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Endocrine consequences of prader-willi syndrome

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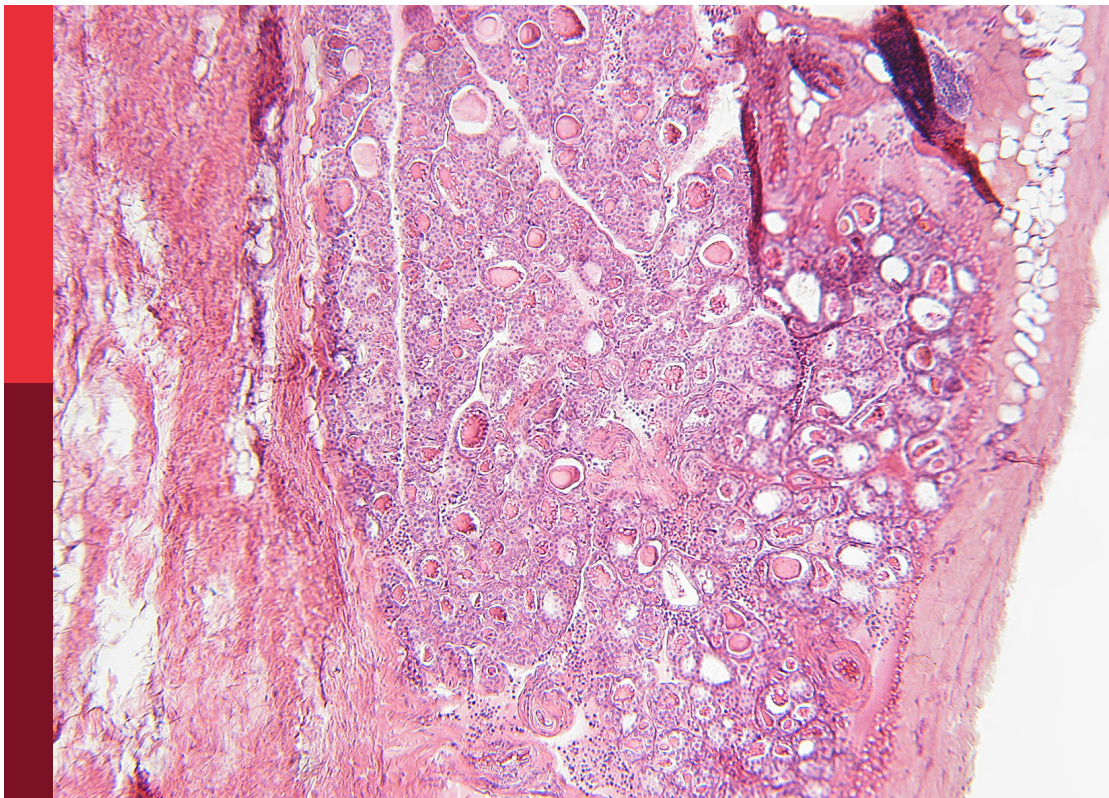

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Endocrine consequences of prader-willi syndrome

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CASE REPORT
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Case Report: Hepatic Adenomatosis in a Patient With Prader–Willi Syndrome

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Prader–Willi syndrome (PWS) is a genetic disorder caused by the lack of expression of genes on the paternally inherited chromosome region 15q11.2-q13. It is a multisystem disorder that is characterized by severe hypotonia with poor suck and feeding difficulties in early infancy, followed in early childhood by excessive eating and gradual development of morbid obesity. The incidence of type 2 diabetes mellitus is high, particularly in obese patients. Non-alcoholic fatty liver disease has also been reported in some patients with PWS. Liver adenomatosis is a benign vascular lesion of the liver, defined by the presence of >10 adenomas, in the otherwise healthy liver parenchyma. We report the first case of a patient with PWS with severe obesity, type 2 diabetes mellitus, and non-alcoholic fatty liver who also developed liver adenomatosis, review the pediatric literature on liver adenomatosis, and discuss the potential underlying mechanisms.

Keywords: hepatic adenomatosis, Prader Willi syndrom, liver adenoma, oral contraception pills, Glycogen Storage Disease

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INTRODUCTION

Prader–Willi syndrome (PWS) is a complex multisystem disorder caused by lack of expression of genes on the paternally inherited chromosome region 15q11.2-q13. In the neonatal period, there is severe hypotonia with poor suck and feeding difficulties followed in infancy or early childhood by excessive eating and gradual development of morbid obesity. The syndrome is considered the most common genetic cause of obesity, occurring in 1:10,000–1:30,000 live births (1). Obesity and its related complications are the most common causes of morbidity and mortality in PWS. The mechanisms underlying the obesity include alterations in hypothalamic pathways that regulate satiety thus resulting in hyperphagia, disruption in hormones regulating appetite and satiety, and reduced energy expenditure (1).

Severe obesity is a strong risk factor for the development of type 2 diabetes mellitus (T2DM) in patients with PWS. T2DM in PWS occurs mostly in adults, but it has also been reported in patients under the age of 18 years (1). The prevalence of metabolic syndrome in obese patients with PWS

seems to be similar to other obese patients (2). Patients with PWS are thought to be at a lower risk of developing non-alcoholic fatty liver disease (NAFLD) because of a higher insulin sensitivity as well as insulinopenia (3, 4) with the staging of the NAFLD depending on body composition (3, 4).

Liver adenomatosis is a benign vascular lesion of the liver, defined by the presence of >10 adenomas, in an otherwise healthy liver parenchyma (5). A variety of associations have been reported with liver adenomatosis including glycogen storage disease types I and IV, transfusion-induced hemosiderosis, Fanconi's anemia, Hurler's disease, severe combined immunodeficiency, familial adenomatous polyposis, and galactosemia (6).

We report the finding of liver adenomatosis in a case of PWS who is known to have severe obesity, T2DM, and NAFLD. We review all the previous reported cases of liver adenomatosis in pediatric patients and discuss the possible underlying mechanisms. Despite several cases reported in the literature, liver adenomatosis remains a poorly understood disease of unknown etiology.

CASE PRESENTATION

We report a 17-year-old obese female patient with PWS who was born in Egypt and then moved to Qatar at the age of 9 years. She was born at term to non-consanguineous parents with a birth weight of 3.5 kg. The pregnancy was uneventful. In the newborn period, there were feeding difficulties, and on day 12 of life, she had a seizure with frothing, cyanosis, and jaw clenching. She was admitted for 10 days to the neonatal intensive care unit (NICU). There was a delay in achievement of motor milestones, with crawling starting at the age of 1 year 8 months and walking at 2 years. There was also a history of speech delay, and the patient started speaking at the age of 5 years. She started to gain weight from the age of 2 years; however, at the age of 12 years, around puberty, hyperphagia and excessive weight gain were noticed. Her parents had difficulty limiting her food intake, as she had a persistent feeling of hunger to the extent of vomiting after eating large quantities. She initially sought endocrine care at the age of 15 years for an obesity workup.

Ultrasound of the liver was done to rule out fatty liver, and this showed multiple lesions in the liver that were thought to be areas of focal fatty sparing. MRI of the liver was done that showed an enlarged liver with diffuse fat infiltration and multiple numerous focal lesions suggestive of multiple adenomas (adenomatosis; **Figure 1**). A follow-up ultrasound in 2021 showed that the liver remained markedly echogenic and heterogeneous. The largest measured adenoma was 13 mm superficially in the left lobe (previously up to 11 mm) and generally was of the order of around 6 mm in diameter. Echocardiography showed normal cardiac function and anatomy. The patient undergoes an MRI of the abdomen every 6 months for the liver Adenomatosis. To date, all MRI scans do not show any significant interval changes in the number or size of the adenomas.

During the coronavirus disease 2019 (COVID-19) quarantine, at the age of 16, she gained weight rapidly, with 17 kg in 1 year. The weight upon presentation was 124.1 kg (99.54 centile), height was 138.8 cm (0.01 centile: -3.73 Z-Score), and body mass index (BMI) was 64.4 kg/m^2. The patient also had symptoms suggestive of sleep apnea with mouth breathing, snoring nights, and occasional daytime sleepiness. A sleep study revealed obstructive sleep apnea; hence, she was started on non-invasive ventilation. She had menarche at the age of 13, with irregular menses since then with prolonged heavy menses. In light of the long-term risks of endometrial hyperplasia and carcinoma due to her secondary amenorrhea, the oncology team recommended a minimum of four withdrawal bleeds per year to reduce the risk. The patient was prescribed medroxyprogesterone; she received a short course, 5 days, once every 3 months. She has received three courses since 2019 (15 days total in 2 years).

When she was 17 years of age, blood glucose values were high (250–300 mg/dl) during multiple clinic follow-ups with polyuria, polydipsia, and nocturia. Her blood gas did not show acidosis. HbA1c was 8.1% with high serum insulin and C-peptide levels along with acanthosis nigricans on the neck, axilla, and inguinal region suggestive of insulin resistance. The patient was started on both long-acting and short-acting insulin for glycemic control. **Table 1** shows the laboratory test results.

In terms of management, metformin was started initially, but a response was not observed. Liraglutide, a glucagon-like peptide 1 receptor (GLP-1) agonist, was tried for a month to help with

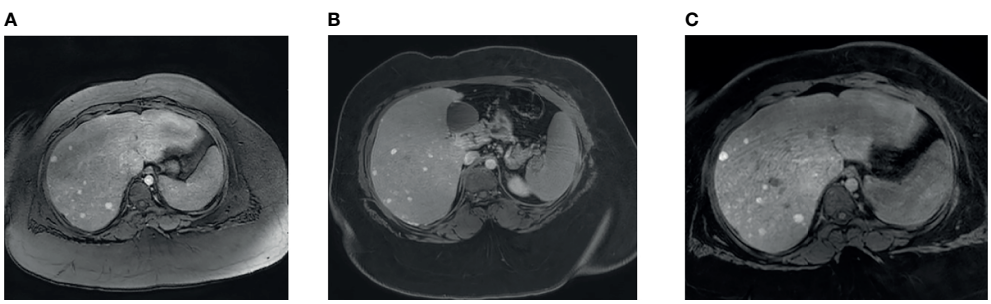

FIGURE 1 | MRI liver findings. **(A)** Contrast enhanced liver MRI image showing multiple small liver lesions showing early arterial enhancement. **(B)** Lesions showing retention of contrast after 6 minute delay. **(C)** Lesions showing retention of contrast after 31 minute delay.

TABLE 1 | Results of laboratory tests done.

Blood test	Blood test result	Reference range
Insulin level	618 pmol/L	20–571
C-peptide	14.9 ng/ml	(0.9–9.4)
HbA1c	8.1%	<6%
ALT	118 IU/L	9–22
AST	95 IU/L	15–28
ALP	141	48–96

ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; HBA1c, Haemoglobin A1c.

weight reduction and reduce insulin resistance. However, she developed a local skin allergy in the form of itchiness on the injection sites, so it was stopped. Her insulin regimen included long-acting insulin (Glargine) 20 units daily and rapid-acting insulin (noveorapid) of 3–4 units three times daily. Despite this, her obesity and T2DM were difficult to manage. A sleeve gastrectomy was done in October 2021, considering the extreme obesity, associated comorbidities, and the relatively poor response to medical therapy for her weight. Her 2-month post-sleeve gastrectomy weight is 113.2 kg (compared to 121 kg). Her blood glucose readings are within range, and she is off all antihyperglycemic medications.

Family History

She is the youngest of three siblings; attended school for children with special needs. Father has T2DM.

Genetic Testing

Methylation studies in 2019, when she was 15 years old, revealed an absence of the paternal allele at 15q11-q13 due to abnormal methylation, thus suggesting a defect in imprinting as the underlying molecular mechanism of the PWS.

Radiology

MRI of the liver was done that showed an enlarged liver with diffuse fat infiltration and multiple numerous focal lesions suggestive of multiple adenomas (adenomatosis; **Figure 1**). A follow-up ultrasound in 2021 showed that the liver remained markedly echogenic and heterogeneous. The largest measured adenoma was 13 mm superficially in the left lobe (previously up to 11 mm) and generally was of the order of around 6 mm in diameter. Echocardiography showed normal cardiac function and anatomy.

DISCUSSION

Hepatic adenomas are benign vascular lesions of the liver that are usually solitary, associated with young women taking oral contraceptives. However, some patients have multiple adenoma, which is called liver adenomatosis. This was first described as a distinct entity in 1985 by Flejou et al. (7), who defined it as the presence of multiple adenomas (>10) that are not associated with steroid medication or glycogen storage disease. Currently, liver adenomatosis is defined as the presence of more than 10 adenomas in an otherwise normal parenchyma (8). The first clinical presentation due to liver adenomatosis is usually abdominal pain (9). Intraperitoneal bleeding, intratumoral hemorrhage, or necrosis producing acute pain are also reported (10). Only one report refers to malignant transformation of adenomas (11). This shows that even if the disease is benign, the risk of hemorrhage remains a concern.

We reviewed all the previous cases of liver adenomatosis in patients under the age of 18 years. **Table 2** summarizes the age of presentation, gender, clinical features of the patients, the use of oral contraceptives, and associated complications. No previous patients with PWS have been reported with liver adenomatosis. The mechanism behind the development of multiple hepatic adenomas is not well established, especially in the pediatric age group. Liver adenomatosis has been reported in association with metabolic conditions, vascular anomalies, with the use of oral contraceptives, and with inactivating mutations in the *HNF1A* gene.

With regard to metabolic disease, there is a strong association reported between liver adenomatosis and glycogen storage disease with 50%–80% of children with type I or III glycogen storage disease developing multiple hepatic adenomas (19, 20). The impairment in glycogenesis and the accumulation of glycogen deposits within the hepatocyte lead to hepatocyte hyperplasia, resulting in multiple adenoma formation. NAFLD is also reported to be associated with liver adenomatosis but mostly in adults (20). The increase in the intracellular lipid content could lead to a hyperplastic reaction with changes in oxidative and inflammatory pathways (21). An alternative mechanism of liver adenomatosis due to NAFLD suggests that the fatty tissue may generate continuous local estrogen through the increased activity of the enzyme aromatase, thus leading to the accelerated rate of hepatocyte growth and possible tumor formation (22). Multiple adenomas have been reported in other metabolic diseases such as diabetes, metabolic syndrome, and obesity in the adult population (8, 23).

The vascular hypothesis of liver adenomatosis is based on the association between reported cases of liver adenomatosis and hepatic vascular abnormalities, assuming that irregular vascular flow can result in the development of liver adenomatosis (**Table 2**). For instance, liver adenomatosis was reported in a 13-year-old male patient by Kawakatsu et al. (13) who had a spontaneous intrahepatic porto-hepatic venous shunt.

The association of liver cell adenomatosis and oral contraceptive or androgenic steroid use is still a point of controversy. Chiche et al. (8) reported that oral contraceptive therapy is not as rarely associated with this liver disease as initially suggested by Flejou et al. (7); 46% of their female patients were on oral contraceptives (9, 15). Adenoma regression was recognized after discontinuing hormonal contraceptives in multiple studies, which suggest that oral contraceptives play a role in the evolution of liver adenomatosis. In our review of the pediatric cases, we did not find a correlation with the use of oral contraceptives and liver adenomatosis, as only 3 out of 13 reported cases used oral contraceptives (**Table 2**).

TABLE 2 | All pediatrics cases of LA reported in literature from years 1981 to 2019.

	Year/ Author	Age in years	Associated conditions	Gender	OCP use	Clinical presentation	Complications
1	Flejou, 1981 (7)	13	None reported	M	No	Abdominal pain after trauma	Intraperitoneal bleeding from ruptured nodule
2	Chen, 1983 (12)	13	None reported	F	No	Hepatomegaly	None reported
3	Lesse, 1988 (11)	16	None reported	M	No	Abdominal pain due to ruptured nodule in the center lobe of the liver after trauma, found multiple adenomas	Malignant transformation
4	Kawakatsu, 1994 (13)	13	None reported	M	No	Pain, jaundice	Intratumoral bleeding
5	Gokhale, 1996 (14)	17	Minimal change GN diagnosed at the same time	F	No	Monthly abdominal pain and headache, resolve spontaneously	Hydronephrosis of the center kidney, cystic center ovary
6	Chiche, 2000 (8)	18	DM, non-insulin-dependent, hypertension on beta blockers	F	1year	Intraparietal bleeding	Extratumoral bleeding, cardiac arrest, and death
7	Chiche, 2000 (8)	14	None reported	M	No	Incidental finding	None reported
8	Chiche, 2000 (8)	17	None reported	F	No	Pain, hepatomegaly	Had segmentectomy, but 14 years later presented with intratumoral hemorrhage
9	Kadir Babaoglu 2010 (15)	7	CHD, Fontan procedure at 2 years of age	F	No	Abdominal distention	None reported
10	Wellen, 2010 (16)	15	None reported	F	Yes	Abdominal pain and weight loss	None reported
11	Timothy, 2019 (24)	15	Kapuki syndrome, medullary nephrocalcinosis, intermittent microhematuria, IgG deficiency, asthma, bilateral conductive hearing loss, and developmental delay	F	1 year	Intermittent abdominal pain, occasional dark urine in the preceding 2 months and unexplained pruritus for 10 years	HCC
12	Marino, 1992 (17)	10	Familial HA (mother and 8-year old brother were diagnosed with HA	F	No	Chronic recurrent episodes of abdominal pain	
13	Oji, 2019 (18)	18	Obesity, BMI 40	M	No	Incidental finding	None reported

OCP, oral contraceptive pills; HCC, hepatocellular carcinoma; GN, glomerulonephritis; HA, hepatic adenoma; CHD, congenital heart disease; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; HBA1c, Haemoglobin A1c.

In some patients with liver adenomatosis, there is a genetic background to the etiology. This involves biallelic inactivating mutations in the transcription factor, *HNF1A*, in the hepatic adenomas by the occurrence of two molecular events: either a germline and a somatic *HNF1A* mutation or two independent somatic events (25). This is usually associated with the Maturity Onset Diabetes of the Young (MODY) in the family history as heterozygous *HNF1A* mutations are a cause of MODY3.

Our patient with PWS has several risk factors for the development of liver adenomatosis. These include severe obesity (BMI of 64.4 mg/m^2), T2DM, NAFLD, and possibly the use of oral contraceptives. Medroxyprogesterone was used for a short period of time, so we do not think that this is a significant risk factor, although we cannot completely rule this out. There was no family history of MODY in this patient. It is difficult to know which of these risk factors is directly linked to the liver adenomatosis but it is likely that the combination of the risk factors is involved. This case highlights that patients with PWS, who have risk factors such as obesity, T2DM, and NAFLD, may develop liver adenomatosis. Physicians should have a low threshold for making this possible diagnosis.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HD and AS collected patient information, recruited the patient, analyzed and interpreted the data, and drafted the article. KH designed the study, obtained funding, and reviewed and edited the article. BH analyzed the data and reviewed and edited the article. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Circulating Irisin in Children and Adolescents With Prader-Willi Syndrome: Relation With Glucose Metabolism

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Irisin is a myokine involved in the browning of white adipose tissue and regulation of energy expenditure, glucose homeostasis and insulin sensitivity. Debated evidence exists on the metabolic role played by irisin in children with overweight or obesity, while few information exist in children with Prader Willi Syndrome (PWS), a condition genetically prone to obesity. Here we assessed serum irisin in relation to the metabolic profile and body composition in children and adolescents with and without PWS. In 25 PWS subjects [age 6.6-17.8y; body mass index standard deviation score (BMI SDS) 2.5 ± 0.3] and 25 age, and BMI-matched controls (age 6.8-18.0y; BMI SDS, 2.8 ± 0.1) we assessed irisin levels and metabolic profile inclusive of oral glucose tolerance test (OGTT), and body composition by dual-energy X-ray absorptiometry (DXA). In PWS, we recorded lower levels of fat-free mass (FFM) ($p < 0.05$), fasting ($p<0.0001$) and 2h post-OGTT insulin ($p<0.05$) and lower insulin resistance as expressed by homeostatic model of insulin resistance (HOMA-IR) ($p<0.0001$). Irisin levels were significantly lower in PWS group than in controls with common obesity ($p<0.05$). In univariate correlation analysis, positive associations linked irisin to insulin $OGTT_0$ ($p<0.05$), insulin $OGTT_{120}$ ($p<0.005$), HOMA-IR ($p<0.05$) and fasting C-peptide ($p<0.05$). In stepwise multivariable regression analysis, irisin levels were independently predicted by insulin $OGTT_{120}$. These results suggest a link between irisin levels and insulin sensitivity in two divergent models of obesity.

Keywords: irisin, PWS, obesity, glucose metabolism, children, adolescents

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INTRODUCTION

Prader-Willi Syndrome (PWS) is a rare genetic disease caused by the lack of expression of paternal genes on chromosome 15q11.2-q13 (1) and it represents one of the most common forms of genetic obesity. The estimated incidence rate is 1 in 25,000 live births (2). Three main genetic mechanism have been detected in PWS: interstitial deletion of the proximal long arm of chromosome 15 (15q11-q13)(DEL15), maternal uniparental disomy of chromosome 15 (UPD15) and imprinting defects (3).

PWS is a complex multisystem disorder, characterized by infantile lethargy and muscle hypotonia followed by hyperphagia and excess weight gain during early child-hood, mildly dysmorphic acro-facial features, kyphoscoliosis, developmental delay with learning and behavioral problems, and a variable number of hypothalamo-pituitary disorders comprising impaired GH secretion with short stature, hypogonadism, hypothyroidism (1, 2, 4, 5). A typical feature of PWS is a lack of satiety due to hypothalamic dysfunction, which generates obsessive craving for food approximately by the age of 2 years and progresses to development of severe obesity in a large proportion of patients unless overeating is promptly controlled by caregivers (6).

With respect to subjects with common obesity, obese children with PWS display distinct phenotypic and metabolic features (1), consisting of higher fat mass (FM), lower fat-free mass (FFM) and impaired muscle function (7, 8). Despite this adverse phenotype, fasting insulin levels and insulin resistance are comparatively lower at all ages in obese patients with PWS (9–11). Furthermore, non-obese children with PWS showed lower insulin and glucose levels than their obese counterpart (12). When plotted against results obtained in non-PWS controls, reasons for higher insulin sensitivity in PWS include a modest accumulation of visceral fat after adjustment for total adiposity (13), an disproportionately elevated ghrelin concentration for the degree of obesity (8, 14), high levels of adiponectin (10) and an impaired GH secretion (15).

In the past few years, attention has focused on irisin, a myokine involved in the cross-talk between muscle and adipose tissue (16, 17) that has been proposed to play a role in the pathophysiology of obesity. Originally described in mice by Böstrom et al. (16), irisin is a 112 amino acid cleavage product of fibronectin type III domain-containing protein 5 (FDNC5), that was shown to stimulate the "browning" of white adipocytes, thus acting to increasing total body energy expenditure, reducing body weight, and reducing insulin resistance (16, 18). While irisin was first identified as a myokine secreted in response to exercise, recent studies in rodents and humans demonstrated that irisin is also expressed and secreted by white adipose tissue (WAT) (17), and there is emerging evidence that irisin could also act as an adipokine (17, 19, 20). As such, in the adult population circulating irisin is positively associated with body weight (21–24) and several measures of adiposity (22, 23), as well as muscle mass (20–22).

On the contrary, scanty and debated information exists on the role played by irisin in relation to pediatric obesity (25). While studies found a positive correlation between irisin and body mass index (BMI), waist circumference (WC) and fat-free mass (26–28), others documented a negative or a lack of correlation between irisin and adiposity measures (29–31). Conceivably, at this stage divergences may depend on variability in hormonal activity, muscle development and body fat accumulation in growing children (32, 33).

With regard to irisin and glucose metabolism, its role is not completely understood both in children and adults (18, 25). Irisin facilitates glucose uptake by skeletal muscle and adipose tissue (by increasing the expression of GLUT4) and improves hepatic glucose metabolism, increasing glycogenesis and reducing gluconeogenesis (18). Also, it contributes to β-islet cell survival and function (34). In obese children, irisin concentrations have been correlated to glucose and insulin levels as well as insulin resistance (27, 28,31, 35). Intriguingly, a cross-sectional study in obese children with and without insulin resistance recorded no difference in serum irisin levels (36), while a negative correlation was observed in healthy children between fasting glucose and irisin levels (37).

