diagnosed with acute lymphoblastic leukemia. **Table 3** shows the prevalence of malignancies for different age groups, demonstrating that the prevalence increased with age: 0-9 years 0.0%, 10-19 years 0.8%, 20-29 years 0.4%, 30-39 years 1.6%, 40-49 years 2.6%, and 50-74 years 2.7%. All patients had different types of malignancies, namely acute lymphoblastic leukemia, intracranial hemangiopericytoma, melanoma, adenocarcinoma of the stomach, biliary cancer, adenocarcinoma of the parotid gland and colon cancer. One patient with a malignancy had a family history of malignancies.

Table 2. Patient characteristics according to history of malignancies

	Number of observations	Malignancy absent n = 699	Malignancy present n = 7	P-value
Age				
Median [IQR]	706	24 [18-34]	39 [22-46]	0.04
Range		0.4-73	[18-55]	
Male gender	706	322 (46%)	4 (57%)	0.7
Genotype				
Deletion		403 (58%)	7 (100%)	0.045[a]
mUPD		236 (34%)	0 (0%)	
ICD	706	13 (2%)	0 (0%)	
mUPD or ICD		20 (3%)	0 (0%)	
Other		8 (1%)	0 (0%)	
Unknown		18 (3%)	0 (0%)	
Country				
The Netherlands		115 (17%)	0 (0%)	
United Kingdom		45 (6%)	0 (0%)	
France	706	91 (13%)	1 (14%)	0.5
Spain		93 (13%)	1 (14%)	
Italy		287 (41%)	3 (43%)	
Australia		68 (10%)	2 (29%)	
GH treatment				
During childhood	706	418 (60%)	2 (29%)	0.1
During adulthood	704	155 (22%)	2 (29%)	1
Childhood and/or adulthood	706	458 (66%)	4 (57%)	0.7
Current	693	226 (32%)	1 (14%)	1
Duration, median [IQR]	396	8 [4-12]	1 [0.6-10]	
Anthropometric measurements				
Height, cm, median [IQR]	16	153 [144-163]	155 [152-162]	0.8[b]
Weight, kg, median [IQR]	16	78 [60-98]	100 [69-127]	0.5[b]
BMI, kg/m², median [IQR]	16	32 [25-42]	36 [28-55]	0.4[b]
Obesity	16	374 (55%)	5 (71%)	0.7[b]
Intoxications				
Alcohol	593	12 (2%)	1 (14%)	0.1
Glasses per week, median [IQR][c]	13	2 [1-4]	1	
Smoking	598	36 (5%)	2 (29%)	0.07
Cigarettes per week, median [IQR][c]	38	70 [42-113]	35 and 49	
Drugs	592	0 (0%)	0 (0%)	
Sex hormone replacement therapy				
Males, n (% of males)	313	149 (48%)	3 (75%)	0.4
Median age at start [IQR]	313	18 [16-25]	23, 30 &30	
Females, n (% of females)	366	186 (51%)	1 (33%)	0.6
Median age at start [IQR]	355	17 [15-20]	13	
Cryptorchidism, n (% of males)	310	250 (81%)	2 (67%)	0.5
Surgery for cryptorchidism, n (% of cryptorchidism)	252	232 (93%)	2 (100%)	
Known family history of malignancy in first degree relatives	567	88 (16%)	1 (20%)	0.6
Mortality	706	25 (4%)	4 (57%)	<0.001
Age of death	706	33 [26-49]	39 [24-46]	
Type 2 diabetes mellitus (T2DM)	648	111 (17%)	2 (29%)	0.4
Only non-insulin antidiabetics, n (% of T2DM)[d]		59 (60%)	0 (0%)	
Only insulin, n (% of T2DM)	100	3 (3%)	0 (0%)	
Both, n (% of T2DM)		35 (35%)	1 (100%)	
None, n (% of T2DM)		2 (2%)	0 (0%)	

Data are displayed as n (%). Abbreviations: Body mass index (BMI), imprinting center defect (ICD), interquartile range (IQR), maternal uniparental disomy (mUPD), type 2 diabetes mellitus (T2DM). [a] P-value calculated for deletion vs non-deletion. [b] For adults only. [c] In patients that smoke/drink alcohol only. [d] Either oral antidiabetics or GLP-1 analogues.

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Table 3. Prevalence of malignancies for different age groups.

	Patients with malignancies / total (%)	Age at diagnosis	Current age	Type of malignancy	Genotype	Family history of malignancy in first degree relatives	WHO 1-year cancer prevalence[a]
0-9 years old	0 / 85 (0%)						0.014%
10-19 years old	1 / 131 (0.8%)	18	†	Acute lymphoblastic leukemia	Deletion, unspecified	None	0.015%
20-29 years old	1 / 247 (0.4%)	22	22	Intracranial hemangiopericytoma	Deletion, unspecified	None	0.040%
30-39 years old	2 / 129 (1.6%)	39	39	Melanoma in neck	Type 1 deletion	None	0.093%
		33	†	Adenocarcinoma of stomach	Type 2 deletion	None	
40-49 years old	2 / 77 (2.6%)	44	†	Biliary cancer	Deletion, unspecified	None	0.22%
		46	†	Adenocarcinoma parotid gland (metastasized)	Deletion, unspecified	None	
50-74 years old	1 / 37 (2.7%)	55	55	Colon cancer (metastasized)	Type 1 deletion	Pancreatic cancer (father)	0.87%

† means deceased. ªThe WHO 1-year cancer prevalence for Europe for both sexes in 2020 (105). It should be noted that these numbers are not directly comparable to our results, as we do not report a 1-year prevalence.
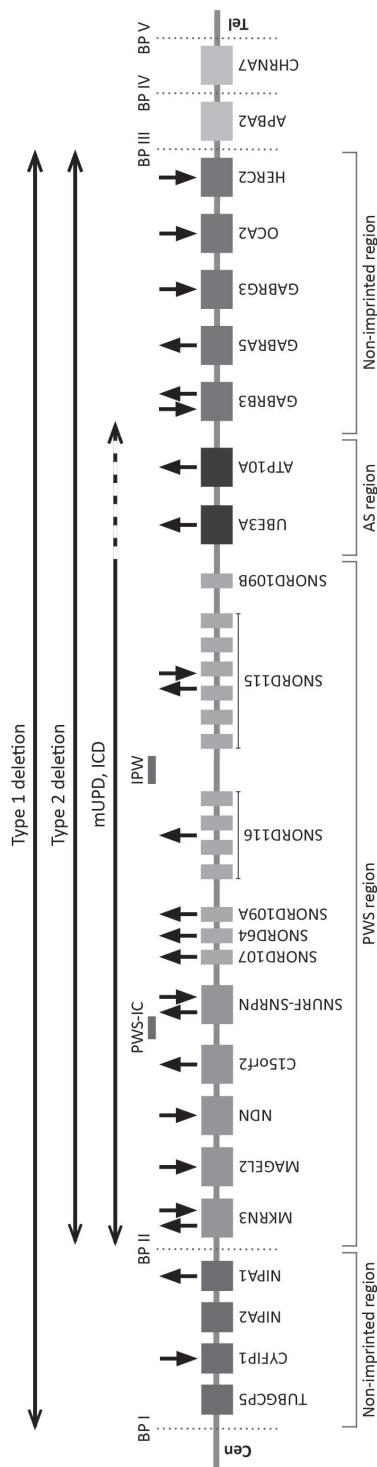
Figure 1. Genes on chromosome 15q11.2-q13 in relation to malignancies

Abbreviations: Angelman syndrome (AS), breakpoint (BP), centromere (Cen), imprinting center (IC), Prader–Willi syndrome (PWS), telomere (Tel). Figure adapted from Cheon et al. (6). Chromosome 15q11.2-q13 can be divided into different regions: a proximal non-imprinted region, the Prader–Willi syndrome (PWS) region that is maternally imprinted, the paternally imprinted region that is also known as the Angelman syndrome region, and a distal non-imprinted region. *Legends*: Horizontal arrows indicate regions of chromosome 15q11.2-q13 affected by the different genotypes of PWS. Solid horizontal arrows indicate diminished or loss of expression, dotted arrows indicate increased expression. ↑: the gene is upregulated in one or multiple different malignancies in ≥ 80% of studies, ↓: the gene is downregulated in one or multiple different malignancies in ≥ 80% of studies, ↑↓: the gene is more often upregulated than downregulated in one or multiple different malignancies, ↓↑: the gene is more often downregulated than upregulated in one or multiple different malignancies, no symbol: we did not find any information about the relationship between this gene and the development of malignancies.

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Literature review

Table S2 shows a literature-based overview of the genes on chromosome 15q11.2-q13 and their relation to malignancies. Genes that were (mainly) upregulated in malignant tumors were: *NIPA1, C15orf2, SNORD107, SNORD64, SNORD109A, SNORD116, UBE3A, ATP10A* and *GABRA5*. *CYFIP1, MAGEL2, NDN, GABRG3, OCA2,* and *HERC2* were downregulated in malignant tumors. *MKRN3, SNURF-SNRPN, SNORD115,* and *GABRB3* were associated with both up- and downregulation in malignant cells. These data are graphically summarized in **Figure 1**.

DISCUSSION

Malignancies were rare in our cohort of 706 patients with PWS. Our cohort included 546 adults of whom 37 were aged over 50 years. Only 7 adults had a malignancy. The malignancies that occurred were all of different origin. This suggests a multifactorial etiology of the malignancies. Therefore, we do not recommend to screen routinely for a particular type of cancer.

Although scarce, there are some studies that have previously investigated the occurrence of cancer in PWS. Patja et al. reported 3 malignancies (acute lymphatic leukemia (ALL), testicular tumor and breast cancer), in a cohort of 56 children and adults with PWS, while the expected number was 1.5 patients. They concluded that there is 'a possibility of increased risk of malignancies among persons with PWS' (20). A questionnaire-based study performed in the United States of America (USA) in patients with PWS aged 0-63 years (with only 2 being older than 50 years) found that 3 children and 5 adults had a malignancy, while 4.8 cases were expected based on the prevalence in the general USA population (difference not significant). Three patients had leukemia, which was significantly more than expected based on the general population (0.075 cases expected) (21). Several case reports describe patients with PWS and cancer, including acute lymphoblastic leukemia (ALL) (22), acute and chronic myeloid leukemia (23), hepatoblastoma (24), medulloblastoma (25), pulmonary carcinoid tumor (26), Wilms tumor (27), intratubular germ cell neoplasia (28) and testicular seminoma (29-31). In one male with PWS and testicular seminoma, loss of methylation of the Prader-Willi syndrome imprinting center (PWS-IC) was found during histological examination, suggesting involvement of genes in the PWS critical region (29).

All seven patients with malignancies had a deletion of the paternal copy of the PWS region, which was also the most common genotype. No malignancies were found in

patients with the genotypes mUPD or ICD. We performed a literature review to explain this finding.

Literature review

Our literature review revealed that various genes on chromosome 15q11.2-13 are up- or downregulated in different types of cancer. However, this relationship appears to be complex with several genes being both up- and downregulated in different types of malignancies.

The proximal non-imprinted region contains *TUBGCP5*, *CYFIP1*, *NIPA2* and *NIPA1*. They are expressed from both the maternal and the paternal allele. While this region is not affected in patients with a type 2 deletion or a mUPD, one copy of these genes is deleted in patients with a type 1 deletion, leading to a decreased expression (32). *CYFIP1* shows reduced expression in various types of human cancers as it acts as an invasion suppressor (33). Therefore, patients with a type 1 deletion might have an increased risk of malignancies. However, as the type of deletion was unknown for most patients, we were unable to investigate whether this was true in our cohort. Of the other genes in the proximal non-imprinted region, *NIPA1* is upregulated in acute myeloid leukemia. We did not find any studies relating *TUBGCP5* or *NIPA2* to malignancies.

Apart from the proximal non-imprinted region, we also studied literature about the genes on the PWS critical region itself. The genes in this region are not expressed in patients with PWS. In patients with a deletion, the paternal allele is absent and the maternal allele is present, but not expressed. In patients with an mUPD or ICD, there are two maternal alleles, which are not expressed. According to our literature review, several genes in the PWS region have been associated with malignancies:

MKRN3 inactivation leads to proliferation and progression of non-small cell lung cancers (34). However, upregulation of *MKRN3* has been found in osteosarcoma and squamous cell carcinoma of the head and neck (35,36).

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NDN, also known as *necdin*, is a tumor suppressor gene that represses cell-cycle-promoting proteins, interacts with p53 and inhibits cell growth (37-40). *NDN* is downregulated in many types of cancer. Lack of expression of this tumor suppressor gene in PWS might therefore, in theory, lead to an increased risk of cancer.

Little is known about the relation between *MAGEL2* and *C15orf2* and malignancies. *MAGEL2* has been associated with down regulation in hepatocellular carcinoma (41) and

C15orf2 was upregulated in acute myeloid leukemia in one study (42), but other types of malignancies have not been investigated.

<u>*SNURF-SNRPN*</u>, due to its relation with the PWS imprinting center (43), has been extensively investigated in order to understand the relationship between epigenetic imprinting and cancer development. Both up- and downregulation of *SNURF-SNRPN* have been reported in different types of malignancies. *SNRPN* might affect cancer development through regulation of the cell cycle, tumor proliferation and apoptosis (44,45).

<u>Small nucleolar RNAs (snoRNAs)</u> are a class of non-coding RNAs (ncRNAs). Some snoR-NAs demonstrate the capability to affect tumorigenesis and metastasis (46). Although evidence is scarce, studies suggest a role of the snoRNAs located on the PWS region in the tumorigenesis of different types of cancer. Most studies report the upregulation of these snoRNAs, in particular <u>*SNORD116*</u> and <u>*SNORD115*</u>, in malignancies. As these genes are not expressed in PWS, this might protect against cancer.

Downstream of the PWS region lies the Angelman syndrome region. This region contains *UBE3A* and *ATP10A*. Patients with an mUPD have increased expression of these genes compared to patients with a deletion or healthy controls (32).

<u>*UBE3A*</u> encodes E3 ligase E6-associated protein (E6AP), which is involved in viral oncogenesis (i.e. human papillomavirus, hepatitis C virus and Epstein-Bar virus-associated malignancies). *UBE3A* is also involved in the non-viral oncogenesis of multiple types of cancer by degradation of the tumor suppressor promyelocytic leukemia protein (PML) and p27^{Kip1}. Thus, upregulation of *UBE3A* is likely related to tumorigenesis (47). This might indicate that patients with an mUPD could have an increased risk of malignancies, which we was not confirmed in our cohort. Little is known about the relationship between <u>*ATP10A*</u> and malignancies.

Next to the Angelman region lies the distal non-imprinted region. The genes in the distal non-imprinted region are deleted on one allele in patients with a paternal deletion, but not affected in patients with an mUPD or ICD.

<u>*GABRB3*, *GABRA5*</u> and <u>*GABRG3*</u> all encode one of the 19 GABA$_A$ receptor subunits (48). The GABA pathway is involved in embryonic stem cell and peripheral neural crest cell proliferation, blunting rapid proliferation, resulting in a more tempered proliferation. This enhances genome integrity (49-51). Multiple studies reported loss of expression or decreased expression of *GABRB3* in malignancies, while some reported increased

expression. *GABRA5* was upregulated in several malignancies and *GABRG3* was down-regulated in colon adenocarcinoma.

OCA2 is involved in pigmentation and eye color. Therefore, alterations in the *OCA2* gene have been associated with melanoma (52-54). Mutations in *OCA2* result in oculocutaneous albinism (55), which is associated with an increased risk of skin cancer (50,56). Additionally, it is downregulated in thyroid carcinoma.

HERC2 is a member of the HERC family. HERCs play a role in replication stress and DNA damage, cell proliferation and migration and immune response (57). *HERC2* is associated with eye color and pigmentation. Genetic variants in this gene have been associated with an increased risk of melanoma (53,58). Additionally, depletion of *HERC2* leads to inhibition of the tumor suppressor p53 (59). Mutations in and downregulation of *HERC2* have been associated with multiple types of malignancies (57). As patients with a deletion have only one copy of *HERC2*, this might lead to an increased risk of malignancies.

Hypopigmentation, which is common in patients with PWS with a deletion (60), is a risk factor for the development of skin cancers (50,56). We report one patient with melanoma, who had a type 1 deletion.

We found several relatively rare types of malignancies in our population such as hemangiopericytoma, parotid gland cancer and biliary cancer. Research regarding the relationship between these rare types of malignancy and the genes on chromosome 15q11.2-13 was scarce and therefore we could not explain this finding.

Besides the direct effects of altered gene expression, various clinical features of PWS may increase or decrease the risk of malignancies, including GH and sex hormone treatment, obesity and use of alcohol and tabacco.

Growth hormone treatment

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Nowadays, most children with PWS are treated with growth hormone (GH) treatment. Multiple observational studies in non-PWS populations did not indicate an increased risk of malignancies later in life after treatment with GH during childhood (61,62). However, the Safety and Appropriateness of Growth Hormone Treatments in Europe (SAGHe) study showed increased incidence and mortality risks for several cancer sites, largely related to second primary malignancies in patients who received GH treatment after cancer treatment. Only the incidence of bone and bladder cancer was also significantly increased in patients without previous cancer who received GH therapy. Additionally, there was a significant increase in incidence of Hodgkin lymphoma with longer follow-

up, also in patients without previous malignancies (63). However, these outcomes might reflect the effect of the underlying condition leading to GH treatment, rather than the effect of GH treatment itself. Therefore, GH treatment is still considered safe with regard to risk of malignancies.

Sex hormone replacement therapy

Many patients with PWS have hypogonadism and are treated with estrogen or testosterone replacement therapy (64-66). In our cohort 49% of males and 51% of females were receiving sex steroid replacement. In the general population, estrogen replacement therapy is associated with an increased risk of malignancies, especially breast cancer (67,68). However, little is known about the risk of estrogen replacement therapy in patients with congenital hypogonadism.

The relationship between testosterone replacement therapy and prostate cancer remains complex. However, testosterone replacement therapy seems to be safe and might even be used to help control prostate cancer through normalization of testosterone concentrations (69). We recommend yearly measurement of prostate specific antigen in males with PWS who receive testosterone replacement therapy, according to the guidelines for the general population (70).

Obesity

Obesity was prevalent in our cohort (55%), especially among adults (65%). However, our definition of obesity was based on BMI only, which might lead to an underestimation of adiposity, due to abnormal body composition with low fat free mass compared to fat mass in patients with PWS (4). There is a clear correlation between obesity and many types of malignancies, with relative risks (RR) ranging from 1 to 3 per 10 kg/m^2 increase in BMI (71). However, obese patients with PWS have reduced visceral adiposity (72) and are more insulin sensitive (72-74) compared to non-PWS obese adults. This may partly protect adults with PWS from the increased risk of malignancies caused by obesity (75).

In obese individuals with PWS, serum leptin concentrations are increased, as is expected in obesity (74,76). Leptin is associated with a higher risk of malignancies, i.e. breast cancer (77), colorectal cancer (78), thyroid cancer (79), and endometrial cancer (80), also after adjustment for obesity. However, while obesity usually suppresses plasma ghrelin, plasma ghrelin concentrations are increased in PWS (81-84). The relation between circulating ghrelin and the risk of malignancies is still controversial (85). Furthermore, obesity is also associated with chronic low-grade systemic inflammation and oxidative stress (86,87), which plays a role in the development of malignancies (88,89). However, there

are contradictory reports as to whether peripheral inflammatory markers and adipocytokines are lower, appropriate or raised for their obesity in patients with PWS (73,90-92).

Use of tobacco and alcohol

Adults with PWS smoke and drink alcohol less often than non-PWS adults. In the general population, tobacco use is associated with lung, laryngeal, pharyngeal, upper digestive tract and oral cancers (93). Alcohol use leads to an increased risk of cancers of the oral cavity, pharynx, esophagus, colon, rectum, liver, larynx and breast (94). While 25% of the general European population are cigarette smokers (95), only 5% of our PWS cohort were cigarette smokers. While almost three-quarters of the European population drinks alcohol, only 2% of our PWS cohort drank alcohol (96). Based on these numbers, tobacco and alcohol-associated malignancies are expected to be less prevalent in patients with PWS.

Population screening for malignancies

Studies have reported a lower participation in population screening programs for breast, cervical and colorectal cancer in adults with an intellectual disability compared to the general population (97-101). Additionally, the consumption of cancer-related healthcare is also lower in adults with an intellectual disability (ID) (100), while the prevalence of cancer seems to be higher than in the non-ID population (101,102). This could be due to underdiagnosis and undertreatment in this patient population (100,101). In our clinical experience, participation in cancer screening programs is also low for patients with PWS, especially for the cervical cancer screening. It is often assumed that cervical cancer screening is not indicated in patients with an intellectual disability as they are not sexually active. However, assumption is not always correct, as these patients can be sexually active as well (66). On the other hand, cervical cancer screening could be traumatic for some patients, depending on their sexual history. Therefore, the decision to screen for cervical cancer should be carefully made for each individual patient. We do recommend participation in national screening programs for breast and colon cancer for all PWS adults, due to the increased cancer risk associated with obesity.

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Cancer treatment and intellectual disability

The diagnosis and treatment of malignances is especially complicated in patients with PWS and ID (103). First, their inability to express their physical complaints could lead to underdiagnosis (101). Second, when a malignancy is diagnosed, it is more difficult to convey this information in an effective way to the patient. Information material designed for patients with ID is often unavailable (104). Physicians for IDs, who are experts in communication with and management of patients with ID, are often unfamiliar with the details of cancer diagnosis and cancer treatment. On the other hand, oncologists often

lack the specific background and education needed for communication with individuals with ID. Therefore, it is important that these specialists work together, to make sure that both effective communication and accurate information is provided to both patients and their parents / caregivers.

Strengths and limitations

Strengths of this study include that we report on malignancies in a large international cohort of patients with PWS, that clinical assessments of patients with PWS were performed by experienced physicians and that we report an elaborate literature review. One limitation is a relatively young age of the participants. Although we were able to collect data on a very large cohort of patients with this rare disease, only 37 subjects were older than 50 years, while most malignancies often occur later in life. This lack of older adults with PWS is related to their limited life expectancy (7). The second limitation is the possibility of underdiagnoses. All patients were subject to a yearly follow-up including medical interview, physical examination, and blood measurements. This reduces the risk of underdiagnosis compared to questionnaire studies that only assess self-reported malignancies. However, underdiagnosis cannot be completely ruled out as we did not perform any specific screening for malignancies. Furthermore, national screening programs for malignancies (e.g. cervical, breast, colon) vary between countries and data on participation in these screening programs was largely unavailable. The third limitation is the risk of survival bias. We collected data on patients that visited or had visited the PWS reference centers in the past. However, it is possible that patients had already died as a result of cancer before visiting one of the PWS reference centers. The fourth limitation is the lack of a control population. We performed a cross-sectional study where we reported whether patients had a past or current diagnosis of a malignancy. We did not have access to similar data in a control population. However, even without comparing our findings to a control population, we believe that it is unlikely that the risk of a certain type of malignancy is increased, as all types of cancer only occurred once. Lastly, our literature review addresses the potential effect of the genes on chromosome 15q11.1-13 on cancer risk. However, most of the literature did not provide insight into the causal relation between the up- or downregulation of these genes and the development of malignancies. Therefore, a causal relationship cannot be proven.

CONCLUSION

In conclusion, cancer is rare in our cohort of 706 patients with PWS. The seven patients with malignancies all had different types of cancer, which suggests a multifactorial etiology. All patients with a malignancy had a paternal deletion. However, the relationship

between the PWS genes and cancer risk is complex. Due to the increased cancer risk associated with obesity, we recommend participation in national screening programs for breast and colon cancer for all adults with PWS. The decision to screen for cervical cancer should be carefully made for each individual patient, depending on sexual history and degree of intellectual disability. In males who receive testosterone replacement therapy, we recommend measurement of prostate specific antigen (PSA) according to the general guidelines for testosterone therapy (70). Additional screening for malignancies is only indicated in case of a clinical suspicion based on unexplained weight loss, loss of appetite, paraneoplastic symptoms, or localizing symptoms.

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SUPPLEMENTARY DATA

Table S2. Relation between the expression of genes on chromosome 15q11.2-q13 and various types of malignancies

Gene	Study	Species	Malignancy type	Differential expression in the corresponding malignancy type
TUBGCP5	No studies found			
CYFIP1	Silva et al. (2009) (1)	Human, mice	Epithelial cancers of lung, breast, bladder and colon	No expression
	Chen et al. (2016) (2)	Human	Acute lymphatic leukemia, lymph node metastasis	Downregulation
	Dziunycz et al. (2017) (3)	Human	Cutaneous squamous cell carcinoma	Downregulation
	Liu et al. (2017) (4)	Human	Diffuse large B-cell lymphoma	Downregulation
	Shi et al. (2018) (5)	Human	Nasopharyngeal carcinoma	Downregulation
NIPA2	No studies found			
NIPA1	Chen et al. (2018) (6)	Human	Acute myeloid leukemia	Upregulation
MKRN3	Li et al. (2008) (7)	Human	Osteosarcoma	Upregulation
	Zhang et al. (2021) (8)	Human	Squamous cell carcinoma of the head and neck	Upregulation
	Li et al. (2021) (9)	Human	Non-small cell lung cancer	Downregulation
MAGEL2	Li et al. (2020) (10)	Human	Hepatocellular carcinoma	Downregulation
NDN	Kobayashi et al. (2002) (11)	Mice	Neuroblastoma	Downregulation
	Kawamata et al. (2003) (12)	Human, mice	Metastasizing esophageal squamous cell carcinoma	Downregulation
	Hoek et al. (2004) (13)	Human	Melanoma	Downregulation
	Moss et al. (2007) (14)	Human	Colon cancer	No expression
	Chapman et al. (2008) (15)	Human	Urothelial carcinoma of the bladder	Downregulation
	Li et al. (2008) (7)	Human	Osteosarcoma	Downregulation
	Hattori et al. (2011) (16)	Human, rat	Mammary carcinoma	Downregulation
	De Faveri et al. (2013) (17)	Human	Urothelial carcinoma	Downregulation
	Harada-Shirado et al. (2014) (18)	Human	Acute myeloid leukemia	No difference
	Ribarska et al. (2014) (19)	Human	Prostate cancer	Downregulation

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Table S2. Relation between the expression of genes on chromosome 15q11.2-q13 and various types of malignancies (*continued*)

Gene	Study	Species	Malignancy type	Differential expression in the corresponding malignancy type
	Chang et al. (2016) (20)	Human	Urothelial carcinoma of upper urinary tract, urothelial carcinoma of urinary bladder	Upregulation
	Chatterjee et al. (2016) (21)	Mice	Leukemic bone marrow	Downregulation
	Yang et al. (2016) (22)	Human	Ovarian cancer	Downregulation
	Hu et al. (2017) (23)	Human	Colorectal cancer	Downregulation
	Przybyl et al. (2017) (24)	Human	Undifferentiated uterine sarcoma	Downregulation
	Liu et al. (2020) (25)	Human	Endometrial cancer	Downregulation
	Li et al. (2022) (26)	Human	Osteosarcoma	Downregulation
C15orf2	Yang et al. (2021) (27)	Human	Cytogenetically abnormal acute myeloid leukemia	Upregulation
SNURF-SNRPN	Hashimoto et al. (1997) (28)	Human	Malignant mixed Müllerian tumor of the uterus	Upregulation
	Kohda et al. (2001) (29)	Human	Lung adenocarcinoma	No difference
	Schneider et al. (2001) (30)	Human	Germ cell tumor	Upregulation
	Albrecht et al. (2004) (31)	Human	Barrett's adenocarcinoma	Upregulation
	Kagawa et al. (2006) (32)	Human	Medulloblastoma	Upregulation
	Korshunov et al. (2007) (33)	Human	Central neurocytoma	Downregulation
	Kou et al. (2008) (34)	Human	Recurrent biparental hydatidiform mole	Upregulation
	Furukawa et al. (2009) (35)	Human	Yolk sac tumor	Upregulation, downregulation
	Benetatos et al. (2010) (36)	Human	Acute myeloid leukemia, myelodysplastic syndrome	Downregulation
	Tanaka et al. (2010) (37)	Human	Hepatocellular carcinoma	Upregulation
	Barault et al. (2013) (38)	Human	Invasive breast carcinoma	Upregulation
	Ichikawa et al. (2013) (39)	Human	Germ cell tumor	Upregulation, downregulation
	Barrow et al. (2015) (40)	Human	Invasive breast cancer	Upregulation, downregulation

Table S2. Relation between the expression of genes on chromosome 15q11.2-q13 and various types of malignancies (*continued*)

Gene	Study	Species	Malignancy type	Differential expression in the corresponding malignancy type
	Devaney et al. (2015) (41)	Human	Prostate cancer	Downregulation
	Jing et al. (2015) (42)	Human	Medulloblastoma	Upregulation
	Sepulveda et al. (2016) (43)	Human	Gastric cancer	Downregulation
	Ito et al. (2016) (44)	Human	Recurrent hydatidiform mole	Upregulation
	Wang et al. (2016) (45)	Human	Ovarian teratoma	Downregulation
	Bretz et al. (2017) (46)	Mice	T-cell lymphoblastic lymphoma	Downregulation
	Vastrad et al. (2017) (47)	Human	Glioma, glioblastoma	Downregulation
	Alur et al. (2019) (48)	Human	Epithelial ovarian cancer	Downregulation
	Zeschnigk et al. (2003) (49)	Human	Uveal melanoma	Downregulation
	Shen et al. (2020) (50)	Human	Bladder, breast, colorectal, esophagus, lung, pancreatic, prostate, skin and thyroid cancer	Upregulation
	Ji et al. (2020) (51)	Human	Colorectal cancer	Upregulation
	Yang et al. (2021) (27)	Human	Cytogenetically abnormal acute myeloid leukemia	No difference
	Zhou et al. (2021) (52)	Human	Lung cancer	Upregulation
	Jiang et al. (2021) (53)	Human	Renal papillary cell carcinoma	Downregulation
	Kwiecinska et al. (2021) (54)	Human	Acute lymphoblastic leukemia	Downregulation
	Liu et al. (2022) (55)	Human	Hepatocellular carcinoma	Upregulation
SNORD107	Vendramini et al. (2017) (56)	Human	ERG-related leukemia	Upregulation
SNORD64	Vendramini et al. (2017) (56)	Human	ERG-related leukemia	Upregulation
SNORD109A	Vendramini et al. (2017) (56)	Human	ERG-related leukemia	Upregulation
SNORD116	Ronchetti et al. (2012) (57)	Human	Multiple myeloma	Upregulation
	Mannoor et al. (2014) (58)	Human	Non-small cell lung cancer	Downregulation

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Table S2. Relation between the expression of genes on chromosome 15q11.2-q13 and various types of malignancies (*continued*)

Gene	Study	Species	Malignancy type	Differential expression in the corresponding malignancy type
	Davanian et al. (2017) (59)	Human	Ameloblastoma	Upregulation
	Vendramini et al. (2017) (56)	Human	ERG-related leukemia	Upregulation
	Kothari et al. (2018) (60)	Human	Atypic ductal hyperplasia, ductal carcinoma in situ	Upregulation
SNORD115	Ronchetti et al. (2012) (57)	Human	Multiple myeloma	Upregulation
	Wang et al. (2015) (61)	Human	Retinoblastoma	No difference
	Jha et al. (2015) (62)	Human	Pediatric high-grade gliomas	Downregulation
	Kothari et al. (2018) (60)	Human	Atypical ductal hyperplasia, ductal carcinoma in situ	Upregulation
SNORD109B	No studies found			
UBE3A	Huibregtse et al. (1991) (63)	Human	Cervical carcinoma	Upregulation
	Cooper et al. (2003) (64)	Human, mice, monkey	Cervical carcinoma	Upregulation
	Deng et al. (2007) (65)	Human	Breast cancer	Upregulation
	Brimer et al. (2007) (66)	Human, mice, monkey	Cervical carcinoma	Upregulation
	Min et al. (2009) (67)	Human	Cervical carcinoma	Upregulation
	Srinivasan et al. (2011)(68)	Human, mice	Prostate cancer	Upregulation
	Wolyniec et al. (2012) (69)	Human, mice	Burkitt lymphoma	Upregulation
	Aguilar-Martinez et al. (2015) (70)	Human	Cervical carcinoma	Upregulation
	Zhou et al. (2015) (71)	Human	Breast cancer	Upregulation
	Mortensen et al. (2015) (72)	Human	Cervical carcinoma	Upregulation
	Gamell et al. (2017) (73)	Human, mice	Non-small cell lung cancer	Downregulation
	Kohli et al. (2018) (74)	Human, mice	Hepatocellular carcinoma	Upregulation

Table S2. Relation between the expression of genes on chromosome 15q11.2-q13 and various types of malignancies (*continued*)

Gene	Study	Species	Malignancy type	Differential expression in the corresponding malignancy type
	Sakharkar et al. (2020) (75)	Human	Bladder urothelial, breast invasive, colon adeno-, esophageal, head and neck squamous cell, kidney renal clear cell, liver hepatocellular, lungadeno-, prostate carcinoma, stomach adeno-, thyroid, and uterine corpus endometrial carcinoma	Up- and downregulation
	Zheng et al. (2021) (76)	Human	Esophageal cancer	Upregulation
ATP10A	Yu et al. (2022) (77)	Human	Multiple myeloma	Upregulation
GABRB3	Sun et al. (2003) (78)	Human	Malignant hepatocyte cells	No expression
	Minuk et al. (2007) (79)	Human, mice	Hepatocellular carcinoma	Downregulation
	Cheung et al. (2008) (80)	Human	Stage IV neuroblastoma	Upregulation
	Zhang et al. (2013) (81)	Human	Non-small cell lung cancers	No difference
	Guerrero-Preston et al. (2014) (82)	Human	Head and neck squamous cell carcinoma	No expression
	Yamamoto et al. (2015) (83)	Human	Neuroblastoma	Downregulation
	Hirase et al. (2016) (84)	Human	Neuroblastoma	Upregulation
	Hsu et al. (2016) (85)	Human	Head and neck squamous cell carcinoma	Downregulation
	Kallay et al. (2019) (86)	Human	Wingless (WNT) subgroup medulloblastoma, sonic hedgehog (SHH) subgroup medulloblastoma, group 3 medulloblastoma, group 4 medulloblastoma	Upregulation
	Yan et al. (2020) (87)	Human	Colon adenocarcinoma	Downregulation
	Yang et al. (2021) (27)	Human	Cytogenetically abnormal acute myeloid leukemia	No difference
GABRA5	Hooper et al. (2014) (88)	Human	Group 3 medulloblastoma	Upregulation
	Sengupta et al. (2014) (89)	Human	Group 3 medulloblastoma	Upregulation

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Table S2. Relation between the expression of genes on chromosome 15q11.2-q13 and various types of malignancies (*continued*)

Gene	Study	Species	Malignancy type	Differential expression in the corresponding malignancy type
	Lambert et al. (2015) (90)	Human	Hepatocellular carcinoma	Upregulation
	Kallay et al. (2019) (86)	Human	Group 3 medulloblastoma	Upregulation
GABRG3	Yan et al. (2020) (87)	Human	Colon adenocarcinoma	Downregulation
OCA2	Rayner et al. (2019) (91)	Human	Amelanotic and hypomelanotic melanoma	No difference
	Bai et al. (2022) (92)	Human	Thyroid carcinoma	Downregulation
HERC2	Wu et al. (2018) (93)	Human	Breast invasive carcinoma, bladder urothelial carcinoma, esophageal carcinoma, lung adenocarcinoma, thyroid carcinoma, kidney clear cell carcinoma, kidney papillary cell carcinoma	Downregulation
	Zhu et al. (2021) (94)	Human	Colorectal cancer	No difference

Abbreviations: ETS-related gene (ERG), erythroblast transformation-specific (ETS).

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The diagnostic journey of a patient with Prader-Willi-Like syndrome and a unique homozygous *SNURF-SNRPN* variant; bio-molecular analysis and review of the literature

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ABSTRACT

Prader–Willi syndrome (PWS) is a rare genetic condition characterized by hypotonia, intellectual disability, and hypothalamic dysfunction, causing pituitary hormone deficiencies and hyperphagia, ultimately leading to obesity. PWS is most often caused by the loss of expression of a cluster of genes on chromosome 15q11.2-13. Patients with Prader–Willi-like syndrome (PWLS) display features of the PWS phenotype without a classical PWS genetic defect. We describe a 46-year-old patient with PWLS, including hypotonia, intellectual disability, hyperphagia, and pituitary hormone deficiencies. Routine genetic tests for PWS were normal, but a homozygous missense variant NM_003097.3(*SNRPN*):c.193C>T, p.(Arg65Trp) was identified. Single nucleotide polymorphism array showed several large regions of homozygosity, caused by high-grade consanguinity between the parents. Our functional analysis, the 'Pipeline for Rapid in silico, in vivo, in vitro Screening of Mutations' (PRiSM) screen, showed that overexpression of *SNRPN-p.Arg65Trp* had a dominant negative effect, strongly suggesting pathogenicity. However, it could not be confirmed that the variant was responsible for the phenotype of the patient. In conclusion, we present a unique homozygous missense variant in *SNURF-SNRPN* in a patient with PWLS. We describe the diagnostic trajectory of this patient and the possible contributors to her phenotype in light of the current literature on the genotype–phenotype relationship in PWS.

INTRODUCTION

Prader–Willi syndrome (PWS) is a rare genetic condition (estimated prevalence of 1:10,000–1:30,000), affecting multiple organ systems (1). In the neonatal period, PWS is characterized by muscular hypotonia and feeding difficulties, which usually require tube feeding. Later in infancy, patients switch to hyperphagia (overeating) due to abnormal satiety response to food intake (1–3). Endocrine features include growth hormone deficiency, hypothyroidism, hypogonadism, and, rarely, central adrenal insufficiency (1,3,4). Both hyperphagia and pituitary dysfunction contribute to abnormal weight gain, which can ultimately lead to obesity. Additionally, temperature regulation and pain registration are often disturbed (1,2,5). The majority of these features can be explained by hypothalamic dysfunction (6). Moreover, patients with PWS often display developmental delay, autism spectrum disorders (ASD), ASD-like behavior (1,7), and challenging behavior (3). The physical appearance is characterized by short stature, obesity, almond-shaped eyes, strabismus, small bitemporal diameter, a thin upper lip, small hands and feet, and tapering fingers (1,3). Commonly used consensus diagnostic criteria are those proposed by Holm et al. (8) in 1993 (**Table 1**).

Table 1. Consensus criteria Prader–Willi syndrome and the score of the index case.

Major Criteria (1 point each)	Score	Minor Criteria (0.5 points each)	Score
Neonatal/infantile hypotonia and poor suck	1	Decreased foetal movement and infantile lethargy	0.5
Feeding problems and failure to thrive as infant	1	Typical behaviour problems	0.5
Weight gain at 1–6 years; obesity; hyperphagia	1	Sleep apnoea	0.5
Characteristic dysmorphic facial features	1	Short stature	0
Hypogonadism with small genitalia, pubertal delay and insufficiency	1	Hypopigmentation	0
Developmental delay/intellectual disability	1	Small hands and feet	0.5
Deletion or other cytogenetic/molecular abnormality of the Prader-Willi chromosome region, including maternal disomy	0	Narrow hands, straight ulnar border	0.5
		Esotropia, myopia	0.5
		Thick, viscous saliva	0
		Speech articulation defects	0.5
		Skin picking	0.5
Total major	6	Total minor	4
Total points index case		**10 points**	
Requirements clinical diagnosis PWS for adults		**8 points, of which 5 points major**	

Major criteria are weighted at one point each and minor criteria at half a point each. For adults, a total score of eight, of which at least five points for the major criteria, is necessary for the clinical diagnosis of PWS (8).

PWS is usually caused by the absence of expression of a cluster of paternally expressed genes on chromosome 15q11.2-q13. The maternal allele is imprinted, and therefore genomic and epigenetic changes lead to PWS only if they occur in the paternally expressed genes. PWS is most commonly caused by a paternal deletion (70–75%), a uniparental maternal disomy 15 (mUPD, 25–30%), an imprinting center defect (ICD, 1–3%), or a paternal chromosomal translocation (rare) (9,10). The so called 'Prader–Willi syndrome critical region' on chromosome 15q11.2-q13 encompasses several genes, including *MKRN3*, *MAGEL2*, *NDN, NPAP1*, *SNURF-SNRPN,* and numerous non-coding RNAs (ncRNAs) including small nucleolar RNAs (*snoRNAs*) (10). The exact function of most of these genes and their exact relation to the PWS phenotype still remains unclear.

In patients with Prader–Willi-like syndrome (PWLS), PWS features are present without the classical PWS genotype (paternal deletion, mUPD, ICD, or translocation of chromosome 15q11.2-13). To provide adequate treatment and genetic counselling to the patients with PWLS and their relatives, it is important to understand the underlying genetic defects and pathways (10). Cases with PWLS provide novel insight into the complex genotype–phenotype relationship of PWS. Many chromosomal alterations involving different chromosomes have been described in relation to PWLS (10–12). Several cases of PWLS with a small deletion in the PWS critical region have been described. Deletions ranged from 80 to 236 kb and most include *SNORD116* and the 'imprinted in Prader–Willi syndrome' (*IPW*) gene (13–19). These case reports suggest that the region encoding *SNORD116* and *IPW* is responsible for the key characteristics of PWS, while other genes in the PWS critical region may have smaller phenotypic contributions.

We now present a patient with PWLS, with a normal result on genetic diagnostic testing for PWS, without abnormalities in *SNORD116* or *IPW*. We describe the fascinating diagnostic trajectory and discuss possible explanations for her phenotype in light of the current knowledge on the genotype–phenotype relationship in PWS.

MATERIALS AND METHODS

The patient and her mother; who is legal representative, gave permission for the use of the clinical information and pictures in the current paper.

Methylation-Specific Multiplex-Ligation Probe Amplification (MS-MPLA)

For the MS-MLPA, we used the Salsa MLPA ME028-B2 PWS/AS probemix (MRC-Holland©), as described by Beygo et al. (20).

Single Nucleotide Polymorphism (SNP) Array

Genomic DNA was extracted from peripheral blood and hybridized to a Human Cy-toSNP-12 array (Illumina, San Diego, CA, USA) as reported before (21). The array was scanned with the Illumina iScan Control. Data were processed using Genome Studio v2.1 software and analyzed with Nexus Copy Number software v5.0 (Biodiscovery, El Segundo, CA, USA).

Next Generation Sequencing (NSG)

Obesity gene panel sequencing was performed at the genetic diagnostics laboratory of the UMC Utrecht to search for pathogenic abnormalities in the exons of 57 protein coding obesity-associated genes, as described previously (22). Identified variants were classified according to the American College of Medical Genetics and Genomics guide-lines for variant classification.

RNA Sequencing

A punch biopsy of the skin was taken from the index case and the mother. RNA se-quencing was performed at the Institute of Human Genetics, University Hospital Essen. In order to understand whether, and how, the new variant affects gene expression, we compared RNA sequencing data from the patient and her mother to healthy controls and patients with genetically confirmed classical PWS.

Effect Predictor Programs

To predict whether the variant could affect protein function, we used Alamut VISUAL PLUS ^{TM} v.2.15 to access multiple protein prediction programs: Align-GVGD (23), Muta-tionTaster (Build NCBI37/Ensembl 69) (24), sorting intolerant from tolerant (SIFT) (25,26), and Polymorphism Phenotyping v2 (Polyphen-2) (27,28).

PR*i*SM Screen

We assessed the effect of the *SNRPN* variant on protein function and its pathogenicity using our in-house developed functional genomics screen "Pipeline for Rapid in silico, in vivo, in vitro Screening of Mutations (PR*i*SM)" (for other studies where this has been used see (29–32)).

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Constructs

To obtain the cDNA sequence from human *SNRPN* (NM_003097.6) a PCR (Phusion high fidelity, Thermo Fisher) was done on the human brain cDNA library, using the follow-ing primers: Fw 5' GGCGCGCCACCATGACTGTTGGCAAGAGTAG 3' and Rev 5' TTAAT-TAACTAAGGTCTTGGTGGACG 3'. The gene was then cloned into our dual promoter expression vector (31–33). Using PCR (Phusion high fidelity, Thermo Fisher) we then

introduced the single nucleotide variant using the following primers: SNRPN– c.193c>t (p.Arg65Trp), Fw 5' CCAGAGCGTGAAGAAAAGTGGGTTTTGGGTCTGGTGT 3' and Rev 5' ACAC-CAGACCCAAAACCCACTTTTCTTCACGCTCTGG 3'. The same expression vector without an inserted gene was used as control for all the in vivo and in vitro experiments (control vector).

Mice

FvB/NHsD females were crossed with FvB/NHsD males (ordered at 8–10 weeks old from Envigo) for the neuronal cultures, whereas for the in utero electroporation female, FvB/NHsD (Envigo) were crossed with male C57Bl6/J (ordered at 8–10 weeks old from Charles River). All mice were kept group-housed in IVC cages (Sealsafe 1145T, Tecniplast) with bedding material (Lignocel BK 8/15 from Rettenmayer) on a 12/12 h light/dark cycle in 21 °C (±1 °C), humidity at 40–70%. Food pellets (801727CRM(P) from Special Dietary Service) and water were available ad libitum. All animal experiments were conducted in accordance with the European Commission Council Directive 2010/63/EU (CCD approval AVD101002017893).

HEK-293T Cell Transfections

We cultured HEK-293T cells (not authenticated) in 6-well plates in DMEM/10% Fetal Calf Serum (FCS)/1% penicillin/streptomycin and transfected them when they were 60% confluent with the empty vector control, *SNRPN-WT* or *SNRPN-p.Arg65Trp* (3 ug per 6-well dish), using polyethylenimine (PEI) according to the manufacturer instructions (Sigma). Then, 4–6 h after transfection, we changed the medium to reduce toxicity.

Western Blot

Two to three days after the HEK-293T cells were transfected, they were harvested and homogenized in lysis buffer (10 mM Tris-HCl 6.8, 2.5% SDS, 2 mM EDTA with added protease inhibitor cocktail (#P8340, Sigma), phosphatase inhibitor cocktail 2 (#P5726, Sigma), and phosphatase inhibitor cocktail 3 (#P0044, Sigma)). The BCA protein assay kit (Pierce) was used to determine the protein concentration. The lysate concentrations were adjusted to 1 mg/mL. Primary antibodies used: SNRPN (#11070-1-AP, 1:1000, ProteinTech) and RFP (#600401379, 1:2000, Rockland; used to detect tdTomato); secondary antibody: goat anti-rabbit (#926-68021, 1:15,000, LI-COR). LI-COR Odyssey Scanner and Odyssey 3.0 software were used to quantify the blots. The intensity of the SNRPN protein band in the different conditions was normalized against tdTomato (RFP signal). For the analysis, 4 replicates were used.

Primary Hippocampal Cultures

We prepared the primary hippocampal neuronal cultures from FvB/NHsD wild-type mice following the previously described procedure (34). Briefly, hippocampi from brains of E16.5 embryos were collected in 10 mL ice cold neurobasal medium (NB, Gibco). After incubation of the hippocampi in pre-warmed trypsin/EDTA solution (Invitrogen) at 37° for 20 min, they were dissociated in 1.5 mL NB medium supplemented with 2% B27, 1% penicillin/streptomycin, and 1% glutamax (Invitrogen). Neurons were then plated on poly-D-lysine (25 mg/mL, Sigma) coated coverslips ($1*10^6$ cells per coverslip) in 12 well plates containing 1 mL of supplemented NB for each coverslip. The plates were stored at 37°/5% CO_2.

Neuronal Transfection and Immunocytochemistry

After three days in vitro (DIV), neurons were transfected with an empty vector control (1.8 ug per coverslip), *SNRPN-WT,* or *SNRPN-p.Arg65Trp* (2.5 ug per coverslip). For transfection, Lipofectamine was used according to the manufacturer's instructions (Invitrogen). Five days post-transfection, neurons were fixed with 4% paraformaldehyde (PFA)/10% sucrose, for the neuronal morphology analysis. Antibody staining was done overnight at 4 °C with MAP2 (1:500, #188004, Synaptic System) and SNRPN (#11070-1-AP, 1:100, ProteinTech) in GDB buffer (0.2% BSA, 0.8 M NaCl, 0.5% Triton X-100, 30 mM phosphate buffer, pH7.4). Secondary antibodies: anti-guinea-pig-Alexa647 (#706-605-148) and anti-rabbit-Alexa488 (#711-545-152) conjugated secondary antibody (1:200, Jackson ImmunoResearch). Mowiol-DABCO (Sigma) mounting medium was used to mount the coverslips. The LSM700 was used to acquire confocal images.