In PWS, circulating irisin level have only been assessed in a few studies (20, 38, 39). A previous study from our group in adult obese subjects with PWS reported significantly lower irisin levels in PWS than in controls with common obesity, while being similar to values recorded in lean subjects (20). In a recent study encompassing children and adults, Faienza et al. showed that serum irisin levels didnot differ between PWS and normal weight subjects but, interestingly, the authors found significantly lower irisin levels both in pediatric and adult PWS subjects carrying DEL15 as compared to controls (40).

Given this background, our study aimed to explore circulating irisin in relation to the metabolic profile and body composition in obese children and adolescents with and without PWS. Secondary aim of the study was to assess the predictors of irisin levels in our cohorts.

MATERIALS AND METHODS

Patients

This study enrolled 50 patients, consisting of 25 PWS children and adolescents (16 M/9 F; age, 6.6-17.8y; BMI SDS, 2.5 ± 0.3) and 25 age, gender and BMI-matched control subjects (11 M/14 F; age, 6.8-18.0y; BMI SDS, 2.8 ± 0.1). PWS subjects were referred to the Center for Prader-Willi Syndrome of the Bambino Gesù Children's Hospital in Rome, Italy. All PWS individuals received a diagnosis based on typical syndromic features confirmed by molecular genetic studies of chromosome 15, including 15q11-q13 deletion in 15 (8 males and 7 females) and UPD15 in the remaining 10 patients (8 males and 2 females).

With respect to hormone replacement therapy, 16 patients with PWS were treated with growth hormone, while 1 PWS patient and one control subject were treated with levothyroxine. For all study participants, exclusion criteria included previously known liver disease, kidney failure, autoimmune diseases, uncontrolled hypothyroidism and/or diabetes mellitus, chronic exposure to anti-inflammatory steroids. Alcohol consumption was investigated, and none was an alcohol drinker. The study

design was conformed to the ethical guidelines of the Declaration of Helsinki (1975) and was firstly approved by the Ethical Research Committees of the Bambino Gesù Children's Hospital (protocol 2092/2020 approved 25 march 2020).

Written informed consent was obtained from all participants by their parents, and from patients, when appropriate. The study protocol was conformed to the guidelines of the European Convention on Human Rights and Biomedicine concerning biomedical research.

Body Measurements

Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using standard methods. Waist circumference (WC) was measured midway between the lowest rib and the top of the iliac crest after expiration; hip measurements were taken at the greatest circumference around the nates. BMI was calculated as weight in kg divided by the square of the height in meters and expressed as standard deviation score, and BMI SDS was calculated according to BMI reference tables (41). The BMI cut-of point of ≥ 2 SDS was used to define obesity (41). Pubertal development was assessed according to Tanner's criteria (42). A dual-energy X-ray absorptiometry using a Hologic QDR Discovery, and the APEX-system software version 13.3 (Hologic Bedford, MA) with fan beam in array mode was performed by the same operator. Quality control scans were performed daily using a simulated L1-4 lumbar spine phantom. The measurements were performed by using standard positioning techniques. Total body scans were obtained to estimate Fat Mass (FM%), Fat Free Mass (FFM%) expressed as percentage of total body weight (total body less head, with the skull excluded from analysisis). Trunk Fat (TFM), and FFM/FM ratios were also calculated. Coefficient of variation were between 0.3285 to 1.038%.

Metabolic Studies

Glucose homeostasis was evaluated by fasting glucose levels, oral glucose tolerance test (OGTT)-derived glucose and insulin levels at time 0 and 120 min, and glycated hemoglobin (HbA1c) levels in all subjects. Glucose tolerance was assessed according to ADA guidelines for children and adolescents (43). Insulin resistance was calculated by the homeostatic model of insulin resistance (HOMA-IR) index: insulin (mIU/L) ×[glucose (mmol/L)/22.5] (44). Blood samples were drawn at 08.00 a.m. under 12 hours fasting conditions then vials were centrifuged, and sera were stored at – 80°C until requested. Serum irisin levels were assessed using a commercially available human ELISA kit EK-067–29 (Phoenix Pharmaceutics, Inc, Burlingame, CA, USA) in accordance with the manufacturer's instructions. This ELISA is specific for human irisin, and quality controls were included in all ELISA measurements with the results falling within the expected range. All samples were analyzed in duplicate. Intra-assay and inter-assay coefficients of variation (CV) of irisin immunoassays were less than 10% and 15% respectively, and minimum detectable concentration was 1.5 ng/mL. Serum leptin concentrations were quantified using a commercially available ELISA kit (Mediagnost GmbH, Reutlingen, Germany) with overall inter- and intra-assay CVs of 6.8–8.3% and 5.5–6.9% respectively.

Serum adiponectin levels were determined by an enzyme linked immunosorbent assay (DRG Instruments GmbH, Marburg, Germany), the detection limit was 1.56–100 ng/ml, sensitivity was 0.2 ng/ml, inter- and intra-assay CV was 2.4–8.4 and 0.9–7.4%, respectively.

Routine laboratory data included levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), glucose, total cholesterol (CHO), high-density (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides (TG) and HbA1c, measured by enzymatic methods (Roche Diagnostics, Mannheim, Germany). Levels of insulin were measured using a Cobas Integra 800 Autoanalyzer (Roche Diagnostics, Indianapolis, IN, USA). A two-site, solid-phase chemiluminescent immunometric assay or competitive immunoassay (Immulite 2000 Analyzer; DPC, Los Angeles, CA) was used to determine C-peptide levels.

Statistical Analysis

Statistical analysis was performed using SPSS version 21 (Somers, NY, USA). Values are expressed as means ± standard error mean (SEM). Data points not normally distributed obtained by the Shapiro–Wilk test were log-transformed to improve the symmetry and homoscedasticity of the distribution. For comparative analysis, ANOVA between the 2 groups was used. Pearson's correlation analysis was used to identify significant associations between variables of interest. Stepwise multivariable regression analysis was used to evaluate the independent association of irisin with metabolic, anthropometric or biochemical parameters.

Two multilinear models were built which included the obese phenotype (common obesity = 0; PWS = 1) in association with parameters of body composition and metabolism (model 1: group, BMI SDs; Glucose $OGTT_0$, Glucose $OGTT_{120}$, Insulin $OGGT_0$, Insulin $OGTT_{120}$ C-Peptide, HOMA-IR; leptin, adiponectin; model 2: group, BMI SDS, Glucose $OGTT_0$, Glucose $OGTT_{120}$, Insulin $OGTT_0$, C-Peptide, HOMA-IR, leptin adiponectin; with the exclusion of predictor from the previous model: Insulin $OGTT_{120}$). β coefficients and significance values obtained from the regression models are reported. A p value < 0.05 was considered as statistically significant.

RESULTS

A summary of anthropometric and biochemical data is reported in **Tables 1**, **2**. BMI standard deviation score (SDS) values were comparable between the two groups and ranged, collectively, between 1.1 and 5.7. Within the PWS group, 14 out of 25 were obese (BMI > 2 SDS) and, overall, 15 were prepubertal and 10 were postpubertal. Among controls, 22 out of 25 were obese and, overall, 14 were prepubertal and 11 were postpubertal. No difference in age and gender distribution were observed between groups.

TABLE 1 | Summary of anthropometric data obtained in PWS patients and controls.

Variables	PWS patients (n=25)	Controls (n=25)	P value
Males/females	16/9	11/14	0.16
Prepubertal/pubertal	15/10	14/11	0.78
Age (years)	11.1 ± 0.6	12.60 ± 0.7	0.16
BMI SDS	2.5 ± 0.3	2.8 ± 0.1	0.31
Weight (kg)	**51.9 ± 3.2**	**71.8 ± 3.5**	**<0.0001**
Height (cm)	**139.0 ± 2.5**	**155.7 ± 3.0**	**<0.0001**
Waist (cm)	**82.3 ± 2.9**	**95.9 ± 2.5**	**0.002**
Hip (cm)	**92.1 ± 2.7**	**102.1 ± 2.3**	**0.013**
Waist-to-hip ratio	**0.89 ± 0.01**	**0.94 ± 0.01**	**0.04**
FM (%)	47.2 ± 4.6	43.6 ± 4.61	0.12
FM (Kg)	28.5 ± 2.1	32.1 ± 2.0	0.31
FFM (%)	50.7± 4.9	53.3± 5.0	0.20
FFM (kg)	**29.8± 1.8**	**39.9± 2.6**	**0.014**
Trunk fat (kg)	12.1± 1.1	14.2± 1.0	0.262
FFM/FM ratio	1.11± 0.05	1.29± 0.1	0.219

Data are expressed as mean ± SEM. Comparison between populations was performed by ANOVA test. Significant differences are shown in bold characters.
BMI SDS, body mass index standard deviation score FM, fat mass; FFM, fat-free mass.

TABLE 2 | Summary of biochemical data obtained in PWS subjects and controls.

Variables	PWS (n=25)	Controls (n=25)	P value
Glucose $OGTT_0$ (mg/dL)	83.3. ± 1.5	83.8. ± 1.8	0.84
Glucose $OGTT_{120}$ (mg/dL)	105.2 **± 5**.6	107.5 ± 5.6	0.78
Insulin $OGTT_0$ (mIU/L)	**10.1 ± 1.0**	**19.0 ± 1.5**	**<0.0001**
Insulin $OGTT_{120}$ (mIU/L)	**39.8 ± 4.4**	**112.8 ± 15.1**	**0.006**
C-Peptide (µg/L)	**1.4 ± 0.1**	**2.1 ± 0.1**	**<0.0001**
HbA1c (%)	5.1 ± 0.3	5.0 ± 0.1	0.35
HOMA-IR	**2.1 ± 0.3**	**4.0 ± 0.4**	**<0.0001**
AST (U/L)	24.6 ± 1.6	24.6 ± 2.1	0.99
ALT (U/L)	23.3 ± 3.2	26.7 ± 4.6	0.55
GGT (U/L)	15.3 ± 1.2	15.4 ± 1.4	0.94
TG (mg/dL)	80.4 ± 9.8	95.4 ± 8.2	0.25
Tot CHO (mg/dL)	165.6 ± 6.1	164.5 ± 6.7	0.91
LDL CHO (mg/dL)	96.1. ± 4.0	96.3 ± 6.0	0.98
HDL CHO (mg/dL)	52.6 ± 2.1	47.4 ± 2.2	0.095
Irisin (ng/mL)	**22.4 ± 0.7**	**25.7 ± 1.1**	**0.013**
Leptin (ng/ml)	31.2 ± 3.7	27.9 ± 2.0	0.42
Adiponectin (µg/ml)	**15.2 ± 1.5**	**9.5 ± 0.9**	**0.001**

Data are expressed as mean ± SEM. Comparison between populations was performed by ANOVA test. Significance is shown in bold characters.
OGTT, Oral Glucose Tolerance Test; $OGTT_0$ and $OGTT_{120}$, OGTT at 0 and 120 min; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model of insulin resistance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transpeptidase; TG, triglycerides CHO, total cholesterol; LDL CHO, low density lipoprotein cholesterol; HDL CHO, high density lipoprotein cholesterol.

Following OGTT, abnormal glucose metabolism was detected in 2 children with PWS (8%) and 4 controls (16%): impaired fasting glucose was found in 1 subject of the control group and no patient with PWS while impaired glucose tolerance was found in 2 patients with PWS and 3 controls. No cases of type 2 diabetes mellitus (T2DM) were diagnosed in either group.

Anthropometric parameters differed between groups, and subjects with PWS showed lower values of FFM ($p < 0.05$), WC ($p < 0.005$) and waist-to-hip ratio ($p<0.05$) with respect to controls.

Children and adolescents with PWS harbored lower insulin $OGTT_0$ ($p < 0.0001$) and insulin $OGTT_{120}$ levels ($p<0.05$) than controls. PWS group also showed lower fasting C-peptide levels ($p<0.0001$), lower insulin resistance expressed as HOMA-IR ($p<0.0001$) and higher adiponectin levels ($p<0.005$) compared to controls (**Table 2**). There were no differences in lipids and liver enzymes between populations.

Analysis of circulating irisin showed measurable levels in all cases. As illustrated in **Figure 1**, the distribution of irisin levels was somewhat more dispersed around the mean in patients with PWS than controls, with the former exhibiting lower concentrations of irisin. When only obese subjects were compared, mean circulating irisin was also significantly lower in the subgroups of obese PWS patients that in obese controls (21.8 ± 1.6 vs 25.9 ± 1.1 ng/ml, $p<0.05$). In more detailed analysis, irisin levels of PWS patients with DEL15 were significantly reduced compared with controls with common obesity (21.8 ± 0.8 ng/ml and 25.8 ± 1.1 ng/ml, $p=0.018$) while PWS patients with UPD15 did not show significant differences compared with controls (23.3 ± 1.0 ng/ml and 25.7 8 ± 1.1 ng/ml, $p=0.2$).

In gender-based analysis, irisin levels did not differ between males and females in PWS (22.08 ± 0.8 and 23.07 ± 1.1 ng/ml, $p=0.5$) and controls (27.1 ± 2.4 and 24.0 ± 1.4 ng/ml $p=0.07$). Analysis by pubertal stage showed no difference in irisin levels between prepubertal and postpubertalsubjects both in the PWS (22.1 ± 0.8 vs 22.9 ± 1.1, $p=0.5$) and control group (26.4 ± 1.5 vs 24.9 ± 1.1, $p=0.5$).

Correlation analysis in separate groups showed no significant association between irisin and the study variables. In merged datasets (**Table 3**), positive associations linked irisin to PWS status, insulin $OGTT_0$ and insulin $OGTT_{120}$ levels, HOMA-IR and fasting C-peptide levels.

After controlling for age, sex and BMI SDS, correlation remained significant between irisin and insulin $OGTT_0$ ($r=0.345$,

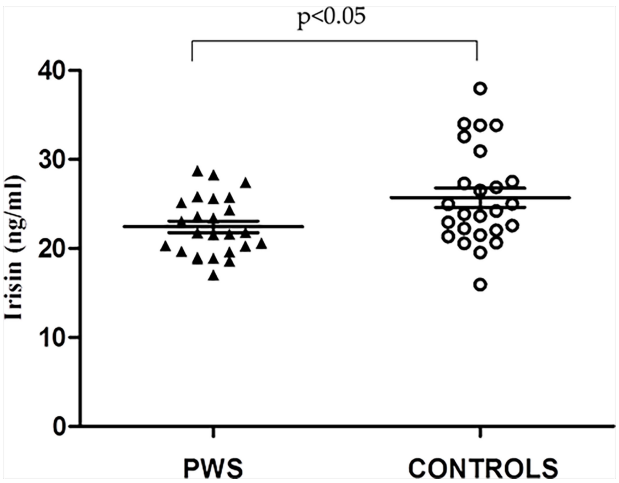

FIGURE 1 | Individual values of circulating irisin levels obtained in Prader Willi Syndrome (PWS) patient (black triangles) and controls (white circles). Lines represent mean ± standard error of the mean (SEM) values.

TABLE 3 | Pearson's correlation analysis between irisin levels and anthropometric and biochemical parameters in the study populations as a whole.

Parameters	Irisin levels	
	r	P value
Age	0.166	0.25
PWS status	**-0.35**	**0.013**
BMI SDS	- 0.06	0.69
FM (%)	- 0.10	0.56
FM (kg)	0.01	0.63
FFM (kg)	0.22	0.20
FFM (%)	0.10	0.60
Glucose $OGTT_0$ (mg/dL)	0.18	0.20
Glucose $OGTT_{120}$ (mg/dL)	0.11	0.47
Insulin $OGTT_0$ (mIU/L)	**0.30**	**0.042**
Insulin $OGTT_{120}$ (mIU/L)	**0.58**	**0.004**
HOMA-IR	**0.30**	**0.045**
C- Peptide (µg/L)	**0.30**	**0.034**
$HbA1_C$ (%)	-0,084	0,57
Leptin (ng/ml)	0.17	0.25
Adiponectin (µg/ml)	- 0.201	0.16

For PWS status: PWS = 1, obese control = 0. Significance is shown in bold characters. BMI SDS, body mass index standard deviation score; FM, fat mass; FFM, fat-free mass. OGTT, Oral Glucose Tolerance Test; $OGTT_0$ and $OGTT_{120}$, OGTT at 0 and 120 min; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model of insulin resistance.

TABLE 4 | Regression coefficients derived from the stepwise multivariable regression analysis conducted in the PWS subjects and obese controls as a whole on irisin as the dependent variable.

Multivariable regression analysis	Included variables			Excluded variables
		β	P value	
Model 1	Insulin $OGTT_{120}$	0,58	0,005	group, BMI SDS, Glucose $OGTT_0$, Glucose $OGTT_{120}$, Insulin $OGTT_0$, C-Peptide, HOMA-IR, leptin, adiponectin
Model 2 (Insulin $OGTT_{120}$ not included)	Group	-0,35	0,025	BMI SDS, Glucose $OGTT_0$, Glucose $OGTT_{120}$, Insulin $OGTT_0$, C-Peptide, HOMA-IR, leptin, adiponectin.

Group (PWS = 1, obese controls = 0) was introduced as independent variable in all models. Other independent variables introduced in the two models: model 1: group, BMI SDS, Glucose OGTT0, Glucose OGTT120, Insulin $OGTT_0$, Insulin $OGTT_{120}$, C-Peptide, HOMA-IR, leptin, adiponectin; model 2: BMI SDS, Glucose OGTT0, Glucose $OGTT_{120}$, Insulin $OGTT_0$, C-Peptide, HOMA-IR, leptin, adiponectin. β standardized coefficients and p values are shown.

HOMA-IR, homeostatic model of insulin resistance; OGTT, Oral Glucose Tolerance Test; $OGTT_0$ and $OGTT_{120}$, OGTT at 0 and 120 min.

p<0.05), insulin $OGTT_{120}$ (r=0.622, p<0.005), HOMA-IR (r= 0.338, p<0.05) and C-peptide (r=0.325, p<0.05). When controlled for groups (PWS and non-PWS), only the correlation between irisin and insulin $OGTT_{120}$ was maintained (r = 0.45, p= 0.041).

Stepwise multivariable regression analysis was performed in the two groups as a whole, and a number of models were explored to analyze the predictors of irisin levels in the study populations. As shown in **Table 4**, analysis documented that irisin levels were only predicted by insulin $OGTT_{120}$ levels (β =0.58, p=0.005). After its removal from the regression equation, the PWS group acted as independent negative predictor of irisin levels (β =-0.35, p=0.025). No variable entered the regression equation in analyses on separate groups.

DISCUSSION

The present study analyzed circulating irisin levels in children and adolescents with and without PWS in relation to metabolic profile and body composition. Current results show that irisin levels are lower in PWS than in controls independent of BMI, and that a strong association links irisin to measures of insulin resistance, particularly insulin $OGTT_{120}$ levels.

It is well known that clinical features of PWS include muscle hypotonia, endocrine disturbances, childhood-onset obesity, and peripheral adiposity (4).

Although this adverse phenotype, obese children and adolescents with PWS have a milder metabolic derangement compared to their BMI-matched controls (11, 45, 46). In particular, PWS children show lower insulin and C-peptide levels, lower insulin resistance, as well as higher adiponectin and HDL cholesterol levels (47). Our study confirmed these peculiarities: in patients with PWS, in fact, we found significantly lower fasting and post-OGTT insulin levels, C-peptide and HOMA-IR together with higher levels of adiponectin with respect to controls matched for BMI SDS, confirming better insulin sensitivity. In addition, HDL cholesterol levels in PWS patients appear to be slightly higher, also not significantly, than in controls.

Irisin, a myokine that induces the browning of WAT and regulates the transcription of thermogenic genes (16), has recently attracted attention for its potential role in obesity and metabolic syndrome *via* regulation of WAT accumulation and body weight control (48).

In children, the direction of the relationship between irisin levels and obesity is debated (25), with some authors reporting unaltered or even decreased irisin levels (28, 48), and others showing higher irisin levels in obese children as compared with healthy controls (28, 35, 49). Potential reasons for such discrepancy involve the role of peripubertal development on the interplay between fat mass, muscle, and fat/muscle mass ratio (25), as well as the potential for irisin to be secreted as a myokine in condition of healthy body weight (16, 50, 51) and as an adipokine in condition of fat accumulation (17, 20, 23).

In PWS, irisin has been primarily studied in adults and its levels have been reported to be lower than in BMI-matched obese controls, while being similar to those observed in normal weight subjects (20, 38, 39). In our population, we found that obese PWS children had lower irisin levels than controls with common obesity, a finding that parallels our previous results in PWS adults (20). Although our study confirmed significant differences in lean mass even in young PWS subjects as compared to their control counterpart, the lack of relationship between irisin and lean mass suggests the negligible role for the latter to explain differences in circulating irisin between groups. In a gender based analysis, irisin concentrations did not appear to differ between

genders both in PWS and controls. While previous studies in children found higher irisin levels in girls, either lean or obese, than boys (22, 30), this difference has not been confirmed in subsequent studies in overweight or obese children (26, 35, 37). Analysis by the pubertal stage showed no difference in irisin levels between prepubertal and postpubertal children with PWS and in controls, which agrees with results from previous studies (28, 52). In particular, pediatric PWS with DEL15 have significantly reduced levels of irisin compared with controls with common obesity. Our results obtained in a young and homogenous populations, confirm previous finding by Faienza at al., who highlighted a potential role for PWS genotype, particularly DEL15 genotype, in decreasing irisin levels significantly in comparison to controls (40). Also, it is to be noted that a previous study showed that different genetic subtypes in PWS had also different endocrine characteristics, for instance growth hormone secretion (15).

Noteworthy, irisin partakes in the regulation of glucose homeostasis and insulin sensitivity by promoting glucose uptake and glycogenolysis, and by reducing gluconeogenesis (18, 34). In children and adolescents, many but not all authors described positive associations between irisin and insulin or glucose levels, and insulin resistance (26,31, 35, 53). In PWS and non-PWS children, our results support evidence of a significant positive association between circulating irisin level and several measures of insulin metabolism, including levels of levels and post-OGTT insulin, fasting peptide-C, and HOMA-IR and expand to the pediatric age previous observations obtained in adults with PWS (20). Insulin $OGGT_{120}$ was the only predictor of irisin levels in the groups as a whole, suggesting a tight and independent association between irisin and post-absorptive insulin secretion. Noticeably, this relationship remained significant even after adjustment for potential confounders such as age, gender, and BMI, confirming previous similar observations (54). Based on evidence that irisin acts as an insulin-sensitizing hormone, facilitates liver and muscle glucose metabolism, and promotes β-cell survival (55), it is possible that irisin reflects a compensatory response to insulin resistance and initial deterioration of glucose tolerance. As such, divergences in irisin between PWS and non-PWS subjects reflect differences in insulin sensitivity, as confirmed by divergences in levels of adiponectin, an adipokine known for its insulin-sensitizing effects (10). Further, Reinher and coworkers reported higher irisin level in obese children with impaired glucose tolerance compared to obese children with normal glucose tolerance and normal-weight children (31). This implies that the lower irisin levels seen in PWS reflects their healthier glucose homeostasis as compared to obese controls, while the association documented between irisin and post-OGTT insulin levels could reflect its association with hyperinsulineamia and insulin resistance. Alternatively, Sesti et al. hypothesized that a decrease in insulin clearance could be the mechanism underlying higher irisin levels in individuals with increasing post-OGTT insulin levels (54), in the attempt to reduce β-cell stress due to increasing insulin resistance (53). Whether these mechanisms are responsible for current observations in children with and without PWS warrant further investigation. Together, we hypothesize that irisin is an active participant in the regulation of insulin resistance rather than an innocent by-stander. It is conceivable that irisin secretion increases in obesity to maximize energy usage and glucose homeostasis, so as to achieve metabolic balance and compensate for the underlying irisin resistance (18, 56).

Some limitations should be acknowledged in our study. First, it was based on cross-sectional analysis and so it is not possible to provide information on changes in irisin levels possibly linked to modifications of clinical parameters such as weight and metabolic balance, therefore, no conclusion regarding cause–effect relationships can be made. The HOMA model is only an assessment of insulin resistance. Clamp studies are actually the "gold standard" for analyzing insulin resistance. However, validation studies demonstrated a good correlation between HOMA and clamp techniques in young participants (57).

Notwithstanding these limitations, this study enrolled a consistent sample of PWS children that were extensively characterized in terms of anthropometric, clinical and metabolic profiling. Also, irisin assessment was carried out with what is currently considered as the best available irisin immunoassay (18).

Together, our findings suggest that circulating irisin levels are lower in PWS compared to BMI-matched children possibly due to differences in body composition and insulin resistance. The potential influence of a genetic component associated with PWS cannot be, however, entirely excluded and needs further assessment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Research Committees of the Bambino Gesù Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Conceptualization, SM and PM; methodology, SM, RV, AlC, and SB; formal analysis, SM and PM; investigation, SM and DF; resources, DF, AnC, and MS; data curation, DF, SB, AlC; writing—original draft preparation, SM; writing—review and editing, DF, CM, MS, GG, GA, AnC, and PM; supervision, PM and MS. All authors have read and agreed to the published version of the manuscript.

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Adrenal insufficiency in patients with Prader-Willi syndrome

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The generalized dysfunction of the hypothalamic-pituitary axis in patients with Prader-Willi syndrome (PWS) is the most likely cause of hypogonadism, inadequate growth hormone secretion, excessive appetite and associated obesity, impaired body temperature regulation, and hypothyroidism. The syndrome is also related to an increased risk of central adrenal insufficiency, although its prevalence remains unknown. The results of the studies in which different methods of pharmacological stimulation were used do not provide conclusive outcomes. As a result, there are no clear guidelines with regard to diagnosis, prevention, or long-term care when adrenal insufficiency is suspected in patients with PWS. Currently, most patients with PWS are treated with recombinant human growth hormone (rhGH). It has been confirmed that rhGH therapy has a positive effect on growth, body composition, body mass index (BMI), and potentially on psychomotor development in children with PWS. Additionally, rhGH may reduce the conversion of cortisone to cortisol through inhibition of 11β-hydroxysteroid dehydrogenase type 1. However, its influence on basal adrenal function and adrenal stress response remains unexplained in children with PWS. This paper reviews the literature related to the hypothalamic-pituitary-adrenal axis dysfunction in the PWS patient population with a focus on children.

KEYWORDS

PWS, Prader-Willi syndrome, adrenal insufficiency, Synacthen, LDSST

Introduction

Prader-Willi syndrome (PWS), which was first described in 1956 (1), is caused by the loss of paternally expressed imprinted genes at chromosome 15q11-q13. One in 10000-30000 live-born neonates is diagnosed with this condition. Due to the phenomenon of genomic imprinting, genes located in this region are expressed only on the paternally-derived chromosome, while the same genes on the maternally-derived chromosome undergo methylation and are not expressed and therefore are silenced. Deletion of a fragment of the paternal chromosome (60% of cases) is the most common mechanism for the loss of expression, followed by disomy of the maternal chromosome (35%) and disruption of the imprinting process, which rarely occurs (up to 5%) (2). The loss of

expression of the paternally-derived genes leads to the occurrence of PWS. DNA methylation testing can detect 99% of cases (3).

Many physical, psychological and behavioral complications are associated with the course of the disease. Clinical manifestations are age-dependent (4), but not gender-dependent. In addition, the syndrome is the leading genetic cause of childhood obesity. Life expectancy depends on the age of diagnosis, the initiation of multi-specialty medical care and appropriate treatment, as well as the occurrence, course, and severity of complications. However, if the weight is well regulated, patients with PWS can reach the seventh decade (5).

The generalized dysfunction of the hypothalamic-pituitary axis is the likely basis for many symptoms and complications associated with PWS. In the life-threatening context, the hypothalamic-pituitary-adrenal (HPA) axis is of crucial importance. Symptoms of central adrenal insufficiency (CAI) are mainly due to glucocorticoid deficiency, which may manifest as reduced weight gain or prolonged jaundice in newborns. General weakness, recurrent infections, headache, muscle and joint pain are reported in older children. In the case of partial glucocorticoid deficiency, the disease may be asymptomatic and manifest only in stressful situations. Underestimation and underdiagnosis of CAI in the context of PWS patients (and children in particular) may lead to severe consequences. Temperature instability is common, with a tendency toward lower body temperature when compared to general population. Fever may be absent even during severe infection (2). This, combined with a decreased cortisol response in stressful situations in CAI patients, can lead to recurrent and life-threatening infections, often with a need for hospitalization, especially since it has been reported that up to 44% of death cases in PWS are due to respiratory tract infections (6). On the other hand, it is well known that glucocorticoid excess, which may be caused by chronic supplementation with glucocorticoids in cases of CAI overdiagnosis, may lead to decreased bone mineral density, osteoporosis, weight gain, hypertension, or immunosuppression. Additionally, it may also lead to hyperglycemia with insulin resistance, which may also be potentially enhanced by the above-mentioned growth hormone treatment therapy. The effect of isolated hypothalamic or pituitary dysfunction on the production of mineralocorticoids, including aldosterone, is limited, and electrolyte disturbances are usually not reported (6).

The diagnosis of CAI is based on the determination of adrenocorticotropic hormone (ACTH) and cortisol levels in the morning. CAI can be suspected when the cortisol level is low (< 83 nmol/L), the ACTH level is normal or decreased, and the electrolyte level is normal. An HPA-axis stimulation test is crucial for establishing the diagnosis. In the context of PWS patients, the choice of the test depends on the experience and capabilities of the research center and the availability of various stimulation methods, e. g. metyrapone is not available in the US at all. It requires overnight hospitalization and increases the risk of adrenal crisis, while the insulin tolerance test (ITT) is related to the risk of significant hypoglycemia, especially in children at risk such as PWS patients. Given the considerable safety profile, corticotropin analogs such as tetracosactrin (Synacthen®), which is a synthetic ACTH, are recommended as first-line stimulation tests, especially the low-dose tetracosactrin stimulation test (LDSST) (1 μg), which is usually very well tolerated (7).

The aim of this study was to review the literature related to the HPA axis disorders in the context of improving the quality of medical care for PWS patients. The aim of the authors was to provide up-to-date information on the prevalence and risks of CAI in PWS patients with a focus on children. Recommendations for CAI screening in PWS children are provided together with the dosage in hydrocortisone replacement therapy when it is detected. The authors believe this may lead to a better understanding of the presented issue among endocrinologists and general practitioners and to a better medical care of PWS patients, especially during illness or when hospitalization is indicated.

Materials and methods

The literature review was based on the available papers published in the PubMed database between 1963 and June 2021. The search criteria used in the Medical Subject Headings (MeSH) were as follows: “Prader-Willi” [All Fields] AND (“adrenal glands”[MeSH Terms] OR (“adrenal”[All Fields] AND “glands”[All Fields]) OR “adrenal glands”[All Fields] OR “adrenals”[All Fields] OR “adrenalitis”[All Fields] OR “adrenally”[All Fields] OR “epinephrine”[MeSH Terms] OR “epinephrine”[All Fields] OR “adrenal” [All Fields])”.

Only original papers in English were analyzed. Case reports or literature reviews were not included in the review.

Results/analysis of selected papers

Based on the criteria, 65 potentially relevant papers were identified. After analyzing the titles, abstracts and whole papers, ten articles (8–17) that met the criteria were included in the detailed analysis. The excluded papers included systematic reviews, case reports and papers that did not provide adequate insight regarding the prevalence, diagnosis or treatment of CAI.

The ten studies performed between 2008 and 2020 presented the results of dynamic tests which were performed to assess the risk of CAI. A total of 445 patients with PWS were enrolled, including 244 children. The studies consisted in the use of stimulation tests and the consecutive assessment of ACTH and/or cortisol secretion. The Synacthen test was used in 5 studies, the metyrapone test was used in 3 studies, the insulin test

was used in 3 studies and the glucagon test (GT) in a single study. In the case of an abnormal test result, a confirmatory test was performed in 3 studies (the Synacthen test was used twice, while the metyrapone test was used once). Table 1 shows cortisol and ACTH levels, the diagnostic criteria adopted by the investigators in the study protocol and the number of patients diagnosed with CAI in a given study. In total, CAI was diagnosed in 38 (8.5%) patients, including 33 children. In this group, the metyrapone test was used in 29 patients, the Synacthen test was used in eight subjects, while the ITT was used in a single patient. Information about the protocols of the stimulation tests with the conclusions drawn by the investigators is given in Table 1.

Discussion

De Lind van Wijngaarden et al. performed one of the first studies on CAI in 2008. Overnight single-dose metyrapone tests were performed in randomly selected 25 children who were in a pediatric intensive care unit. Because metyrapone inhibits cortisol synthesis, it causes an increased demand for ACTH production, a situation mimicking stress. Based on the above and the fact that salivary cortisol levels were considered normal, de Lind van Wijngaarden et al. suggested that CAI became apparent only during stressful conditions when an increased demand for cortisol occurred. Salivary cortisol levels were found not to be useful in identifying PWS patients at risk of CAI. All patients with PWS should be treated with hydrocortisone during stressful situations if it is not possible to exclude CAI using the metyrapone test (12).

A similar study was conducted one year later. It was based on the assessment of the prevalence and severity of the sleep apnea syndrome during sleep. Poorer results were found, especially in children with CAI, which could confirm a common cause of the abnormality in the form of hypothalamic dysfunction. The authors found no significant differences in ACTH or cortisol levels between children who were later diagnosed with CAI and those who were not diagnosed with it (15).

In turn, in 2010, Nyunt et al. conducted their study on a group of 41 children with PWS. Following Synacthen administration, a normal response to stimulation was reported in all children. According to Nyunt et al., a high percentage of patients with CAI in a study conducted by de Lind van Wijngaarden et al. could be explained by adopting the level of ACTH, which is an unstable compound, as a criterion for the diagnosis of CAI. It would be necessary to extend the diagnosis of CAI by performing an overnight metyrapone test or the insulin-induced hypoglycemia test (10).

In 2011, Corrias et al. conducted a study on 84 children with PWS. In the first phase, decreased cortisol levels were found in 12 patients as a result of the low-dose tetracosactrin stimulation test (LDSST). This test was repeated in one patient. The test with a standard dose was performed in nine patients. Finally, CAI was confirmed in 4 patients. Corrias et al. recommended the LDSST and the assessment of morning cortisol and ACTH levels as the element of the primary diagnosis of the function of the HPA axis in children with PWS. The morning cortisol level was found to be of poor diagnostic value in diagnosing CAI even though basal cortisol levels were lower in the CAI group, but in both groups the values largely overlapped. Corrias et al. did not provide clear cut-off values indicating the need for further investigation with a LDSST. Substitution treatment with hydrocortisone seems to be warranted in moderate and severe stressful situations in all subjects with PWS who were not tested or were diagnosed with CAI (8).

In 2013, Grugni et al. conducted a study on 53 adults with PWS. The research plan was similar to the one in the above study. In the first phase, decreased cortisol levels were obtained after stimulation in eight patients. The tests were repeated and four patients showed persistent suboptimal responses. Grugni et al. stressed the recommendation to test each PWS patient for CAI. However, according to them, caution should be employed about establishing a diagnosis of CAI based solely on a single stimulation test. Once a decreased cortisol level is obtained using the low-dose short Synacthen test (LDSST) and in the case of no contraindications, the insulin-induced hypoglycemia test or the metyrapone test should be confirmatory. It is also possible to repeat LDSST or perform tests using the standard dose of Synacthen. Grugni et al. speculated that if LDSST had been performed in all patients with the basal cortisol cut-off of <250 nmol/l, no patient with CAI would have been missed. However, basal cortisol is overall a poor predictor of potential CAI (9).

In 2011, Farholt et al. performed a study in which all patients had a normal response to stimulation. Sixty-five patients with PWS were examined using the standard Synacthen test or the ITT. As opposed to de Lind van Wijngaarden et al., Farholt et al. were of the opinion that routine prophylactic treatment with hydrocortisone during acute illness in PWS was of concern due to many adverse effects such as obesity and hypertension. They recommended such treatment if it was clinically indicated. They found no significant gender effect on cortisol levels during stimulation (11).

In 2015, V. Beauloye et al., retrospectively reviewed cortisol response in 20 PWS children following either an ITT or a GT. Only a single patient showed insufficient cortisol response after ITT. Basal cortisol levels did not differ significantly between children with PWS and the controls. An overnight polysomnographic study was also conducted, but the results did not support the hypothesis of an increased risk of the sleep apnea syndrome in PWS as suggested by R. F. De Lind Van Wijngaarden et al. The link between CAI and the cases of sudden deaths in PWS was considered unlikely (15, 17).

In 2017, Obrynba et al. conducted a study on 21 patients with PWS. All patients underwent the Synacthen test, followed

TABLE 1 Summary of the protocols and the results of the stimulation tests used by the investigators.

Study (author; year; citation)	Patients(n; department; age)	Morning cortisol level prior to testing	Method(s) (test/dose)	Level considered normal (min cut-off)	Diagnosis of CAI	Conclusions	Authors' further comments
R. F. De Lind Van Wijngaarden et al., 2008 (12)	25 children hospitalized in the ICU	Median value of157.2 nmol/l (52.4–446.9) considered normal by the authors	A single metyrapone test (30 mg/kg)	ACTH ≥ 33 pmol/l at 0730 h	15 (60%)	A high prevalence of CAI in PWS may explain cases of sudden deaths, especially during infection.	CAI can develop over time. Therefore, a single normal test result does not rule out the occurrence of CAI in the future. Further studies are warranted.
R. F. De Lind Van Wijngaarden et al., 2009 (15)	20 children hospitalized in the ICU	Not assessed	A single metyrapone test (30 mg/kg)	ACTH ≥ 33 pmol/l at 0730 h	13 (65%)	Increased risk of the sleep apnea syndrome in PWS, especially in diagnosed CAI.	Changes in respiratory parameters can be even greater in stressful situations than during stimulation tests.
O. Nyunt et al., 2010 (10)	41 children	Median value of 223 (± 116) nmol/l considered normal by the authors	Synacthen test(1 μg)	Cortisol > 500 nmol/l measured at 30 min	0	Results contrary to the above studies; unstable level of ACTH in the blood sample which is subject to greater changes compared to cortisol.	It would be necessary to extend the diagnosis of CAI by performing the metyrapone test or the insulin-induced hypoglycemia test.
S. Farholt et al., 2011 (11)	65 patients (including 18 children < 17 years of age)	Median value of 194 (58-1020) nmol/l considered normal by the authors	Synacthen test in 57 patients (0.25 mg/m^2) and the insulin tolerance test in 8 patients	Cortisol > 500 nmol/l measured at 30 min or increase in cortisol of ≥ 250 nmol/l	0	CAI rare in PWS in adults and children. Routine prophylactic treatment with hydrocortisone should be performed only if clinically indicated.	No significant gender effect on cortisol levels during stimulation.
A. Corrias et al., 2011 (8)	84 children	Median value of 341.7 (± 172.8) nmol/l considered normal by the authors	First, Synacthen test (1 μg), followed by the confirmatory Synacthen test (0.25 mg/m^2).	Cortisol > 500 nmol/l measured at 30 min or increase in cortisol of ≥ 250 nmol/l.	4 (4.8%)	Recommended tests: LDSST and the assessment of morning cortisol and ACTH levels in all children with PWS as the element of the primary diagnosis of the function of the HPA axis.	In the case of normal stimulation tests, a repeat diagnosis should be performed periodically. However, further studies are warranted to determine the frequency of diagnostic procedures.
G. Grugni et al., 2013 (9)	53 adult patients	Median value of 336.6 (± 140.7) nmol/l considered normal by the authors	First, Synacthen test (1 μg), followed by the confirmatory Synacthen test (0.25 mg/m^2)	Cortisol ≥ 500 nmol/l measured at 30 min	4 (7.5%)	Maintaining the above recommendations. A regular repeat diagnosis every 2 - 4 years is recommended.	Cooperation between centers and the development of diagnostic standards for CAI in PWS are essential to obtain more reliable data.
V. Beauloye et al., 2015 (17)	20 children	Considered normal by the authors; exact numerical values were not provided	The insulin tolerance test in 6 patients, glucagon test	Cortisol > 550 nmol/l measured at 30 min or increase	1 (5%)	CAI is rare in PWS.Increased risk of the sleep apnea	The study has methodological limitations due to its retrospective

(Continued)

TABLE 1 Continued

Study (author; year; citation)	Patients(n; department; age)	Morning cortisol level prior to testing	Method(s) (test/dose)	Level considered normal (min cut-off)	Diagnosis of CAI	Conclusions	Authors' further comments
			in 13 patients, both tests in a single patient	in cortisol of > 250 nmol/l		syndrome in PWS was not confirmed.	nature and multicenter data collection.
Kathryn S. Obrynba et al., 2017 (14)	21 patients aged 4-53 years	Not assessed	Synacthen test (1 μg/m^2, max. 1 μg) followed by the metyrapone test (30 mg/kg, max 3g)	Synacthen test: cortisol ≥ 427.6 nmol/l;The metyrapone test:11-deoxycortisol ≥ 200 nmol/l at 0800 h, regardless of the cortisol level	0	Clinically significant CAI is rare in PWS.The metyrapone test is a preferred method for the diagnosis of CAI in PWS.	Metyrapone is poorly available.The test requires close observation to monitor for potential adverse effects.
Yuji Oto et al., 2018 (16)	36 children	Median value of496.6 (391.7 – 653) nmol/l considered normal by the authors	Insulin tolerance test (0.1 IU/kg)	Morning level of ACTH 1.1-11 pmol/land cortisol > 165.6 nmol/lPeak (stimulation) ACTH level > 11 pmol/l and peak cortisol > 499.3 nmol/l or increase in cortisol by ≥ 9.1 μg/dl (251 nmol/l)	0	Delayed peak cortisol level in PWS compared to the controls -probably due to the hypothalamic dysfunction.	Further studies are warranted to confirm the findings.
AGW Rosenberg et al., 2020 (13)	82 adult patients	Median value of 325.5 (126.0 – 764.0) for the metyrapone test;median value of 172.5 (93.0 – 545.0) nmol/l, 185.5 (175.0 – 265.0) nmol/l, 233.0 (119.0 – 502.0) nmol/l, 229.0 (102.0 – 384.0) nmol/l for the ITT, depending on the center; all values considered normal by the authors	The multiple-dose metyrapone test (750 mg) or the insulin tolerance test (0.15 IU/kg)	Metyrapone test - 11-deoxycortisol > 230 nmol/l;insulin test – cortisol > 500 nmol/l, > 450 nmol/l for the British patients	1 (1.2%)	CAI is rare in adults with PWS. Routine treatment with hydrocortisone is not recommended in stressful situations or perioperatively.	Only 28% of patients were treated chronically with rhGH. Growth hormone deficiency can potentially mask adrenal insufficiency.

by the metyrapone. A third of patients who underwent the Synacthen test had peak cortisol below the level considered normal. However, one subject of the 21 patients had an inconclusive result of an overnight metyrapone test. This patient had normal stimulation test results in the first test. The authors found no difference in mean peak cortisol during the LDSST between patients treated with GH and those who did not undergo such treatment. According to Obrynba et al., the Synacthen test may lead to overdiagnosis of CAI, and the metyrapone test is the preferred diagnostic method. However, it is not widely available and requires close observation to monitor for potential adverse effects of transient adrenal insufficiency (14).

In 2018, Oto et al. conducted a study on 36 PWS children using ITT. None of the subjects was diagnosed with CAI. However, two-thirds showed delayed peak cortisol levels compared to the controls. According to Oto et al., the delay related to the response to ITT may signify the existence of a central obstacle in adjustment of the HPA axis of unknown origin and is related to latent CAI. Higher basal cortisol was observed in PWS patients when compared to control children. However, the difference was insignificant. Further research is required on the possible relationship between the altered pattern of cortisol secretion and hypoglycemia and cases of sudden death in PWS (16).

In their most recent study, Rosenberg et al. (2020) (13) investigated 82 adult patients with PWS. The multiple-dose metyrapone test was given to 46 subjects. Thirty-six patients underwent ITT. None of the patients underwent two tests. An abnormal response indicating CAI was found in one patient. Rosenberg et al. stressed that in the study population only 28% of subjects were treated chronically with rhGH. While growth hormone deficiency can potentially mask adrenal insufficiency, CAI is almost non-existent in adult PWS patients.

As presented, the authors of the above studies did not provide clear morning cortisol cut-offs to perform stimulation tests. These tests were performed in patients with basal cortisol considered normal by the authors. As mentioned before, basal cortisol is of limited value in detecting potential CAI cases because of its low sensitivity and specificity.

The analysis of stimulation tests shows that a high percentage of CAI in PWS patients was found by de Lind van Wijngaarden et al. (28 out of 38, 73.6%), most of whom (33; 86%) were children. There could be an increased annual sudden death rate in PWS patients with non-detected CAI and more severe cases do not make it to adulthood. However, the percentage of CAI cases in PWS subjects was much lower in research performed later. Four studies involving a total of 163 PWS patients, including 95 children, found that CAI was not diagnosed in any subject (10, 11, 14, 16). The differences in the results may be due to the type of a stimulation test and different diagnostic criteria. As mentioned above, the criteria for the diagnosis of CAI adopted by de Lind van Wijngaarden et al. were based on the concentration of ACTH, which is highly unstable after collection due to proteolytic degradation (18). In addition, there are some doubts about the ACTH concentration accepted as normal. According to some authors, the adopted cut-off point is too high, which leads to overdiagnosis of CAI (10, 13). Furthermore, according to Rosenberg et al., a single-dose metyrapone test has limited diagnostic usefulness due to the short suppression time of the HPA axis, which does not give sufficient time for the increase in adrenal steroid production. The metyrapone test was also carried out by Obrynba et al. in 2017. The study also included children, but CAI was not diagnosed in any patient. However, 11-deoxycortisol levels were used to determine cut-off values for CAI diagnosis (14). If we compare the studies of Corrias et al. (2011) and Grugni et al. (2013) on two different groups (children and adults, respectively) CAI was diagnosed more frequently in adults than in children (7.5% vs 4.8%) (8, 9). It is unclear whether the differences are related to the age of the patients or the adopted stimulation test and cut-off points.

Early detection of CAI is crucial due to the need for the rapid introduction of hydrocortisone replacement therapy. As mentioned earlier, glucocorticoid deficiency can be associated with many adverse effects and complications, which may not be found in non-stressful situations. Furthermore, many authors have suggested that some deaths could potentially be related to undetected CAI.

Due to the differences in the study results, no clear guidelines have been developed for the diagnosis, management, or long-term care of patients with PWS or CAI. Some centers inform patients with PWS of the possibility of adrenal insufficiency and recommend low-dose hydrocortisone treatment in the home setting when the infection starts or exacerbates (19). However, Heksch et al. recommended screening tests for CAI before major surgery and glucocorticoid replacement of 30-50 mg/m^2/day divided three times daily during mild or moderate insufficiency, and 75-100 mg/m^2/dose given immediately prior to surgery or anesthesia. However, the authors did not recommend specific tests and reported that the selection of the optimal test remained unclear (20). The insulin test is considered the gold standard for diagnosing adrenal insufficiency (21). However, it is related to the risk of significant hypoglycemia, especially in children at risk. In turn, the metyrapone test increases the risk of adrenal crisis (8). Furthermore, some studies suggest a high number of false-positive results when the Synacthen test is used, although it is considered an alternative to the insulin test (14). The type of genetic defect does not seem to affect the risk of CAI (12).

There is also an important issue of different cortisol assays used in laboratories. Newer cortisol assays have much improved specificity, and cortisol values are approximately 20% lower when compared to previous assays. Lowering the cortisol response threshold of 500 nmol/l by 20% for CAI detection has been suggested. However, it requires further research. Significant variability is observed depending on the type of cortisol assay used. Therefore, it is recommended to check the reference ranges with the laboratory (22). Since the exact assays used worldwide are currently not standardized as regards CAI, it is still unclear which exact cut-off values should be adopted.