At least 10 confocal images (20X objective, 0.5 zoom, 1024 × 1024 pixels) of different transfected neurons (identified by the red staining from the tdTomato) were obtained from each condition, with at least two independent experimental replications. Total neurite length and arborization (the number of branching of each primary neurite) was analyzed, using the NeuronJ plugin of ImageJ. All values were normalized against the mean values of the empty vector control. Analysis was done by an experimenter blinded for the transfection conditions.

In Utero Electroporation

The in utero electroporation was done as described previously (32). In short, pregnant FvB/NHsD mice at E14.5 of gestation were anesthetized, and the uterus was exposed. Through the uterus wall, the DNA construct (1.5–3 ug/uL, diluted in fast green (0.05%)) was injected in the lateral ventricle of the embryos, using a glass pipette controlled by a Picospritzer® III device. Using tweezer-type electrodes connected to a pulse generator (ECM 830, BTX Harvard Apparatus), five electrical square pulses of 45 V (50 ms per pulse

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and 150 ms inter-pulse interval (ipi)) were delivered. The positive pole of the tweezers was placed on top of the developing somatosensory cortex. Plasmids injected: empty vector control, *SNRPN-WT,* or *SNRPN-p.Arg65Trp*. After birth, pups (M/F) were sacrificed at P1 for histochemical processing.

Immunohistochemistry

Mice (deeply anesthetized with an overdose of Nembutal) underwent transcardial per-fusion with 4% paraformaldehyde (PFA) and the brains were post-fixed in 4% PFA. After embedding the brains in gelatin and cryoprotecting them in 30% sucrose in 0.1 M phos-phate buffer (PB) for 2–4 h, they were frozen on dry ice, and sectioned coronally using a freezing microtome (50 µm thick). Free-floating sections were blocked in PBS containing 10% normal horse serum (NHS) and 0.5% Triton X-100 for one hour and then incubated with primary antibody RFP (#600401379, 1:2000, Rockland) in PBS containing 2% NHS, 0.5% Triton X-100, at ambient temperature overnight. Secondary antibody used: Cy3 donkey-anti-rabbit (1:400, Jackson ImmunoResearch) diluted in PBS containing 2% NHS, 0.5% Triton-X 100. 4',6-diamidino-2-phenylindole solution (DAPI, 1:10,000, Invitrogen) was used as a counterstain and Mowiol was used to mount the sections on glass. Images were acquired using a LSM700 confocal microscope (Zeiss) with a 10X objective.

Confocal images (10X objective, 0.5 zoom, 1024 × 1024 pixels) obtained from 2–3 non-consecutive sections from at least 3 successfully targeted animals per condition were used for the neuronal migration analysis (previously described (31–33)).

Statistical Analysis

We assumed normally distributed data. For the in vitro and in vivo overexpression experiments, statistical difference was determined using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test for multiple comparisons. Two-tailed unpaired *t*-test (dual comparison) was used for the Western blot analysis. We analyzed neuronal migration based on the proportion of electroporated cells that migrated to the cortical plate at P1 (defined as the most proximal 40% of dorsoventral distance between the pia and ventricle (first four of ten equally spaced bins)).

Literature Review

We performed a search on Embase, Medline, the Web of Science Core Collection, Cochrane Central Register of Controlled Trials, and Google Scholar for case reports of patients with genetic alterations (e.g., translocations, deletions, genetic variants) in the PWS critical region on the paternal chromosome that affected only part of the PWS criti-cal region. We included case reports and case series that provide a description of the genotype and phenotype of each individual case. We included articles about patients

with and without features of PWS. Exclusion criteria were articles that were not available online, articles that were not available in English, patients with genetic alterations of the PWS critical region on the maternal chromosome only, case reports where the entire PWS critical region or the PWS-imprinting center (IC) was affected, case reports where the affected region extended beyond the PWS critical region, and case reports with insufficient genotyping or phenotyping. For the full search strategy, see **Table S2**.

RESULTS

Patient Description

The female patient presented at our hospital at the age of 46 years, after she had been referred for treatment of morbid-obesity. She fulfilled the criteria for the clinical diagnosis of PWS, as is shown in **Table 1**.

Medical History

There were little fetal movements during pregnancy. The index case was born after a pregnancy of 40 weeks with a birth weight of three kilograms (−0.5 SD). Postnatally, she was cyanotic and hypotonic. Her neonatal feeding difficulties required tube feeding for which she was hospitalized for several months. Her feeding difficulties persisted until she was 1.5 years old, after which she developed hyperphagia, resulting in obesity at the age of four. When she was twelve years old, her weight was 125 kg according to her mother. Secondary to her obesity, she had developed type 2 diabetes mellitus and dyslipidaemia, for which she was treated with metformin and simvastatin, respectively. Psychomotor development was severely delayed; she started walking at the age of eight years and talking at the age of 40 years. She received special education. She had primary amenorrhea.

Anamnesis

The patient had clear hyperphagia: She could easily eat four full plates of food. Her mother, who was the primary caregiver, reported challenging behavior including temper tantrums, stealing food, skin picking, and trichotillomania.

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Family History

The parents of the index case were first-degree relatives. The mother of the index case had many (half-) siblings, of which most were illiterate or had great difficulty reading and writing, mainly caused by lack of education during childhood. Although both the mother of the index case and multiple siblings had short stature and were overweight, none of the family members had the same phenotype as the index case. However, the

mother had lost contact with most relatives and, therefore, family health history was incomplete. The mother of the patient was 157 cm tall and weighed 91 kg (body mass index (BMI) 37 kg/m^2).

Physical Examination

Physical examination revealed a typical PWS appearance of the index case, with obesity (BMI 34 kg/m^2), hypotonia, kyphosis, high-arched feet (requiring orthopedic shoes), and small hands with tapering fingers. Facial features included narrow bitemporal diameter, almond-shaped eyes, and strabismus (**Figure 1**). She had some breast formation, but exact Tanner stage for breast development was hard to assess due to her obesity. She had Tanner stage 3 pubic hair development. Her height was 158 cm (−2 SD, (35)), her weight 85 kg, and her head circumference 56.5 cm (+0.7 SD).

Figure 1. Appearance of the index case.
Picture of the patient's body, and left hand (**a**) compared to the hand of a healthy female control (**b**).

Biochemistry and Imaging

Laboratory results revealed central hypogonadism and a lowered Insulin-like growth factor (IGF)1 level (**Table S1**). Peak growth hormone value during growth hormone releasing hormone (GHRH) and arginine stimulation test was 2.9 µg/L. This is lower than the cut-off of 4.2 µg/L in obese subjects (36), confirming the diagnosis growth hormone deficiency. Cortisol and thyroid hormone levels were normal. Dual-energy X-ray absorptiometry (DEXA) scan showed osteopenia of the lumbar vertebrae and osteoporosis of the femoral neck.

Polysomnography

Polysomnography showed mild sleep apnea with an apnea–hypopnea index of 6.2 per hour (37).

Features not corresponding to PWS

Apart from the typical PWS features, the patient also had features not corresponding to PWS, like celiac disease, tinnitus, hearing loss (requiring a hearing aid), cataract (requiring lens implantation), and arthralgia of the hands and feet. Atypical findings during physical examination were a remarkable overbite, diastemata, multiple naevi, and a small palpebral fissure width. Her relatively tall stature (158 cm, while most PWS females have a height below 150 cm) was also atypical for PWS. Additionally, MRI of the brain also revealed abnormalities not specific for PWS, including septo-optic dysplasia (SOD), with several midline defects, including partial agenesis of the corpus callosum and the septum pellucidum, an atrophic left optic nerve, but the pituitary gland had a normal size (38). In addition, there was partial agenesis of the sagittal sinus and most likely agenesis of the falx cerebri. Lastly, there was a small lesion in the left-posterior part of the pituitary gland with a maximum diameter of 5 mm, most likely a pituitary incidentaloma. Her clinical, behavioral, and dysmorphic features are shown in **Table 2**.

As the patient fulfilled the consensus criteria for Prader–Willi syndrome (**Table 1**), we performed genetic diagnostic testing for PWS. After routine PWS methylation tests, using methylation-specific multiplex-ligation probe amplification (MS-MLPA), ruled out the presence of a deletion, mUPD, or ICD of chromosome 15q11.2-13, we performed additional genetic testing. Obesity gene panel analysis revealed a homozygous variant NM_003097.3(SNRPN):c.193C>T, p.(Arg65Trp) in *SNURF-SNRPN* (**Figure 2**), which was classified as a variant of uncertain clinical significance (class 3 according to the American College of Medical Genetics and Genomics guidelines). This variant was mentioned in the supplementary table (individual 111) of the article by Kleinendorst et al. (22). The mother was heterozygous for the same variant. The father was not available for DNA analysis. Hemizygosity in the index patient was excluded by MS-MLPA analysis. Single nucleotide polymorphism array (SNP) array showed several large regions of homozygosity (ROH), caused by high-grade consanguinity between the parents (**Figure 3**). The exact coordinates of the breakpoints can be found in **Table S3**.

SNURF-SNRPN was located in one of the ROH. In silico analysis using variant effect predictor programs Align-GVGD (23), MutationTaster (Build NCBI37/Ensembl 69) (24), sorting intolerant from tolerant (SIFT) (25,26), and Polymorphism Phenotyping v2 (Polyphen-2) (27,28) predicted that NM_003097.3(*SNRPN*):c.193C>T, p.(Arg65Trp) is deleterious. The variant was not present in the 1000 Genomes Project (Phase 3 release) (42) or in the

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Table 2. Clinical features, dysmorphic features, and challenging behavior associated with PWS and presence in the index case.

Clinical Features (1–3,5,6,39–41)	Present in Index Case	Prevalent in PWS
Poor foetal movement	yes	yes
Hypotonia during infancy	yes	yes
Feeding problems during infancy	yes	yes
Abnormal pubertal development	yes	yes
Developmental delay		
Speech	yes	yes
Psychomotor	yes	yes
Intellectual disability	yes	yes
Sleep related breathing disorder	yes	yes
Osteoporosis	yes	yes
Diabetes mellitus type 2	yes	yes
Hypertension	no	yes
Abnormal pain registration	no	yes
Pituitary hormone deficiencies		
Hypogonadism	yes	yes
Growth hormone deficiency	yes	yes
Hypothyroidism	no	yes
Vitamin D deficiency	yes	yes
Bowel problems		
Obstipation	yes	yes
Abnormal temperature regulation	no	yes
Leg edema	no	yes
Foot problems	yes	yes
Challenging behaviour		
Skin picking	yes	yes
Hair pulling	yes	yes
Temper tantrums	yes	yes
Stealing food	yes	yes
Obesity	yes	yes
Hyperphagia	yes	yes
Dysmorphic features		
Narrow temple distance	yes	yes
Narrow nasal bridge	no	yes
Almond-shaped eyes	yes	yes
Small palpebral fissure length	yes	no
Strabismus	yes	yes
Thin upper lip	no	yes
Low hair line	yes	no
Small chin	yes	no
Broad nose	yes	no
Small hands and feet	yes	yes
Scoliosis	no	yes
Kyphosis	yes	yes
Short stature (height below −2 SD)	no	yes

Abbreviations: insulin-like growth factor 1 (IGF-1), growth hormone (GH), standard deviation (SD).
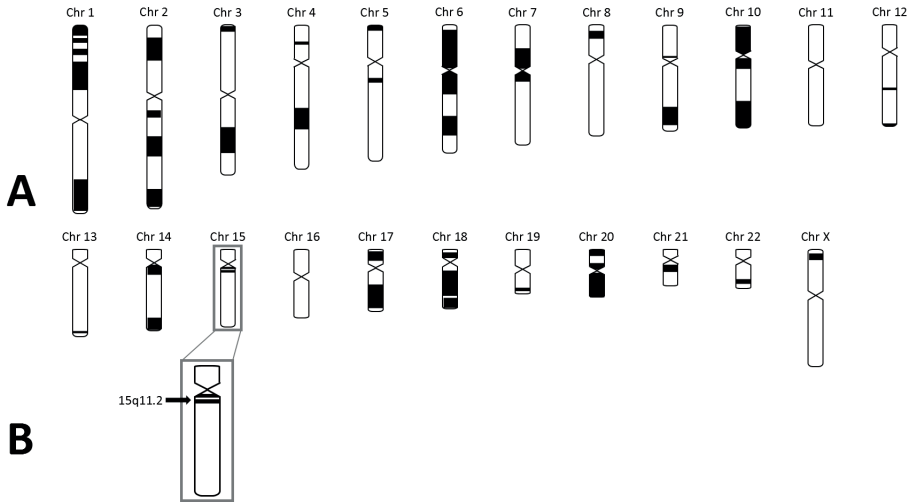
genome aggregation database (gnomAD v2.1.1) (43). The protein structure of SNRPN and the location of the changed amino acid is depicted in **Figure 4**. RNA sequencing analysis showed equal expression of *SNRPN* and all other genes in the PWS critical region for the index case, the mother, and four healthy, Caucasian volunteers (one male and three females) that served as controls. RNA sequencing confirmed that the mother of the patient had the variant on her paternal chromosome 15.

Figure 2. The Prader–Willi syndrome region and NM_003097.3(*SNRPN*):c.193C>T, p.(Arg65Trp).
(**A**) The arrow indicates the location of the variant in the *SNURF-SNRPN* gene. (**B**) The cross indicates the location of the affected nucleotide and the amino acid. (**C**) The structural formula of arginine and tryptophan.

Functional analysis showed that the NM_003097.3(*SNRPN*):c.193C>T, p.(Arg65Trp) variant did not cause instability of the protein, showing similar expression levels to *SNRPN-WT* (P = 0.16, two-tailed unpaired Student's *t*-test) (**Figure 5a**). Additionally, overexpression of *SNRPN-WT* or *SNRPN-p.Arg65Trp* in a small subset of neurons during embryological development did not alter the migration pattern of the neurons transfected (One-Way ANOVA: $F_{(2,29)} = 0.97$, P = 0.39) (**Figure 5b**). However, whereas overexpression of *SNRPN-WT* showed a small trend in reducing neuronal maturation (Neurite length: One-Way ANOVA: $F_{(2,45)} = 6.29$, P = 0.003; *SNRPN-WT* versus empty vector control: P = 0.0627, Tukey's multiple comparison test; Arborization: One-Way ANOVA: $F_{(2,45)} = 5.66$, P = 0.006; *SNRPN-WT* versus empty vector control: P = 0.44, Tukey's multiple comparison test), overexpression of the *SNRPN-p.Arg65Trp* variant significantly affected neuronal maturation in vitro, resulting in a decreased neurite length and arborization (Neurite length: *SNRPN-p.Arg65Trp* versus empty vector control: P = 0.003, Tukey's multiple comparison test; Arborization: *SNRPN-p.Arg65Trp* versus empty vector

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Figure 3. SNP array results in the index case.
(**A**) Black parts represent homozygous regions in the index case. The number of homozygous regions is suggestive for high-grade consanguinity. (**B**) The black arrow represents 15q11.2 in which *SNURF-SNRPN* is located. As shown, *SNURF-SNRPN* lies within a homozygous region.
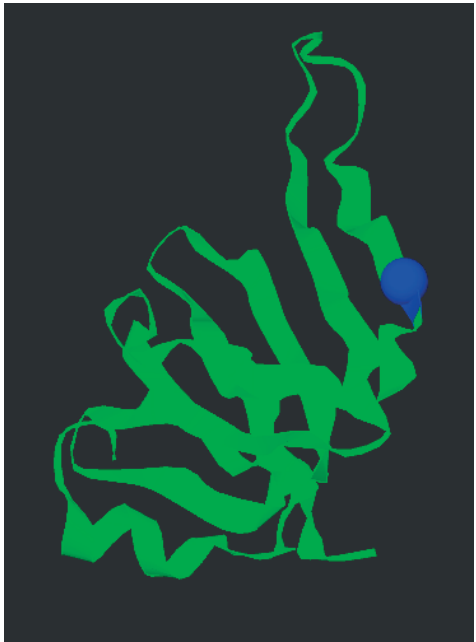
Figure 4. SNRPN protein structure and location p.(Arg65Trp) variant.
The protein structure of SNPRN is given in green, the p.(Arg65Trp) variant is depicted with the blue sphere. This figure was generated using mutation3D (44).

control: P = 0.004, Tukey's multiple comparison test) (**Figure 5c**). Taken together, these results suggest that the *SNRPN-p.Arg65Trp* variant does affect the function of *SNRPN*, suggesting pathogenicity.
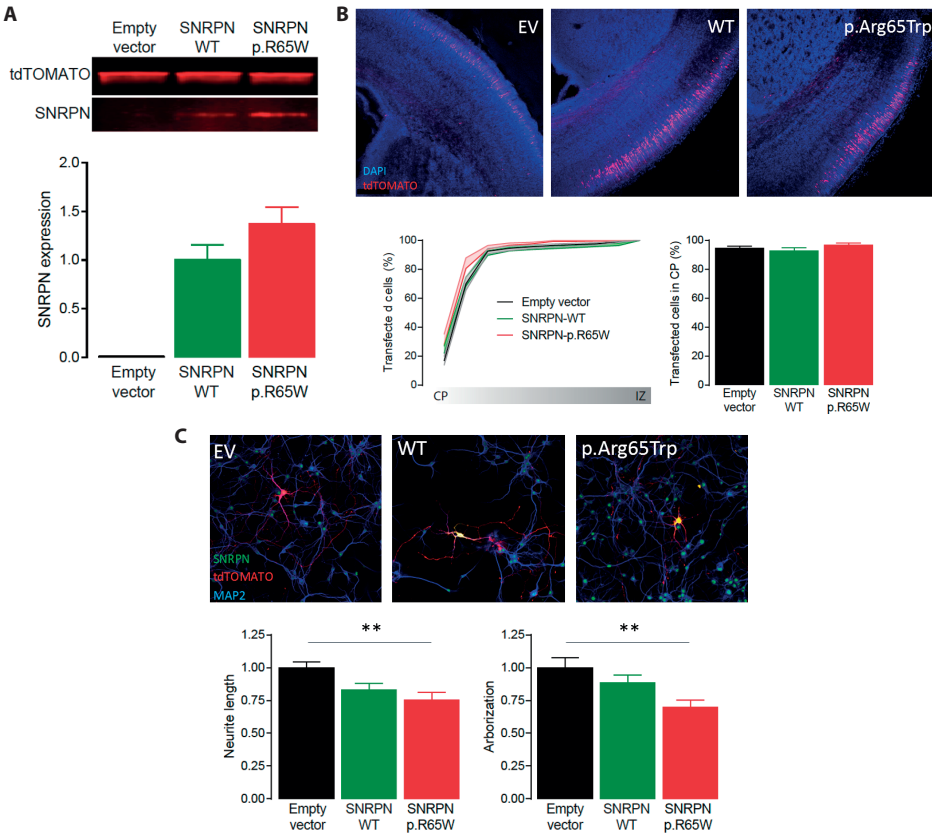
Figure 5. (a) Western blot analysis of transfected HEK293T cells show that *SNRPN* expression is normal. Abbreviations: Empty vector (EV), wild type (WT). Top: Western blot of transfected HEK293T cells showing clear overexpression of *SNRPN-WT* and *SNRPN-p.R65W*. Bottom: Quantification of the Western blots showing that the *SNRPN-p.R65W* missense variant does not alter the expression levels of *SNRPN* (n = 4 per condition). **(b)** In utero electroporation of the different *SNRPN* constructs does not affect neuronal migration in vivo. Abbreviations: Empty vector (EV), wild type (WT). Top: Representative images from in utero electroporated postnatal brains on day 1. These images show that the far majority of the transfected cells (tdTOMATO+) migrated out to the cortical plate (CP; the outer layer of the cortex). Bottom left: Cumulative distribution of the transfected neurons at P1 from the cortical plate (CP; the outer layer of the cortex) to the intermediate zone (IZ; inner layer of the cortex). Bottom right: Percentage of neurons that migrated out to the superficial layers of the cortex (sum of bins 1 through 4). Number of pictures/animals for each condition = 9/3. **(c)** Expression of the different *SNRPN* constructs in mouse primary hippocampal neurons reveal that overexpression of the p.Arg65Trp variant significantly affects neuronal maturation in vitro. Abbreviations: Empty vector (EV), wild type (WT). Top: Representative images of primary hippocampal neurons transfected with Empty vector control, *SNRPN-WT* or *SNRPN-p.Arg65Trp* (tdTO-MATO+). Neurons are stained for SNRPN (green) and MAP2 (blue). Bottom: Quantitative analysis of the total neurite length and arborization in the different conditions. Number of neurons/number of batches for each condition = 20/2; ** P < 0.01.

Literature Review

To interpret our findings in the light of the available literature, we performed a thorough literature search for case reports or case series of patients with genetic alterations (e.g., translocations, deletions, genetic variants) in the PWS critical region on the paternal chromosome that affected only part of the PWS critical region. After deduplication, we found 4693 articles, of which the titles and abstracts were reviewed. After full-text screening, we included 22 cases with PWLS. Additionally, we found 29 case reports that described abnormalities in only *MAGEL2* (associated with Schaaf–Yang syndrome) and 42 case reports with abnormalities in only *MKRN3* (associated with central precocious puberty), which are not included in the table. Most of the 22 cases with PWLS had a translocation or a deletion, leading to a loss of function of one or more genes of the PWS critical region. Additionally, we found one case report of a patient with a small duplication in exon 1 of *SNURF*, which introduced a premature stop codon in *SNURF*. The results of this literature review are summarized in **Table 3** and **Figure 6**.

DISCUSSION

We here present the puzzling diagnostic trajectory of a 46-year-old female patient with PWLS. After genetic testing excluded the classical PWS genetic defects (deletion, mUPD, ICD, or translocation of chromosome 15q11.2-13), we performed additional genetic analyses. Obesity gene panel analysis of 57 obesity-associated genes revealed a homozygous variant NM_003097.3(SNRPN):c.193C>T, p.(Arg65Trp) in exon 6 of the *SNURF-SNRPN* gene, changing CGG into TGG (**Figure 2**). This variant was not present in the 1000 Genomes Project (Phase 3 release) (42) or in the genome aggregation database (gnomAD v2.1.1) (43). The variant changes the non-aromatic and positively charged arginine into the aromatic, non-polar tryptophan (Arg65Trp), which is deleterious according to various variant effect predictor programs. The fact that this homozygous missense variant is located in the PWS critical region suggests a relation with the PWS phenotype of the patient.

While the patient's phenotype included PWS features, she also had atypical features, including brain lesions at MRI. Although morphological abnormalities in the brain of patients with PWS have been described (67–71), the brain lesions found in our patient were atypical for PWS. SNP array analysis showed multiple large ROH spread across different chromosomes, suggesting that (multiple) autosomal recessive diagnoses could contribute to the complete phenotype in this patient. Additionally, the fact that the mother of the patient had the same variant on her paternal chromosome 15 without having PWLS (she had obesity but did not fulfil any other diagnostic criteria for PWS),

suggests that this variant is probably not the only explanation for the phenotype of the index case.

As she had dysgenesis of midline brain structures and optic nerve hypoplasia at MRI with hypothalamic-pituitary dysfunction (38), she fulfilled the criteria for SOD. SOD has a wide variability of clinical features, including some features that were present in our patient, like developmental delay (72), dysmorphic features, and autism-like behavior (73). However, our patient also had many symptoms not associated with SOD, including hyperphagia, hypotonia, and kyphosis. Although the etiology remains unclear in most cases, SOD has been associated with pathogenic variants in *HESX1, OTX2, SOX2*, and *SOX3* (38,74,75). One of the ROH of the index patient included *SOX3*, but none of the ROH included *HESX1*, *OTX2*, or *SOX2*.

The index case has a remarkable family history with a large pedigree. Most family members were short, overweight, and/or illiterate. The latter is remarkable in the context of 10% inadequate literacy in the total Dutch 16–65 year old population (76). The high prevalence of short stature, obesity and/or illiteracy in this family is suggestive of a genetic component. Unfortunately, other family members were unavailable for genetic analysis.