Table 2 includes all stimulation tests that are currently used in the diagnosis of CAI. LDSST is presented as in the Endocrine Society guidelines. Different interpretation values depend on the laboratory assay used (6, 23).

Based on the literature review, primary adrenal insufficiency (PAI) has not been reported in PWS patients. It should be suspected in case of decreased basal cortisol levels with elevated ACTH levels. Standard dose Synacthen stimulation test (SDSST) with 250 µg of tetracosactrin is recommended to confirm the diagnosis (7). Cortisol value of ≥ 500 nmol/l at 0, 30 or 60 min. excludes PAI.

Conclusions

In the 10 studies, CAI was diagnosed in 38 (8.5%) patients, including 33 children. The metyrapone test was performed in 29 subjects, LDSST in 8 patients and ITT in one subject (8, 9, 12, 13, 15, 17). After reviewing the papers included in this literature review and after a detailed review of the advantages and disadvantages of specific stimulation tests, it may seem reasonable to exclude CAI in each patient with PWS due to the likely increased risk of the HPA axis dysfunction, obligatorily when the symptoms that may indicate it are present (Table 3) (7). As mentioned before, it has been speculated that if LDSST had been performed in all patients with the basal cortisol cut-off of <250 nmol/l, no patient with CAI would have been missed (9). However, this value is quite high as it includes cortisol values that are within the normal serum cortisol range. With the newer cortisol assays we recommend mandatory CAI exclusion by

TABLE 2 All stimulation tests used to diagnose central adrenal insufficiency in children (6, 22).

Test	Advantages	Disadvantages	Interpretation of the results
LDSST 1 ug synthetic ACTH/Synacthen	- relatively easily available and simple to perform - quite high sensitivity, an acceptable equivalent of the insulin test - high safety profile	- a potentially high number of a false positive diagnosis of CAI - low specificity, a negative result does not rule out CAI	Cortisol level > 500 nmol/l measured at 30 min rules out the diagnosis of CAI (23)
Insulin test 0.05 – 0.15 U/kg	- high sensitivity and a high diagnostic value - considered the gold standard in the diagnosis of adrenal insufficiency	- the risk of significant hypoglycemia, especially in children at risk - poorly tolerated by patients - necessary experience to perform the test	Cortisol level > 500 (≥ 550 (6)) nmol/l rules out the diagnosis of CAI (23)
Metyrapone test 30 mg/kg	- easy to perform - high sensitivity	- increases the risk of adrenal crisis - limited availability (unavailable in the USA) leads to the lack of standardization - according to some authors, a single test is of limited diagnostic value	ACTH level >17 pmol/l (23) and/or 11-deoxycortisol level >200 nmol/l rules out the diagnosis of CAI (6, 23)

stimulation tests in patients with the basal cortisol cut-off of < 138 nmol/l (5 μg/dL). Basal morning cortisol should be assessed regularly every 3 – 6 months in PWS patients as soon as diagnosis is made. Patients should be closely monitored for CAI symptoms [Table 3] and in case of their occurrence, even despite normal basal cortisol levels, CAI should be excluded by performing stimulation tests.

LDSST is the proposed first-line stimulation test since it is simple to perform and is characterized by a low risk of adverse effects. When the test result does not exclude CAI (cortisol level < 500 nmol/L at 30 min.), the insulin test would be the optimal choice to confirm the diagnosis. It is possible provided the center has adequate experience in its use and there are no clinical contraindications for performing such a test (cardiovascular/neurological conditions, untreated hypothyroidism, glycogen storage disease). The metyrapone test or a repeat LDSST is an alternative to the insulin test.

Currently, most patients with PWS are treated with rhGH and there is no consensus on the need for HPA axis testing before starting the rhGH therapy (24). Such therapy has a positive effect on growth, body composition, BMI, and potentially on psychomotor development in children with the syndrome (25). Additionally, rhGH may reduce the conversion of cortisone to cortisol through inhibition of 11β-hydroxysteroid dehydrogenase type 1. However, the influence of therapy with rhGH on basal adrenal function and adrenal stress response remains unexplained in children with PWS (26). No statistically significant differences were demonstrated between the prevalence of CAI in patients treated with rhGH and those who were not on such therapy (14). In addition, no effect of rhGH therapy was observed on the increased mortality rate in patients with PWS (27), which is estimated at 3% annually (28), and its most common cause is related to exacerbation of respiratory infections (29). In addition, post-mortem studies found below-average-sized adrenal glands in some children, which could indicate undiagnosed subclinical adrenal insufficiency (30).

TABLE 3 Symptoms of adrenal insufficiency in children which indicate the need for the early assessment of hypothalamic-pituitary-adrenal axis function (7).

Clinical symptoms	Laboratory symptoms
- abnormal weight gain, weight loss - vomiting - recurrent infections - excessive fatigue - muscle and joint pain - headache and dizziness	- hypoglycemia - hyponatremia - normokalemia (CAI) - hyperkalemia (chronic kidney disease) - anemia - lymphocytosis - eosinophilia

Hypogonadotropic hypogonadism is typical of PWS patients due to the hypothalamic dysfunction. Adrenarche is clinically defined as the start of pubarche or axillarche with other symptoms such as change in the body odor or acne. Premature adrenarche, which occurs in about 14 - 30% of PWS patients, also indicates HPA dysfunction, but rarely progresses to precocious puberty, although such cases were reported in both sexes (31, 32). It is possible that premature adrenarche is yet another clinical feature of PWS not necessarily connected with hypogonadotropic hypogonadism as their timings do not seem to be correlated (32). Based on this speculation, it seems unlikely that CAI prevalence is correlated with premature adrenarche or pubarche.

- It is recommended that the hydrocortisone dose is increased for up to 3 days then decreased back to the basic replacement dose, but the exact length of increased hydrocortisone dose is dependent on the patient's clinical state.

In children with PWS, it seems reasonable to perform the diagnosis of CAI at least once. It should be repeated if symptoms occur. The proposed hydrocortisone replacement doses are given in Table 4 (7, 20). At present, however, there are no

TABLE 4 Proposed replacement doses of hydrocortisone in children in central adrenal insufficiency, depending on the stressful situation, with the authors' modification (7, 20).

Situation	Daily dose
Basic replacement	7.5 – 15 mg/m^2 in 2 - 4 divided doses
Infection/stress	30 – 50 mg/m^2 in 3 divided doses (20) OR: 2 – 3 times the basic replacement dose*
Surgery, patients in a more severe condition	2 mg/kg before surgery, then following depending on the weight of the patient: - ≤ 10 kg - 25 mg/24h - > 10-20 kg - 50 mg/24h - > 20 kg - 100 mg/24h prepubertal or 150mg/24h pubertal (7) OR: 0 – 3 yrs: bolus of 25 mg + 25 mg/24h in 3 divided doses 3 – 12 yrs: bolus of 50 mg + 50 mg/24h in 3 divided doses > 12 yrs: bolus of 100 mg + 100 mg/24h in 3 divided doses*

universal recommendations on the necessity, method, or frequency of conducting/repeating the diagnostic procedures for CAI in PWS. Additionally, there are no guidelines for replacement. Further research is warranted to develop a generally accepted diagnostic and therapeutic plan. The prevalence and the mechanism of occurrence of CAI in children with PWS are still unexplained. To date, there have been no specific diagnostic criteria for CAI in PWS, and the opinions of different authors about its prevalence are inconclusive.

Author contributions

MK identified potential papers, analyzed selected papers, and took the lead in writing the manuscript. AG supervised the project, assessed the performed analysis and recommended changes, contributed to the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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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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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 cardiopulmonary 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), hypogonadism (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 diagnosing and treating CV problems in this vulnerable patient population.

KEYWORDS

Prader-Willi syndrome, cardiovascular system, comorbidity, heart failure, cardiovascular abnormalities

Introduction

Prader-Willi syndrome (PWS) is a complex genetic disorder caused by the loss of function 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 compositon 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.

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.

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.

Comparison to controls

In Tables 1, 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.

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/m^2) 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

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/m^2)	Type 2 diabetes mellitus	Hyper-tension	Hyperchol-esterolemia	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.

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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 number of controls with the outcome / total number of controls for whom the outcome was known (%) for the group of control patients.

[a] Signs of pulmonary hypertension include: right axis deviation on electrocardiogram, signs of increased systolic right ventricle pressure on echocardiogram (in absence of pulmonary valve stenosis): increased tricuspid regurgitation velocity, enlarged vena cava and/or right ventricle dilatation or hypertrophy. [b] Number of controls differs as a result of missing values.

pressure, were present in most patients. Left ventricle function was usually normal.

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, hypercholesterolemia, 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 untreated. 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

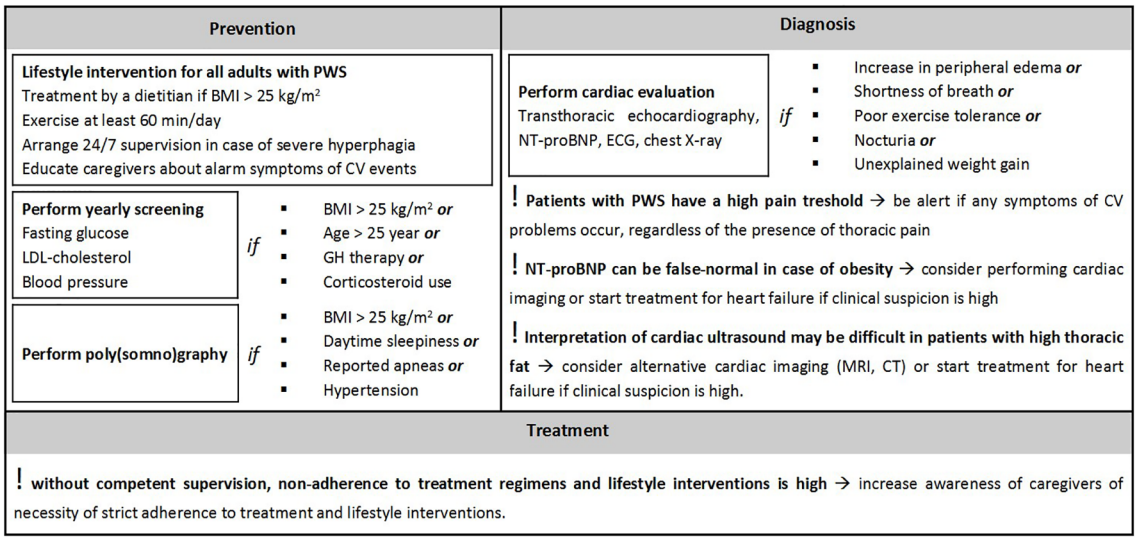

FIGURE 1
Recommendations for prevention, diagnosis and treatment of cardiovascular events in adults with Prader-Willi syndrome. Abbreviations: 24 hours a day, 7 days a week (24/7), body mass index (BMI), computed tomography (CT), cardiovascular (CV), electrocardiogram (ECG), growth hormone (GH), low-density 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).

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 physicial 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 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.

Data availability statement

The datasets presented in this article are not readily available in order to protect the privacy of the patients participating in this study. As Prader–Willi syndrome is a rare syndrome, individual patient data could be traced back to the individual. Requests to access the datasets should be directed to Laura de Graaff, l.degraaff@erasmusmc.nl.

Ethics statement

The studies involving human participants were reviewed and approved by the medical ethical committee of the Erasmus MC. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Conceptualization, KP and LG. Methodology, KP and LG. Formal analysis, KP. Investigation, KP, PW and LG. Resources, LG. Writing—original draft preparation, KP and PW. Writing—review and editing, all authors. Visualization, KP. Supervision, LG

and AL. Project administration, KP and LG. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1145066/full#supplementary-material

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A rare occurrence of non-classic congenital adrenal hyperplasia and type 1 diabetes mellitus in a girl with Prader-Willi Syndrome: Case report and review of the literature

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Prader–Willi syndrome (PWS) is a rare genetic disorder resulting from lack of expression of the paternally derived chromosome 15q11–13, associated with several complications, including pubertal disorders, short stature, hyperphagia, obesity, glucose metabolism abnormalities, scoliosis, obstructive sleep apnea syndrome (OSAS) and behavioral problems. We report the case of a girl affected by PWS who presented at the age of 5.9 with premature pubarche, accelerated linear growth and advanced bone age (BA). She was subsequently diagnosed with non-classic congenital adrenal hyperplasia (CAH) confirmed by genetic analysis. Considering the clinical, biochemical, and genetic findings, hydrocortisone therapy was started to prevent rapid BA acceleration and severe compromission of final height. During infancy, short stature and low levels of insulin-like growth factor-1 (IGF-1) for age and gender led to suspicion of growth hormone deficiency (GHD), confirmed by stimulation testing (arginine and clonidine). rhGH therapy was administered and continued until final height was reached. During endocrinological follow up she developed impaired glucose tolerance with positive markers of β-cell autoimmunity (anti-glutamic acid decarboxylase antibodies, GAD Ab), which evolved over time into type 1 diabetes mellitus and insulin therapy with a basal-bolus scheme and an appropriate diet were needed.

KEYWORDS

Prader-Willi Syndrome, adrenarche, premature pubarche, pubertal development, growth hormone deficiency, congenital adrenal hyperplasia, diabetes mellitus

1 Introduction

1.1 Prader-Willi Syndrome

Prader-Willi Syndrome (PWS) is a rare disease and the most frequent form of syndromic obesity, with a prevalence rate of 1 in 10,000 to 30,000 live births (1, 2). This genetic disorder is caused by the absence of the paternally expressed imprinted genes in the critical region of chromosome 15 at the locus 15q11.2-q13, which can derive from paternal deletion (del15) (65-75% of individuals), maternal uniparental disomy (UPD15) (20-30%), or an imprinting defect (ID15) (1-3%) (1, 2).

PWS is characterized by a multitude of signs and symptoms, many of which possibly linked to hypothalamic dysfunction (3), including: neonatal hypotonia accompanied by failure to thrive, followed by hyperphagia in early childhood and gradual development of morbid obesity, dysmorphic features (characteristic facial appearance with turricephaly, small hands and feet, scoliosis and kyphosis), intellectual disability, behavioral problems and multiple endocrine abnormalities, including GH deficiency (GHD) and hypogonadism (2).

1.1.1 Adrenarche and pubertal development in PWS

Early adrenarche and higher prevalence of premature pubarche (PP) have been described in PWS, although data on prevalence and age of onset are mostly based on case series (4). The estimated prevalence of PP in PWS is approximately 30% in girls and 16% in boys (4). Adrenarche is defined as the increase in production of adrenal androgens, most specifically dehydroepiandrosterone sulphate (DHEA-S) (5). It is a gradual process characterized by the appearance of adult body odor, acne and blackheads, oily hair and pubic and/or axillary hair (5) which usually occurs after the age of 8 years in girls and 9 years in boys. Early adrenarche and PP in children with PWS possibly result from earlier maturation of the zona reticularis of the adrenal glands, independently of hypothalamic-pituitary-adrenal axis activation (5, 6) which is heterogeneous in PWS. Nevertheless, PWS is characterised by a slower pubertal pace, with a higher age at Tanner stage P5 in both sexes. In girls a higher age at menarche with respect to the general population is described, whereas a minority may present with pubertal arrest and/or primary amenorrhea (7, 8). Males with PWS often present hypothalamic hypogonadism, although recently primary testicular dysfunction has been advocated as a major contributor to abnormal pubertal development (9, 10). Although puberty and sexual maturation are usually delayed or incomplete in PWS, rare occurrences of true precocious puberty are described (11, 12).

1.1.2 Diabetes mellitus in PWS

Diabetes mellitus can affects PWS patients and it typically presents with a type 2 (T2DM) phenotype (13), with a reported prevalence of 7-24% (14–16). Nonetheless, rare cases presenting as diabetes type 1 (T1DM), or a monogenic diabetes (maturity onset diabetes of the young, MODY), have also been described (17–19). Furthermore, occasional reports of islet autoantibodies in diabetic PWS subjects were associated with a T2DM phenotype (20). In a group of 23 diabetic PWS patients with age at onset <20 years (13.4 ± 3.9 years), β-cell antibodies were found in 40% of subjects (21). Besides these scarce reports, islet autoantibodies and other markers of autoimmune islet cell destruction in PWS have not been extensively studied.

In PWS it has been traditionally assumed that abnormal glucose metabolism develops as a consequence of obesity. In accordance, non-obese subjects show lower insulin and glucose values compared to obese PWS individuals, both in children and in adults (22–24). Nevertheless, the relationship between obesity and the development of diabetes is not completely elucidated and may be different than in individuals with simple obesity.

1.2 Congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) represents a heterogeneous group of autosomal recessive disorders caused by deficient adrenal corticosteroid biosynthesis (25, 26). It arises from enzymatic defects in the adrenal steroidogenic pathway or in the electron-providing factor, (P450 oxidoreductase - POR). Between 90% and 95% of cases of CAH are caused by 21-hydroxylase deficiency (21OHD) encoded by the *CYP21A2* gene (25, 26) located on the short arm of chromosome 6 (6p21.3).

In Western societies, the incidence of classic 21OHD (present at birth with or without salt-wasting and cortisol deficiency) varies from 1 in 10.000-15.000 live births. Non-classic (or late-onset) 21OHD is more common, with an incidence of about 1 in 500 to 1.000 live births (27). The condition arises because of defective conversion of 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol. Defective cortisol biosynthesis results in reduced hypothalamic-pituitary negative feedback and increased ACTH secretion; consequently, adrenal androgens are produced in excess. The genotype-phenotype correlation in CAH due to the 21OHD is well established and the clinical phenotype is dependent

Abbreviations: 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; Ab-Tg, anti-thyroglobulin antibodies; Ab-TPO, thyroid peroxidase antibodies; BA, bone age; BMI, body mass index; CA, chronological age; CAH, congenital adrenal hyperplasia; del, deletion; DHEA-S, dehydroepiandrosterone sulphate; E2, oestradiol; GAD, anti-glutamic acid decarboxylase antibodies; GH, growth hormone; GHD, growth hormone deficiency; GHRH, growth hormone releasing hormone; GnRH, gonadotropin-releasing hormone; HGT, Hemoglucotest; HPG, hypothalamus-pituitary-gonadal axis; HV, height velocity; IAA, anti-insulin antibodies; IA2, anti-tyrosine phosphatase antibodies; ID, imprinting defect; IGF-1, insulin-like growth factor-1; IGT, impaired glucose tolerance; MODY, maturity onset diabetes of the young; MPH, mid parental height; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification analysis; oGTT, oral glucose tolerance test; OSAS, obstructive sleep apnea syndrome; PCOS, polycystic ovary syndrome; PP, premature pubarche; PWS, Prader-Willi Syndrome; SDS, standard deviation scores; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; UPD, uniparental disomy.

on residual enzymatic activity provided by the less severely mutated allele.

Several clinical variants have been identified from genital ambiguity in newborns girls to precocious pseudopuberty in both males and females. During adolescence and adulthood, females can present with a phenotype similar to polycystic ovary syndrome (PCOS) with hirsutism, primary or secondary amenorrhea, or anovulatory infertility (28). In the salt-wasting form the patients also present concomitant cortisol and aldosterone deficiency; it usually occurs after the first 2 weeks of life and neonates show hypotension, poor feeding, vomiting, lethargy, and sepsis-like symptoms.

The remaining 5-10% of CAH forms are caused by mutations in other steroidogenic enzymes involved in adrenal steroidbiosynthesis, such as: *CYP11B1*, *CYP17A1* and *HSD3B2*, encoding respectively 11β-hydroxylase, 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase type 2.

Association between PWS and adrenal congenital hyperplasia is very rare and only sporadic case reports are described in literature (29, 30).

2 Case report

We report the case of C.B., a 16 years-old girl affected by PWS, who presented PP in the context of CAH by 21OHD and later developed T1DM.She was born at 37 weeks by cesarean section, her birth weight was 2500 g (-0,89 standard deviation scores [SDS]). During infancy she presented with severe hypotonia, respiratory distress, weak cry, small hands and feet, which led to the suspicion of PWS. At 12 months of age the diagnosis was confirmed by methylation-specific multiplex ligation-dependent probe amplification analysis (MS-MLPA) and chromosome 15 DNA polymorphism analysis conducted on the proband and her parents, demonstrated the presence of maternal UPD. During childhood she presented neurodevelopmental disorders comprising learning and intellectual disability, language delay and impairment. Neuropsychiatric evaluation did not report behavioral disorders, although skin picking of the four limbs was found on pre-existing injuries. Food seeking behavior was controlled through parental training, providing a strict schedule of meals and avoiding the intake of sweet and high calorie foods from infancy; this is particularly relevant since the hyperphagia phase in PWS usually occurs between 2 and 4 years of age, and is then followed by an abnormal interest towards food (31). At the age of 2.4 years she presented short stature (Table 1) alongside reduced levels of insulin-like growth factor-1 (IGF-1) for age and gender (31 ng/mL, n.v. 49-289) and growth hormone (GH) stimulation tests with arginine (0.5 g/Kg iv) and clonidine (150 μg/m^2 orally) were performed, leading to the diagnosis of GHD (Table 2). According to the 2006 Italian Drug Agency (AIFA) Note 39 criteria (32) rhGH therapy was started at a dose of 0.033 mg/Kg/d (0.7 mg/m^2/d); thereafter the dose was titrated according to IGF-1 levels, keeping it within <2 SDS, according to guidelines for GHD treatment in PWS (31, 33). Clinical follow up was established every six months and polysomnography were performed once a year to exclude obstructive sleep apnea syndrome (OSAS) which can worsen during rhGH therapy. A controlled diet with the support of a nutrition specialist permitted to control the patient's body mass index (BMI) keeping it in the normal weight range (Figure 1). Regular growth with a height velocity (HV) of 6-7 cm/yr was registered until the age of 5.5 (75° centile; +0.74 SDS), alongside pre-pubertal Tanner stage 1 by physical examination. At 5.5 years of age pubic hair growth was noted, without thelarche, and at the age of 5.9 an endocrinological evaluation was requested (Tables 1, 2). Six months later, elevated 17OHP and DHEA-S levels were confirmed, HV attested at 10.4 cm/yr (>97° centile; +4.7 SDS) and physical examination confirmed a Tanner stage Ph2-Pb1, with no evidence of axillary hair. Stimulation test for CAH with tetracosactide 250 μg IV was conducted showing basal and stimulated level of 17OHP >10 ng/mL, both diagnostic for 21OHD (35). The patient and her parents were referred for genetic testing (MLPA confirmed by Sanger sequencing) for non-classic CAH due to 21OHD with evidence of compound heterozygosity for *CYP21A2* mutation: V281L, inherited from the mother, and A(C)656G [intron 2], inherited from the father. At the age of 7.7 years the patient confirmed accelerated HV (10 cm/aa; + 5.2 SDS), physical examination revealed Tanner stage Ph3-Pb2, initial axillary hair and clitoral enlargement; bone age (BA) was advanced corresponding to 9.5 years, according to Greulich&Pyle method, and pelvic ultrasound showed infantile uterine morphometry, with maximum ovarian volume of 1.2 mL and a thin endometrium. The evidence of pubertal progression prompted the execution of a stimulation test with 100 μg IV gonadotropin-releasing hormone (GnRH test), which showed a FSH and LH peak in accordance with pubertal activation of the hypothalamic-pituitary-gonadal axis (Table 2). According with the biochemical and clinical evidence of hyperandrogenism which led to the linear growth acceleration, and considering the risk of premature epiphyseal closure, therapy with hydrocortisone 20 mg/m^2/d was started in order to promptly suppress androgen levels. Eight months after the start of therapy, Tanner stage was Ph3-Pb2, suppressed levels steroid hormones were observed, HV declined to 5 cm/yr and a left-hand X-ray documented a BA of 10.5 years (Tables 1, 2). An oral glucose tolerance test (oGTT 1.75 g/Kg) showed an impaired glucose tolerance (IGT), and therefore rhGH therapy and hydrocortisone therapy were reduced to 0.021 mg/Kg/d and 14 mg/m^2/d, respectively). At the age of 9.0 the patient presented HV 3.9 cm/yr (3rd centile; -1.9 SDS) and Tanner stage did not change. Considering the patient having entered an appropriate age for pubertal start (>8 years-old), the HV and pubertal stage stability, hydrocortisone therapy was reduced to 11.5 mg/m^2/d and rhGH therapy increased to 0.026 mg/Kg/d, to allow linear growth and spontaneous pubertal progression.