Interestingly, *SNURF-SNRPN* is a bi-cistronic gene, which is transcribed exclusively from the paternally inherited chromosome. *SNURF-SNRPN* is expressed in several tissues and shows the highest RNA expression in the brain and heart, followed by endocrine tissues, male reproductive tissues, and blood (77). *SNURF-SNRPN* encodes two proteins: SNURF and SNRPN (also called SmN) (78). The function of SNURF is unknown (78). SNRPN is involved in mRNA splicing and predominantly expressed in neurons, especially in central neurons (79–81). SNRPN influences neurite outgrowth, neuron migration, and distribution of dendritic spines in mice (82). However, the precise role of SNRPN in the development of the PWS phenotype remains largely unknown. Apart from encoding SNURF and SNRPN proteins, *SNURF-SNRPN* transcription is necessary for production of downstream ncRNAs implicated in many PWS traits (83,84).

Imprinting at 15q11.2-13 is regulated by a bipartite imprinting center defined as the smaller regions of overlap (SRO) found in patients with imprinting defects (85–90). The Angelman syndrome-SRO (AS-SRO) functions to silence paternally expressed genes on the maternal allele. The PWS-SRO functions somatically to activate paternally expressed genes on the paternal allele (91–93). The PWS-SRO spans *SNURF-SNRPN* exon 1 (94,95). The missense variant in this patient is in exon 6, about 20 kb downstream of the PWS-SRO

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Table 3. Literature review.

Case and Reference	Sensitivity (46)	Greger et al. (1993) & Hamabe et al. (1991) (47,48)	Schulze et al. (1996) (49)	Sun et al. (1996) (50)	Conroy et al. (1997) (51)	Kuslich et al. (1999) (52)	Wirth et al. (2001) (53)
Genetic abnormality		1.5 Mb deletion paternally transmitted	Balanced translocation t(9;15)(q21;q12–13)	Balanced translocation t(15;19)(q12;q13.41)	Balanced translocation t(2;15)(q37.2;q11.2)	Balanced translocation t(4;15)(q27;q11.2)	Balanced translocation t(X;15)(q28;q12)
Location		*SNORD115* to *GABRB3* intron 3	*SNRPN* exon 20/intron 20 (located between *SNORD108* and *SNORD109A*)	*SNRPN* intron 2	*SNRPN* exon 20/intron 20 (located between *SNORD108* and *SNORD109A*)	*SNRPN* intron 2	*SNRPN* exon 20/intron 20 (located between *SNORD108* and *SNORD109A*)
Gender		3 family members	male	male	male	male	female
Age at last examination (years)		no phenotype when paternally transmitted (causes Angelman syndrome when maternally transmitted)	29	3	4	11	20
Weight (kg)			89	34	>95 th percentile	NA	72
Height (cm)			169	103	50–75 th percentile	NA	151
Major criteria							
Neonatal hypotonia	98		+	+	+	+	-
Feeding problems/FTT	96		+	+	+	+	-
Excessive weight gain 1–6 years	95		- (at 7 years)	+	+	+	+
Characteristic facial features	49		+	+	+	+	-
Hypogonadism/genital hypoplasia/delayed or incomplete puberty	96		+	+	NA	+	+
Developmental delay/intellectual disability/learning problems	98		+	+	+	+	+
Hyperphagia/food foraging/obsession with food	93		NA	+	+	+	+

Table 3. Literature review. (*continued*)

Case and Reference	Sensitivity (46)	Greger et al. (1993) & Hamabe et al. (1991) (47,48)	Schulze et al. (1996) (49)	Sun et al. (1996) (50)	Conroy et al. (1997) (51)	Kuslich et al. (1999) (52)	Wirth et al. (2001) (53)
Minor criteria							
Decreased foetal movements/infantile lethargy	89	NA	NA	+	+	+	–
Challenging behaviour	82	+	+	+	+	+	+
Sleep disturbance or sleep apnoea	37	+	+	–	NA	+	–
Short stature	86	+	+	–	– –	NA	+
Hypopigmentation	47	+	+	–	NA	–	–
Small hands/feet for height and age	75	+	+	+	–	–	–
Narrow hand, straight ulnar border	69	NA	NA	NA	NA	–	NA
Eye abnormalities (esotropia, myopia)	49	+	+	–	+	–	+
Thick, viscous saliva	83	NA	NA	–	+	+	NA
Speech articulation defects	93	–	–	–	+	+	–
Skin picking	61	+	+	–	+	+	–

Excessive weight gain was only scored as present if it occurred when the case was 1 to 6 years old. Challenging behavior was scored as '+' when at least one typical challenging behavior was present (temper tantrums, violent outbursts and obsessive/compulsive behavior; tendency to be argumentative, oppositional, rigid, manipulative, possessive, and stubborn; perseverating, stealing, and lying). Abbreviations: Body mass index (BMI), growth hormone therapy (GHT), failure to thrive (FTT), up to and including (UTAI), +: present, –: absent, +/–: somewhat present, N/A: not described, /: and/or.

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Table 3. Literature review (continued).

Case and Reference	Maina et al. (2007) id05 and Whittington et al. (2002) case 1 (54,55)	Bürger et al. (2002) & Runte et al. (2005) (56,57)	Schüle et al. (2005) (58)	Sahoo et al. (2008) (14)	Calounova et al. (2008) patient 2 (59)	de Smith et al. (2009) (15)
Genetic abnormality	Deletion of maximum 1.8 Mb	~570 kb deletion paternally transmitted	Balanced translocation t(4;15)(q27;11.2)	175 kb deletion	9.5 Mb deletion	187 kb deletion
Location	Includes: *CYFIP1* and *MKRN3*, but not *SNRPN*	*SNORD115* UTAI *UBE3A*	*SNRPN* intron 17 (=between *SNORD108* and *SNORD109A*)	*SNORD109A* UTAI *SNORD115-24*	*NPAP1* UTAI *AVEN*	*SNURF-SNRPN* exon 2 UTAI *IPW*
Gender	male		male	male	female	male
Age at last examination (years)	22	3 family members	22	4	18	19
Weight (kg)	NA		90	63	79	109
Height (cm)	NA		164	115	149	167.5
Major criteria						
Neonatal hypotonia	+	no phenotype when paternally transmitted (causes Angelman syndrome when maternally transmitted)	+	+	+	+
Feeding problems/FTT	+		+	+	+	+
Excessive weight gain 1–6 years	+		–(at 8 years)	+	NA	+
Characteristic facial features	NA		–	+	NA	+/–
Hypogonadism/genital hypoplasia/delayed or incomplete puberty	+		+	+	+	+
Developmental delay/intellectual disability/ learning problems	+		+/–	+	+	+
Hyperphagia/food foraging/obsession with food	+		+	+	NA	+

Table 3. Literature review (continued). (*continued*)

Case and Reference	Maina et al. (2007) id05 and Whittington et al. (2002) case 1 (54,55)	Bürger et al. (2002) & Runte et al. (2005) et al. (2005) (56,57)	Schüle et al. (2005) (58)	Sahoo et al. (2008) (14)	Calounova et al. (2008) patient 2 (59)	de Smith et al. (2009) (15)
Minor criteria						
Decreased foetal movements/infantile lethargy	+		+	+	NA	NA
Challenging behaviour	+		+	+	+	+
Sleep disturbance or sleep apnoea	+		+	+	NA	NA
Short stature	+		+	−	+	−
Hypopigmentation	+		−	NA	NA	NA
Small hands/feet for height and age	+		+	+	NA	+
Narrow hand, straight ulnar border	NA		−	NA	NA	NA
Eye abnormalities (esotropia, myopia)	+		+	−	+	NA
Thick, viscous saliva	+		−	−	NA	NA
Speech articulation defects	+		−	+	NA	NA
Skin picking	+		+	+	NA	+

Excessive weight gain was only scored as present if it occurred when the case was 1 to 6 years old. Challenging behavior was scored as '+' when at least one typical challenging behavior was present (temper tantrums, violent outbursts and obsessive/compulsive behavior; tendency to be argumentative, oppositional, rigid, manipulative, possessive, and stubborn; perseverating, stealing, and lying). Abbreviations: Body mass index (BMI), growth hormone therapy (GHT), failure to thrive (FTT), up to and including (UTAI), +: present, −: absent, +/−: somewhat present, N/A: not described, /: and/or.
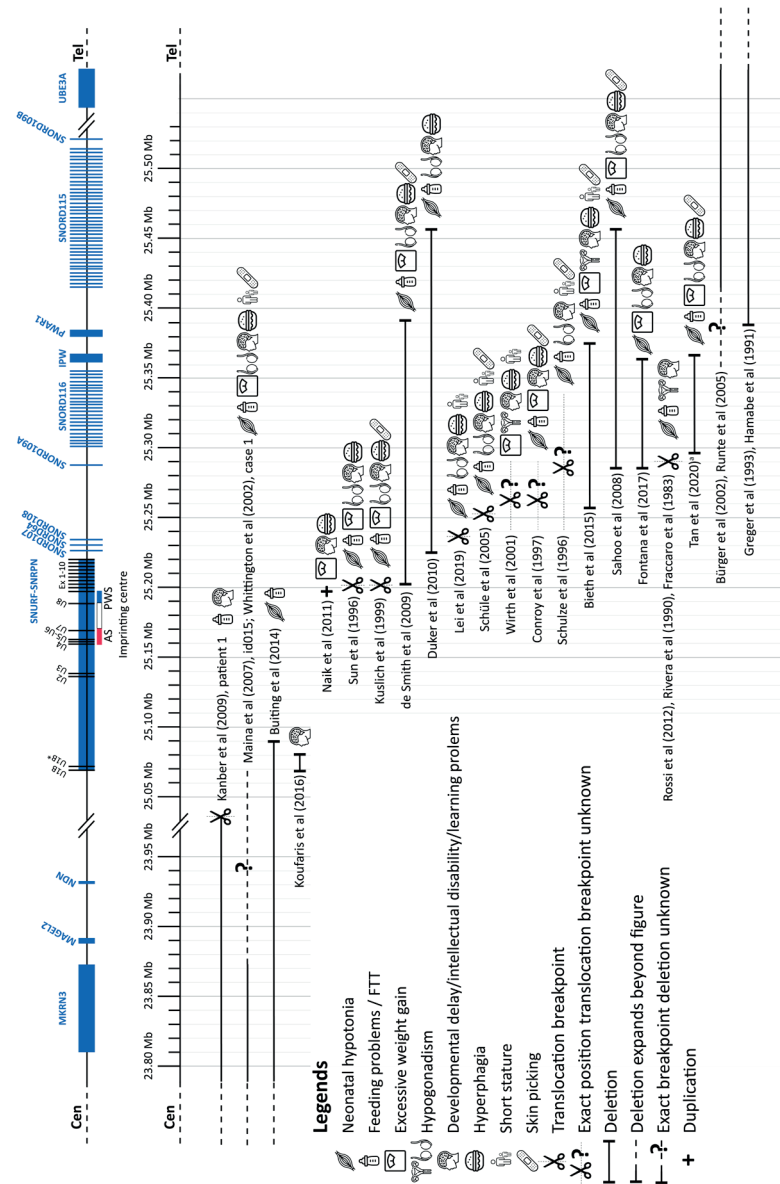
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Table 3. Literature review (continued).

Case and Reference	Patient 1 in Kanber et al. (2009) (60)	Duker et al. (2010) (16)	Naik et al. (2011) (61)	Rossi et al. (2012) & Rivera et al. (1990) & Fraccaro et al. (1983) (45,62,63)	Buiting et al. (2014) (64)	Bieth et al. (2015) (17)
Genetic abnormality	Unbalanced translocation t(X;15) (q28;q11.2)	236 kb deletion	25 bp duplication	Jumping translocation with a major t(15;18) (q13;q23) and a minor t(X;15) (q28;q13) cell line	~3.9 Mb deletion	118 kb deletion
Location	Breakpoint: between *NDN* and *SNURF-SNRPN* Deletion: *CYFIP1* UTAI *NDN*	3' end of *SNRPN* (exon 10) UTAI *SNORD155-24*	Exon 1 *SNURF/ SNRPN*	Between *SNORD108* & *SNORD116*	*CHEK2P2* UTAI *SNRPN* exon U1B*	*SNORD109A* UTAI *IPW*
Gender	female	male	female	female	male	female
Age at last examination (years)	12	11	11	10 months	3	23
Weight (kg)	75	94	63.5	9.9	17.3	75
Height (cm)	160	10–25th centile	170	70	103	155 (6m GHT)
Major criteria						
Neonatal hypotonia	−	+	−	+	Asymptomatic apart from delayed motor skills and transient muscular hypotonia associated with mild feeding difficulties in infancy	+
Feeding problems/FTT	+	+	−	+		+
Excessive weight gain 1–6 years	NA	−(7 months)	+	NA		+
Characteristic facial features	−	−	+	+		+
Hypogonadism/genital hypoplasia/delayed or incomplete puberty	−	+	NA	+		+
Developmental delay/intellectual disability/learning problems	+	+	+	+		+
Hyperphagia/food foraging/obsession with food	−	+	+/−	NA		+

Table 3. Literature review (continued). (*continued*)

Case and Reference	Patient 1 in Kanber et al. (2009) (60)	Duker et al. (2010) (16)	Naik et al. (2011) (61)	Rossi et al. (2012) & Rivera et al. (1990) & Fraccaro et al. (1983) (45,62,63)	Buiting et al. (2014) (64)	Bieth et al. (2015) (17)
Minor criteria						
Decreased foetal movements/infantile lethargy	–	+	–	+		NA
Challenging behaviour	–	+	+	NA		+
Sleep disturbance or sleep apnoea	–	+	NA	NA		+
Short stature	–	NA	–	NA		+
Hypopigmentation	–	–	NA	+		NA
Small hands/feet for height and age	–	–	NA	NA		+
Narrow hand, straight ulnar border	NA	NA	NA	NA		NA
Eye abnormalities (esotropia, myopia)	NA	+	NA	NA		+
Thick, viscous saliva	–	+	NA	NA		+
Speech articulation defects	–	+	NA	NA		NA
Skin picking	–	–	NA	NA		+

Excessive weight gain was only scored as present if it occurred when the case was 1 to 6 years old. Challenging behavior was scored as '+' when at least one typical challenging behavior was present (temper tantrums, violent outbursts and obsessive/compulsive behavior; tendency to be argumentative, oppositional, rigid, manipulative, possessive, and stubborn; perseverating, stealing, and lying). Abbreviations: Body mass index (BMI), growth hormone therapy (GHT), failure to thrive (FTT), up to and including (UTAI), +: present, –: absent, +/–: somewhat present, N/A: not described, /: and/or.

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Table 3. Literature review (continued).

Case and reference	Koufaris et al. (2016) (65)	Fontana et al. (2017) (19)	Lei et al. (2019) (66)	Tan et al. (2020) (13)
Genetic abnormality	13 kb deletion	~ 81 kb deletion	Balanced translocation t(15;19)(q11.2;q13.3)	71 kb deletion
Location	U1B and U1B* upstream exons of *SNRPN*	*SNORD109A* to exon 3 *IPW*	Between *PAR5* (including *SNORD108*) and *PAR6*	At least *SNORD116* and *IPW*
Gender	NA	male	male	male
Age at last examination (years)	child	18	13	17
Weight (kg)	overweight	76	45	BMI: 28.45 kg/m^2
Height (cm)	NA	174	132	181
Major criteria				
Neonatal hypotonia	Overweight child with mild intellectual disability and neurodevelopmental delay	+	+	+
Feeding problems/FTT		NA	+	+
Excessive weight gain 1-6 years		+	– (7 months)	+
Characteristic facial features		–	NA	+
Hypogonadism/genital hypoplasia/delayed or incomplete puberty		+	+	–
Developmental delay/intellectual disability/learning problems		+/–	+	+
Hyperphagia/food foraging/obsession with food		+	+	+

Table 3. Literature review. (*continued*)

Case and reference	Koufaris et al. (2016) (65)	Fontana et al. (2017) (19)	Lei et al. (2019) (66)	Tan et al. (2020) (13)
Minor criteria				
Decreased foetal movements/infantile lethargy	NA	NA	+	+
Challenging behaviour	NA	NA	+	+
Sleep disturbance or sleep apnoea	NA	NA	–	+
Short stature	–	–	+	–
Hypopigmentation	NA	NA	–	NA
Small hands/feet for height and age	–	–	–	–
Narrow hand, straight ulnar border	NA	NA	–	NA
Eye abnormalities (esotropia, myopia)	+	+	–	–
Thick, viscous saliva	NA	NA	–	NA
Speech articulation defects	NA	NA	–	NA
Skin picking	NA	NA	–	+

Excessive weight gain was only scored as present if it occurred when the case was 1 to 6 years old. Challenging behaviour was scored as '+' when at least one typical challenging behavior was present (temper tantrums, violent outbursts and obsessive/compulsive behavior; tendency to be argumentative, oppositional, rigid, manipulative, possessive, and stubborn; perseverating, stealing, and lying). Abbreviations: Body mass index (BMI), growth hormone therapy (GHT), failure to thrive (FTT), up to and including (UTAI), +: present, –: absent, +/–: somewhat present, N/A: not described, /: and/or.

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Figure 6. Overview cases literature review. Abbreviations: Angelman syndrome (AS), failure to thrive (FTT), Prader–Willi syndrome (PWS). [a] Exact breakpoints for this case are not available, depicted is the minimal deleted region.

This figure shows an overview of the cases we found during our literature review. For each case, the affected region is depicted, followed by symbols representing the clinical phenotype of the case. Genes are represented at scale with physical distance in Mb. SnoRNAs are depicted as vertical lines and other genes as boxes. Deletions are depicted according to the genomic deletion coordinates for build hg19. Excessive weight gain was only scored as present if it occurred when the case was 1 to 6 years old. Figure adapted from Fontana et al. (19) and Tan et al. (13). This figure has been designed using resources from Flaticon.com (from surang, freepik, smashicons, dinosoftlabs and monkik), accessed on 1 March 2021.

and is therefore very unlikely to generate an ICD. Additionally, in our patient, imprinting and expression of the genes in the PWS critical region was normal, excluding an ICD.

The PWS critical region encompasses several genes, including *MKRN3*, *MAGEL2*, *NDN*, *SNURF-SNRPN,* and ncRNAs including snoRNAs like *SNORD115* and *SNORD116* (10). Two individuals with deletions of *MKRN3, MAGEL2,* and *NDN* have been described (59,64). These patients lacked many typical PWS features, suggesting that deletions of these genes cannot cause the full PWS phenotype on their own. However, patients with a heterozygous truncating pathogenic variant in the paternally derived allele of the *MAGEL2* gene have Schaaf–Yang syndrome, which has clinical overlap with PWS, including intellectual disability, autism spectrum disorder, neonatal hypotonia, and infantile feeding problems (96,97). Patients with Schaaf–Yang syndrome also display non-PWS features, for example distal joint contractures. *SNORD115* is unlikely to play a role in the PWS phenotype, as individuals with a paternally inherited deletion of *SNORD115* do not have PWS features (46,47,55,56). *SNORD116* is suspected to be a strong contributor to the PWS phenotype (98). Several cases with PWS features and microdeletions or translocations resulting in lack of expression of *SNORD116* and *IPW* indicate an important role of *SNORD116* and *IPW* (13–19,48–52,57,66), **Table 3**. However, *IPW* is a non-protein coding RNA, which is minimally conserved between human and mouse genomes (99,100). Mice with a targeted deletion of *SNORD116* show cognitive deficits, abnormal growth and feeding (101,102), while mice with paternal deletions of portions of *SNURF* or *SNRPN* seem to have a normal phenotype (103,104). It has been suggested that individuals with translocations have a milder phenotype when only *SNORD116* is affected, compared to individuals in whom *SNURF-SNRPN* expression is also abolished. *SNORD116* deletions seem to cause most of the major features of PWS, but they may be less prominent (57). It should be noted that this is hard to investigate as all patients are assessed by different physicians, making it hard to objectively assess patients (84). Lastly, *SNURF* could contribute to the PWS phenotype, as is demonstrated by Naik et al. (60), who presented a case with a small duplication in exon 1 of *SNURF-SNRPN*, which is predicted to only affect *SNURF* expression, although it cannot be ruled out that *SNRPN* is also affected. This patient had developmental delay, challenging behavior, and increased appetite, but also tall stature (170 cm (+3.26 SD)) and a large head circumference (57 cm (+2.69 SD)), which are not PWS features and were also not present in the index case that we present.

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SNORD116 is produced from the same primary transcript as *SNURF-SNRPN* (83,84) and therefore variants in *SNURF-SNRPN* could cause a PWS phenotype through a change of expression of *SNORD116*. However, the RNA sequencing in our patient showed a normal expression of *SNORD116*. This rules out that the PWLS was caused by lack of *SNORD116* expression.

PWS is generally thought to result from loss of expression of the paternal genes on the PWS critical region, as the maternal PWS critical region is imprinted and therefore not expressed. The mother of the index case had the same variant as the index case on her paternal chromosome 15. She did not have a documented intellectual disability or specific PWS features, but she was illiterate and had obesity. This suggests that the *SNRPN-p.Arg65Trp* variant might not always be pathogenic (incomplete penetrance) or might result in a mild phenotype. There is some evidence from mice that expression of the maternal allele of chromosome 15q11.2-13 can be detected when the paternal allele is not active (105,106). However, findings in mice are poor predictors of the human situation and therefore the loss of silencing found by Rieusset et al. (105) cannot be extrapolated to humans.

A previous report by Iourov et al. (107) suggested that homozygosity (without mUPD) of part of 15q11.2 could be related to a mild PWLS phenotype, mainly characterized by developmental delay and/or intellectual disability. According to SNP array, homozygosity of 15q11.2 was also present in our patient and could have influenced her phenotype. However, the phenotype of our index case was specific to PWS, whereas the five cases described by Iourov et al. lacked most major phenotypic features of PWS. They did not present hypothalamic dysfunction leading to hyperphagia, pituitary hormone deficiencies, abnormal temperature regulation, or inadequate pain registration.

In our patient, RNA sequencing showed that expression of *SNURF-SNRPN* and the other genes in the PWS critical region were not affected. However, the change from arginine to tryptophan caused by the homozygous *SNRPN* variant was predicted to severely affect protein function. In order to understand whether the variant found in this patient indeed is pathogenic and whether it causes a loss of function or a gain of function, we made use of the unbiased functional genomics screen PR*i*SM. This showed that the variant does not cause protein instability, nor behaves differently from *SNRPN-WT* in the migration assay. However, whereas overexpression of *SNRPN-WT* resulted in a small but non-significant trend in reduced neural development in vitro, *SNRPN-p.Arg65Trp* significantly reduced neural development upon overexpression primary hippocampal neurons. This suggests that the missense variant might alter the function of SNRPN, causing a dominant negative effect on neuronal maturation when overexpressed. These results show that this variant does not cause a loss of function, rather it might suggest that it causes a gain of function. However, future studies are needed to confirm its functional impact.

The *SNRPB'/B* genes encode the proteins small nuclear ribonucleoprotein-associated polypeptide B' and B (SmB'/B). They are closely related to *SNRPN*, which encodes the protein SNRPN, also called small nuclear ribonucleoprotein-associated polypeptide N

(SmN). Normally, SNRPN replaces SmB'/B in the brain. It has been demonstrated that the loss of SNRPN in PWS brain tissue causes a compensatory feedback loop that drastically upregulates the levels of SmB'/B. It has been suggested that upregulation of SmB'/B in PWS reduces the severity of the syndrome (108). However, in the presence of a non-functional SNRPN protein, this compensation mechanism might not be induced, leading to more severe phenotypic features compared to cases where SNRPN is completely absent. RNA sequencing in this patient showed that *SNRPB'/B* expression in fibroblasts was normal.

CONCLUSIONS

In conclusion, the finding of this homozygous missense *SNURF-SNRPN* variant in a patient with virtually all clinical features of PWS suggests that this variant might have caused her PWLS with combined pituitary hormone deficiency (CPHD). Additionally, functional analysis suggested that the variant might affect the function of SNRPN. However, the large ROHs that were found throughout the genome suggests other autosomal recessive conditions that could contribute to her complete phenotype. The fascinating trajectory of genetic and functional analyses performed in this patient shows that the finding of a unique variant in the PWS critical region, in a patient with PWLS, does not necessarily prove a causal relationship between the two.