At this time the patient first presented a positive autoimmune screening for glucose metabolism disorders, with an anti-glutamic acid decarboxylase (GAD) titer of 54 U/mL (n.v. <0.9), with normal levels of fasting glucose and insulin.

During the follow-up hydrocortisone therapy was gradually reduced and then stopped at the age of 11.8. rhGH therapy was continued following IGF-1 levels and HV, gradually increased up to 0.034 mg/Kg/d and then stopped when final stature was reached at the age of 14.7 years (HV <2cm/yr). After five months from the stop of rhGH therapy, a retesting with Growth Hormone Releasing Hormone (GHRH) + arginine was performed and persistent

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TABLE 1 Clinical features during endocrinological follow up.

Years	2,4[Δ]	5.9	6.3¤	7.7*	8.4	9[#]	15.7^
	-	-	-	-	-	-	-
Auxological parameters							
Height (cm)	81 (-2.16 SDS)	109.3 (-1.04 SDS)	114.5 (-0.57 SDS)	126 (+0.14 SDS)	128.2 (-0.2 SDS)	130.5 (-0.36 SDS)	151 (-1.75 SDS)
Weight (Kg)	10.5 (-1.73 SDS)	17.2 (-1.35 SDS)	20.1 (-0.68 SDS)	27 (+0.06 SDS)	31.2 (+0.33 SDS)	33.6 (+0.35 SDS)	47 (-1.13 SDS)
BMI (Kg/m^2)	16 (+0.04 SDS)	14.1 (-1 SDS)	15.3 (-0.45 SDS)	17 (+0.16 SDS)	19 (+0.7 SDS)	19.8 (+0.8 SDS)	20.6 (-0.15 SDS)
HV (cm/yrs)	5.6 (-2.03 SDS)	6.5 (+0.33 SDS)	10.4 (+4.7 SDS)	10 (+5.2 SDS)	5 (-0.63 SDS)	3.9 (-1.9 SDS)	< 2
Pubertal Stage							
Breast	1	1	1	2	2	2	3
Pubic hair	1	2	2	3	3	3	5

[Δ]rhGH was started (0,33mg/Kg·d); ¤Patient and her parents were referred for genetic testing for non-classic CAH; *Hydrocortisone was started (20 mg/m^2); #Hydrocortisone was reduced (11.5 mg/m^2) and rhGH was increased; ^Hydrocortisone and rhGH were gradually reduced and stopped at the ages of 11.8 and 14.7 years, respectively.
BMI, body mass index; CAH, congenital adrenal hyperplasia; HV, height velocity; SDS, standard deviation score.

GHD was excluded according to the current Italian Drug Agency (AIFA) Note 39 criteria (36). During the endocrinological evaluation at the age of 15.7 the patient presented: height 151 cm (-1.76 SDS), weight 47 kg (-1.13 SDS), BMI 20.6 (-0.15 SDS), Tanner stage Ph5-Pb3; biochemical and hormonal exams showed a mature hypothalamus-pituitary-gonadal axis (HPG) (Table 2), C-peptide attested at 1.14 ng/mL (in the lower part of reference range) and the IGT condition was confirmed.

At the age of 16.2 she presented with polyuria and polydipsia, weight loss and a fasting Hemoglucotest (HGT) >200 mg/dL. She referred to the emergency room, where she presented glucose levels of 382 mg/dL, HbA1c of 13.7%, C-peptide of 0.41 ng/mL, Na 141 mEq/L, K 4.8 mEq/L, glycosuria and ketonuria and an arterial blood gas analysis documented metabolic acidosis: pH 7.29, base excess -5.9, HCO3- 20.7 mmol/L, lactate 1.3 mmol/L, anion gap 19.3 mEq/L. Anti-insulin (IAA), anti-tyrosine phosphatase (IA2) and anti-ZnT8 antibodies were undetectable, whereas anti-GAD rose to 191 U/mL. Other exams showed normal thyroid function although with positive anti-thyroglobulin (Ab-Tg) and thyroid peroxidase antibodies (Ab-TPO), and a negative screening for celiac disease. Diagnosis of T1DM was made, insulin therapy with a basal-bolus scheme and an appropriate diet was started. The patient is currently followed at our Endocrine Center with a specific therapeutic program consisting in clinical and biochemical follow-up.

Written informed consent was obtained from the parents for the publication of this case report including clinical, biochemical and radiological data.

3 Discussion

Early adrenarche and higher prevalence of PP have been described in patients with PWS and in some of these cases a CAH diagnosis might be present (37). One study conducted on 120 PWS children between the ages of 2 and 17 years, described earlier adrenarche, with serum DHEA-S levels significantly higher in PWS girls and boys compared to healthy controls in the age groups from 3 to 10 years (37). Median age at onset of pubarche (Tanner stage Ph2) in children with PWS was 9.0 years in girls and 10.3 years in boys (37).

Our patient presented adrenarche and pubarche at the age of 5.5 years. In contrast with the literature, she showed fast progression in pubic hair growth; biochemical evaluation demonstrated a very high level of adrenal androgens, including 17OHP. At the first endocrinological evaluation the oestradiol (E2) concentration was also elevated, although with prepubertal levels of LH and FSH, which was interpreted as a condition of pseudopuberty due to excess of androgens and their consequent aromatization. For this reason, standard dose ACTH-stimulation test for 17OHP was performed.

PWS is a rare disease (estimated prevalence of 1:10.000-30.000) (1, 2), whereas CAH in its non-classic form is quite more common (1 in 500 to 1.000 live births in western countries) (27); as such their association may sporadically occur in the population. Nonetheless, the presence of both conditions is rare and to our knowledge only two cases are described in literature (29, 30). Patients with the non-classic form of CAH may present in early childhood with sexual precocity, pubic hair development or growth acceleration due to premature androgen excess. If left untreated, the sex steroid production stimulates premature epiphyseal closure, and final adult height is invariably reduced (38, 39). Additionally, central precocious puberty may develop in patients with CAH, possibly due to androgen activation of the HPG axis, thus exacerbating premature epiphyseal fusion (40, 41). Glucocorticoid therapy remains the standard treatment for patients with CAH. Some studies report a significant improvement in final adult height outcome in CAH children who are treated with GH, alone or in combination with GnRH analogues (42–44). rhGH therapy may counter the deceleration in growth velocity often associated with glucocorticoid therapy, whereas GnRH analogues suppress the

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TABLE 2 Hormonal and radiological exams during endocrinological follow up.

Years	2.4$^{\Delta}$	5.9	6.3¤	7.7*	8.4	9$^{\#}$	15.7^
	-	-	-	-	-	-	-
Hormonal exams							
LH (mUI/mL)	-	<0.2	-	0.1	-	-	0.7
FSH (mUI/mL)	-	<0.2	-	3.4	-	-	6.1
17OHP (ng/mL)	-	7	10.7	14.9	0.1	-	-
DHEA-S (ng/mL)	-	971	2420	2040	<150	-	-
Δ4 (ng/mL)	-	-	1.44	-	<0.3	-	-
Te (ng/dL)	-	30	58.9	104	37.7	-	-
E2 (pg/mL)	-	42.9	-	28.3	17	-	23.5
ACTH (pg/mL)	-	24.7	-	25.2	-	-	-
Cortisol (mcg/ dL)	-	11.2	-	10.1	-	-	-
TSH (mU/L)	6.14	2.85	5.26	2.8	-	-	4.13
FT4 (ng/dL)	0.93	1.24	1.08	1.02	-	-	1.28
IGF-1 (ng/mL)	31	296	283	381	561	350	192
ACTH Tests							
17OHP (ng/mL) – 0’	-	-	10.7	-	-	-	-
17OHP (ng/mL) – 60’	-	-	14.9	-	-	-	-
GH Test							
Arg. GH (ng/mL) - Peack	2.9	-	-	-	-	-	-
Clon. GH (ng/mL) - Peack	3.88	-	-	-	-	-	-
GHRH + Arg. GH (ng/mL)- Peack	-	-	-	-	-	-	14.4
LHRH Test							
LH (mUI/mL) – Basal	-	-	-	0.1	-	-	-
LH (mUI/mL) – Peack	-	-	-	7.53	-	-	-
FSH (mUI/mL) – Basal	-	-	-	3.4	-	-	-
FSH (mUI/mL) – Peack	-	-	-	13.24	-	-	-
Radiological exams							
BA	1.3	-	7.1	9.5	10.5	11	-

$^{\Delta}$rhGH was started (0.33mg/Kg·d); ¤Patient and her parents were referred for genetic testing for non-classic CAH; *Hydrocortisone (20 mg/m^2) was started; #Hydrocortisone was reduced (11.5 mg/m^2) and rhGH was increased; ^Hydrocortisone and rhGH were gradually reduced and stopped at the age of 11.8 and 14.7 years, respectively.
17OHP, 17-hydrossiprogesterone; CAH, congenital adrenal hyperplasia; DHEA-S, dehydroepiandrosterone; Δ4, delta-4-androstenedione; Te, testosterone; E2, estradiol; BA, bone age.

HPG axis to prevent premature epiphyseal closure. However, since the addition of GnRH analogues has not been clearly shown to benefit GH therapy in increasing height gain, their use should be based on the age of the patient and the social impact of precocious puberty. In our patient genetic testing for 21OHD was performed with confirmation of non-classic CAH and as such hydrocortisone therapy was started. The use of corticosteroid therapy was decided based on advanced bone age, biochemical hyperandrogenism and increasing growth velocity to prevent rapid BA acceleration and severe compromission of final height, a common feature of PWS worsened by CAH (29, 44). The very high levels of adrenal androgens in our patient led us to the decision to start oral hydrocortisone at a dosage (20 mg/m^2/d) above the recommended dose interval for non-classic CAH (10-15 mg/m^2/d) (45) in order to suppress adrenal hyperandrogenism and prevent the effects of androgen excess on final height.

Our patient underwent clonidine and arginine GH stimulation testing, which revealed a condition of GHD (46). rhGH therapy was started at the age of 2.7 years within the recommended dose range for PWS. The prevalence of GHD in PWS is reported around 50%, nevertheless rhGH therapy produces several benefits in these subjects: increase in final height, improved body composition,

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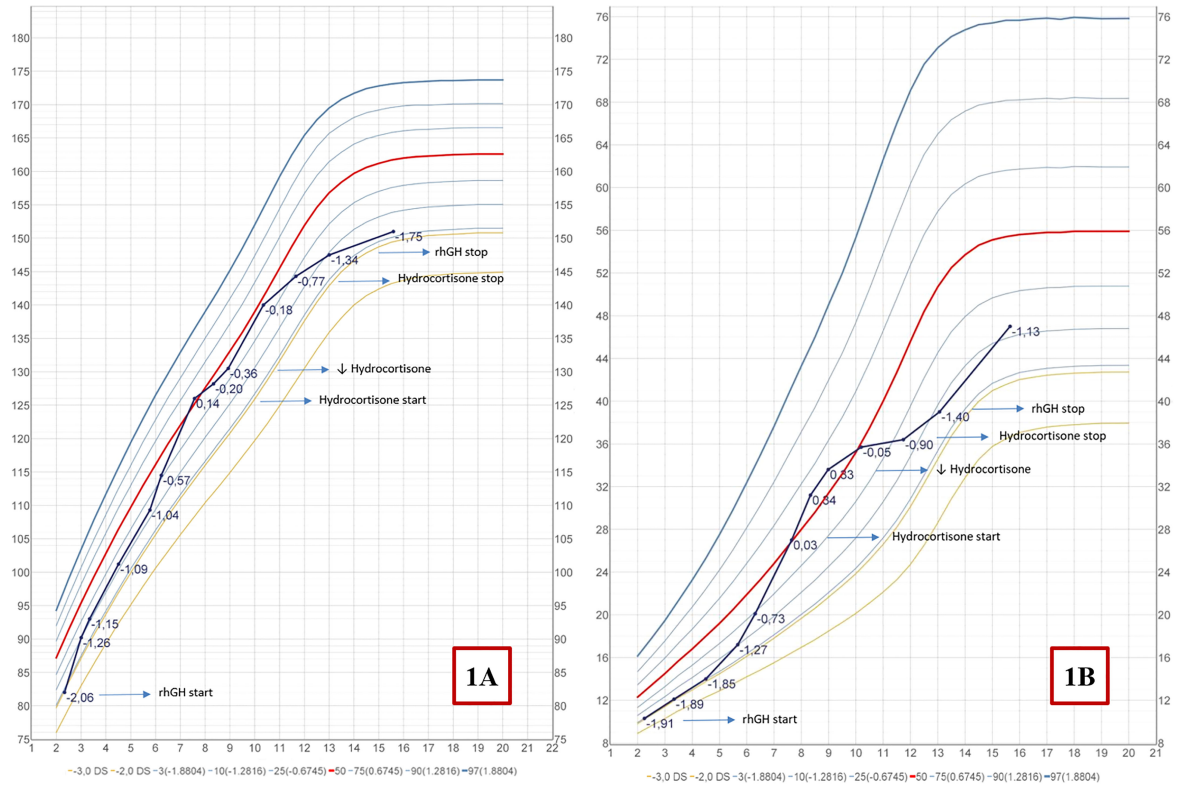

FIGURE 1
Italian growth chart for height **(A)** and weight **(B)** (34).

muscle thickness, bone mineral density, cognitive function and behavior in PWS subjects (31, 47–49). Furthermore, rhGH therapy also influences sexual development due to the existence of a crosstalk between the HPG and the somatotropic axes (50) during development from infancy through puberty, transition age and, finally, in adult life (51–55).

Our patient did not show advanced clinical pubertal features and the pelvic ultrasound examination documented an infantile uterine feature, which prompted to avoid GnRH analogue therapy.

Autoimmune diseases are not described as a typical feature of PWS. Although thyroid dysfunction may also be observed in the syndrome, this is not the result of thyroid autoimmunity (56, 57). Nevertheless, an increased systemic low-grade inflammation was described in these patients. One paper observed that PWS subjects present overactivation of the innate immune system, independent of central adiposity and insulin-resistance (58). Among the factors that may influence this phenomenon, there are the GHD and hypogonadism described in PWS. Another frequent comorbidity in PWS patients is OSAS which may also contribute towards increased low-grade inflammation (59, 60). Nevertheless, despite their severe obesity, lower visceral fat mass, hypoinsulinemia and increased insulin sensitivity have been described in PWS patients by most (61, 62), although not all studies (22–24, 63), compared to non-syndromic obese patients (61, 62). According to early reports, lower fasting insulin levels and reduced insulin resistance (measured by HOMA-Index) have been reported at all ages in obese patients with PWS (64–66). A familial component in insulin resistance, as in the general population, could also contribute to the individual subject affected by PWS whereas the different genotypes (*del* or UPD15) do not appear to influence the development of altered glucose homeostasis in PWS (16, 63). Data about insulin secretion in PWS, however, are still under debate.

In our patient we observed persistent IGT although with a normal body weight, which led to a reduction of rhGH and hydrocortisone dosages. An initial improvement in glucose homeostasis was followed at the age of 9 by a positive autoimmune screening for anti-GAD with normal fasting glucose and insulin levels. At the age of 16.2 years the typical symptoms of diabetes mellitus emerged and diabetic ketoacidosis occurred as typical of type 1 phenotype (T1DM); concurrently she presented anti-thyroid antibodies, but a negative antibody screening for celiac disease. Despite the relationship between obesity and the development of diabetes being unclear in PWS, in our case it was most probable that the patient presented a genetic predisposition for autoimmune diseases, although in the absence of family history.

In conclusion, the present is the third case report of a subject with CAH associated with PWS, but the first confirmed by genetic analysis. Although CAH is a common disease, and PP a common feature in PWS, 17OHP measurement should be performed in all PWS children experiencing PP and early adrenarche to exclude a possible concurrent 21OHD. Furthermore, although PWS is most typically associated with a T2DM phenotype, anti-islet antibodies and C-peptide levels should be assayed at the occurrence of glucose metabolism impairments in children and adolescents affected by

PWS to timely diagnose the sporadic T1DM cases and guide follow-up concerning disease progression and the need for insulin therapy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the parents for the publication of this case report including clinical, biochemical and radiological data.

Author contributions

AA collected data, drafted the initial manuscript, and critically reviewed and revised the manuscript. SB and MM contributed to the original draft of the manuscript. AC and MC critically reviewed and revised the manuscript for important intellectual content. DF coordinated and supervised data collection and manuscript drafting and revised the manuscript for intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Central precocious puberty in Prader-Willi syndrome: a narrative review

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Prader-Willi syndrome (PWS, OMIM176270) is a rare genetic disorder with recognizable dysmorphic features and multisystemic consequences such as endocrine, neurocognitive and metabolic ones. Although most patients with Prader-Willi syndrome exhibit hypogonadotropic hypogonadism, there is variability regarding sexual maturation, with precocious puberty occurring in rare cases. Our aim is to elaborate a thorough review of Prader-Willi patients with central precocious puberty, in order to raise awareness of such cases and to enhance our knowledge regarding the diagnosis and prompt treatment of this particular PWS patients.

KEYWORDS

Prader-Willi syndrome, precocious puberty, endocrine, genetic, metabolic

Introduction

Prader-Willi syndrome (PWS, OMIM176270) is a rare genetic disorder with recognizable dysmorphic features and multisystemic consequences such as endocrine, neurocognitive and metabolic ones (1, 2).

Although described in 1956 for the first time by Swiss endocrinologists Prader, Labhart and Willi (1), based on clinical aspects, genetic confirmation was possible only since 1980, when high resolution chromosome analysis led to the discovery of the chromosomal deletion of 15q11-g13

(3–5). Most PWS patients display a paternal microdeletion of the long arm of chromosome 15 (70%), whereas 20-35% have maternal uniparental disomy 15 (mUPD), and even fewer patients have an imprinting center defect (ICD) or an unbalanced translocation (0,1%) (6).

With an estimated prevalence of 1 in 10.000-30.000 live births, Prader-Willi syndrome represents the most common genetic cause of obesity (7–9) and the first recognized disorder of human genetic imprinting (10). PWS displays great clinical variability

throughout life (1), ranging from hypotonia and failure to thrive during infancy, to morbid obesity, dysmorphic features, short stature and behavioral problems such as hyperphagia and aggressive behavior (11). Patients require multidisciplinary approach, including close endocrinologic follow-up throughout their lives (12) given the hypothalamic-pituitary dysfunction that characterizes these patients. This disfunction leads in turn to multiple endocrinopathies, the most common ones being growth hormone deficiency (GHD), hypogonadism and hypothyroidism (12, 13).

Genital anomalies are common in both female and male PWS patients and are represented by pubertal development disorders (14). Hypogonadism represents a major clinical diagnostic criteria of PWS, according to Holm and Cassidy (13). In Prader-Willi syndrome puberty is most often delayed and incomplete. While PWS male patients usually present cryptorchidism and remain in a Tanner genital stage II or III (14), most female PWS patients have amenorrhea or oligomenorrhea, with only a few undergoing spontaneous menarche (15). However, exceptional cases leading up to pregnancy have been described (6), isolated premature pubarche is reported in 14% of cases (1). Despite most PWS patients having hypogonadism, a few cases of central precocious puberty (CPP) have been reported (16).

Our aim is to elaborate a thorough review of Prader-Willi patients that display central precocious puberty, in order to raise awareness of such cases and to enhance our knowledge regarding the diagnosis and prompt treatment of this particular PWS patients.

Materials and methods

Search strategy

- The literature review was based on the available papers written in English published in the electronic databases PubMed and Embase database between 1979 and January 2023.
- The search criteria used in the Medical Subject Headings (MeSH) included te following terms: “Prader-Willi” [All Fields] AND (“precocious puberty)”[MeSH Terms]
- RCTs reported as literature reviews, case reports and conference abstracts with relevant outcome data were included in the review. Articles and abstracts regarding only premature adrenarche, or cases without genetic confirmation were excluded from the study.
- Duplicates were removed.

Presentation of the results

The following data were collected from each article/abstract if available: age and gender, genetic background, Tanner Pubertal Stage, GnRH stimulation test, determination of basal and peak Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) serum levels, Testosterone, bone age, ultrasound of genitalia, stabilization or regression of Tanner Pubertal Stage under GnRH (gonadotropin releasing hormone) treatment according to dose and duration.

Patients were included if they fulfilled diagnostic criteria for CPP (i.e. age at pubertal onset, bone age, Tanner stage, significant value of LH, FSH and Testosterone GnRH stimulation test).

Results

We sought to summarize the following outcome measures from the reviewed articles:

- suppression of physical signs of puberty: breast development in females, testicular volume in males, genital development – based on modified Tanner staging
- incremental growth rate: cm/year
- suppression of GnRHa – stimulated LH: peak LH value
- incremental change in bone age

We identified a total of 22 children with CPP and genetically confirmed Prader-Willi syndrome mostly in case reports and abstracts, summarizing 13 children from case reports and case studies [13-22] and 9 children from Embase indexed abstracts [23-27]. There were 13 (59%) male patients and 9 (41%) female cases, with mean age at CPP onset of 7,64 +/- 1,01 years (5 – 9 years). At diagnosis they were Tanner stage II-III, mean basal LH was 1,43 +/- 1,46 UI/L (0,3 – 4,6 UI/L), and mean stimulated peak LH was 13,67 +/- 6,45 UI/L (4,6 – 29,7 UI/L). Mean basal FSH was 1,43 +/- 1,46 UI/L (0,3 – 4,6 UI/L) stimulated peak FSH was 13,67 +/- 6,45 UI/L (4,6 – 29,7 UI/L).

Auxological parameters and response to GnRH of the PWS patients with precocious puberty are displayed below in Tables 1, 2.

Discussions

Pubertal timing is a complex process, with genetic, epigenetic, endocrine, metabolic and lifestyle factors playing a role in acquiring secondary sexual characteristics, gonadal maturation and progression of linear growth (28, 29). It normally coincides with the pulsatile release of GnRH from the hypothalamic neurons (30). Normal pulsatile GnRH secretion is initially observed during fetal and neonatal periods (31, 32), suppressed during childhood, only to resume at the age of puberty, under action of Kisspeptin (33).

The first symptoms of precocious puberty are regarded in girls as onset of breast development before the age of 8 years, and in boys as testicular enlargement ≥ 4 ml before the age of 9 years (29), in accordance with the mean age at CPP onset in the reviewed cases (7,64 +/- 1,01 years). The underlying mechanism can be central (GnRH- dependent) or peripheral (GnRH-independent). In Prader-Willi cases associated with precocious puberty only central precocious puberty has been described (13–27).

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TABLE 1 Characteristics of PWS patients with central precocious puberty retrieved from case presentations/case studies.