ACKNOWLEDGEMENTS

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Discussion and conclusions

In this thesis, we described health problems in adults with Prader-Willi syndrome (PWS) and provided clinical recommendations to avoid underdiagnosis and undertreatment. Additionally, we aimed to provide insight into the genotype-phenotype relationship to facilitate future research into new treatments. In this chapter, we will discuss the results and implications of the studies presented. We will also discuss future developments and perspectives. In **Figure 1** we provide an overview of the complex interplay of health problems in PWS in relation to the chapters of this thesis.

SYSTEMATIC SCREENING

PWS is a complex syndrome, leading to a variety of health problems. Underdiagnosis of these health problems is common, due to unfamiliarity of physicians with the syndrome, the specific behavioral phenotype, intellectual disability, and high pain threshold. In **Chapter 2**, we provide an overview of undiagnosed health problems in our cohort of adults with PWS. Of all patients included, 61% had at least one undiagnosed health problem and almost a quarter had multiple undiagnosed health problems. The most frequently missed comorbidities were hypogonadism, vitamin D deficiency, and scoliosis. Several health problems were very common in our cohort of adults with PWS: hypogonadism was present in 100% of males and 93% of females, scoliosis was present in 74%, vitamin D deficiency in 78%, hypercholesterolemia in 19%, hypertension in 18%, type 2 diabetes mellitus (DM2) in 17%, and hypothyroidism in 17%. In our literature review we found that the prevalence of these health problems in previous studies was highly variable. This could be related to lack of systematic screening and small sample sizes in some studies. Two large previous studies also systematically screened for health problems in adults with PWS (1, 2). Compared to these studies, we found a lower prevalence of DM2, scoliosis, and hypothyroidism. The lower prevalence of DM2 could be related to a lower BMI and higher number of patients that had received GH treatment during childhood in our cohort.

We showed that health problems are often missed in adults with PWS. To avoid undiagnosed and untreated health problems in PWS, we propose to perform a yearly systematic health screening in all adults with PWS. However, to avoid unnecessary testing, certain routine check-ups should only be carried out in older adults or adults with obesity, as shown in **Figure 2**. As highlighted in **Chapter 3** of this thesis, a multidisciplinary approach is crucial. Ideally, an endocrinologist, a physician for intellectual disabilities (ID physician), a dietitian, a physiotherapist, and a behavioral expert should work together to optimize care for adults with PWS. If needed, other specialists with knowledge of PWS should be available.
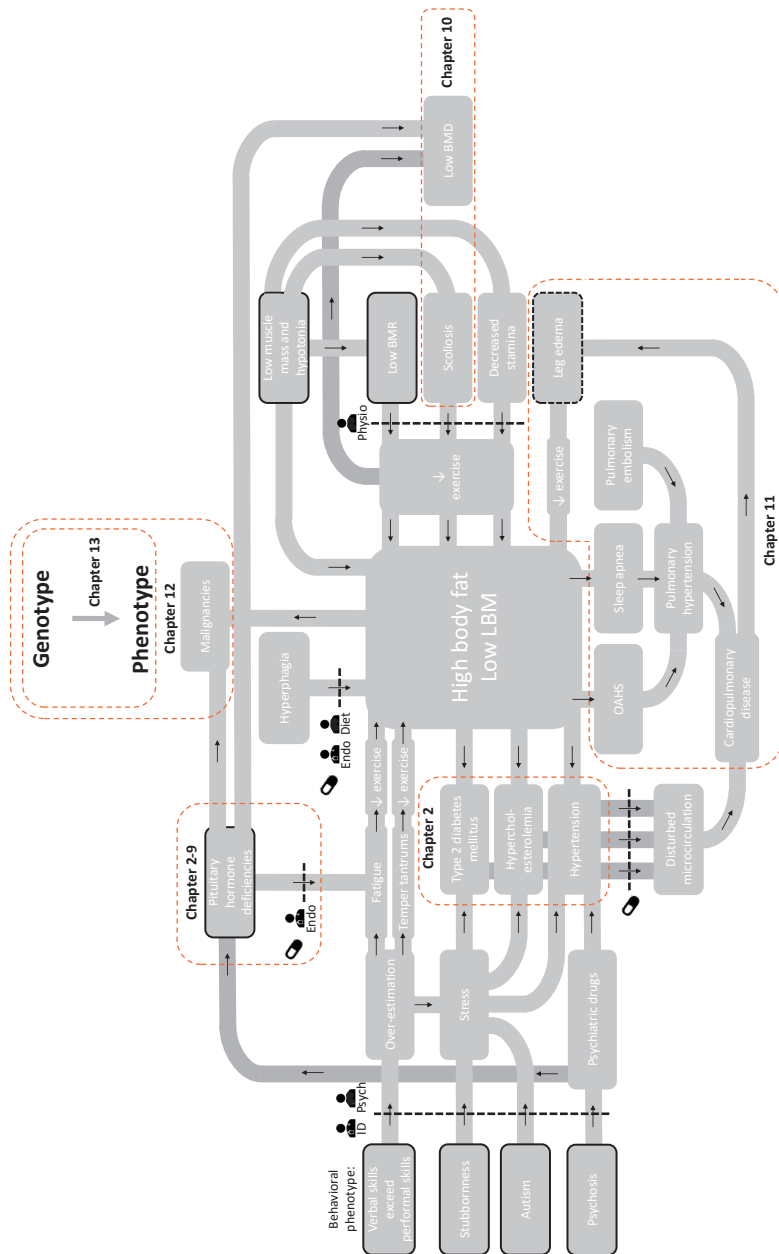
Figure 1. Overview of chapters in this thesis in relation to the complex interplay of health problems in PWS.

Abbreviations: decreased (↓), bone mineral density (BMD), basal metabolic rate (BMR), dietitian (diet), endocrinologist (endo), physician for people with intellectual disabilities (ID), lean body mass (LBM), obesity associated hypoventilation syndrome (OAHS), physiotherapist (physio), Prader-Willi syndrome (PWS), psychologist (psych). Legend: black arrows indicate a cause-and-effect relationship; dotted lines indicate an intervention; the pictogram of medication stands for an intervention with medication; black borders indicate that the factor is inherent to the syndrome; dotted black border indicates that the factor is inherent to the syndrome, but can be aggravated by other factors.

| New patients & yearly controls
Interview
Physical examination
Biochemical measurements | | ▪ | If creatinine > 93 μmol/L (males), > 76 μmol/L (females) **or**
eGFR < 93 ml/min/1.73m² (males), < 98 ml/min/1.73m² (females)
→ perform nephrologic analysis |

| Vitamin D / calcium | | ▪
▪
▪ | Start vitamin D supplementation if vitamin D level < 50 nmol/L
Add calcium supplementation if dairy intake < 3 units per day
Start vitamin D + calcium in case of known osteopenia/osteoporosis |

Start hormone replacement therapy — *if*

Females
- Amenorrhea **or**
- Oligomenorrhea
(do not rely on estradiol levels)

Males
- Sparse body hair **or**
- Underdeveloped genitals **or**
- Daytime sleepiness and testosterone < 10 nmol/L

Perform poly(somno)graphy — *if*
- Daytime sleepiness **or**
- Reported apneas **or**
- BMI > 25 kg/m² **or**
- Hypertension **or**
- Plan to start GH treatment

Perform neuropsychological assessment — *if*
- High blood pressure **or**
- Temper tantrums **or**
- Fatigue / daytime sleepiness

Perform DEXA — *if*
- Hypogonadism **or**
- Fractures **or**
- Corticosteroid use

Perform spine X ray — *if*
- Gibbus deformity at physical examination

If Cobb angle > 10 degrees and:
- Asymptomatic → refer to physiotherapist to prevent progression
- Symptomatic (pain, dyspnea, reduced mobility) → refer to orthopedic surgeon

Perform cardiac evaluation — *if*
- Nycturia **or**
- Progressive pitting edema **or**
- Shortness of breath **or**
- Poor exercise tolerance
(do not rely on NT-proBNP if BMI > 25 kg/m²)

Perform yearly screening
Fasting glucose
LDL-cholesterol
Blood pressure — *if*
- BMI > 25 kg/m² **or**
- Age > 25 year **or**
- GH therapy **or**
- Corticosteroid use

Exclude central adrenal insufficiency by ITT or MTP — *if*
- Apathy **or**
- Fainting **or**
- Observed hypotension during acute infections / stressful events **or**
- Other clinical signs of adrenal insufficiency

Figure 2. General algorithm for a systematic health screening in adults with PWS.
Abbreviations: above (>), below (<), body mass index (BMI), dual energy X-ray absorptiometry (DEXA), follicle-stimulating hormone (FSH), free thyroxin (FT4), growth hormone (GH), insulin tolerance test (ITT), low density lipoprotein (LDL), luteinizing hormone (LH), kilogram (kg), meter (m), metyrapone test (MTP), Prader-Willi syndrome (PWS), sex hormone binding globulin (SHBG).

PITUITARY HORMONE DEFICIENCIES IN PWS

Due to hypothalamic dysfunction, pituitary hormone deficiencies are common in patients with PWS (3). In **Chapters 3 to 9** we focus on pituitary hormones in adults with PWS and provide practical guidelines to prevent underdiagnosis and undertreatment.

Growth hormone

The reported prevalence of growth hormone (GH) deficiency in adults with PWS varies between 0 and 38% (4, 5). However, even in PWS patients without GH deficiency, GH treatment is beneficial to maintain body composition, both in children and in adults (6-15). In **Chapter 3** we show the long-term effect of GH treatment during childhood on health problems during adulthood. However, as all had received GH treatment in a multidisciplinary (MD) setting, we were not able to distinguish the effect of GH treatment from the effect of MD care. The combination of GH treatment and MD care (GHMDc) during childhood was associated with a significantly lower BMI, a lower prevalence of DM2, and less missed diagnoses during adulthood. This remained significant after correction for age. However, as there was limited overlap in age between the group that had received GHMDc and those who did not, these results should be interpreted with caution.

Hypogonadism

As shown in **Chapter 2**, hypogonadism is prevalent in men and women with PWS. Adequate treatment of hypogonadism is needed to avoid osteoporosis, fatigue, and unfavorable effects on body composition and cardiovascular health (16-24). In **Chapter 4 and 5** we discuss this health problem in more detail. **Chapter 4** focusses on hypogonadism in men with PWS. We found a high prevalence (98%) of hypogonadism in our cohort of adult males with PWS, which is in line with previous studies. Hypogonadism can be primary or central in origin. However, as the maturation of the Leydig and Sertoli cells in PWS occurs independently, a combination of both forms may also occur (25). Previous studies have shown variable results regarding the occurrence of these different types of hypogonadism with most studies reporting a high prevalence of central hypogonadism (26-29), one reporting that the majority had primary hypogonadism (30) and only one study that reported a combined form of hypogonadism (25). In our study, hypogonadism was central in origin in 21%, primary in 21% and a combination of hypothalamic and testicular dysfunction in 55%. Like many health problems in PWS, hypogonadism often remained undiagnosed unless systematically screened for. In our population, half of the cases of hypogonadism had been missed until we performed the systematic screening. Untreated hypogonadism was related to a significantly higher BMI and a lower serum hemoglobin. However, treatment of hypogonadism is often suboptimal in men with

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PWS. After being diagnosed with hypogonadism, only 30% of the males in our cohort reached testosterone levels within the normal range. In the other 70%, testosterone treatment could either not be initiated at all (16%), could not be further increased (due to challenging behavior in 32% or unknown reasons in 16%), or the optimizing of the testosterone dosage was still in progress (5%). Additionally, testosterone dose had to be decreased at some point in 38%, due to challenging behavior. After dose reduction, the challenging behavior decreased in 61%. This suggests a causative relation between testosterone replacement therapy and challenging behavior in adults with PWS. This is in contrast with the previous randomized controlled trial by Kido et al. (29), which did not find a difference in aggressive behavior after two years of testosterone replacement therapy. This difference could be related to different treatment regimens, as most patients in our cohort used testosterone gel while the patients in the study by Kido et al. received low-dose testosterone enanthate injections. Additionally, Kido et al. excluded patients based on the degree of behavioral challenges baseline, while we included all adults that visited our outpatient clinic.

Based on our results, the literature review and an expert panel discussion, we defined practical recommendations for the treatment of hypogonadism in males with PWS. Because of the high prevalence of hypogonadism, we recommend measuring serum testosterone and sex hormone binding (SHBG) and assess clinical signs of hypogonadism in all males with PWS during a yearly visit. Different treatment modes can be used: transdermal gel, short-acting injections, and long-acting injections. Regardless of the mode, we recommend starting testosterone replacement therapy at a low dose and to gradually increase the dosage, depending on serum testosterone concentrations and the occurrence of clinical and adverse effects. When unacceptable side effects occur, we recommend decreasing the dosage to the last dose where adverse effects were acceptably mild. Lastly, as non-compliance is frequent, it is important to ask about compliance and discuss reasons for non-compliance. When non-compliance is suspected or confirmed, alternative modes of administration may be used. For the full algorithm see **Figure 3**.

Chapter 5 focusses on hypogonadism in women with PWS. Like in men, hypogonadism is common in females with PWS and is present in 94%. This high prevalence was confirmed by our literature review, with most studies reporting a prevalence above 80%. In females with PWS, hypogonadism is most often central in origin (26-28, 30, 32), although cases with primary hypogonadism (27, 30, 32) and combined (32) forms of hypogonadism have also been reported. We found central hypogonadism in 26% and primary hypogonadism in 4% of females with PWS. However, in 70% of our patients the type of hypogonadism could not be classified due to discrepant LH and FSH values, suggesting a combined form of hypogonadism, like our results in men with PWS (**Chapter 4**).
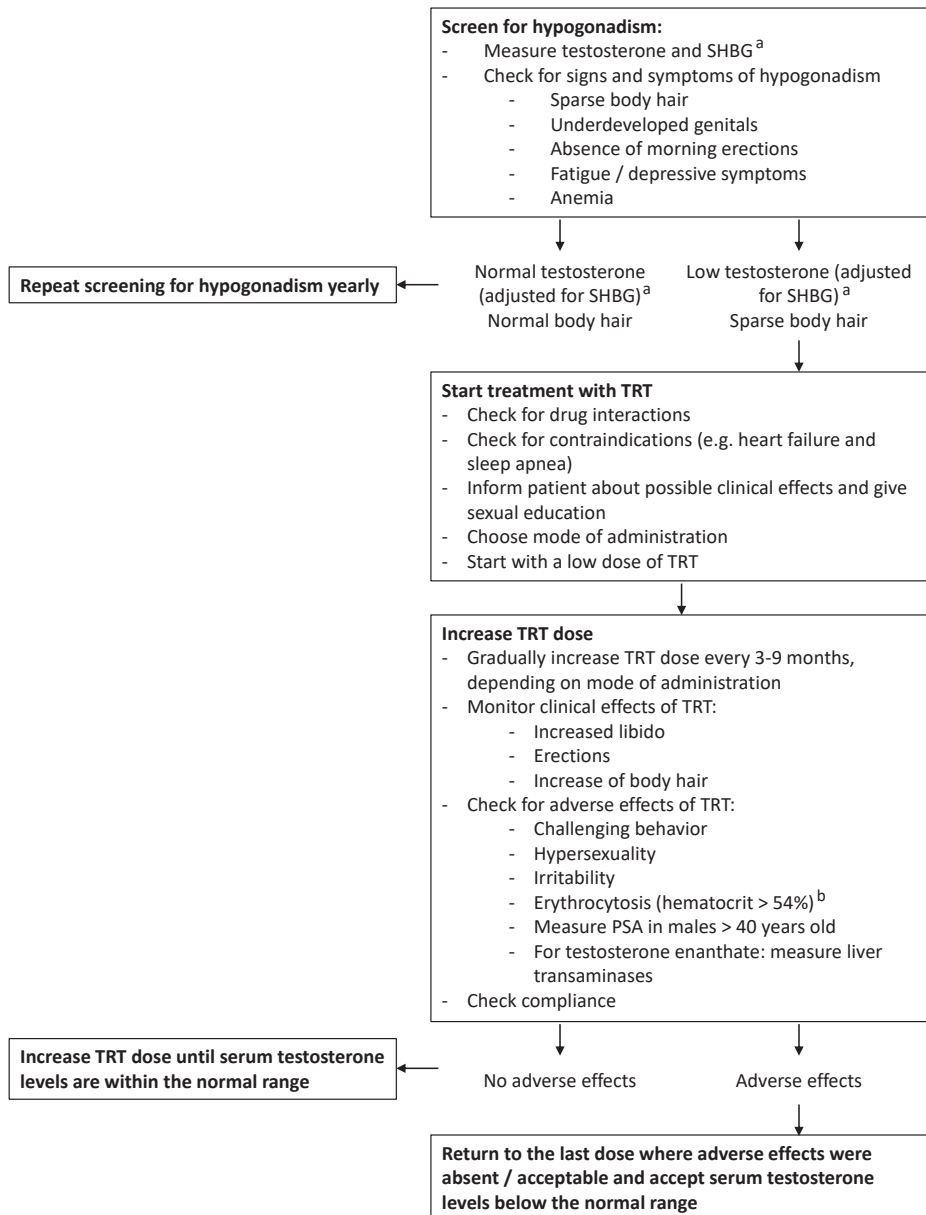
Figure 3. Recommendations for hypogonadism in men with PWS.
Abbreviations: prostate-specific antigen (PSA), sex hormone binding globulin (SHBG), testosterone replacement therapy (TRT). [a] Instead of total testosterone and SHBG, free testosterone can also be measured to diagnose hypogonadism in males with PWS. [b] Based on the Endocrine Society Clinical Practice Guideline for testosterone therapy in men with hypogonadism (31).

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Obesity is associated with disturbances of the menstrual cycle, making it irregular or even absent (33). To investigate the effect of BMI in our cohort of women with PWS, we looked at the relationship between BMI and LH, FSH, estradiol, and SHBG. This showed that only LH was negatively related to BMI. Estradiol was not significantly related to BMI, but this could be the result of a lack of power as estradiol values were only available for the 34 patients who did not receive oral contraceptives (OAC) or hormone replacement therapy (HRT). Additionally, we report that three patients developed a spontaneous menstrual cycle after significant weight loss and that one of them even developed a regular menstrual cycle. This suggests an important role for obesity in the development of hypogonadism in women with PWS. The effect of obesity on hypogonadism may be mediated by changes in fasting insulin, SHBG, and free androgens (34). However, as seven patients with a BMI below 25 kg/m^2 also had hypogonadism, obesity cannot be the only cause of hypogonadism in women with PWS. Remarkably, we found that many women with an absent or irregular menstrual cycle had estradiol levels within the normal range. A possible explanation is increased aromatase activity in adipose tissue, resulting in increased estradiol levels despite hypothalamic, pituitary, or ovarian dysfunction (35, 36).

Another potential cause of hypogonadism is hyperprolactinemia. As described in **Chapter 8**, hyperprolactinemia is present in 22% of patients with PWS and often related to use of medication like antipsychotics. As hyperprolactinemia might also induce hypogonadism, is important to measure prolactin to exclude this as a cause of hypogonadism.

Underdiagnosis was common for hypogonadism, with 34% of women with hypogonadism being undiagnosed before our systematic screening. Of the women that had been previously diagnosed, 23% were still untreated when they presented at our outpatient clinic. We tried to initiate treatment in all women, but 28% remained untreated due to caregivers' fear of hygienic difficulties or due to adverse effects. Indeed, several adverse effects were reported. Of eighteen patients receiving HRT, two reported an increase in challenging behavior and two reported spotting. In the 26 patients that used OAC, adverse effects that were spontaneously reported were spotting (n = 3), hygienic difficulties (n = 2), headache (n = 2), abdominal complaints (n = 2), weight gain (n = 1), hair loss (n = 1), and increase in challenging behavior with psychotic symptoms (n = 2). It is important to prepare patients and caregivers for these potential adverse effects and to instruct the caregivers to be alert to early symptoms of psychosis.

To avoid the negative effects of undiagnosed and untreated female hypogonadism on bone health (37, 38), body composition (39), muscle function (22), psychological well-being (16, 21), and cardiovascular risk (20, 24), we provide a practical algorithm for

the screening and treatment of hypogonadism in women with PWS, see **Figure 4**. We recommend to screen for hypogonadism in all females with PWS and to start treatment when the menstrual cycle is absent or irregular.
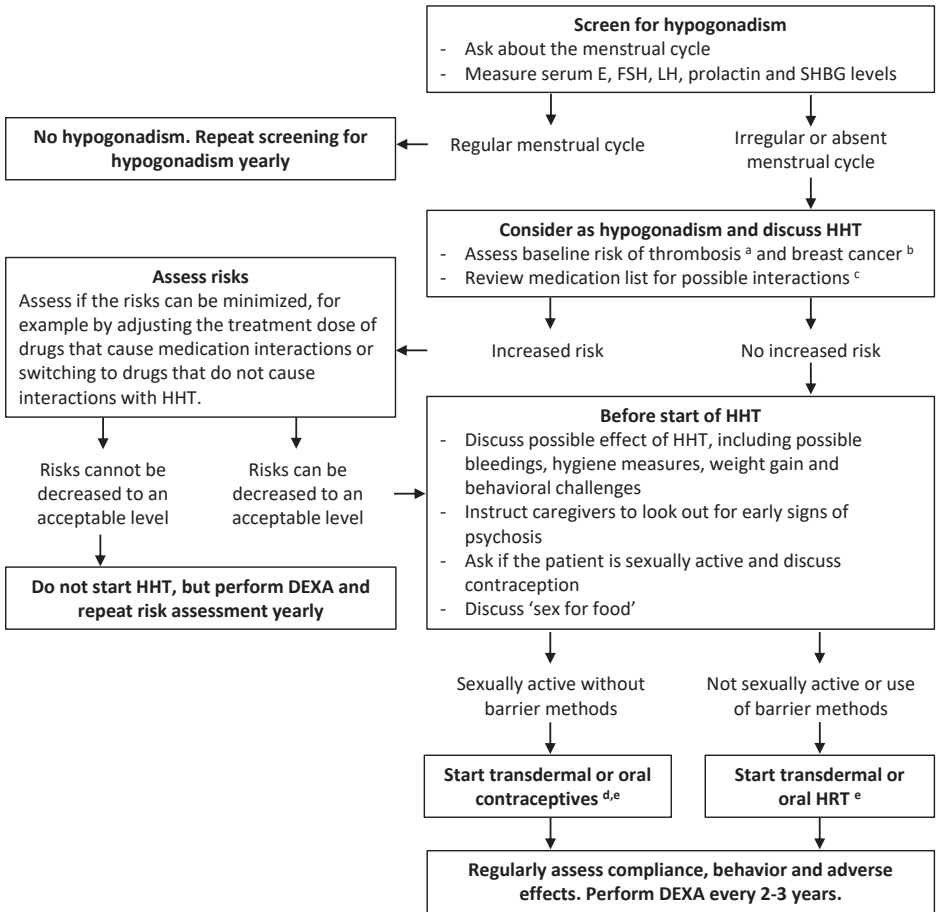
Figure 4. Recommendations for the treatment of hypogonadism in females with PWS, based on the results of the cohort study, a review of the literature, and the expert opinion of an international panel of PWS-experts.

Hypogonadism hormone treatment includes oral contraceptives and hormone replacement therapy. Abbreviations: dual-energy X-ray absorptiometry (DEXA), estradiol (E), follicle stimulating hormone (FSH), hypogonadism hormone therapy (HHT), hormone replacement therapy (HRT), luteinizing hormone (LH), sex hormone binding globulin (SHBG). [a] Relevant risk factors for thrombosis include: previous thrombotic events, increased age, being overweight or obese, smoking, and immobility. [b] Relevant risk factors for breast-cancer include: breast or ovarian cancer in first degree relatives before the age of 50, genetic mutations (e.g., BRCA), being overweight or obese, and alcohol abuse. [c] For example interactions with psychotropic medication and recombinant human growth hormone treatment. [d] Start the combined oral contraceptive pill or transdermal patches containing both estrogen and progestogen. An IUD combined with oral or topical estrogens may be considered. However, general anesthesia or sedation may be necessary as insertion can be traumatic in patients with an intellectual disability; (past) sexual abuse should be excluded first. [e] Transdermal administration is preferred due to the lower risk of thrombosis, however oral preparations could be preferred in patients with skin picking.

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It is often assumed that patients with intellectual disabilities are not sexually active, however, six women in our cohort were in a relation with sexual intercourse. This highlights the importance of adequate contraception in this patient population, which is often but not always infertile. When a patient is sexually active, transdermal or oral contraceptives can be started. When a patient is not sexually active, low dose hormone replacement therapy should be preferred, as this is less thrombogenic (40-42).

During the screening for hypogonadism and the discussion of the sexual history, special attention should be paid to the occurrence of sexual abuse. Shockingly, sexual abuse occurs in one in three adults with an intellectual disability (43). Women with PWS may be especially vulnerable to sexual abuse as they also display hyperphagia, which may lead to 'sex for food' (receiving food in exchange for sexual acts) by caregivers and fellow residents (44). This should be specifically asked for, as patients seldomly mention this spontaneously.

Hypothyroidism

Another prevalent pituitary hormone deficiency in adults with PWS is hypothyroidism. Treatment of hypothyroidism is essential to avoid fatigue, mood impairment, muscle weakness, and negative cardiovascular effects (45, 46). In **Chapter 6**, we assessed thyroid function in our cohort of 122 adults with PWS. Hypothyroidism was present in 17%. This prevalence was slightly higher than that found in most studies in our literature review, of which most reported a prevalence between 0% and 16%. However, two large French studies also found a high prevalence of hypothyroidism of 26% (1, 2). Hypothyroidism can be central or primary in origin. Hypothyroidism was central in origin in 80% and caused by primary thyroid dysfunction in 20%, which was in line with previous studies that also showed that central hypothyroidism was the most frequent.

We explored the relationship between thyroid-stimulating hormone (TSH), free thyroxine (fT4) and triiodothyronine (T3) in patients without hypothyroidism and several other variables. TSH and fT4 did not show any significant relationships with patient characteristics. However, T3 concentrations were higher in patients who received GH treatment and lower in older individuals and in patients who used psychotropic medication. Forty percent of adults received psychotropic drugs, which can influence the synthesis and metabolism of thyroid hormones and may cause altered deiodination of thyroxine (T4) to T3 by stimulating deiodinase activity (47-49). As psychotropic drugs may alter T4 concentrations, we recommend reevaluating the treatment dose of thyroid hormone treatment when psychotropic medications are initiated. Use of GH treatment was also frequent with one third receiving GH treatment. The fact that we found higher T3 values in patients with GH treatment might be explained by increased peripheral conversion

of T4 to T3 caused by GH (50-52). It is currently believed that GH treatment does not induce hypothyroidism but can unmask previously unnoticed hypothyroidism (53-57). Therefore, we recommend measuring TSH and fT4 concentrations three to four months after the start of GH treatment, to detect unmasked hypothyroidism.

Contrary to our findings for hypogonadism, underdiagnosis in hypothyroidism was less common. Of the 21 patients with hypothyroidism, 19 had already been diagnosed before our systematic heath screening. However, we still recommend performing a systematic screening for hypothyroidism in all adults with PWS, as they are especially vulnerable for the negative effects of hypothyroidism. Hypothyroidism can cause symptoms like arthralgia, lethargy, exertion fatigue, shortness of breath, and muscle problems (45, 58). This further decreases the already impaired exercise tolerance in adults with PWS and might aggravate obesity. Additionally, as highlighted in **Chapter 11**, patients with PWS have an increased cardiovascular risk. Hypothyroidism might further increase this risk by causing exercise intolerance, obesity, and direct negative cardiovascular effects (46). Finally, hypothyroidism may further impair cognitive and psychological health in these already vulnerable patients (59).

Diagnosis of hypothyroidism in PWS is complicated because TSH cannot be used as a marker. In non-PWS adults, TSH is often the first step to screen for hypothyroidism. Only when TSH is abnormal, free T4 (fT4) is determined. However, hypothyroidism in adults with PWS is often central in origin. As TSH might be normal in patients with central hypothyroidism, measurement of TSH is not sufficient in adults with PWS, but fT4 should also be measured, see **Figure 5** for the summary of our recommendations.
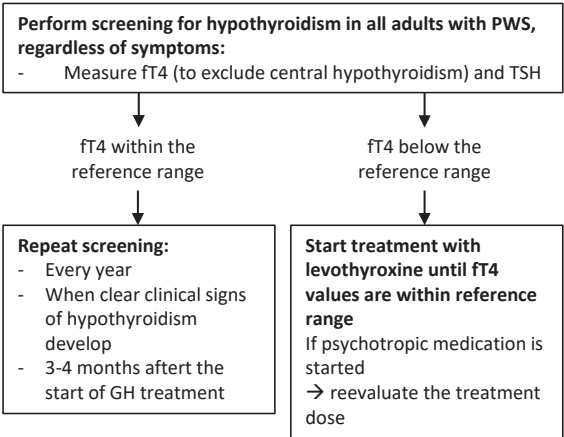
Figure 5. Recommendations for the screening and treatment of hypothyroidism in adults with Prader-Willi syndrome. Abbreviations: free thyroxine (fT4), thyroid stimulating hormone (TSH)

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Central adrenal insufficiency

In **Chapter 7** we describe central adrenal insufficiency (CAI) in a large international cohort of adults with PWS, all treated by PWS-experts of the INfoRMEd-PWS network. Before this study was performed, it was believed that patients with PWS had a high risk of central adrenal insufficiency, because de Lind van Wijngaarden et al. (60) had reported a prevalence of CAI of 60% in children with PWS. Therefore, the standard advice to parents and caregivers of Dutch patients with PWS is to administer hydrocortisone during stressful situations. However, this often leads to frequent administration of hydrocortisone, as illness is hard to define in patients with PWS (due to their high pain threshold and their reduced ability to mount a fever) (3, 61). Additionally, due to the behavioral phenotype of PWS, psychological stress leads to frequent hydrocortisone administration in some patients (3). Frequent administration of hydrocortisone may lead to weight gain, osteoporosis, type 2 diabetes mellitus, and hypertension (62), which are already common health problems in adults with PWS (61).

To minimize the side effects of frequent hydrocortisone use, while avoiding the risks of untreated CAI, we decided to perform a large international study to find the true prevalence of CAI. In our study, we used the insulin tolerance test (ITT) and multiple dose metyrapone test (MTP), the most reliable tests for the diagnosis of CAI.

Of the 82 patients included, only one patient had CAI (1.2%). Thus, CAI is rare in adults with PWS. Additionally, we found that 200 patients had undergone surgery without receiving hydrocortisone, without any signs of CAI or adrenal crisis, further strengthening our conclusion. These findings were in sharp contradiction with the previous study by de Lind van Wijngaarden et al. (60) in children with PWS. This difference could have several explanations. First, in de study by de Lind van Wijngaarden et al. (60) the diagnosis of CAI was based on ACTH levels during MTP, which may lead to false positive results (63). To avoid this, we used 11-deoxycortisol levels during MTP, which is the most reliable marker for CAI in adults. Second, in the study by de Lind van Wijngaarden et al. (60), a single dose MTP was used, which briefly suppresses the hypothalamic-pituitary-adrenal (HPA) axis while we used a multiple-dose MTP, which suppressed the HPA axis for 24 hours and might therefore give the patient more time to produce an adequate response. However, this is an unlikely explanation as Obrynba et al. (64) also used the single dose MTP and found a prevalence of CAI of 0%. Third, there might be a difference in the prevalence of CAI between children and adults due to increased mortality in children with CAI. However, this was not likely based on mortality figures of children with PWS (65). Fourth, the difference could be related to GH treatment. GH deficiency may mask CAI by increasing activity of 11β-hydroxysteroid dehydrogenase type 1, the enzyme that converts cortisone to cortisol (66). In the pediatric study, all patients received GH treat-

ment, while this was only 28% in our study. This may have led to a lower percentage of CAI in our study. However, this is unlikely as GH deficiency was excluded in our patients who underwent ITT and there was no difference in peak cortisol levels between patients with and without GH treatment.

To conclude, we strongly recommend refraining from routine hydrocortisone stress-dose administration in adults with PWS. Only when there is a clinical suspicion of CAI, we recommend performing ITT or MTP to rule out CAI.

Hyperprolactinemia

In **Chapter 8**, we discuss hyperprolactinemia in patients with PWS. Stress and use of psychotropic medication, both risk factors for hyperprolactinemia, are common in PWS (67). If left untreated, hyperprolactinemia may cause hypogonadism, metabolic abnormalities, and increased cardiovascular risk (68-70). However, the prevalence and clinical significance of hyperprolactinemia was previously unknown. Therefore, we investigated hyperprolactinemia in a large international cohort of adults with PWS. To avoid bias when calculating the prevalence of hyperprolactinemia, we only analysed prolactin levels of patients from centers where prolactin was measured routinely. Hyperprolactinemia was present in 22% and was generally mild (80%) or moderate (13%). Only 7% had severe hyperprolactinemia. In patients with hyperprolactinemia, 62% used medication (56% antipsychotics) that might have caused the hyperprolactinemia (71). However, measurements before the start of these drugs were not available and therefore, it was unclear whether these drugs were the (only) cause of the hyperprolactinemia. One patient had severely elevated prolactin values caused by a microprolactinoma. However, prolactinoma seems to be only a rare cause of hyperprolactinemia in PWS, as pituitary imaging was normal in eleven other patients who had undergone radiological examination. In the literature, only one other patient with PWS and a microprolactinoma has been reported (72). Macroprolactin was measured in 17 patients and not present in any of them. Therefore, macroprolactin was probably not the cause of hyperprolactinemia.

Patients with the genetic subtype mUPD had significantly higher prolactin levels compared to patients with a deletion. This is probably explained by the higher risk of psychosis in patients with an mUPD, leading to more frequent use of antipsychotics (73). Similar to the non-PWS population, women had higher prolactin levels than men (74). Clinical symptoms of hyperprolactinemia like galactorrhea and gynecomastia were not observed in our cohort.

Hyperprolactinemia can cause hypogonadism, leading to increased cardiovascular risk and reduced bone mineral density (68-70). Independent of the effect on hypogonadism,

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prolactin itself also has unfavorable metabolic effects as it promotes obesity, metabolic syndrome, and impairment in glucose and lipid metabolism (75). As obesity, cardiovascular problems, and osteoporosis are already frequent among PWS individuals without hyperprolactinemia, further aggravation of these problems by hyperprolactinemia should be avoided. Therefore, we recommend to screen for hyperprolactinemia by measuring prolactin in all patients. As treatment with antipsychotics is a common cause of hyperprolactinemia, we recommend repeating prolactin measurements after the initiation of new antipsychotics. When prolactin levels exceed 100 ng/mL in absence of a known cause (like use of psychotropic medication), we recommend performing pituitary imaging to exclude prolactinoma, as these high levels of prolactin are rare in patients with PWS. In case of repeated moderately increased prolactin levels without a known cause, pituitary imaging should also be considered.

It is important to keep in mind that treatment of hyperprolactinemia with dopamine agonists can trigger or worsen impulse control disorders and psychotic illness (76). Therefore, early symptoms of psychosis should be monitored closely after initiation of dopamine agonists, as patients with PWS already have an increased risk of psychosis.

Hyponatremia

So far, we have investigated and discussed the hormones secreted by the *anterior* pituitary. However, due to hypothalamic dysfunction and frequently used medications, hormones released in the *posterior* pituitary might also be disturbed in patients with PWS. One of them is antidiuretic hormone (ADH), also called vasopressin. ADH plays an important role in the osmotic balance and sodium homeostasis. Increased ADH can cause hyponatremia. In **Chapter 9**, we explore the prevalence and etiology of hyponatremia in individuals with PWS.

Four cases with severe hyponatremia, all requiring admission to the intensive care unit and leading to death in one case, raised the question whether specific measures should be taken to avoid hyponatremia in patients with PWS. However, little was known about hyponatremia in patients with PWS. Therefore, we investigated the prevalence and causes of hyponatremia in an international cohort of patients with PWS.

One of the possible causes of hyponatremia in PWS is excessive fluid intake (EFI). Although the most prominent feature of PWS is hyperphagia, EFI has also been observed. It is important to notice that EFI is often denied by the patients. Psychotropic drugs, often prescribed in PWS individuals, may also cause hyponatremia as they may cause an increase in ADH (syndrome of inappropriate ADH or SIADH). Prescription of desmopressin (a synthetic analogue of ADH) may also cause hyponatremia. Desmopressin is not

seldomly prescribed to treat nycturia, a problem that could be caused by a (missed) underlying heart problem or EFI. Finally, hyponatremia can also be caused by diuretics or CAI.

Of 1326 children and adults with PWS, 34 (2.6%) had at least one episode of mild or moderate hyponatremia (sodium concentration between 125 and 135 mmol/L) and seven (0.5%) had at least one episode of severe hyponatremia (sodium concentration below 125 mmol/L). Two of the patients with mild or moderate hyponatremia were children, but severe hyponatremia only occurred in adults. Possible causes for severe hyponatremia included: use of desmopressin (n = 2), excessive fluid intake (n = 2), treatment with diuretics combined with salt restriction (n = 1), and CAI combined with use of sertraline (n = 1). In one patient, the cause was unknown. Five out of 7 patients with severe hyponatremia had the genetic subtype mUPD, which is remarkable as this is not the most common genetic subtype. The overrepresentation of the genetic subtype mUPD among patients with hyponatremia might be related to the differences in behavioral phenotype between patients with a deletion and mUPD. This may lead to more excessive fluid intake and use of desmopressin for nocturnal enuresis in patients with mUPD (73). All cases of severe hyponatremia resulted in clinical symptoms, ranging from confusion to seizures and coma. In patients with mild or moderate hyponatremia, no symptoms occurred. Possible causes of mild hyponatremia were: use of psychotropic medication (32%), excessive fluid intake (24%), hyperglycemia (12%), diuretics (9%), use of desmopressin (6%), or unknown (29%). As information about urine electrolytes and osmolarity were not available, SIADH could not be excluded in the 'unknown' group. The large number of cases in whom excessive fluid intake seemed to be related to the development of hyponatremia was in accordance with previous studies reporting frequent excessive fluid intake in patients with PWS, leading to water intoxication and hospitalization (77, 78).

To avoid life-threatening complications of severe hyponatremia, we recommend monitoring serum sodium during the routine follow-up of patients with PWS, especially in those who have a history of excessive fluid intake or use of psychotropic drugs. To decrease the risk of excessive fluid intake, patients with PWS and their caregivers should be informed about the dangers of (secretly) drinking large amounts of water. The 'water intoxication alert' published by the PWS association USA could be provided to caregivers of patients with PWS for additional background information. To decrease the risk of hyponatremia caused by desmopressin, the use of this drug should preferably be avoided. If no other treatment options are available, fluid intake should be restricted for at least one hour before and eight hours after administration (79). Lastly, medication that may cause SIADH should be used with caution in patients with PWS.

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Bone health in PWS

Another important endocrine problem in adults with PWS is osteoporosis. Due to hypo-gonadism, hypotonia (3), vitamin D deficiency (80), and low physical activity (81), adults with PWS have an increased risk of osteoporosis and subsequent osteoporotic fractures. In **Chapter 10** we studied bone health in adults with PWS. We assessed osteopenia, osteoporosis, fractures, and risk factors for impaired bone health in our international co-hort of 354 adults with PWS. Osteoporosis (14%) and osteopenia (54%) were prevalent. This was in line with previous studies in our literature review that reported a prevalence of osteoporosis of 2-26%. Only one previous study reported osteopenia in adults with PWS and found a high prevalence of osteopenia of 40% for the lumbar spine and 67% for the total hip (82).

To provide practical recommendations, we assessed modifiable risk factors for osteopo-rosis in adults with PWS. The most frequent were hypogonadism (93% in males and 80% in females), insufficient dairy intake (86%), insufficient physical exercise (40%) and use of corticosteroids (10%). As discussed in **Chapter 4 and 5**, the treatment of hypogonad-ism in both males and females is often complicated by behavioral problems, leading to undertreatment. In line with this observation insufficient treatment of hypogonadism was common in the cohort studied in **Chapter 10**, both among males (25%) and females (20%). Vitamin D deficiency was more common in adults with PWS compared to controls according to our literature review (83, 84) and might therefore also be an important risk factor for osteoporosis in patients with PWS.

When we look at the non-modifiable risk factors, males were significantly more often di-agnosed with osteoporosis than females (P = 0.005), also after correction for age, height, and weight (P <0.001). While this is in contrast with findings in the general population (85), it is in agreement with earlier studies that reported lower BMD Z-scores and T-scores in males than in females with PWS (82, 86). This might be related to the presence of untreated hypogonadism in males and the low age in our population (median 31 years), resulting in a low number of post-menopausal women.

Osteoporosis leads to an increased risk of osteoporotic fractures. Due to the PWS specific behavioral phenotype, including temper tantrums (87), patients with PWS may already have an increased risk of fractures due to mechanical trauma. In our cohort, 10 patients (3%) had previously had at least one vertebral fracture. In six of these patients, these fractures were not caused by mechanical trauma and were therefore likely spontaneous fracture due to low BMD. We also looked at non-vertebral fractures. Fifty-nine (17%) of all patients had suffered at least one non-vertebral fracture. In 51 of them, there had

been an adequate mechanical trauma, but in eight of them the fracture seemed to have occurred spontaneously.

Fourteen patients had multiple fractures, either at the same time or at different points in life. Both vertebral and non-vertebral fractures were significantly associated with osteoporosis.

GH treatment is often prescribed to patients with PWS and has many health benefits. However, GH treatment was not associated with a decreased risk of osteoporosis or fractures after correction for age, sex, height, and weight. Although one study in our literature review reported an increase in BMD in patients who received GH treatment compared to controls (88), most studies did not find a significant association (78, 84, 89, 90). One study even found a decrease in BMD after 12 months of GH treatment (86). Thus, it is unlikely that GH treatment has clinically relevant effects on BMD.

We found a high prevalence of osteoporosis and osteopenia in a cohort of relatively young patients with PWS. To reduce the long-term risk of osteoporosis on fractures, we defined practical recommendations for the prevention, detection, and treatment of osteoporosis in adults with PWS, see **Figure 6**. We recommend performing a dual-energy X-ray absorptiometry (DEXA) in all adults with PWS. DEXA scans should be repeated every five years in patients with a normal BMD and every two years in patients with osteopenia or osteoporosis. As fractures might be missed in patients with PWS due to intellectual disability and a high pain threshold (3), vertebral fracture assessment (VFA) should also be performed to screen for unnoticed osteoporotic vertebral fractures.

One of the preventive measures that should be taken to reduce the risk of osteoporotic fractures is to avoid routine prescription of corticosteroid stress doses in adults with PWS. Prescription of corticosteroid stress doses is still part of standard patient care in some countries. However, as described in **Chapter 7**, CAI is rare in adults with PWS and corticosteroids are not indicated unless CAI is diagnosed by MTP or ITT. We also recommend being aware of the effects of psychotropic medication on BMD. Due to behavioral challenges and psychosis, many patients with PWS use psychotropic drugs (73, 91-93). As highlighted in **Chapter 8** of this thesis, psychotropic medication is a frequent cause of hyperprolactinemia, which is associated with hypogonadism and osteoporosis (94). In the general population, many types of psychotropic medications have been associated with an increased risk of (osteoporotic) fractures (94-99). However, the relation between antipsychotics that do not cause hyperprolactinemia and the risk of osteoporosis remains unclear (100). In spite of the important risks of psychotropic medication for bone

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health, we recommend continuing psychotropic treatment (regardless of BMD), as long as the psychiatrist and/or ID physician believe this is indicated.
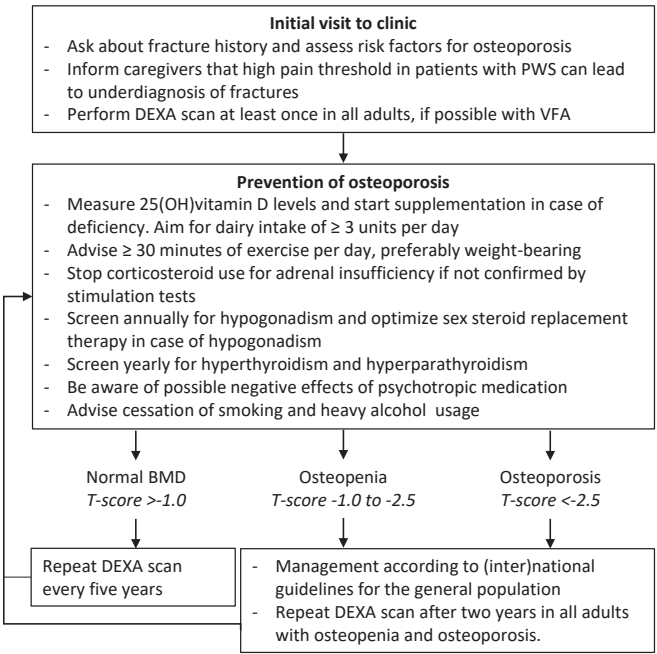
Figure 6. Recommendations for the prevention, detection, and treatment of osteoporosis in adults with PWS. Abbreviations: dual-energy X-ray absorptiometry (DEXA), Prader–Willi syndrome (PWS), vertebral fracture assessment (VFA).

Another frequent bone-related health problem in patients with PWS is scoliosis. As described in **Chapter 10**, scoliosis was present in 263 (80%) patients of our international cohort of 354 adults with PWS. This high prevalence of scoliosis might be related to a combination of hypotonia of (paravertebral) muscles, lack of physical activity, and obesity (101-103). In our literature review we found a highly variable prevalence of scoliosis, ranging from 5% to 100%. However, when only articles that performed a systematic screening for osteoporosis were considered, the prevalence ranged between 47% and 100%.

GH treatment was previously thought to increase scoliosis by increasing growth velocity (104). However, we did not find a relationship between scoliosis and GH treatment. In our literature review we found one study by Sode-Carlsen et al. (105) which found that after two years of GH treatment, 16% of patients receiving GH treatment had an increase of the Cobb angle and 8% had a decrease of the Cobb angle. However, there was no control group and the increase in Cobb angle may very well have reflected the

natural progression of scoliosis in PWS. Other studies in our literature review did not find a relationship between scoliosis and GH treatment (102, 106, 107). Therefore, we conclude that GH treatment is safe regarding the development of scoliosis in individuals with PWS.

Scoliosis can cause back pain and severe scoliosis might even lead to adverse cardio-pulmonary effects (108, 109). To avoid or manage these negative consequences, we designed an algorithm for the screening and treatment of scoliosis, see **Figure 7**. The presence of scoliosis should be assessed yearly during physical examination. In case of doubt or progression or a new gibbus deformity during physical examination, a standing full spine posterior-anterior X-ray should be performed. When the Cobb angle is 10 degrees or more and symptoms of scoliosis are present, an orthopedic surgeon should be consulted. When symptoms are absent, conservative (non-surgical) treatment should be initiated including physiotherapy.

Initial visit to clinic
- Ask for previous diagnosis and/or surgery for scoliosis
- Assess the presence of gibbus malformation

No gibbus deformity *or* stable pre-existent scoliosis

(Mild) gibbus deformity *or* worserning of pre-existent scoliosis

Previous operation

Yearly monitoring

Perform total spine X-ray

Follow-up and management by orthopedic surgeon

No scoliosis
Cobb angle <10°

Scoliosis
Cobb angle ≥ 10°

Assess back complaints or symptoms of scoliosis[a]

Refer to orthopedic surgeon for close monitoring and consider surgical intervention

Present

Absent

Start conservative (non-surgical) treatment
- Monitor back complaints
- Instruct caregivers to contact the clinic if symptoms appear or worsen
- Refer to a physiotherapist for trunk stabilisation exercises, muscle strengthening and/or spinal stretching exercises
- Advise regular hanging from a pull-up bar[b]
- Consider brace therapy in case of pain during specific activities

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Figure 7. Recommendations for the detection, monitoring of progression, and treatment of scoliosis in adults with PWS. Growth hormone treatment can be administered safely in patients with scoliosis without back complaints. Abbreviations: less than (<), more than or equal to (≥). [a] Symptoms of scoliosis include: reduced mobility, breathing difficulties, and change in posture. [b] Based on expert opinion, we recommend regular hanging from a pull-up bar to lengthen the back muscles and relieve scoliosis related symptoms. To our knowledge, the efficacy has not yet been proven in clinical trials.

CARDIOVASCULAR DISEASE

As described in **Chapter 2**, a complex interplay of PWS-related health problems eventually leads to obesity and cardiovascular risk factors like hypertension, hypercholesterolemia, and type 2 diabetes mellitus (DM2). This results in a high risk of cardiovascular (CV) disease. Prevention, diagnosis, and treatment of cardiovascular problems can be extremely challenging in PWS. PWS-specific patient characteristics, comorbidities, and behavioral challenges can lead to ineffective lifestyle interventions, missed or delayed diagnoses and suboptimal treatment. To increase awareness of the diagnostic and therapeutic pitfalls, we describe four patients with severe CV disease in **Chapter 11**.