Reference	Gender	Age at CPP onset (years)	Clinical signs	Growth velocity (cm/year)	Basal/stimulated peak LH	Basal/stimulated peak FSH	Oestradiol/Testosterone (before/during LNRH)	Bone age (years)	Cerebral MRI
Crinò et al. (13), *2008*	M	8,9	Tanner stage II	7	0,4/15,3	5,8/10,9	6.98mmol/L/0,61-1,6 mmol/L	10,6	Gliotic ischemia - left subcortical parietooccipital area
Ludwig et al. (17), 2016	M	8,8	Tanner stage II	NM	0,5/15,8	5,6/NM	54.7 ng/dL/18,7 ng/dL	9	No abnormalities
Monai et al. (18), 2019	M	8,2	Tanner stage II Testicular volume - 3 mL	7,1	1,1/11,1	5,2/7	0.45 nmol/L/NM	9,8	NM
Monai et al. (18), 2019	M	7,2	Tanner stage III (PH) Testicular volume - 4 mL	NM	NM/8,5	NM/3	1.25 nmol/L/NM	NM	NM
Kobayashi et al. (16), 2022	M	7,2	Tanner stage III (PH) Testicular volume - 6 mL	NM	0,5/29,7	5/22,6	0,41 ng/mL/NM	12,5	No abnormalities
Lee et al. (15), 2013	F	8,2	Genitalia stage (Tanner) III Pubic hair (Tanner) I	7	1/10,3	1,7/9,2	15 pg/mL (↑)/N	10,5	NM
Linnemann et al. (19), 1999	M	6,6	Tanner stage II (PH) Testicular volume - 5 mL	NM	0,5/5	1,6/3,6	2.4 nmol/L(↑) /2.1 nmol/L (↑)	9,1	Flat, small pituitary gland
Pusz et al. (20), 2008	F	5	Tanner stage III	NM	4,6	14,8	23 pg/mL (N)	13,6 (9,4)	No abnormalities
Crino et al. (21), 2003	F	7,2	NM	NM	NM	NM	NM	NM	NM
Crino et al. (21), 2003	F	7,4	NM	NM	NM	NM	NM	NM	NM
Crino et al. (21), 2003	M	8	NM	NM	NM	NM	NM	NM	NM
Eldar-Geva et al. (14), 2010	F	< 8	NM	NM	3,3/13,5	10,7/14,7	NM	NM	NM
Hirsch et al. (22), 2009	M	7	NM	NM	2,1/16,3	3,5/5,3	NM	11 (8,5)	5-mm diameter pineal cyst

(Continued)

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TABLE 1 Continued

Reference	Gender	Age at CPP onset (years)	Clinical signs	Growth velocity (cm/year)	Basal/stimulated peak LH	Basal/stimulated peak FSH	Oestradiol/Testosterone (before/during LNRH)	Bone age (years)	Cerebral MRI
Reference	Gender	Age at start of GnRH analogs (years)	Type/dose of GnRH product			Age at discontinuation of GnRH analogs (years)	Stabilization/regression of Tanner Pubertal Stage with GnRH analogs		
Crinò et al. (13), *2008*	M	8,9	Leuprorelin 3,75mg IM/28days			11,3	Good clinical and hormonal response already evident after 4 months		
Ludwig et al. (17), 2016	M	8,8	Leuprorelin 3,75mg IM/28days, than 11,25mg/3m			13	Satisfactory response, no progression of bone age and pubertal Tanner stage III, spontaneous resolution of testicular size discrepancy		
Monai et al. (18), 2019	M	8,2	Leuprorelin 11.25 mg S.Q./3m			12,3	still prepubertal at 12,8 years		
Monai et al. (18), 2019	M	7,4	Leuprorelin 3,75mg/28days			11,5	normal progression in growth and puberty, at 14 years he was fully virilized with testicular volume of 10 mL		
Kobayashi et al. (16), 2022	M	7,5	Leuprorelin acetate 0.94 mg S.Q./28days			11,3	Right and left testicular volume - 10 mL and 15 mL, respectively, Tanner stage 5 pubic hair		
Lee et al. (15), 2013	F	8,2	Leuprorelin 3.75 mg S.Q./28 days			NM	Regression of breast development noted after 3 months, stabilization of bone age		
Linnemann et al. (19), 1999	M	NO	NO			NO	Testicular volume and pubic hair developed slowly		
Pusz et al. (20), 2008	F	8,8	LHRH analog Depot 11.25 mg/28 days for months While on monthly LHRH analog she had persistent unexplained fevers and was changed to daily LHRH analog injection of 0.3 mg.			NM	Menstruation was suppressed, breast development regressed to Tanner II		
Crino et al. (21), 2003	F	7,2	NM			NM	Tanner stage 4 breast and pubic hair, menstruation at 10.2 years		
Crino et al. (21), 2003	F	7,4	NM			NM	Tanner stage 3 breast, Tanner stage 2 pubic hair		
Crino et al. (21), 2003	M	8	NM			NM	Tanner stage 2 genitalia, Tanner stage 3 pubic hair		
Eldar-Geva et al. (14), 2010	F	< 8	NM			NM	NM		
Hirsch et al. (22), 2009	M	7	NM			NM	NM		
Reference	Gender	Age at diagnostic (years)	Comorbidities			GH treatment	Years of follow- up		
Crinò et al. (13), *2008*	M	5,2	Mild perinatal hypoxic damage Seizures at 4 years			1O years -> 15,3 years	8		
Ludwig et al. (17), 2016	M	6	NM			7 years -> NM	7		

(Continued)

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TABLE 1 Continued

Reference	Gender	Age at CPP onset (years)	Clinical signs	Growth velocity (cm/year)	Basal/stimulated peak LH	Basal/stimulated peak FSH	Oestradiol/Testosterone (before/during LNRH)	Bone age (years)	Cerebral MRI
Monai et al. (18), 2019	M	< 1	NM			8 months -> NM		4,5	
Monai et al. (18), 2019	M	< 1	NM			2 years -> NM		NM	
Kobayashi et al. (16), 2022	M	10	NM			yes		8	
Lee et al. (15), 2013	F	< 1	NM			3 years -> last follow-up		1,3	
Linnemann et al. (19), 1999	M	5	Forceps extraction because of intrauterine asphyxia			NM		NM	
Pusz et al. (20), 2008	F	8	Hypothyroidism, osteopenia, Raynaud's phenomenon			yes		9,4	
Crino et al. (21), 2003	F	NM	NM			yes		5, 6	
Crino et al. (21), 2003	F	NM	NM			yes		3	
Crino et al. (21), 2003	M	NM	NM			yes		2,6	
Eldar-Geva et al. (14), 2010	F	NM	NM			NM		NM	
Hirsch et al. (22), 2009	M	NM	NM			NM		NM	

NM, not mentioned.

TABLE 2 Characteristics of PWS patients with central precocious puberty retrieved from case conference abstracts.

Reference	Gender	Age at diagnostic	Comorbidities	GH treatment	Years of follow-up
Pellegrin et al (23), 2016	M	NM	Hypothyroidism since age 2	1,5 years -> NM	NM
Papagianni et al (24), 2016	F	2 months	NM	NM	NM
Karachaliou et al (25), 2013	M	NM	hypobetalipoproteinemia	NM	NM
Cheon et al. (26), 2017	2M/3F	NM	NM	NM	NM
Lu et al. (27), 2019	M	NM	Epilepsy since age 4	yes	NM

(Continued)

TABLE 2 Continued

Reference	Gender	Age at diagnostic	Comorbidities	GH treatment	Years of follow-up
Reference	Gende r	Age at start of GnRH analogs (years)	Type/dose of GnRh product	Age at discontin uation of GnRH analogs (years)	Stabilization/ regression of Tanner Pubertal Stage with GnRH analogs
Pellegrin et al (23), 2016	M	8,5	LHRH analogue	NM	good clinical and hormonal response
Papagianni et al (24), 2016	F	7,6	LHRH analogue	NM	NM
Karachaliou et al (25), 2013	M	9	NM	NM	suppressed pubertal development
Cheon et al. (26), 2017	2M/3F	NM	NM	NM	good clinical and hormonal response
Lu et al. (27), 2019	M	NM	NM	NM	NM

Reference	Gender	Age at CPP onset (years)	Clinical signs	Growt h velocity (cm/ye ar)	Basal/ stimulate d peak LH	Basal/ stimulate d peak FSH	Oestradiol/ Testosterone (before/duri ng LNRH)	Bone age (years)	Cerebral MRI
Pellegrin et al (23), 2016	M	8,5	Tanner stage II for pubic hair and genitalia volume of testis 5 ml	9.4	NM/11.6 mUI/ml	NM/10.8 mUI/ml	1.15 ng/ml	10	Mild hydrocephalus (neonatal intraventricular haemorrhage), normal pituitary gland
Papagianni et al. (24), 2016	F	7,6	Tanner stage: B2A1P2M0	NM	NM/17.4 mIU/ml	NM/13.4 mIU/ml	↑	NM	normal
Karachaliou et al. (25), 2013	M	9	BMISDS: 1.61, pubic hair (PH) 2, testes 5ml	7,5	NM/ ↑	NM/ ↑	NM	10	normal
Cheon et al. (26), 2017	2M/3F	NM	Tanner stage II for breast/testicular development and a Tanner stage I for pubic hair development	8.7 ± 0.6	0.3 ± 0.3 IU/L /11.8 ± 8.0 IU/L	3.7 ± 2.9 IU/Ml /NM	NM	↑ by 2.6 ± 0.5 years	Normal pituitary MRI
Lu et al. (27), 2019	M	~ 6	increased testicular volume and growth velocity	NM	NM/20.51 mIU/mL	NM	3.32 nmol/L	↑	NM

NM, not mentioned. ↑ elevated.

Laboratory and imagistic evaluation

The most sensitive biomarker in CPP is LH, as it is untraceable before the first stages of puberty (34). Therefore determining plasma LH after exogenous GnRH or LHRH stimulation represents the reference test for diagnosing central precocious puberty (35–38). Yet peak cut-off values beyond which puberty is activated remain controversial (35, 36, 38). Values between 5 and 10 IU/L are considered acceptable cut-off points, using Chemiluminescent immunoassay (36, 38). Further assessment includes assessment of bone age (usually advanced as compared to chronological age), pelvic or testicular ultrasonography (to aid in identifying signs of peripheral precocious puberty) and brain magnetic resonance imaging in order to evaluate the hypothalamus and pituitary glands, and to exclude other brain anomalies (39). 9 cases describe imagistic evaluation through cerebral MRI, out of which 3 had abnormalities: one male patient with ischemic cerebral lesions, one with pituitary hypoplasia and

one with a small pineal cyst. It can be argued that such cerebral lesions account in part for the precocious puberal onset, but additional causes related to this patients remain obscure.

Evolution and therapeutic approach

Premature activation of the hypothalamic–pituitary–gonadal (HPG) axis can have several repercussions in children and adolescents, from height deficit caused by premature fusion of epiphyses through accelerated bone maturation, to premature sexual maturation that cause body changes (34, 40). Accumulating evidence points out to the absolute or relative growth hormone deficiency in most cases of PWS patients (41–48), according to standard testing protocols (49). As such, adult height prognosis is additionally compromised in children with Prader-Willi syndrome that exhibit central precocious puberty. This reinforces the interest of detecting any central precocious puberty in Prader-Willi patients. Central precocious puberty also occurs in other diseases that associate hypopituitarism, among which lesions of the central nervous system, such as intracranial malignancies and cranial radiotherapy (50–53), severe head injury (53), arachnoid cyst and septo-optic dysplasia (54–58). Other possible situations involve genetic causes such as, combined pituitary hormone deficiency due to POU1F1 gene mutation (59, 60), Kabuki syndrome (61, 62), Williams–Beuren syndrome (63, 64), Mayer-Rokitansky-Kuster-Hauser syndrome (65) and developmental defect of the hypothalamic–pituitary area (66). The possible common etiological mechanisms in both situations should also be the subject of further studies, as the underlying mechanism of remains unclear (59).

Also, there is increased risk for metabolic comorbidities such as obesity, type 2 diabetes and for cardiovascular events (34, 67, 68). As such, there is increased need for prompt treatment. GnRH analogues are the cornerstone of treatment, and they are administered either intramuscular (1 administration every 28 days) or by subcutaneous implant (69). They have been used effectively in cases of CPP, reducing plasma gonadotrophins, gonadal steroids and peptides (34, 70). Adequate suppression of gonadotrophins can be seen after 3 – 4 months of treatment (71).

Future therapeutic options that are being evaluated as an alternative option include newer kisspeptin and neurokinin B antagonists (69, 72, 73). Clinical trials investigating the short term efficacy of GnRH analogs confirm the fact that this treatment is well tolerated and safe (71, 74–76). Also, case reports regarding PWS patients with central precocious puberty highlight the possible benefit of combined therapy (gonadotropin-releasing hormone agonist and recombinant human growth hormone) on final height, while restoring appropriate pubertal progression (16, 17).

Study limitations

Due to the exceedingly rare cases of PWS patients with precocious puberty, reviewed cases stemmed from case reports and Embase registered conference abstracts. Yet, it is our belief that such cases promote a better understanding of the variety of sexual maturation disorders in PWS patients.

Conclusions

Although most patients with Prader-Willi syndrome exhibit hypogonadotropic hypogonadism, there is variability regarding sexual maturation, with precocious puberty occurring in rare cases. Recent years have brought major improvement in scientific knowledge regarding PWS, but there is still a need for further studies to assess the pathophysiology implicated in timing and progression of pubertal onset (15), and to implement clinical guidelines (12). GnRH agonist therapy seems to be efficient and safe in such cases, although long term follow-up is of need to better address the issue (71, 74–76).

Author contributions

DMN, ACS identified potential papers, D-MN and ACS wrote original draft, D-MN, A-CS, NM, IJ, A-IM and LV analyzed selected papers, and took the lead in writing the manuscript. OM supervised the project and recommended changes. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Acute stress response of the HPA-axis in children with Prader-Willi syndrome: new insights and consequences for clinical practice

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Background: Prader-Willi syndrome (PWS) is associated with hypothalamic dysfunction. It has been reported that the HPA axis might show a delayed response during acute stress, and it is unknown whether the response of the HPA-axis during acute stress changes with age in children with PWS.

Aim: To investigate the HPA-axis response during an overnight single-dose metyrapone (MTP) test in children with PWS and to assess if the response changes with age, whether it is delayed and if it changes with repeated testing over time. In addition, we evaluated different cut-off points of ACTH and 11-DOC levels to assess stress-related central adrenal insufficiency (CAI).

Methods: An overnight single-dose MTP test was performed in 93 children with PWS. Over time, 30 children had a second test and 11 children a third one. Children were divided into age groups (0-2 years, 2-4 years, 4-8 years and > 8 years).

Results: Most children did not have their lowest cortisol level at 7.30h, but at 04.00h. Their ACTH and 11-DOC peaks appeared several hours later, suggesting a delayed response. When evaluated according to a subnormal ACTH peak (13-33 pmol/L) more children had an subnormal response compared to evaluation based on a subnormal 11-doc peak (< 200 nmol/L). The percentage of children with a subnormal ACTH response ranged from 22.2 to 70.0% between the age groups, while the percentage of a subnormal 11-DOC response ranged from 7.7 to 20.6%. When using the ACTH peak for diagnosing acute-stress-related CAI, differences between age groups and with repeated testing over time were found, whereas there was no age difference when using the 11-DOC peak.

Conclusion: Early morning ACTH or 11-DOC levels are not appropriate to determine acute stress-related CAI in children with PWS, thus multiple measurements throughout the night are needed for an accurate interpretation.

Our data suggest a delayed response of the HPA-axis during acute stress. Using the 11-DOC peak for the test interpretation is less age-dependent than the ACTH peak. Repeated testing of the HPA-axis over time is not required, unless clinically indicated.

KEYWORDS

acute-stress response, central adrenal insufficiency, hypothalamic - pituitary - adrenal axis, children, Prader-Willi syndrome, hydrocortisone

1 Introduction

Prader-Willi syndrome (PWS) is a rare syndrome caused by the lack of expression of genes in the PWS region on the paternally derived chromosome 15, due to a paternal deletion, maternal uniparental disomy (mUPD), an imprinting center defect (ICD) or paternal chromosomal translocation (1, 2). Clinical findings characterizing PWS are abnormal body composition, muscular hypotonia, developmental delay, behavioral problems, hyperphagia, obesity, short stature (2), and endocrine problems, like hypothyroidism (3), growth hormone deficiency (4) and hypogonadism (5). In addition, dysregulation of body temperature and pain is also often found in PWS. Hypothalamic dysfunction is likely to be the cause of most of these symptoms.

The mortality rate in subjects with PWS is high (3% per year until the age of 30 years) (6). A large percentage of deaths in adults is associated with the complications of obesity, but children had mostly a mild viral illness or upper respiratory tract infection as the cause of death. Also, sudden, unexpected deaths are described (7–9). As small adrenal glands were found during autopsy in children with PWS after an unexpected death (10, 11), it has been suggested that acute stress-related central adrenal insufficiency (CAI) might be partly responsible for these unexplained deaths.

In literature, there is no agreement on the prevalence of CAI during acute stress in PWS (12). Several studies, in children and adults, using different test methods and definitions of CAI, have shown contradictory results. A previous study by our group reported a prevalence of acute stress-related CAI of 60% in 25 children with PWS (median age 9.7 years) during an overnight single-dose metyrapone (MTP) test, based on an adrenocorticotropic hormone (ACTH) peak cutoff of < 33 pmol/L, according to Steiner et al (13). Since then, the use of this ACTH level as a cutoff for the diagnosis of CAI has been questioned (14) and other cut-off points were used (15). Several studies using other methods to diagnose CAI, found a prevalence in PWS ranging from 0-15% (14, 16–22). However, most of these studies used a low-dose ACTH test, which is considered an appropriate method for diagnosing primary and secondary adrenal insufficiency, but less adequate for diagnosing tertiary adrenal insufficiency, originating from (partial) hypothalamic insufficiency (14, 23). Recently, a study in adults found a prevalence of CAI of 1.2% using the multiple-dose MTP test, but this test does not test the acute stress-related response of the HPA-axis (24). We previously established that children with PWS are able to produce adequate cortisol levels in daily life (13, 25) and do not suffer from CAI requiring daily hydrocortisone treatment. However, based on the unexpected sudden deaths of children with PWS, the question remained whether these children are able to generate a sufficiently fast response of the HPA-axis during severe acute stress. This can be tested with an overnight, single-dose MTP test or an insulin tolerance test (ITT), although the latter is not performed in our clinic anymore, because of the risk of severe side effects. Interestingly, one study in 36 children with PWS found a delayed response of cortisol during an insulin tolerance test (ITT) in 64% of them in comparison with healthy children (16), which indicated that the response of the HPA-axis during acute stress is delayed.

The clinical consequences of under- or over diagnosing acute stress-related CAI are significant. Underdiagnosing can lead to potentially life-threatening situations such as an adrenal crisis during acute stress, and over diagnosing can lead to overuse of hydrocortisone, which might lead to unnecessary side effects. It is, therefore, important to expand the knowledge about acute stress-related adrenal insufficiency in PWS and to create recommendations on the necessity, frequency and repeating the diagnostic procedures for CAI in PWS (12). It has been reported that there is a negative correlation between age and the HPA-axis (18, 21), which suggest that the response of the HPA-axis during acute stress could change with age.

We analyzed the ACTH, 11-deoxycortisol (11-DOC) and cortisol levels at different time-points during an overnight single-dose MTP test in 93 Dutch children with PWS and investigated whether the response to the single-dose MTP test changes with age, using two different cut-off points of acute stress-related CAI based on either the ACTH or 11-DOC peak. In addition, we investigated whether the response of ACTH and 11-DOC after a single-dose of MTP is normal or delayed in (young) children with PWS. With this study, we aimed to increase our insights in the response of the HPA axis during acute stress in children with PWS and whether this would have consequences for clinical practice. We hypothesized that the hormonal responses after a single-dose of MTP are different between children and adults with PWS because these changes are associated with age. In addition, we hypothesized that only few children with PWS have CAI requiring daily hydrocortisone treatment or an increased dose during mild to moderate stress, but that the response of the HPA-axis during severe acute stress as tested during the single-dose MTP test, would be delayed in children with PWS.

2 Materials and methods

2.1 Patients

All participants were diagnosed with PWS, confirmed by methylation analysis of the PWS region, and participated in the Dutch PWS Cohort study (26, 27).

2.2 Design

In this prospective study, all children underwent at least one single-dose MTP test during an overnight stay at the Pediatric Intensive Care Unit of the Erasmus Medical Center/Sophia Children's Hospital (Rotterdam, The Netherlands). MTP tests were performed between March 2006 and June 2022. When a child was ill at the time of the MTP test, the test was not performed. All children visited the Dutch PWS Reference Center in Rotterdam and received multidisciplinary care by the PWS team in collaboration with pediatric endocrinologists and pediatricians in other Dutch hospitals. The Dutch PWS Cohort study was approved by Medical Ethics Committee of the Erasmus University Medical Center. Written informed consent was obtained from parents and children older than 12 years. Assent was obtained from children younger than 12 years. The study was conducted according to the guidelines of the Declaration of Helsinki II (28).

2.3 Overnight single-dose metyrapone test

Metyrapone was administered at 23.30h, in a dose of 30mg/kg (maximum dose of 3 g) at the Pediatric Intensive Care Unit of the Erasmus Medical Center/Sophia Children's Hospital (Rotterdam, The Netherlands). The GH dose was given around 19.00-20.00h in the evening prior to the start of the MTP test, thus approximately 3-4 hours before the first blood draw. Metyrapone blocks the synthesis of cortisol by inhibiting 11-β-hydroxylase type 1, which converts 11-deoxycortisol to cortisol. The decline in plasma cortisol stimulates ACTH production, which causes 11-deoxycortisol to accumulate before the enzyme blockade. In four healthy adults, the maximal decrease in cortisol levels was found at 2 hours after oral administration of MTP and maintained for 7 hours post-dose (40mg/kg) (29). After oral administration, metyrapone is absorbed and eliminated rapidly from the circulation (elimination half-life ≈ 2h). Metyrapol (the active metabolite of metyrapone) has an elimination half-life of ≈ 4h (30). In other studies, serum levels of ACTH, 11-DOC and cortisol were only measured at 7.30h in the morning (31–33), but in our study, blood samples for the analysis of ACTH, 11-deoxycortisol and cortisol were obtained at 23.30h, 04.00h, 06.00h and 07.30h. The tubes for ACTH determination were immediately stored on ice after the blood draw. During the MTP test, blood pressure, heart rate and oxygen saturation were measured. After the fasting blood sampling at 07.30h, a single dose of 25mg hydrocortisone was orally administered. The MTP results were defined inconclusive if cortisol was not suppressed (cortisol < 200 nmol/L) during the metyrapone test (MTP) at any blood draw (04.00h, 06.00h, 07.30h). We used two measures to diagnose CAI. According to Steiner et al (32), a patient has CAI requiring daily hydrocortisone treatment, if the ACTH peak is < 13 pmol/L at all timepoints (04.00h, 06.00h, 07.30h) and a subnormal response, not requiring daily hydrocortisone treatment, if the ACTH peak is between 13 – 33 pmol/L, and normal if ACTH peak is > 33 at either 04.00h, 06.00h or 07.30h (32). Based on the 11-deoxycortisol levels, an 11-DOC peak lower than 200 nmol/L at all timepoints was defined as subnormal (34).

2.4 Assays

Tubes for adrenocorticotropic hormone (ACTH) determination were immediately stored on ice after the blood draw. Blood samples were measured in the Biochemical and Endocrine laboratories of the Erasmus University Medical Center, Rotterdam. ACTH and cortisol levels were analyzed with Siemens Immulite 2000XPi and 11-deoxycortisol (11-DOC) with UPLC-MSMS (Waters TQS, Etten-Leur, The Netherlands).

2.5 Statistics

Statistical analyses were performed using IBM SPSS Statistics 25. Categorical variables were reported using frequencies (N (%)) and continuous variables were expressed as median ± interquartile range (IQR), as not all variables had a normal distribution. Correlation between age and the results of the MTP test were analyzed with Spearman's Rho. Differences between the different age-categories were analyzed by Kruskall-Wallis tests. Categorical data were compared by Chi-Square tests. The longitudinal changes in patients who were tested multiple times were analyzed with Wilxocon signed rank test, McNemar test, Friedman test or the Cochran's Q test. Level of significance was set at a p-value of 0.05.

3 Results

We performed 166 tests, of which 13 (7.8%) had incomplete lab results. Of the 153 complete tests, 17 (10.9%) were inconclusive, due to inadequate suppression of the cortisol levels (<200 nmol/L) at 04.00h, 06.00h or 07.30h. In total, 136 tests were included in this study. In 93 children, we performed one test, in 30 children we also performed a second test, in 11, a third one and in 2, a fourth one.

3.1 Baseline characteristics

Table 1 presents the baseline characteristics of the 93 children, including 58 (62.4%) boys. Of all children, 50 had a paternal deletion and 34 a mUPD. The median (IQR) age at the first MTP test was 0.97 (0.48; 4.81) years.

TABLE 1 Baseline characteristics.

Number	93
Male (%)	58 (62.4)
Genotype (%) Deletion mUPD Other	50 (53.8) 34 (36.6) 9 (9.6)
Age (years)	0.97 (0.48; 4.81)
Age at start GH (years)	0.70 (0.40; 1.91)

Data presented as n (%) or as median (IQR).

3.2 ACTH, 11-DOC and cortisol levels during the overnight singe-dose MTP test in various age groups

An inverse correlation between age and serum levels of ACTH was found at time point 06.00h (r=-0.246, p=0.004), but there was no correlation between age and 11-DOC levels at any time point. An inverse correlation was also found between age and cortisol levels at time points 04.00h, 06.00h and 07.30h (r=-0.346, -0.328 and -0.390, resp. (all p-values <0.001). Based on the correlation between age and the results of the MTP test, we decided to present our results per age group.

Table 2 presents the results of the MTP test per age group. At baseline (23.30h), there were no differences between the age groups. At 06.00h, a difference in ACTH levels was found between the age groups, with the highest ACTH levels found in children aged 0-2 years. At 04.00h and 06.00h, children aged > 8 years had lower cortisol levels than children aged 0-2 years (p<0.001). At 07.30h, cortisol levels were still suppressed in children aged > 8 years, while in children aged 0-2 years, the cortisol levels had increased > 200 nmol/L. At none of the timepoints a difference in 11-DOC levels was found between the age groups.

Figure 1 presents the timing of the lowest cortisol levels and ACTH and 11-DOC peak levels per age group. The lowest cortisol levels were found at 04.00h in all age groups, however, 88.5% of children did not have their ACTH peak and 83.8% not their 11-DOC peak at the same time, indicating a delayed response of the HPA-axis after the MTP administration.

Seventy-five percent of the children aged > 8 years had their ACTH peak at 07.30h and 76% had their 11-DOC peak at 07.30h. Sixty percent of the children aged 0-2 years had their ACTH peak at 06.00h and 50% of them had their 11-DOC peak at 06.00h.

Timing of the peak ACTH and 11-DOC and lowest cortisol levels were neither significantly different between boys and girls nor between the different genetic subtypes (all p-values > 0.206)

3.3 The use of different cut-off points to diagnose subnormal acute stress-related response per age group

Table 3 presents an overview of the subnormal acute stress-related response based on the different cut-off points of ACTH and 11-DOC peak levels after a single MTP administration per age group. Since most ACTH and 11-DOC peaks were not at 07.30h (as presented in Table 2; Figure 1), we investigated the ACTH and 11-DOC levels at all time points during the entire overnight MTP test. An abnormal ACTH peak (< 13 pmol/L) was not significantly different between the age groups as it was found in 1 only child. The prevalence of a subnormal ACTH peak (13 – 33 pmol/L) was different between the age groups (p=0.007), being 22.2% in children aged 0-2 years and 70.0% in children aged > 8 years. The prevalence of a subnormal 11-DOC peak (< 200 nmol/L) was 20.6% in children aged 0-2 years. 7.7% in those aged 4-8 years and 10.0% in those aged > 8 years (p = 0.641).

TABLE 2 Results of the MTP test in children with PWS based on age groups.

	23.30h			04.00h			06.00h			07.30h		
	ACTH (pmol/L)	11-DOC (nmol/L)	Cortisol (nmol/L)	ACTH (pmol/L)	11-DOC (nmol/L)	Cortisol (nmol/L)	ACTH (pmol/L)	11-DOC (nmol/L)	Cortisol (nmol/L)	ACTH (pmol/L)	11-DOC (nmol/L)	Cortisol (nmol/L)
Age 0-2 year	4.0 (2.8; 6.2)	1.8 (0.6; 6.5)	131 (79; 263)	16.9 (11.3; 30.0)	236.2 (155.3; 277.6)	70 (40; 113)*	42.8 (25.0; 72.4)#	299.0 (195.9; 343.0)	236 (117; 349)*	32.2 (13.8; 77.8)	269.4 (166.5; 345.0)	315 (196; 441)*
Age 2-4 year	3.0 (1.8; 4.4)	2.1 (0.5; 5.4)	196 (107; 361)	11.2 (6.6; 22.1)	181.9 (83.2; 242.8)	53 (28; 74)*	20.8 (15.6; 44.0)#	223.1 (150.0; 307.0)	218 (97; 334)*	21.8 (9.7; 64.2)	234.2 (122.8; 276.8)	323 (214; 416)*
Age 4-8 year	3.7 (2.8; 5.1)	4.0 (0.9; 11.8)	209 (121; 329)	13.5 (8.2; 37.2)	199.0 (137.6; 277.8)	38 (14; 78)*	31.8 (20.2; 55.3)#	266.0 (220.5; 343.5)	113 (72; 309)*	26.1 (11.0; 84.6)	281.0 (186.5; 377)	173 (91.3; 269.0)*
Age > 8 years	4.0 (3.1; 6.4)	1.1 (0.5; 6.3)	110 (49; 243)	19.2 (8.7; 48.4)	204 (143.3; 254.2)	14 (14; 29)*	28.6 (14.7; 78.1)#	261.1 (187.0; 302.8)	43 (14; 90)*	40.8 (18.5; 80.6)	280 (219.2; 402.9)	81 (27; 154)*

Data presented as median (IQR), * p-value < 0.001, # p<0.05 for the difference between the age groups in ACTH, cortisol or 11-DOC levels at 04.00h/06.00h/07.30h.

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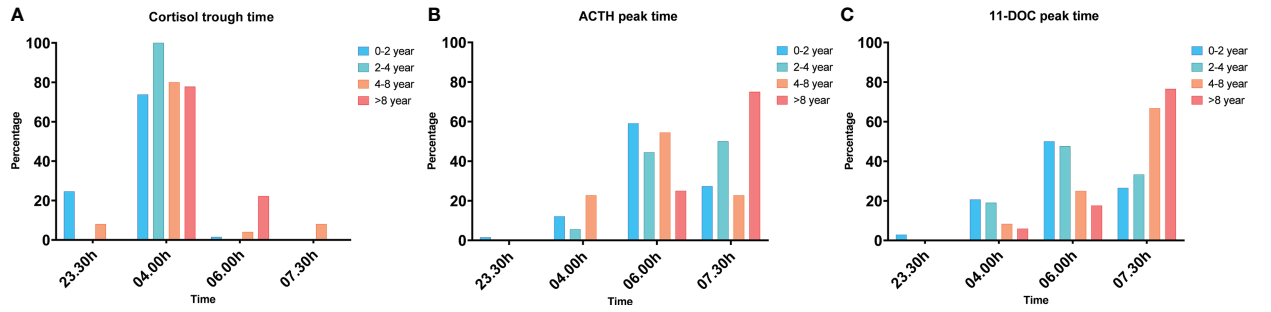

FIGURE 1
The percentage of children that had their cortisol trough time **(A)**, ACTH peak time **(B)** and 11-DOC peak time **(C)** per timepoint, in response to the overnight, single-dose MTP test, presented per age group. The cortisol trough time, ACTH peak time and 11-DOC peak time were significantly different between the age groups (p<0.001, p=0.025, p=0.007).

3.4 Results of repeated MTP tests over time

Table 4 presents the results of the repeated MTP tests. Thirty children had 2 tests over time and 11 children had 3 tests. The prevalence of an abnormal ACTH peak < 13 pmol/L was very low and not different over time, but a higher prevalence of a subnormal ACTH peak between 13-33 pmol/L was found at the second test, when children were older. No differences in ACTH peak levels were found between the first, second and third test in 11 children with 3 MTP tests. The prevalence of an subnormal response based on a 11-DOC peak < 200 nmol/L (10-13%) did not change over time.

At the first test, 18 children were still prior to the start of GH treatment, but all children started GH before the second test. A 11-DOC peak < 200 nmol/L was found in one of these eighteen patients at the first MTP test and in three patients at the second MTP test (p=0.625). An ACTH peak of < 13 nmol/L was found in none of the patients at the first test and in one patient at the second MTP test (p=0.999).

4 Discussion

This is the largest study to date investigating acute stress-related CAI in young children with PWS with the overnight single-dose MTP test. Most children had their lowest cortisol levels at 04.00h, but their ACTH and 11-DOC peak levels appeared several hours later at 06.00h or 07.30h, which might indicate a delayed response of the hypothalamic–pituitary–adrenal axis to acute stress in children with PWS. As most (young) children with PWS did not have their ACTH and 11-DOC peak levels and lowest cortisol levels at 07.30h, multiple measurements during the overnight, single-dose MTP test appear to be needed in children with PWS for an adequate interpretation of the HPA-axis response to a single-dose of MTP. The prevalence of a subnormal ACTH peak (13 - 33 pmol/L) during the overnight single-dose MTP was different between the age groups (ranged from 22.2 to 70.0%), but the prevalence of a subnormal 11-DOC peak (< 200 nmol/L) did not significantly change with age (ranged from 7.7 to 20.6%). In addition, the prevalence of a subnormal stress response based on the 11-DOC peak did not change with repeated MTP testing over time. We, therefore, conclude that the 11-DOC peak provides more consistent information than the ACTH peak to interpret the single-dose MTP test. We also showed that repeated MTP testing over time provides similar results.

Our study gives insight in the prevalence of acute stress-related CAI in children with PWS. Steiner et al. showed that an ACTH plasma level < 33 pmol/L as well as 11-DOC levels < 200 pmol/L are cut-off points with a high sensitivity of diagnosing CAI (32). However, they also showed that patients with an ACTH peak level between 13-33 pmol/L did not require daily substitution with hydrocortisone treatment and mentioned that an ACTH peak level < 33 pmol/L could lead to overdiagnosis of CAI. They concluded that plasma ACTH levels of 13 pmol/L would probably be sufficient to produce cortisol levels within the normal range (32). ACTH levels between 13-33 pmol/L, however, indicated a subnormal response during the MTP test, not requiring daily hydrocortisone treatment (32), but this would not exclude the

TABLE 3 ACTH and 11-DOC peak levels and per age group.

	0-2 year (n=63)	2-4 year (n=7)	4-8 year (n=13)	>8 years (n=10)	p-value
Peak ACTH < 13 pmol/L	1 (1.6)	0	0	0	0.923
Peak ACTH 13-33 pmol/L	14 (22.2)	2 (28.6)	7 (53.8)	7 (70.0)	0.007
Peak ACTH > 33 pmol/L	48 (76.2)	5 (71.4)	6 (46.2)	3 (30.0)	0.005
Peak 11-DOC <200 nmol/L	13 (20.6)	1 (14.3)	1 (7.7)	1 (10.0)	0.641

Data presented as n (%).

TABLE 4 Longitudinal changes in ACTH and 11-DOC peak levels.

	First MTP (n=30)	Second MTP (n=30)	p-value*	Third MTP (n=11)	p-value**
Age (years)	0.75 (0.37; 2.69)	3.05 (1.26; 5.66)	<0.001	5.55 (3.55; 9.35)	<0.001
Peak ACTH < 13 pmol/L	1 (3.3)	1 (3.3)	0.999	0	0.368
Peak ACTH 13-33 pmol/L	4 (13.3)	17 (56.7)	<0.001	4 (36.4)	0.325
Peak ACTH > 33 pmol/L	25 (83.3)	12 (40.0)	<0.001	7 (63.6)	0.197
Peak 11-DOC (<200 nmol/L)	4 (13.3)	3 (10.7)	0.999	1 (10.0)	0.368

Data presented as n (%).
*=p-value for the difference between first and second MTP test.
**=p-value for the difference between all three tests (for n=11).

need for hydrocortisone medication during stressful events. We investigated all three ACTH cut-off levels in the present study. Younger children (aged 0-2 years) were more likely to have a normal ACTH peak compared to older children, who were more likely to have a subnormal ACTH peak after MTP, while no correlation between age and 11-DOC was found. Although the prevalence of a subnormal ACTH peak changed with age, the prevalence of a 11-DOC peak < 200 nmol/L did not change with age, showing that a sufficient 11-DOC peak was reached even with a lower ACTH peak level in the older children. This might suggest that the response of the hypothalamic-pituitary-adrenal (HPA) axis improves with age and that the adrenal gland becomes more sensitive to ACTH during childhood. However, further research is needed to gain more insight in the potential changes in the HPA-axis during puberty. We found that the prevalence of a subnormal 11-DOC peak < 200 nmol/L decreased from 22.2% in children aged 0-2 years to 11.1% in children aged > 8 years, albeit not significantly. We, therefore, conclude that using the cut-off point of 11-DOC peak < 200 nmol/L is more consistent and probably more appropriate than using the ACTH peak as cut-off point.

In other studies, serum levels of ACTH, 11-DOC and cortisol were only measured at 7.30h in the morning (31–33) and the cut-off point of 11-DOC for diagnosing CAI in adults is only based on the measurement at 07.30h (32, 34). We found that the majority of the very young children had their ACTH and 11-DOC peaks at 06.00h. If only one measurement at 07.30h would have been performed, many young children would have been wrongly diagnosed. In addition, several tests would have been issued as inconclusive, as most very young children had cortisol levels > 200 nmol/L at 07.30h, while their cortisol was sufficiently suppressed at an earlier time point, mostly at 04.00h. Therefore, multiple measurements during the overnight, single-dose MTP test are needed for an accurate interpretation of the test in young children with PWS.

Almost all children had their lowest cortisol level measured at 04.00h, but almost none of them had their ACTH or 11-DOC peak at that same time. Metyrapone is quickly absorbed after oral administration (30), and maximal suppression of cortisol is found after 2 hours (29). In times of acute stress, the amygdala activates the HPA axis by signaling the hypothalamus to release CRH (35), which triggers the release of ACTH from the anterior pituitary, which in turn stimulates the adrenal cortex to release cortisol (36). This is a fast process, as approximately 15 minutes after the onset of stress, cortisol levels are rising (37, 38). We would, therefore, have expected to find high levels of ACTH and 11-DOC at 04.00h, at 4.5 hours after the single-dose MTP. Our results are in line with the findings of Oto et al., who found a delayed peak in ACTH and cortisol levels during an insulin tolerance test (ITT) in children with PWS compared to healthy children (16). Together, these findings might indicate that there is a delayed response of ACTH and 11-DOC in children with PWS during severe acute stress, when a fast response of ACTH is required. It might also explain the difference in results of our previous study (13) compared to the study performed in adults (24). In that study, the 24-hour multiple dose MTP test was used, leading to a much longer period for the HPA axis to respond with adequate levels of 11-DOC at 24 hours after the start. We have previously established that most children with PWS are able to produce adequate cortisol levels in daily life (13, 25), thus not requiring treatment with hydrocortisone in everyday life. But a delayed response of the HPA-axis during acute stress, as found by Oto et al. and suggested by our study, can result in symptoms similar to acute stress-related CAI. Although this delayed response of the HPA axis would formally not be marked as an insufficient result of the MTP test, treatment during severe acute stress may be needed.

Notably, although all children included in our study had at least one cortisol measurement < 200 nmol/L, the early morning cortisol levels were higher, especially in the very young children. According to the interpretation of the MTP test in adults, results are inconclusive when cortisol levels are not suppressed at 07.30h. We measured the hormone levels multiple times during the night, to provide more insight in the suppression of cortisol levels and the peak ACTH and 11-DOC levels. We found that cortisol levels were lowest at 04.00h in almost all children. Interestingly, although all children had a sufficient suppression of cortisol after the single-dose of metyrapone, cortisol levels increased again after 04.00h in the very young children, which resulted in cortisol levels > 200 nmol/L at 07.30h. It might be that the very young children have a faster metabolism of metyrapone and therefore a faster elimination of metyrapone and metyrapol, and that the HPA axis is already restored in the early morning. Another explanation might be that the dose of 30mg/kg is too low for very young children to maintain a sufficiently long blockade of 11-β-hydroxylase type 1. Current dosing is calculated as mg/kg, which results in a relatively low

dose in very young children (with a small body size), compared to older children. For example, a young child of 5 kg with a body surface area (BSA) of 0.3 m2 receives 150 mg metyrapone, which corresponds to 500 mg/m2, while an older child of 50 kg with a BSA of 1.6 m2 receives 1500 mg metyrapone, corresponding with a much higher dose of 938 mg/m2. Dosing metyrapone in mg/m2 might solve this. However, this needs to be investigated further and the dose should not increase the maximum of 3000mg. As most infants with PWS were tube fed and received the MTP *via* the tube, these very young children had a guaranteed intake of MTP. We recommend to perform multiple measurements during the night, when the overnight single-dose MTP test is performed to study acute stress-related CAI in young children, as the HPA axis might be restored in the early morning in young children with PWS.

This is the first study describing the results of repeated overnight single-dose MTP tests over time in children with PWS. As a negative correlation between age and peak cortisol levels to the low dose ACTH test was previously described (18, 21), there was a need for investigating changes in the function of the HPA axis over time. Our results show no difference over time in the prevalence of an insufficient 11-DOC peak level. Repeating the MTP tests over time is, therefore, not required, unless there is a clinical indication.

There are, to our knowledge, no overnight, singe-dose MTP test results reported in healthy young children and thus no reference values of peak ACTH and 11-DOC levels for the pediatric population. Although our results suggest a delayed response of the HPA-axis during acute stress, in line with Oto et al. (16), we could not prove this hypothesis due to the lack of an adequate control group. The use of age-matched healthy children as a control group would have been the first-choice to compare the response of the HPA-axis in (young) children with PWS. However, it would have been unethical to perform the MTP test in healthy children, since the test is invasive and requires overnight admission at the paediatric ICU because of a potential risk of an adrenal crisis during the night. Since data about the MTP test in children are very scarce, we used the published cut-off levels for the test in healthy adults. As there are hormonal differences between children, adolescents and adults, our results should interpreted with caution. However, all studies describing the results of the overnight single-dose MTP test in adults have only drawn blood at 07.30h, so it is unknown how ACTH and 11-DOC levels changed during the night. Although our study provides more insight into the HPA axis response to a single-dose of MTP as a proxy for acute stress in children with PWS, further research, also on the cut-off levels in healthy children and adolescents, is warranted.

In our previous study, the prevalence of acute stress-related CAI was 60% (13). This prevalence was based on an ACTH peak < 33 pmol/L as the cut-off, recommended by Steiner et al (32). As it might be that the use of a cut-off point of ACTH < 33 pmol/L leads to overdiagnosis of stress-related CAI, we decided to differentiate between an abnormal ACTH response (< 13 pmol/L at all timepoints) requiring daily hydrocortisone treatment and subnormal (ACTH response of 13-33 pmol/L), not requiring daily hydrocortisone treatment and also evaluated a cut-off point of 11-DOC < 200 nmol/L. In the present study, we found only 1 child with an abnormal response of ACTH, but a subnormal response was found in a higher percentage of children. Over the years, we observed a decline in the need for hydrocortisone stress-dose medication during mild to moderate illness in the children followed in our PWS Reference Center. Nonetheless, it remains important to be aware that acute stress-related CAI may be part of the PWS phenotype during childhood, due to a delayed acute stress response of ACTH.

Untreated growth hormone deficiency may mask symptoms of CAI, as low serum insulin-like growth factor-1 levels lead to higher levels of the enzyme that converts cortisone to cortisol (39). In 18 patients who were prior to the start of GH, we repeated the MTP tests during GH treatment and found no change in the prevalence of acute stress-related CAI. Other studies in PWS also found no difference between GH-treated patients and non-GH treated patients (14, 24). These findings suggest that GH treatment in children with PWS has no influence on the HPA-axis.

In conclusion, sing 11-DOC for the interpretation of the MTP test seems more accurate and less age-dependent than ACTH peak levels. The percentage of a subnormal ACTH response ranged from 22.2 to 70.0% between the age groups and the percentage of a subnormal 11-DOC response from 7.7 to 20.6%. Multiple laboratory measurements during the night after MTP administration are needed, as most younger children had their lowest cortisol level at 04.00h and their ACTH and 11-DOC peak at 06.00h. epeated testing for CAI should not be standard procedure, but should only be performed when there is a clinical suspicion of CAI. The increase in ACTH and 11-DOC during MTP test was found several hours after the lowest cortisol levels, suggesting a delayed response of ACTH during the overnight MTP test. As this delayed response of ACTH might lead to symptoms of CAI during acute stress, it seems safer to treat children with PWS with hydrocortisone during surgical procedures and severe acute illness (for example when the child is admitted to the Intensive Care Unit), while standard use of hydrocortisone during mild to moderate stressful conditions in daily life might not be indicated.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Review Committee of the Erasmus MC. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

LG and AH-K were responsible for conceptualization, analysis was performed by LG, GK and AH-K were responsible for the

methodology, supervision was done by GK and AH-K. Writing – original draft performed by LG, review and editing performed by LG, GK, AJ, DT-T AND AH-K. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Long-term effects of GH therapy in adult patients with Prader-Willi syndrome: a longitudinal study

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Introduction: Prader-Willi syndrome (PWS) is a complex disorder resulting from the failure of expression of paternal alleles in the PWS region of chromosome 15. The PWS phenotype resembles that observed in the classic non-PWS GH deficiency (GHD), including short stature, excessive fat mass, and reduced muscle mass. To date, a small number of studies on the long-term effects of GH treatment are available in adult subjects with PWS.

Methods: In this longitudinal study, 12 obese subjects with PWS (GHD/non-GHD 6/6) were treated for a median of 17 years, with a median GH dose of 0.35 mg/day. The median age was 27.1 years. Anthropometric, body composition, hormonal, biochemical, and blood pressure variables were analyzed in all subjects.

Results: Waist circumference was significantly lower at the end of the treatment period (p-value=0.0449), while body mass index (BMI) did not differ significantly. Compared to the baseline, a highly significant reduction of Fat Mass % (FM%) was observed (p-value=0.0005). IGF-I SDS values significantly increased during GH therapy (p-value=0.0005). A slight impairment of glucose homeostasis was observed after GH therapy, with an increase in the median fasting glucose levels, while insulin, HOMA-IR, and HbA1c values remained unchanged. Considering GH secretory status, both subjects with and without GHD showed a significant increase in IGF-I SDS and a reduction of FM% after GH therapy (p-value= 0.0313 for all).

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Discussion: Our results indicate that long-term GH treatment has beneficial effects on body composition and body fat distribution in adults with PWS associated with obesity. However, the increase in glucose values during GH therapy should be considered, and continuous surveillance of glucose metabolism is mandatory during long-term GH therapy, especially in subjects with obesity.

KEYWORDS

Prader-Willi syndrome, adults, GH deficiency, GH therapy, obesity

Introduction

Prader-Willi syndrome (PWS) is a rare multisystemic disorder resulting from the lack of expression of paternally inherited imprinted genes in the q11–13 region on chromosome 15. The main genetic mechanisms responsible for PWS are an interstitial deletion of the proximal long arm of the paternal chromosome 15 (del15) (60–70%), maternal uniparental disomy for chromosome 15 (UPD15) (25–35%), or imprinting defects and other chromosome 15 abnormalities (1–4%) (1).

The PWS phenotype is currently thought to be related to a complex hypothalamic dysfunction. The clinical picture is characterized by neonatal hypotonia, poor feeding, and lack of appetite in infancy, followed by hyperphagia leading most subjects to develop morbid obesity from early childhood (if uncontrolled), abnormal body composition, dysmorphic features, behavioral disorders, cognitive impairment and reduced longitudinal growth (2).

Multiple endocrine abnormalities are commonly observed in PWS, including GH/IGF-I axis dysfunction, hypogonadism, central adrenal insufficiency, premature adrenarche, and hypothyroidism (3). The coexistence of an altered GH response to different stimuli and reduced IGF-I levels was found both in children and adult subjects with PWS, with a prevalence of subjects diagnosed with GH deficiency (GHD) that differed between studies in relation to the sample size, weight status, genotypes, and type of GH stimulation test (4). In this light, the PWS phenotype has some common features of classic non-PWS GHD. Apart from short stature, subjects with PWS and GHD are reported to have excessive body fat, decreased muscle mass, reduced muscle strength, hypokinetic cardiac features, impaired bone mineral density, and psychological impairment (5, 6). As a result, GH therapy in children with genetically confirmed PWS without prior demonstration of GHD was approved in the United States in 2000 and in Europe in 2001. Conversely, the Consensus Guidelines for GH therapy in PWS recommended the determination of the presence of GHD after attainment of final height, and in many countries testing before starting the treatment of PWS adults is required (7).