All patients described were younger than 40 years during their first CV event, indicating that CV disease may already develop at a young age. This high risk of CV problems at a young age, has also been described in previous studies (110-114). However, practical guidelines to prevent or detect CV problems were unavailable.

In the cases we describe, several CV risk factors were present at this young age: obesity (n = 4), DM2 (n = 2), hypertension (n = 2), hypogonadism (n = 3), and sleep apnea (n = 2). To prevent CV disease, screening and early treatment of these risk factors are essential. Additionally, like the general population, a healthy lifestyle is important to improve CV health (**Figure 8**). The latter is often challenging in adults with PWS, due to intellectual disability, the complex behavioral phenotype, and lack of proper supervision.

Figure 8. Recommendations for prevention, diagnosis, and treatment of cardiovascular events in adults with Prader-Willi syndrome.
Abbreviations: 24 hours a day, 7 days a week (24/7), body mass index (BMI), computed tomography (CT), cardiovascular (CV), growth hormone (GH), low-density lipoprotein (LDL), minutes (min), magnetic resonance imaging (MRI), Prader-Willi syndrome (PWS).

Not only the behavioral complexity is an issue in PWS. Also, the pathogenesis may be more multifaceted than in the non-PWS population. In our cases with PWS, pulmonary hypertension played a key role in the pathogenesis of CV disease. Pulmonary hypertension resulted in a dilated right ventricle and dilated vena cava inferior in three patients, which are both signs of increased right hart pressure. However, left ventricle function was usually normal.

When CV disease develops, early detection and treatment can prevent further aggravation and complications. In patients with PWS, the diagnoses of CV disease can be complicated by the high pain threshold (3), the obesity-related false negative NT-proBNP values (115), and the obesity-related impaired diagnostic value of cardiac ultrasound (116). Additionally, the informative value of the physical examination may be poor in PWS. While peripheral edema is an important marker for CV disease in the general population, peripheral edema is present in one third of patients with PWS without known CV disease. Therefore, this is not a reliable marker of CV problems in PWS. One important observation from our case series was that all patients with CV events showed progressive pitting edema shortly before or during their CV event. Therefore, progression of edema should be considered an alarm symptom which requires immediate action. As all cases with CV events presented with orthopnea or progressive dyspnea, shortness of breath should also be seen as an alarm symptom and not just as lack of stamina.

Both the treatment of CV disease and CV risk factors may be complicated by non-adherence. In the patients we describe, insufficient supervision has led to non-adherence to CPAP, food restrictions, exercise instructions, diuretics, and salt and water restriction. In one patient, this non-adherence resulted in the ingestion of a large amount of water and salty food, which eventually led to his death. Continuous supervision is often needed to ensure compliance with medication and adherence to medical advice. Use of CPAP is often problematic but can be improved by stepwise introduction of the mask and consultation of a behavioral expert in case of fear or refusal to use the mask.

We show that the prevention, diagnostic trajectory, and treatment of CV problems in adults with PWS can be extremely challenging. Therefore, we recommend treating these patients in a multidisciplinary team and to collaborate closely with the local team of the residential home where the patient lives.

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MALIGNANCIES

Cancer risk is an important subject of study in PWS as obesity is a well-known risk factor for many types of malignancies (117). Moreover, women with PWS often use estrogen replacement therapy, which has been associated with an increased risk of breast cancer in the general population (118, 119).

We hypothesized that the risk of malignancies might be increased in patients with PWS due to the presence of these risk factors and due to direct genetic effects. However, we found that malignancies were rare in our international cohort of children and adults with PWS (**Chapter 12**), which is reassuring. Of 706 patients, only seven adults (age range 18-55 years old) had ever been diagnosed with a malignancy. Although our study had some limitations (described in **Chapter 12**), we believe our data is sufficient to advise against additional screening in adults with PWS. The fact that all patients had different types of malignancies (acute lymphoblastic leukemia, intracranial hemangiopericytoma, melanoma, stomach adenocarcinoma, biliary cancer, parotid adenocarcinoma, and colon cancer) suggests that the development of malignancies in PWS is multifactorial and that screening for a specific type of cancer is not indicated.

One remarkable finding of our study was the relationship between genetic subtype and cancer risk. To our surprise, all patients with a malignancy had a paternal deletion, compared with only 58% in patients without a malignancy (P = 0.045). We performed a literature review to explain this phenomenon. The literature review showed that several genes on chromosome 15q11.2-q13 are related to malignancies. However, this relationship is complex and therefore it is not possible to give a clear explanation for the relationship between cancer risk and the genetic subtype of PWS.

Although we do not recommend performing any additional screening, we do recommend low-threshold diagnostics (biochemical analysis and imaging) in case of unexplained weight loss, loss of appetite, localizing symptoms and / or symptoms suggestive of paraneoplastic syndrome. Additionally, we recommend participation in national screening programs for breast cancer, prostate cancer, and cervical cancer, as participation rates of national screening programs are often low in patients with an intellectual disability (120-124). Special attention should be paid to the cervical cancer screening. It is often assumed that patients with PWS are not sexually active. However, as we showed in **Chapter 5**, this is not always the case. As cervical cancer screening can be traumatic for some patients with intellectual disabilities, the decision to participate in the cervical cancer screening should be made on a case-by-case basis.

GENOTYPE-PHENOTYPE RELATION IN PWS

In most cases, PWS is caused by the absence of expression of the paternally expressed PWS critical region due to a paternal deletion (70-75%), a uniparental maternal disomy 15 (mUPD, 25-30%), an imprinting center defect (ICD, 1-3%) or a paternal chromosomal translocation (rare) (125, 126). The PWS critical region is located on chromosome 15q11.2-q13 and encompasses several genes, including *MKRN3*, *MAGEL2, NDN, NPAP1, SNURF-SNRPN,* and numerous non-coding RNAs (ncRNAs) including small nucleolar RNAs (*snoRNAs*) (126). However, the exact contribution of each of these genes to the PWS phenotype is still uncertain. In cases with Prader-Willi-like syndrome (PWLS), clinical features of PWS are present without one of the classical genetic detects. These cases may provide unique insight into the genotype-phenotype relation. In **Chapter 13**, we describe a female with PWLS and discuss her genetic results considering the current knowledge about the genotype-phenotype relationship. Our index patient fulfilled the clinical criteria for PWS. While regular diagnostic tests for PWS were negative, additional testing (obesity gene panel analysis) revealed a homozygous missense variant in exon 6 of the *SNRPN* gene (NM_003097.3(*SNRPN*):c.193C>T, p.(Arg65Trp)). According to various variant effect predictor programs, this variant was deleterious. Additionally, single nucleotide polymorphism array (SNP) array showed several large regions of homozygosity (ROH), caused by high-grade consanguinity between the parents. This suggests that other autosomal recessive diagnoses might also have contributed to the phenotype of this patient.

The mother of the patient had the same variant on her paternal (i.e. expressed) chromosome 15 without having PWLS. She was illiterate and had obesity but did not fulfill any other diagnostic criteria for PWS. Incomplete penetrance and / or the additional presence of large ROHs in the index case might explain the phenotypic differences. However, they might also be explained by the fact that the mother had the variant only on the paternal allele, while both alleles were affected in the index case. It is generally assumed that only the paternal allele of chromosome 15q11.2-13 is expressed. However, there is some murine evidence that the maternal allele can also be expressed (127, 128). Thus, it is possible that the maternal allele may have contributed to the phenotype of the patient. Interestingly, most other family members of the index case were short, overweight, and/or illiterate, which is suggestive of a genetic component. Unfortunately, the other family members were not available for genetic analysis.

Functional analysis of the variant showed that, while overexpression of *SNRPN-WT* resulted in a small, non-significant reduction in neural development *in vitro*, overexpression of the *SNRPN-p.Arg65Trp* variant significantly reduced neural maturation, resulting

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in a decreased arborization and neurite length. Although future studies are needed to confirm the functional impact, these results might indicate a gain of function, rather than a loss of function, of *SNRPN-p.Arg65Trp*.

To explain our results, we performed a review of previously reported cases with PWLS with abnormalities on chromosome 15q11.2-13. As several cases with PWLS and lack of expression of *SNORD116* have been described, *SNORD116* might be a strong contributor to the PWS phenotype, (129-142). In accordance with the results in humans, mice with *SNORD116* deletions show cognitive deficits and abnormal growth and feeding (143, 144). Although alterations in *SNURF-SNRPN* might hypothetically cause a PWS pheno-type through a change of expression of *SNORD116* (145, 146), *SNORD116* expression was normal in our patient. Mice with paternal deletions of portions of *SNURF* or *SNRPN* seem to have a normal phenotype (147, 148), while humans with isolated deletions of *SNURF* or *SNRPN* have not been reported. Thus, we are the first to report a patient with PWLS without alterations in *SNORD116*, but with a homozygous alteration of *SNRPN*.

Apart from the diagnostic journey, this case shows us the importance of improving healthcare for individuals with rare genetic syndromes. Although the dysmorphisms and intellectual disability clearly showed that this patient had a genetic syndrome, genetic analysis and multidisciplinary care had only been initiated at the age of 46 years. By that time, the patient already had several undiagnosed and untreated comorbidities.

UNDERDIAGNOSIS AND UNDERTREATMENT

One of the recurring problems in this thesis is underdiagnosis and undertreatment. Patients with PWS are vulnerable to underdiagnosis for several reasons. First, due to intellectual disability and the PWS-specific behavioral phenotype, patients are often un-able to express what they feel. This means that the presentation of health problems may be atypical. Second, patients with PWS tend to have a high pain threshold, a disturbed temperature regulation, and are usually unable to vomit (149-152). Third, there is a com-plex interplay between psychological and somatic problems in PWS. This makes it hard for physicians to recognize the symptoms of individual health problems. Fourth, due to the rarity of the syndrome (153), physicians are often unfamiliar with the syndrome and its comorbidities, which may lead to doctors' delay. Fifth, many PWS-associated health problems cause symptoms that are already part of the syndrome itself, like weight gain and leg edema (3). This can lead to underdiagnosis, as physicians may see the symptom as 'just part of the syndrome' and therefore refrain from additional diagnostics.

Thus, while some aspects of the syndrome are well-known, these 'visible' health problems are only the tip of the iceberg. In this thesis we shaped the rest of the iceberg, highlighting the unknown health problems that are often missed (**Figure 9**). We showed that a systematic health screening can prevent underdiagnosis and undertreatment of commonly missed health problems like hypogonadism, hypothyroidism, hyperprolactinemia, hyponatremia, scoliosis, osteoporosis, hypertension, hypercholesterolemia, type 2 diabetes mellitus, and cardiovascular disease. We provide practical recommendations to prevent, detect, and treat these health problems. Following these recommendations will prevent underdiagnosis, undertreatment, and long-term complications. This will reduce patient burden and healthcare costs, improving quality of healthcare for this vulnerable patient population.

Cognitive impairment

Behavioral challenges

Psychosis

Poor exercise tolerance

Hyperphagia

Obesity

(Chapter 4 and 5) Hypogonadism

(Chapter 6) Hypothyroidism

(Chapter 8) Hyperprolactinemia

(Chapter 9) Hyponatremia

(Chapter 10) Osteoporosis

Hypertension (Chapter 2)

Hypercholesterolemia (Chapter 2)

Type 2 Diabetes Mellitus (Chapter 2)

Scoliosis (Chapter 10)

Cardiovascular disease (Chapter 11)

Figure 9. Iceberg of undiagnosed health problems in PWS. Above the water health problems that were already well known. Below the water important health problems that we identified in this thesis. Image adapted from StockVector, ID 1153638118, Shutterstock, kmsdesen.

CONCLUSIONS, FUTURE PERSPECTIVES, AND NEW RESEARCH QUESTIONS

The studies presented in this thesis fill a major gap in knowledge about the health problems that occur in adults with PWS. Our clinical research has resulted in practical tools that will improve healthcare for this vulnerable and complex patient population. Our genetic studies provide new insight into the genotype-phenotype relationship. However, this research has also led to new research questions.

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Genotype-phenotype

In this thesis we shed new light on the genotype-phenotype relationship in PWS. However, uncertainties regarding this genotype-phenotype relation remain. Case reports of affected and non-affected individuals with abnormalities in the PWS critical region are needed to gain more insight into the genotype-phenotype correlation. Apart from clinical cases, mouse models will help us understand the role of the different genes in the PWS region in the development of the PWS-phenotype. Eventually this might lead to new therapeutic approaches, such as gene therapy.

Growth hormone treatment

Growth hormone treatment has many beneficial effects and has drastically improved the health of patients with PWS. However, robust research into the effects of GH on quality of life of adults with PWS is lacking. Also, more studies about the optimal treatment dose are needed.

Growth hormone treatment dosage is usually based on total IGF-1 values. However, new evidence suggests that IGF-1 might be less reliable in patients with PWS (154, 155). New trials focusing on the optimization of growth hormone dosage are needed to further improve healthcare for patients with PWS. Especially the use of assays measuring bioavailable IGF might prevent the negative consequences of under- and overdosage of GH treatment.

Coronary sclerosis

Patients with PWS have a high cardiovascular risk. In this thesis, we describe one patient that had already developed coronary sclerosis by the age of 39. As patients with PWS have a high pain threshold, they do not report chest pain and coronary sclerosis may remain undetected. Future imaging studies into the prevalence of coronary sclerosis is needed to determine whether screening and early intervention is indicated to prevent myocardial damage.

Malignancies

Our research shows that malignancies are rare in patients with PWS. However, few patients in our cohort were older than 50 years old. As life expectancy of patients with PWS is increasing, it is likely that the number of older adults with PWS will increase. Therefore, this study should be repeated when larger cohorts of older patients with PWS become available. Also, to avoid survival bias, longitudinal follow-up is needed.

Kidney function

In this thesis, we showed that creatinine levels are often low in adults with PWS, due to low muscle mass. Therefore, 'normal' creatinine levels that fall within the non-PWS reference ranges, are actually too high for their low muscle mass. As a result, impaired kidney function may remain undetected in adults with PWS. However, due to their impaired microvascular function and the high prevalence of hypertension and type 2 diabetes mellitus, patients with PWS may be at risk to develop kidney failure (156). Studies investigating kidney function and albuminuria are needed to investigate this health problem and provide guidelines to avoid underdiagnoses.

Accelerated aging

We describe the presence of cardiovascular disease, an age-related disorder, at an exceptionally young age in PWS. Apart from cardiovascular aging, our clinical impression is that adults with PWS show premature aging in general. This is in line with research showing accelerated brain aging in PWS (157). The exact mechanism behind this accelerated aging is still largely unknown. Future research should focus on the etiology of accelerated aging process on a cellular level. Quantifying clinical aging parameters and relating geriatric assessment scores to cellular processes will help us understand premature aging in PWS. This, in turn, will aid in the development of future strategies to slow down (premature) aging.

Insulin sensitivity and adipose tissue

Research into rare disorders like PWS can help us understand common health problems in the general population. In particular, research in patients with PWS may help us uncover new molecular mechanisms involved in insulin sensitivity and fat metabolism. Patients with PWS are more insulin sensitive and have less lipid profile disorders than one might expect based on their degree of obesity. Thus, patients with PWS are relatively protected against the negative metabolic effects of obesity. The exact mechanisms behind this protection remains unclear (158). Detailed analysis of blood and adipose tissue of patients with PWS is needed to understand the underlying mechanisms. This may lead to new treatment strategies for insulin resistance and metabolic syndrome in the future.

Taken together, remarkable clinical, biochemical, and cellular findings in patients with PWS may help us understand and improve common health problems in the general population, like aging and metabolic syndrome.

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Systematic screening and multidisciplinary care

In this thesis we showed that PWS is a complex genetic syndrome that affects multiple organ systems. As a consequence, PWS can cause severe medical, psychological and so-

cial problems. However, the detection and treatment of these health problems is often complicated. Comorbidities may remain undetected due to the PWS-specific behavioral phenotype and physicians' unfamiliarity with this rare and complex syndrome. Moreover, adherence to treatment is often poor, due to intellectual disability and challenging behavior. To minimize underdiagnosis and undertreatment, we provide recommendations for a systematic approach to detect and treat health problems. Besides treatment, special attention should be paid to prevention. For example, weight management and treatment of cardiovascular risk factors should be part of the standard clinical care for all adults with PWS. Lastly, psychological and social consequences of the syndrome should also be taken care of. To accurately address all aspects of the syndrome, the treatment of adults with PWS should ideally be performed by a multidisciplinary (MD) team. This team should include an endocrinologist, a dietitian, a physiotherapist, a behavioral expert or psychologist, and a physician for intellectual disabilities (ID physician) or psychiatrist. Other specialists, such as the cardiologist, urologist, gynecologist, neurologist and / or the orthopedic surgeon should be consulted if necessary. It is preferable to gather a stable team of dedicated healthcare professionals, in order to build knowledge and experience on the syndrome.

If all adults with PWS are systematically screened for health problems and treated in a MD setting, we believe that many complications can be prevented and that the quality of life of these vulnerable patients can be improved. Therefore, in 2015, the Center for adults with Rare Genetic Syndromes was launched at the Erasmus University Medical Center. This specialized, multidisciplinary center offers a systematic health screening and multidisciplinary treatment, provided by an experienced team specialized in rare genetic syndromes. To date, around 1100 adults with rare genetic syndromes are treated in our center. In the long term, health outcomes like quality of life and mortality will be analyzed in order to investigate the long term benefits of a systematic health screening and MD care. This will help us to further improve healthcare for adults with rare genetic syndromes.

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SUMMARY

This thesis focusses on improving detection and treatment of health problems in adults with Prader-Willi syndrome (PWS). Based on the results of our national and international cohort studies, we present practical recommendations with the aim to improve health-care for adults with PWS. By showing the diagnostic journey of a patient with a rare new genetic variant, we also provided new insight into the genotype-phenotype relationship. We will now summarize our findings.

Chapter 1 provides a general introduction on PWS and the pituitary hormone axes studied in this thesis.

Chapter 2 gives a general overview of health problems in adults with PWS. Health problems that were common in our cohort of adults with PWS were: hypogonadism (100% in males and 93% in females), scoliosis (74%), vitamin D deficiency (78%), hypercholesterolemia (19%), hypertension (18%), type 2 diabetes mellitus (DM2, 17%), and hypothyroidism (17%). Moreover, we showed that many health problems remained undetected unless systematically screened for. To avoid the long-term complications of undetected and thus untreated comorbidities, we recommend performing a systematic health screening in all adults with PWS.

In **Chapter 3**, we describe the effect of multidisciplinary (MD) care and growth hormone (GH) treatment during childhood on health outcomes during adulthood. The combination of GH treatment and MD care was associated with lower body mass index (BMI), lower prevalence of DM2, and less undiagnosed health problems. This association remained significant after correction for age. Based on these results, we recommend providing GH treatment (unless contra-indications for GH are present) and MD care to all children and adults with PWS.

Chapter 4 focusses on hypogonadism in men with PWS. Almost all men with PWS in our cohort had hypogonadism (98%). This high prevalence was confirmed by our literature review. We found central hypogonadism in 21%, primary hypogonadism in 21%, and a combination of central and primary hypogonadism in 55%. Half of men with hypogonadism remained undiagnosed unless systematically screened for. Untreated hypogonadism was related to a significantly higher BMI and a lower serum hemoglobin. Treatment of hypogonadism with testosterone replacement therapy (TRT) was complicated by challenging behavior, resulting in inadequate testosterone levels. Based on our results, the literature review, and an expert panel discussion, we recommend performing a yearly screening for hypogonadism in all men with PWS. To avoid behavioral problems, we

recommend starting TRT at a low dose and increase the dosage gradually, depending on serum testosterone concentrations and the clinical (beneficial and adverse) effects. Lastly, we showed that non-compliance is frequent. It is important to ask about compliance and discuss reasons for non-compliance.

In **Chapter 5** we describe hypogonadism in women with PWS. The prevalence of hypogonadism in females was 94%. In our literature review we also found a high prevalence of hypogonadism in females with PWS, with most studies reporting a prevalence of 80% or higher. Central hypogonadism was the most common form of hypogonadism (26%) and primary hypogonadism was found in 4%. However, in 70% of our patients the type of hypogonadism could not be classified due to discrepant luteinizing hormone (LH) and follicle-stimulating hormone (FSH) values, suggesting a combined form of hypogonadism. Before our systematic screening, hypogonadism was undiagnosed in 34%. Therefore, we recommend systematic screening for hypogonadism in all females with PWS and to initiate treatment for hypogonadism in all females without a regular menstrual cycle. When a patient is sexually active, contraceptives should be started in transdermal or oral form. When a patient is not sexually active, low dose hormone replacement therapy is preferred. However, adverse effects are common. In our cohort, two patients reported an increase in challenging behavior with psychotic symptoms after the start of oral contraceptives. Therefore, it is important to prepare patients and caregivers for these potential adverse effects and to instruct the caregivers to be alert to early symptoms of psychosis.

Chapter 6 focusses on thyroid function in adults with PWS. Hypothyroidism was present in 17%, compared to only 3% in non-PWS adults. Most patients had central hypothyroidism (80%), although primary hypothyroidism was also present (20%). Additionally, we showed that triiodothyronine (T3) concentrations were higher in patients who received GH treatment and were lower older individuals and in patients who used psychotropic medication. Based on our results, we recommend measuring thyroid-stimulating hormone (TSH) and free thyroxine (fT4) in all adults with PWS. Moreover, as psychotropic medication can influence thyroid hormone metabolism, we recommend reevaluating the treatment dose of thyroid hormone treatment when psychotropic medications are initiated. Lastly, as GH treatment might unmask hypothyroidism, we recommend measuring TSH and fT4 concentrations three to four months after the start of GH treatment.

In **Chapter 7** we show that, contrary to what was previously believed, central adrenal insufficiency (CAI) is rare in adults with PWS. In 81 patients who underwent dynamic testing of the pituitary-adrenal axis, only one had (borderline) CAI. Of 200 patients with PWS who underwent surgery without receiving hydrocortisone, none showed any signs

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of CAI or adrenal crisis. Based on these results, we strongly recommend refraining from routine hydrocortisone stress-dose administration in adults with PWS. Only when there is a clinical suspicion of CAI, we recommend performing an insulin tolerance test or metyrapone test to rule out CAI.

In **Chapter 8** we show that hyperprolactinemia was present in 22% of adults with PWS. Hyperprolactinemia was often mild (80%) or moderate (13%), and only severe in 7%. Hyperprolactinemia was often related to use of medication (62%), in particular antipsychotics (56%). The genetic subtype maternal uniparental disomy (mUPD) was associated with higher prolactin levels compared to patients with a deletion. This is probably related to the higher prevalence of psychosis in mUPD carriers, leading to more frequent use of antipsychotics. No clinical effects of hyperprolactinemia were observed. Based on our results, we recommend to screen for hyperprolactinemia by measuring prolactin in all patients. As treatment with antipsychotics is a common cause of hyperprolactinemia, we recommend repeating prolactin measurements after the initiation of new antipsychotics.

In **Chapter 9**, we discuss hyponatremia in PWS. Of our cohort, 2.6% had at least one episode of mild or moderate hyponatremia and 0.5% had at least one episode of severe hyponatremia. Of 36 cases with hyponatremia, only 2 were children, who both had mild hyponatremia. Adults tended to have more severe hyponatremia, resulting in clinical symptoms ranging from confusion to seizures and coma. Possible causes for severe hyponatremia included the use of desmopressin, excessive fluid intake, and treatment with diuretics combined with salt restriction. In patients with mild or moderate hyponatremia, no symptoms occurred. Possible causes were: use of psychotropic medication that is known to cause syndrome of inappropriate antidiuretic hormone secretion (SIADH) (32%), excessive fluid intake (24%), hyperglycemia (12%), diuretics (9%), desmopressin (6%) or unknown (29%). To avoid life-threatening complications of severe hyponatremia, we recommend monitoring serum sodium in patients with PWS, especially in those who have a history of excessive fluid intake or use psychotropic medication. Additionally, physicians should inform caregivers about the dangers of (secretly) drinking large amounts of water. Treatment with desmopressin or medication that may cause SIADH should be used with extra caution in patients with PWS.

Chapter 10 focusses on bone health in adults with PWS. Osteoporosis was present in 14% and osteopenia in 54%. Prevalent modifiable risk factors for osteoporosis were: hypogonadism (93% in males, 80% in females), insufficient dairy intake (86%), insufficient physical exercise (40%), and use of corticosteroids (10%). Additionally, vitamin D deficiency was more common in adults with PWS compared to controls according to

our literature review. Three percent of our cohort had previously suffered at least one vertebral fracture. In six, the vertebral fracture occurred without adequate mechanical trauma. Moreover, 17% of our cohort had previously suffered a non-vertebral fracture, often after mechanical trauma. GH treatment was not related to osteoporosis, osteopenia, or fractures. Based on these results, we recommend taking measures to prevent osteoporosis and to screen for osteoporosis by dual-energy X-ray absorptiometry scan in all adults with PWS. As scoliosis was also prevalent (80%), we recommend to screen for scoliosis by yearly assessment of gibbus deformity during physical examination.

In **Chapter 11**, we describe four patients with severe cardiovascular (CV) disease, who were all younger than 40 years old. Pulmonary hypertension played a key role in the development of CV disease in these patients. Several risk factors for CV disease were present: obesity (n = 4), DM2 (n = 2), hypogonadism (n = 3), hypertension (n = 2), and sleep apnea (n = 2). Screening and early treatment of these risk factors are essential to prevent CV disease. Once CV disease develops, the diagnostic process can be challenging due to high pain threshold, (obesity-related) false negative NT-proBNP values and (obesity-related) impaired diagnostic value of cardiac ultrasound. In addition, edema is not a reliable marker of CV problems in PWS as it is present in one third of patients with PWS without known CV disease. However, all four patients with CV events showed progressive pitting edema shortly before or during their CV event. Therefore, progression of edema should be considered an alarm symptom which requires immediate action. Moreover, as all cases with CV events presented with orthopnea or progressive dyspnea, shortness of breath should also be seen as an alarm symptom. When CV disease is diagnosed, treatment could be complicated by non-adherence due to intellectual disability. Therefore, competent supervision is essential in the treatment of CV disease in adults with PWS.

Chapter 12 shows that malignancies are rare in children and adults with PWS. Seven patients in our cohort of 706 patients with PWS had ever been diagnosed with a malignancy. All had different types of malignancies, which indicates that the development of malignancies in PWS is multifactorial and that screening for a specific type of cancer is not indicated. However, as obesity is often present and significantly increases cancer risk, we do recommend participation of adults with PWS in national screening programs. To our surprise, all patients with a malignancy had deletion as the genetic subtype, which was significantly more than in the patients without a malignancy. We performed a literature review to investigate the relationship between the genes on chromosome 15q11-13 and malignancies. However, this relationship is complex and therefore it is not possible to give a clear explanation for the relationship between cancer risk and the genetic subtype of PWS.

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In **Chapter 13** we present a patient with a Prader-Willi-like syndrome (PWLS) phenotype and a unique homozygous variant in the *SNRPN* gene, *SNRPN-p.Arg65Trp*. According to our literature review, *SNORD116* seemed the most important contributor to the PWS phenotype. However, we show that, based on genetic and functional testing, a homozygous variant of *SNRPN* may have contributed to the PWLS phenotype in our index case.

Finally in **Chapter 14**, the results of the studies presented in this thesis are discussed to the background of past and current scientific and societal developments. This discussion is followed by recommendations for future research.

SAMENVATTING

Dit proefschrift beschrijft ons onderzoek naar gezondheidsproblemen bij volwassenen met Prader-Willi syndroom (PWS).

De medische zorg voor volwassenen met PWS laat veel te wensen over. Hoewel er veel onderzoek is gedaan naar kinderen met PWS, is er weinig bekend over de gezondheidsproblemen die op volwassen leeftijd optreden. Zo weten we niet wat de beste aanpak is voor het opsporen en behandelen van deze gezondheidsproblemen. Hierdoor gaat er veel mis bij volwassenen met PWS. Aan de ene kant worden belangrijke gezondheidsproblemen over het hoofd gezien, aan de andere kant worden onnodige medische onderzoeken en behandelingen gestart. Dit brengt onnodig leed en onnodige zorgkosten met zich mee.

Op basis van de resultaten van onze nationale en internationale cohortstudies, hebben we praktische aanbevelingen geformuleerd om de medische zorg voor volwassenen met PWS te verbeteren. Ook laten we het diagnostische traject zien van een patiënt met een zeldzame, nieuwe genetische variant om nieuwe inzichten in de genotype-fenotype relatie van PWS te geven. We zullen hieronder onze bevindingen samenvatten.

In **Hoofdstuk 1** geven we een algemene introductie over PWS en de hormoon-assen die bestudeerd worden in dit proefschrift.

In **Hoofdstuk 2** geven we een overzicht van de gezondheidsproblemen die voorkomen bij volwassenen met PWS. De meest voorkomende gezondheidsproblemen in ons cohort waren: hypogonadisme (100% bij mannen en 93% bij vrouwen), scoliose (74%), vitamine D-deficiëntie (78%), hypercholesterolemie (19%), hypertensie (18%), type 2 diabetes mellitus (DM2, 17%) en hypothyreoïdie (17%). We laten ook zien dat veel gezondheidsproblemen worden gemist als er geen systematische screening wordt verricht. Om de lange termijn complicaties van onderdiagnostiek en onderbehandeling te voorkomen, raden we aan om een systematische gezondheidsscreening uit te voeren in alle volwassenen met PWS.

In **Hoofdstuk 3** beschrijven we het effect van multidisciplinaire (MD) zorg en groeihormoon (GH) behandeling in de kinderjaren op gezondheidsuitkomsten bij volwassenen met PWS. De combinatie van GH-behandeling en MD zorg leidde tot een significant lagere body mass index (BMI), minder DM2 en minder onderdiagnostiek van gezondheidsproblemen, ook na correctie voor leeftijd. Daarom raden wij GH-behandeling en MD zorg aan voor alle kinderen en volwassenen met PWS.

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In **Hoofdstuk 4** focussen we op hypogonadisme bij mannen met PWS. 98% van de mannen in ons cohort had hypogonadisme, wat overeenkwam met eerdere studies. 21% had centraal hypogonadisme, 21% primair hypogonadisme en 55% een combinatie van beide. Zonder systematische gezondheidsscreening had de helft van de mannen met PWS in ons cohort niet-onderkend hypogonadisme. De behandeling van hypogonadisme met testosteron suppletie werd vaak bemoeilijkt door het optreden van moeilijk verstaanbaar gedrag (MVG, voorheen 'gedragsproblemen' genoemd), waardoor de optimale testosteron dosering niet werd behaald. On-onderkend en onbehandeld hypogonadisme was geassocieerd met een hogere BMI en een lager serum hemoglobine. Op basis van deze resultaten adviseren we om jaarlijks te screenen voor hypogonadisme in alle mannen met PWS. Om MVG te voorkomen, raden we aan om te beginnen met een lage dosis testosteron suppletie en deze geleidelijk op te hogen, afhankelijk van de serum testosteron concentratie, klinische effecten en bijwerkingen. Tenslotte laten we zien dat therapieontrouw frequent voorkomt. Het is daarom belangrijk om therapietrouw en redenen voor therapieontrouw bespreekbaar te maken.

In **Hoofdstuk 5** laten we zien dat hypogonadisme ook vaak voorkomt bij vrouwen met PWS (94%). Ons literatuuroverzicht laat zien dat de meeste eerdere studies ook een prevalentie van minstens 80% vonden. De meest voorkomende vorm was centraal hypogonadisme (26%), maar primair hypogonadisme kwam ook voor (4%). In 70% kon er op basis van waardes van luteïniserend hormoon (LH) en follikelstimulerend hormoon (FSH) geen onderscheid worden gemaakt tussen centraal en perifeer hypogonadisme. Dit suggereert dat er ook een gecombineerde vorm van hypogonadisme voorkomt. Zonder systematische gezondheidsscreening bleef bij 34% van de vrouwen met PWS het hypogonadisme onopgemerkt. Daarom raden we aan om in alle vrouwen met PWS te screenen op hypogonadisme en om behandeling te starten bij vrouwen die geen reguliere menstruatiecyclus hebben. Als een vrouw met PWS seksueel actief is, heeft het de voorkeur om te starten met transdermale of orale anticonceptie. Als een vrouw niet seksueel actief is, heeft een lagere dosis hormoon substitutie) de voorkeur, omdat het minder risico geeft op trombose. Veel vrouwen gaven aan bijwerkingen te hebben. Na start van de orale anticonceptie kregen twee patiënten een toename in MVG met zelfs psychotische symptomen. Daarom is het belangrijk om patiënten en ouders/verzorgers in te lichten over deze bijwerkingen en om de verzorgers opmerkzaam te maken op de vroege tekenen van psychose.

In **Hoofdstuk 6** gaan we in op de werking van de schildklier bij volwassenen met PWS. Hypothyreoïdie kwam voor bij 17%, tegenover 3% in de niet-PWS populatie. Bij 80% was de hypothyreoïdie centraal, bij 20% primair. We lieten ook zien dat triiodothyronine (T3) concentraties hoger waren bij patiënten die GH-behandeling kregen en lager waren bij

oudere mensen en bij mensen die psychofarmaca gebruikten. We adviseren om jaarlijks thyroid-stimulerend hormoon (TSH) en vrij thyroxine (T4) te bepalen bij alle volwassenen met PWS, om hypothyreoïdie tijdig op te sporen. Bovendien raden we aan om de dosering levothyroxine te heroverwegen wanneer er wordt gestart met psychofarmaca, omdat psychofarmaca het schildkliermetabolisme kunnen beïnvloeden. Tenslotte raden we aan om drie tot vier maanden na de start van GH-behandeling opnieuw TSH en vrij T4 te meten, omdat GH-behandeling een latente hypothyroïdie kan ontmaskeren.

In **Hoofdstuk 7** laten we zien dat, in tegenstelling tot wat eerder werd geloofd, centrale bijnierschorsinsufficiëntie (BNI) zeldzaam is bij volwassenen met PWS (1.2%). Ook laten we zien dat tweehonderd patiënten, die operaties ondergingen zonder toediening van hydrocortison, geen enkel klinisch teken van BNI vertoonden. Het op grote schaal voorschrijven van hydrocortison stress schema's, zoals nu in sommige landen gebeurt, is dus niet nodig. Sterker nog, het kan nadelig zijn voor de patiënt omdat het een verhoogde kans geeft op overgewicht, diabetes, osteoporose en hypertensie. Dit zijn problemen waar volwassenen met PWS van nature al meer last van hebben. Daarom adviseren we om geen hydrocortison stress-doseringen voor te schrijven voor volwassenen met PWS. Als er een klinische verdenking is op BNI, adviseren we om een insuline tolerantie test of een metyrapontest uit te voeren.

In **Hoofdstuk 8** tonen we aan dat een vijfde van de patiënten met PWS hyperprolactinemie heeft. Hyperprolactinemie is meestal mild (80%) of matig (13%), maar in 7% ernstig. Het is vaak gerelateerd aan het gebruik van medicatie (62%) zoals antipsychotica (56%). Patiënten met het genetische subtype maternale uniparentele disomie (mUPD) hebben hogere prolactine concentraties dan patiënten met een deletie. Dit wordt waarschijnlijk veroorzaakt door de hogere prevalentie van psychoses bij patiënten met een mUPD, wat leidt tot frequenter gebruik van antipsychotica. Er werden geen klinische effecten gezien van hyperprolactinemie. Op basis van onze resultaten adviseren we om te screenen op hyperprolactinemie, door het serum prolactine te meten in alle patiënten met PWS. Ook adviseren we om de prolactine concentratie opnieuw te meten na start van nieuwe psychofarmaca, omdat dit hyperprolactinemie kan veroorzaken.

Hoofdstuk 9 gaat over hyponatriëmie bij PWS. In ons cohort hadden 2.6% van de mensen tenminste één keer milde of matige hyponatriëmie gehad en 0.5% had tenminste één keer ernstige hyponatriëmie gehad. Van de 36 mensen met hyponatriëmie waren er twee onder de 18 jaar oud. Beiden hadden een milde hyponatriëmie. Iedereen met ernstige hyponatriëmie had klinische symptomen, variërend van verwardheid tot epileptische insulten en coma. Oorzaken voor ernstige hyponatriëmie waren: gebruik van desmopressine, polydipsie (overmatig veel drinken) en gebruik van diuretica in combi-

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natie met een zoutbeperking. Geen van de patiënten met milde of matige hyponatri-emie had klinische symptomen. Oorzaken van milde en matige hyponatriëmie waren: psychofarmaca die het syndroom van inadequate secretie van antidiuretisch hormoon (SIADH) kunnen veroorzaken (32%), polydipsie (24%), hyperglycemie (12%), diuretica (9%), desmopressine (6%) en onbekend (29%). Om levensbedreigende complicaties van ernstige hyponatriëmie te voorkomen, raden we aan om regelmatig het serum natrium te bepalen bij patiënten met PWS, in het bijzonder bij patiënten die psychofarmaca ge-bruiken of een voorgeschiedenis hebben met polydipsie. We raden artsen ook aan om de verzorgers van mensen met PWS te waarschuwen voor de gevaren van polydipsie. Tenslotte moeten desmopressine en medicijnen die SIADH kunnen veroorzaken, waar mogelijk worden gemeden bij patiënten met PWS.

Hoofdstuk 10 gaat over botgezondheid bij volwassenen met PWS. 14% had osteopo-rose en 54% had osteopenie. Risicofactoren voor osteoporose waren: hypogonadisme (93% bij mannen, 80% bij vrouwen), onvoldoende zuivel intake (86%), onvoldoende lichaamsbeweging (40%) en gebruik van corticosteroïden (10%). In ons literatuuronder-zoek vonden we ook dat vitamine D-deficiëntie vaker voorkomt bij volwassenen met PWS in vergelijking met gezonde controles. 3% van ons cohort had ooit een wervel-fractuur gehad. Bij 6 mensen ontstond de wervelfractuur spontaan. 17% had ooit een niet-wervel fractuur gehad, vaak veroorzaakt door trauma. GH-behandeling was niet geassocieerd met osteoporose, osteopenie of het ontstaan van fracturen. We adviseren om preventieve maatregelen te nemen om osteoporose te voorkomen en om te scree-nen op osteoporose middels een dual-energy X-ray absorptiometry (DEXA) scan bij alle volwassenen met PWS. Vier op de vijf volwassenen met PWS had een scoliose. We raden aan om hierop te screenen, door jaarlijks tijdens lichamelijk onderzoek te beoordelen of patiënt een gibbus (uitbochting van de rug aan de kant van de scoliose) heeft.

In **Hoofdstuk 11** beschrijven we vier patiënten met PWS en ernstige cardiovasculaire (CV) ziekte. Alle vier waren ze jonger dan 40 jaar. Pulmonaire hypertensie speelde een belangrijke rol bij het ontstaan van CV-ziekte bij alle vier de casus. Risicofactoren voor CV-ziekte waren: obesitas (n = 4), diabetes mellitus type 2 (n = 2), hypogonadisme (n = 3), hypertensie (n = 2) en slaap apnoe (n = 2). Screening en behandeling van deze risico-factoren is belangrijk om CV-ziekte te voorkomen. Wanneer CV-ziekte toch optreedt, is dit lastig vast te stellen. Dit komt doordat mensen met PWS een hoge pijngrens hebben en doordat NT-proBNP waarden vals-normaal kunnen zijn door overgewicht. Daarnaast is bij overgewicht de echo van het hart minder betrouwbaar. Tenslotte is perifeer oe-deem geen betrouwbare marker voor CV-ziekte bij patiënten met PWS, omdat dit ook aanwezig is bij een derde van de patiënten met PWS die geen CV-aandoening hebben. Opvallend was dat alle vier de beschreven patiënten progressief pitting oedeem hadden

vlak voor of tijdens het ontstaan van hun CV-probleem. *Progressie* van oedeem is dus een alarmsymptoom, waarop direct actie ondernomen moet worden. Een ander alarm symptoom is kortademigheid, omdat alle patiënten zich presenteerden met orthopnoe of dyspnoe. Wanneer de CV-ziekte eenmaal gediagnosticeerd is, wordt de behandeling ervan bemoeilijkt door therapieontrouw, wat vaker voorkomt bij patiënten met een verstandelijke beperking. Daarom is adequate supervisie essentieel bij de behandeling van CV-ziekte bij volwassenen met PWS.

In **Hoofdstuk 12** laten we zien dat kanker zeldzaam is bij kinderen en volwassenen met PWS. Zeven van de 706 patiënten hadden kanker (gehad). Ze hadden allemaal een ander soort kanker, waardoor screenen op één soort kanker niet zinvol is. Echter, omdat obesitas veel voorkomt bij patiënten met PWS en een belangrijke risicofactor is voor kanker, adviseren we om deel te nemen aan het bevolkingsonderzoek. Verrassend genoeg hadden alle patiënten met een maligniteit een deletie als genetisch subtype, wat significant meer was dan in de groep patiënten zonder kanker. Daarom hebben we een literatuuronderzoek gedaan naar de relatie tussen genen op chromosoom 15q11-13 en kanker. Deze relatie bleek complex, waardoor het niet mogelijk was om een duidelijke verklaring te geven voor de relatie tussen het genetisch subtype en kanker.

In **Hoofdstuk 13** presenteren we een patiënt met een PWS fenotype en een unieke, homozygote variant van het *SNRPN* gen, *SNRPN-p.Arg65Trp*. In onze literatuurreview vonden we dat *SNORD116* de belangrijkste bijdrage leverde aan het PWS fenotype. Genetisch onderzoek en functionele testen lieten zien dat deze nieuwe homozygote *SNPRN* variant mogelijk heeft bijgedragen aan het PWS fenotype van deze patiënt.

Als laatste bespreken we in **Hoofdstuk 14** de resultaten van onze onderzoeken in relatie tot bestaande en nieuwe wetenschappelijke en maatschappelijke ontwikkelingen. Ons onderzoek heeft veel kennis opgeleverd over het voorkomen en behandelen van gezondheidsproblemen bij volwassenen met PWS. Hiermee hebben we een groot kennishiaat gevuld, waarmee de medische zorg voor volwassenen met PWS zal worden verbeterd. Dit zal onnodig lijden en onnodige zorgkosten voorkomen. Natuurlijk hebben onze bevindingen ook weer nieuwe onderzoeksvragen opgeleverd. Daarom eindigen we hoofdstuk 14 met een reeks aanbevelingen voor toekomstig onderzoek.

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Pellikaan K*, Elizabeth MSM*, Hokken-Koelega ACS, Sanders RJ, van der Lely AJ, van den Berg SAA, de Graaff LCG. Reference intervals for bioavailable IGF-I values in Dutch healthy children, adolescents and young adults. In preparation.

* These authors contributed equally to this study.

PHD PORTFOLIO

Name PhD student: Karlijn Pellikaan
Erasmus MC department: Internal Medicine, Section Endocrinology
Promotor: prof. dr. A.J. van der Lelij
Copromotor: dr. L.C.G. de Graaff

Summary of PhD training

	Year	Workload (ECTS)
Courses		
Study Design (NIHES)	2017	4.3
Biostatistical Methods I: Basic Principles (NIHES)	2017	5.7
Biostatistical Methods II: Classical Regression Models (NIHES)	2017	4.3
Principles of Research in Medicine and Epidemiology (NIHES)	2017	0.7
Clinical Translation to Epidemiology (NIHES)	2017	2.0
Clinical Epidemiology (NIHES)	2017	3.7
Methods of Public Health Research (NIHES)	2017	0.7
Clinical Trials (NIHES)	2017	0.7
The Practice of Epidemiologic Analysis (NIHES)	2017	0.7
Fundamentals of Medical Decision Making (NIHES)	2017	0.7
Value Based Healthcare, from theory to implementation (NIHES)	2017	0.7
Advances in Genomics Research (NIHES)	2017	0.4
Advanced topics in Decision-making in Medicine (NIHES)	2018	2.4
Preventing Failed Interventions in Behavorial Research (NIHES)	2018	1.4
Psychopharmacology (NIHES)	2018	1.4
Quality of Life Measurement (NIHES)	2018	0.9
Intermediate Course in R (NIHES)	2018	1.4
Repeated Measurements in Clinical Studies (NIHES)	2018	1.4
Advanced Decision Science Modeling (NIHES)	2018	1.4
Principles in Causal Inference (NIHES)	2018	1.4
Review of Mathematics and Introduction to Statistics (NIHES)	2018	1.4
Inleiding in de Gezondheidspsychologie (Open universiteit)	2018	5.0
Test- en Toetstheorie (Open universiteit)	2018	4.3
Basiscursus Regelgeving en Organisatie voor Klinisch onderzoekers (BROK)	2019	1.5
Pharmaco-epidemiology and Drug Safety (NIHES)	2019	1.9
Scientific Writing in English for Publication (NIHES)	2019	2.0
Principles of Epidemiologic Data-analysis (NIHES)	2019	0.7
Advanced Analysis of Prognosis Studies (NIHES)	2019	0.9
Advanced Clinical Trials (NIHES)	2019	1.9

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	Year	Workload (ECTS)
Scientific Integrity (NIHES)	2019	0.3
Course OpenClinica (Erasmus MC)	2019	0.2
Courses LimeSurvey and GemsTracker (Erasmus MC)	2020	0.2
Scientific Integrity for PhD students (Erasmus MC)	2020	0.3
Systematic Literature Retrieval I and I (Medical library Erasmus MC)	2020	0.2
(Inter)national Conferences and Presentations		
European Congress of Endocrinology (ECE), Barcelona, Spain *(Poster presentation)*	2018	1.0
European Young Endocrine Scientists (EYES) meeting, Poznań, Poland *(Oral presentation)*	2018	1.5
Jonge Nederlandse Vereniging voor Endocrinologie (JNVE) meeting, Nijmegen, the Netherlands *(Oral presentation)*	2018	1.0
Jonge onderzoekersdag EAA, Amsterdam, the Netherlands *(Oral Presentation)*	2018	0.5
Dutch Endocrine Meeting (DEM), Noordwijkerhout, the Netherlands *(Oral Presentation)*	2019	1.0
European Congress of Endocrinology (ECE), Lyon, France *(Poster presentation)*	2019	1.0
European Society for Paediatric Endocrinology (ESPE) meeting, Vienna, Austria *(Poster presentation)*	2019	1.0
Science Days Internal Medicine, Sint-Michielsgestel, the Netherlands *(Poster presentation)*	2020	0.5
European Congress of Endocrinology (ECE), online *(Poster presentation)*	2020	1.0
International Congress of Endocrinology (ICE), online *(Oral presentation)*	2021	1.5
ENDO, online *(Poster presentation)*	2021	1.0
European Congress of Endocrinology (ECE), online *(Poster presentation)*	2021	1.0
ENDO, Atlanta, USA *(Oral presentation, Outstanding Abstract Award)*	2022	1.5
International Prader-Willi syndrome Organisation (IPWSO) Conference, Limerick, Ireland *(Oral Presentation)*	2022	1.5
Seminars		
EAA biweekly research meetings, Erasmus MC	2020-2021	1.0
Teaching activities		
Supervision five medical students	2019-2021	5.0
Lecturing		
Contactdag Prader-Willi Stichting, Lelystad, the Netherlands *(Oral Presentation)*	2018	0.5
Other activities		
Endocrine Society Early Career Forum	2021	0.9
Peer reviewer for an international scientific journal	2022	0.3

ABOUT THE AUTHOR

Karlijn Pellikaan was born on June 17th 1996 in Zwijndrecht, the Netherlands. She graduated from secondary school in 2014 (DevelsteinCollege) after which she started her bachelor in Medicine at the Erasmus University Rotterdam. After she finished her bachelor in Medicine, she started a two-year research master in Clinical Research at the Erasmus MC Netherlands Institute for Health Sciences (NIHES). During this master, she also completed a premaster in Health Psychology at the Open Universiteit. In 2019 she started as a fulltime PhD-student at the Center for adults with Rare Genetic Syndromes / Center of Reference for Prader-Willi Syndrome, at the Department of Internal Medicine-Endocrinology of the Erasmus University Medical Center, Rotterdam, the Netherlands. The results of this PhD-project are presented in this thesis. In September 2021 she started her master in Medicine at the Erasmus University Rotterdam.

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