In children with PWS, GH therapy has consistently been shown to improve growth, body composition, metabolic aspects, muscular function, and cognitive development (8). It is notable that these positive effects were observed in children with PWS, both with and without proven GHD (7). As far as adult subjects are concerned, previous data demonstrated a positive effect of GH treatment on body composition, skeletal muscle characteristics, motor performance, heart function, peak respiratory flow, metabolic markers, and psychological well-being, in the absence of major safety issues (6, 7, 9). More recently, a systematic review by Frixou et al. (10) and a meta-analysis by Rosenberg et al. (11) have confirmed that GH administration in adults with PWS was able to significantly improve body composition, without safety concerns. However, most of the studies available were characterized by a short duration, while long-term surveillance of the benefits and risks of GH therapy was mandatory for the PWS population. In this context, data from a cross-sectional study of GH treatment for a median of twenty years showed that all adults with PWS had a normal body composition (12).

With this background, in this longitudinal study, we evaluated the effects of 17 years of GH treatment on body composition, weight status, and metabolic homeostasis in a group of obese adults with PWS. Furthermore, we compared individuals with and without GHD to determine whether GH secretory status predicts metabolic response to GH therapy.

Materials and methods

Study population

Twelve obese subjects with a genetically confirmed diagnosis of PWS (seven men and five women; median age 27.1 years, range 18.5–37 years) were consecutively enrolled in the present study. All subjects were Caucasian, showing typical PWS clinical phenotype. Eleven subjects had del15, while UPD15 was found in the remaining individual.

Three men and two women had previously undergone GH treatment, in all cases suspended for at least 2 years before starting the present study. No patient was treated with anti-obesity drugs during the entire study period. Two men had undergone biliopancreatic diversion 10 and 13 years earlier, respectively. All subjects showed normal findings in the main laboratory test, and none had impaired renal or hepatic function or had central adrenal insufficiency, as assessed by the low-dose short synacthen test. During the entire study period, 11 subjects were living with their families at home, while one man was in a mixed residential hostel.

The study was approved by the Ethical Committee of Istituto Auxologico Italiano, IRCCS, Milan, Italy (ref. no. 001C726_2017; acronym: EpidAduPWS), and all subjects and their parents or legal guardians gave their written informed consent to participate in the study. The study was performed in accordance with the Declaration of Helsinki and with the 2005 Additional Protocol to the European Convention of Human Rights and Medicine concerning Biomedical Research.

Endocrine characterization and study design

At baseline, stimulated GH secretion was evaluated by dynamic testing with a standard GHRH+arginine test. Tests started at 8:30 am after overnight fasting, with the patient recumbent. In addition, basal IGF-1 serum levels were determined. Analyses were performed at the Department of Clinical Chemistry, Istituto Auxologico Italiano of Piancavallo – Verbania. GH levels were measured by chemiluminescence (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA), as previously described (5). Baseline samples were analyzed for IGF-I determination by using the Liaison XL kit (DiaSorin, Saluggia, Italy), while the more recent samples were analyzed by the chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA). IGF-I levels were expressed as SDS, adjusting for age and gender, by using the Apps (IGF1 SD_score), as reported by Chanson et al. (13).

To define the GH deficiency (GHD) status, the BMI-dependent diagnostic cut-off limits of GH peak response (GHp) for subjects with obesity (< 4.2 μg/L) (14), combined with IGF-I level < − 2 standard deviation score (SDS), were considered.

After baseline examination, the subjects received GH treatment (Genotropin; Pfizer, Rome, Italy) with a median starting dose of 0.30 mg/day for the first month. Subsequently, the dose was adjusted in order to maintain serum total IGF-1 within ±2 SDS from an age-matched reference value to avoid overdosing. At the end of the study, the median GH dose was 0.35 mg/day. All injections of GH were supervised by caregivers. No problems concerning compliance with the injections were reported.

All outcome variables were determined before starting GH therapy and at the end of the study period when the subjects were hospitalized for clinical measurements and functional testing. During the study period, the subjects were regularly followed every 6 months both as in-patients and out-patients, as previously described (15). At discharge from every visit, the subjects and their caregivers received individualized counseling on nutrition and physical activity. The dietary regimen remained unchanged during the follow-up (mean daily energy intake: 1200 Kcal/day). All our subjects performed regular physical exercise (on average 6 hours/week).

Anthropometric measurements

Physical examination included the determination of height, weight, and waist circumference (WC) by the same trained operators. All subjects were examined wearing light underwear, in fasting conditions after voiding. Standing height was measured using a Harpenden Stadiometer (Holtain Limited, Crymych, Dyfed, UK). Body weight was measured to the nearest 0.1 kg, using standard equipment. WC was determined in a standing position midway between the lowest rib and the top of the iliac crest after gentle expiration, with a non-elastic flexible tape measure.

Blood pressure measurements and body composition

Diastolic and systolic blood pressure (BP) were measured to the nearest 2 mmHg in the supine position after 5 min rest, using a standard mercury sphygmomanometer with an appropriately sized cuff. The average of three measurements on different days was used.

Dual-energy X-ray absorptiometry (DXA) was used for measurements of fat mass percentage (FM%) and lean body mass (LBM), using a GE-Lunar Prodigy scanner (GE Medical Systems, Milwaukee, WI, USA). No sedation was required.

Metabolic evaluations

Blood samples were drawn fasting in the morning using venipuncture for determination of glycemia, insulin, hemoglobin A1c (HbA1c)), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Routine laboratory data were measured using enzymatic methods (Roche Diagnostics, Mannheim, Germany). Insulin resistance (IR) was measured using homeostasis model assessment (HOMA-IR) (16).

Definitions

Body Mass Index (BMI) was defined as the weight (kg)/height $(m)^2$. The BMI cutoff point of 30 kg/m^2 was used to define obesity (17).

According to the literature (18), metabolic syndrome (MetS) was defined in the presence of three abnormal findings out of the following five parameters: central obesity, high systolic BP and/or diastolic BP, high TG, low HDL-C, and altered glucose metabolism.

Statistical analysis

Continuous variables are shown as the median, while categorical ones as absolute and relative frequencies. Wilcoxon signed rank sum test was performed to compare the values pre- and post-treatment of the continuous variables as well as the McNemar test for categorical variables. The comparisons between GHD and non-GHD were performed by the Wilcoxon rank test for continuous variables and the Fisher test for categorical ones.

Spearman coefficient (or point-biserial correlation in case of a dichotomous variable) was applied to estimate the correlation between change of anthropometric and body composition variables with selected variables at baseline (age, sex, BMI, weight, GH dosage, IGF-I SDS).

All analyses were performed using the Statistical Analysis System Software (version 9.4; SAS Institute, Cary, NC, USA). Statistical significance was set at the 0.05 level. All p-values were two-sided.

Results

Six subjects with PWS (2 women) fulfilled the combined criteria for GHD, while the remaining six showed a normal GH-stimulated secretion. IGF-1 SDS values were pathological in 9 subjects.

At baseline, all subjects had central obesity [BMI range 32.8–53.2 kg/m^2, waist range 87-126 cm (women) and 103-134 cm (men)]. Three women and one man were undergoing sex steroid replacement therapy. Two subjects suffered from central hypothyroidism (1 man) and were biochemically euthyroid on thyroxine substitution. Behavioral abnormalities were present in all subjects, and four of them (3 men) were treated with neuroleptics. MetS was detected in 4 subjects (3 men).

At the end of the study, central obesity was found in all subjects [BMI range 32.4–48.1 kg/m^2, waist range 93-122 cm (women) and 109-132 cm (men)]. Three women continued sex steroid replacement therapy, while testosterone was withdrawn in the man due to worsening behavior. Five subjects (4 men) were undergoing therapy with neuroleptics. MetS was found in 3 subjects (2 men).

The clinical, laboratory, and instrumental characteristics of our study population, at baseline and after a median of 17 years of GH therapy, are shown in Tables 1 and 2, respectively.

As expected, IGF-I SDS values significantly increased during GH therapy, thus leading to age-normalized IGF-1 levels in 11 out of 12 subjects. Waist circumference was significantly lower after 17 years of GH treatment (p-value=0.0449), while BMI did not differ significantly.

Compared to the baseline, a highly significant reduction of FM % was observed (p-value= 0.0005). LBM showed a slight increase, but the difference was not significant (p-value= 0.0557).

Both systolic and diastolic BP were unchanged during the study. At baseline, three subjects (2 men) were treated for hypertension, while five subjects (4 men) were receiving anti-hypertensive therapy at the end of the study period.

As far as metabolic parameters are concerned, fasting glycemia was significantly higher at the end of the study (p-value= 0.0142), while insulin, HOMA-IR, and HbA1c values were unchanged. Median TC, HDL-C, and TG values remained stable throughout the study. Considered individually, at baseline one woman had type 2 diabetes mellitus (T2DM) and was treated with insulin, while no other patient showed an alteration of glucose metabolism. During the period of follow-up, the woman with T2DM discontinued insulin therapy. At the end of the study, two women were treated with oral antidiabetic drugs for T2DM, while two additional men had impaired fasting glucose. Normal insulin levels were observed in all subjects at any time during the study. HbA1c values were elevated in the woman with diabetes at baseline and in three additional subjects (2 men) after GH therapy. HOMA-IR was elevated in five subjects (2 women) at the beginning of the study and in four individuals (2 women) at follow-up. At baseline, three subjects (2 women) had slight hypercholesterolemia, five subjects (1 woman) showed low HDL-C levels and hypertriglyceridemia was found in one man. None of them was taking therapy for dyslipidemia. After GH therapy, slight hypercholesterolemia was observed in one woman and low HDL-C values in three men, while one man underwent treatment for hypercholesterolemia.

Finally, no correlation was found between body composition variables (BMI and FM%) and age, sex, BMI, weight, GH dosage, and IGF-I SDS measured at baseline (data not shown).

No major side effects related to GH therapy were found. Transient edema was observed in three subjects within the first month of therapy. In all cases, symptoms resolved with a temporary reduction of GH dose. None of the subjects developed cardiovascular diseases or cancer during the study period.

Comparison between subjects with PWS (with and without GHD)

The clinical, laboratory, and instrumental data obtained at baseline and at the end of the study in subjects with PWS with different GH secretory statuses are reported in Tables 3 and 4, respectively. Both subjects with and without GHD showed a

TABLE 1 Clinical characteristics of 12 adults with PWS at baseline and after GH therapy.

		Baseline	After GH therapy	p-value
Age (years)		27.1	44.65	
Sex F/M *n (%)*		5 (42%) – 7 (58%)		
Genetic *n (%)*	UPD15 del15	1 (8%) 11 (92%)		
Height (cm)		155.35		
Weight (kg)		97.9	90.7	0.0771‡
BMI (kg/m^2)		42.54	37.01	0.0771‡
WC (cm)		124	112	0.0449‡

‡ = Wilcoxon-Mann-Whitney test.
PWS, Prader-Willi syndrome; UPD15, maternal uniparental disomy for chromosome 15; del15, deletion of the paternal chromosome 15; BMI, body mass index; WC, waist circumference. Data are expressed as median or n (%).

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TABLE 2 Laboratory and instrumental characteristics of 12 adults with PWS at baseline and after GH therapy.

	Baseline	After GH therapy	p-value
IGF SDS	-3.60	0.45	0.0005‡
Glycemia (mmol/L)	4.36	4.97	0.0142‡
Insulin (mIU/L)	10.15	9.4	0.6914‡
HOMA-IR	2.25	2.34	0.6221‡
HbA1c (%)	5.6	5.75	0.7344‡
Systolic BP (mm/Hg)	120	120	0.7266‡
Diastolic BP (mm/Hg)	80	80	1.0000‡
TC (mg/dl)	185	170.5	0.1763‡
HDL-C (mg/dl)	48	50.5	0.7744‡
TG (mg/dl)	103	107.5	0.8501‡
FM (%)	56.45	47.45	0.0005‡
LBM (kg)	44.65	48.25	0.0557‡
MetS *n (%)*	4 (33%)	3 (25%)	0.3173 ‖

‡ = Wilcoxon-Mann-Whitney test;
‖ = McNemar test.
PWS, Prader-Willi syndrome; SDS, standard deviation score; HbA1c, hemoglobin A1c; BP, blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; FM, fat mass; LBM, lean body mass; MetS, metabolic syndrome.
Data are expressed as median or n (%).

significant increase in IGF-I SDS and a reduction of FM% after GH therapy (p= 0.0313 for all). No other difference occurred at any time during the study.

As expected, the comparison between PWS subjects with and without GHD showed that the median GH peak at baseline was significantly higher in individuals with normal GH-stimulated levels (p-value = 0.0051). IGF-I SDS differed significantly between PWS with and without GHD at study entry (-3.5 vs -1.11, p=0.0202) but not after GH therapy (p-value = 0.4712). Comparison of individuals with and without GHD did not reveal any significant difference for the remaining parameters at baseline or after GH treatment.

Discussion

Adult subjects with PWS associated with obesity displays distinct phenotypic characteristics compared to subjects suffering from non-syndromic obesity. In fact, obesity in PWS is characterized by a marked

TABLE 3 Clinical characteristics of subjects with PWS (with and without GHD), at baseline and after GH therapy.

		GHD (N = 6)			non-GHD (N = 6)		GHD vs non-GHD	
	Baseline	After GH therapy	p-value	Baseline	After GH therapy	p-value	p-value at baseline	p-value after GH therapy
Age (years)	28.95	48.5		24	37		0.1275‡	-
Sex Female *n (%)*	2 (33.3%)			3 (50%)			1.0000†	-
Genetic *n (%)* UPD15/del15	1/5			0/6			1.0000†	-
Height (cm)	157.15			155.35			0.5752‡	-
Weight (kg)	97.9	89.8	0.4375!!!!	99.75	90.7	0.2188!!	1.0000‡	1.0000‡
BMI (kg/m^2)	42.6	35.6	0.4375!!	42.31	39.09	0.2188!!	1.0000‡	0.2980‡
WC (cm)	124	112	0.5625!!	125	112	0.0625!!	0.5182‡	0.9361‡

!! = Wilcoxon signed rank sum test; ‡ = Wilcoxon-Mann-Whitney test; † = Fisher test.
PWS, Prader-Willi syndrome; GHD, GH deficiency; UPD15, maternal uniparental disomy for chromosome 15; del15, deletion of the paternal chromosome 15; BMI, body mass index; WC, waist circumference.
Data are expressed as median or n (%).

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TABLE 4 Laboratory and instrumental characteristics of subjects with PWS (with and without GHD), at baseline and after GH therapy.

		GHD (N = 6)			non-GHD (N = 6)		GHD vs non-GHD	
	Baseline	After GH therapy	p-value	Baseline	After GH therapy	p-value	p-value at baseline	p-value after GH therapy
GH peak (μg/L)	2.8			6.0			0.0051‡	-
IGF-I SDS	-4.1	-0.20	0.0313!!	-2.00	1.20	0.0313!!	0.0247‡	0.6304‡
Glycemia (mmol/L)	4.3	5.55	0.0625!!	4.5	4.77	0.2500!!	1.0000‡	0.4217‡
Insulin (mIU/L)	10.15	7.9	0.4375!!	11.05	11.31	1.0000!!	0.9362‡	0.5752‡
HOMA-IR	2.25	1.93	0.5625!!	2.11	2.36	1.0000!!	1.0000‡	1.0000‡
HbA1c (%)	5.8	6.1	0.8438!!	5.4	5.6	0.2500!!	0.1697‡	0.4712‡
Systolic BP (mm/Hg)	125	120	0.1250!!	120	120	0.6250!!	0.2268‡	0.2406‡
Diastolic BP (mm/Hg)	80	80	0.5000!!	70	80	0.5000!!	0.2034‡	0.9233‡
TC (mg/dl)	184	175.5	0.2188!!	185	170	0.6875!!	0.9361‡	0.7479‡
HDL-C (mg/dl)	40	38.5	1.0000!!	55.5	55	0.6875!!	0.5211‡	0.0916‡
TG (mg/dl)	125.5	117	0.6875!!	78.5	103	0.4375!!	0.0776‡	0.5745‡
FM (%)	54.75	46.5	0.0313!!	56.45	48.85	0.0313!!	0.9362‡	0.2980‡
LBM (kg)	44.5	48.6	0.1875!!	47.55	47.85	0.4375!!	0.6889‡	0.8102‡
MetS *n (%)*	3 (50%)	3 (50%)	n.a.‖	1 (17%)	0 (0%)	n.a.‖	0.5455†	0.1818†

!! = Wilcoxon signed rank sum test; ‖ = McNemar test; ‡ = Wilcoxon-Mann-Whitney test; † = Fisher test;
PWS, Prader-Willi syndrome; GHD, GH deficiency; SDS, standard deviation score; HbA1c, hemoglobin A1c; BP, blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; FM, fat mass; LBM, lean body mass; MetS, metabolic syndrome;.n.a, not applicable.
Data are expressed as median or n (%).

increase in fat mass associated with a decrease in lean mass, lower trunk-to-appendicular fat mass ratio, and preferential subcutaneous fat distribution (19–22). In addition, these subjects show increased adipocyte volume relative to fat mass and lower expression of genes related to fibrosis and metabolic derangement (22).

GH exerts anabolic effects on several tissues and plays a critical role in regulating metabolism, which results in a marked increase in lipolysis. The benefits of GH therapy in non-PWS adults include improvements in body composition, motor performance, bone characteristics, lipid profile, and quality of life (23). It has been demonstrated that GH treatment yields positive effects on exercise capacity, psychological well-being, and body composition also in adult subjects with PWS, leading to an increase in lean body mass and a reduction in total fat mass (9–11, 24). However, few studies evaluating GH therapy of adult subjects with PWS are currently available, and most of them are of short duration.

The present study longitudinally evaluated the effects of 17 years of GH replacement on body composition in a group of 12 adults with PWS associated with obesity. After a median of 17 years, a significant reduction of FM% and WC were observed. These results were obtained during a multidisciplinary metabolic rehabilitation program, characterized over time by unchanged dietary prescriptions and constant physical activity, under the supervision of a trained staff (15). Our findings confirmed the observations made by other authors, performed with a cross-sectional design, showing a positive effect of long-term GH therapy on the body composition of adults with PWS (12, 25). Differently from the previous studies, however, our investigation enrolled subjects with morbid obesity, demonstrating that the prolonged administration of GH exerted the same benefits in subjects with severe weight excess.

Compared to the general population, there is strong evidence of a reduced life expectancy for subjects with PWS (26). The majority of deaths seem to be the direct or indirect consequences of abnormal body composition. The high fat mass and low muscle mass lead to obesity and its severe complications, including respiratory insufficiency and heart failure, which are associated with the high mortality rate of people with PWS (27, 28). Thus, it is conceivable that some of the comorbidities associated with obesity may be, at least in part, counteracted by the favorable effects on body composition of GH therapy in adults with PWS. However, randomized placebo-controlled studies showing reduced morbidity and mortality in adults with PWS receiving GH therapy are still lacking. For this purpose, a very large number of subjects with PWS is needed.

Our results showed that the improvements resulting from GH administration do not depend on the GH-stimulated secretory pattern. In fact, GH therapy was able to induce a significant reduction of FM% in both subjects with and without GH, as well as an increase in IGF-I levels. Moreover, no significant difference was observed between the two groups for the vast majority of the parameters considered at any time in the study. These findings can probably be explained by the difficulties of confirming GHD in

subjects with hypothalamic diseases, such as PWS, as adequate tests are still lacking. In this context, the GHRH-arginine test can lead to falsely normal GH responses, while the insulin tolerance test is often contraindicated because of the cumbersome procedure and potential side effects (11, 29). Altogether, these results support the need for the continuation of GH treatment in adult subjects with PWS, irrespective of their baseline GH status (30).

In our investigation, GH therapy slightly impaired glucose homeostasis, resulting in a fasting glucose increase, while insulin, HOMA-IR, and HbA1c values remained unchanged. Considered individually, glucose metabolism worsened in three additional subjects after GH therapy, whereas the woman with T2DM at baseline discontinued insulin therapy during the study. In this context, close monitoring of glucose metabolism is mandatory during GH treatment in subjects with T2DM or in those predisposed to developing diabetes. However, the worsening of glucose metabolism could be the consequence of the natural clinical history of the disease in subjects with severe obesity, rather than (or in addition) the direct effects of GH therapy. In fact, it has been previously shown that glucose metabolism disorders appear more common in adult subjects with PWS associated with obesity (31). Nevertheless, the lack of a control group is an obstacle in allowing us from addressing the question of whether GH therapy per se causes impaired glucose metabolism. Further longitudinal studies are needed to better understand this crucial point.

The strengths of this study are represented by the long-term duration of GH therapy and its longitudinal design. In addition, all subjects were recruited and followed by a single center, with the same well-trained operators and the same laboratory, which makes the interpretation of the data more reliable than those obtained with a multicenter study. Moreover, body composition was determined using DXA, which represents the gold standard for its evaluation. On the other hand, there are some limitations to our study. The main weakness is related to the small number of the study population, thus resulting in limited strength of the statistical analysis. However, it must be considered that PWS is a rare pathological condition, and enrolment of these subjects is extremely difficult. Another weak point is the lack of an appropriate control group of non-GH-treated patients. In addition, we only considered subjects with PWS associated with obesity, so our data may not be generalizable to all subjects with this syndrome. Furthermore, body fat distribution was evaluated with WC and it can be argued that this might not be the most correct analysis of visceral fat. Nevertheless, WC is commonly used as a surrogate measure of visceral adipose tissue, representing a simple index to monitor changes during interventional studies (32). Finally, our findings were obtained in a tertiary care center with further specialization on PWS, only in Caucasian subjects, and may not be generalizable to other contexts.

In conclusion, our findings show that 17 years of GH therapy has beneficial effects on body composition and body fat distribution in adults with PWS associated with obesity, regardless of whether or not GHD is present. These results seem to support the concept of continuing GH treatment in adults with genetically confirmed PWS without testing for GH secretion, as in pediatric age (30). In our investigation, however, GH therapy slightly impaired glucose homeostasis. The potential clinical consequences of these effects should be considered in the adult setting of PWS, and continued surveillance of glucose metabolism and diabetes risk should be the top priority during long-term GH therapy (7). In this perspective, the relevance of our data remains to be fully established in studies of larger cohorts.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of Istituto Auxologico Italiano, IRCCS, Milan, Italy. The patients and their parents or legal guardians provided their written informed consent to participate in this study.

Author contributions

GG and AC designed the study. AS dealt with all the administrative aspects and relations with the ethical committee. GG and LG conducted the study. DS and AZ analyzed the data. GG, AS, and AC interpreted the data and drafted the manuscript. AS and GZ contributed to the conceptualization of the findings and provided critical revisions to the manuscript. All authors have read and agreed to the published version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The relationship between endogenous oxytocin and vasopressin levels and the Prader-Willi syndrome behaviour phenotype

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Background: Oxytocin and vasopressin systems are altered in Prader Willi syndrome (PWS). However, investigations into endogenous oxytocin and vasopressin levels as well as clinical trials evaluating the effect of exogenous oxytocin on PWS symptoms have had mixed results. It is also unknown whether endogenous oxytocin and vasopressin levels are associated with certain PWS behaviours.

Method: We compared plasma oxytocin and vasopressin and saliva oxytocin levels in 30 adolescents and adults with PWS to 30 typically developing age-matched controls. We also compared neuropeptide levels between gender and genetic subtypes within the PWS cohort and examined the relationship between neuropeptide levels and PWS behaviours.

Results: While we did not measure a group difference in plasma or saliva oxytocin levels, plasma vasopressin was significantly lower in individuals with PWS compared to controls. Within the PWS cohort, saliva oxytocin levels were higher in females compared to males and individuals with the mUPD compared to the deletion genetic subtype. We also found the neuropeptides correlated with different PWS behaviours for males and females and for genetic subtypes. For the deletion group, higher plasma and saliva oxytocin levels were related to fewer behaviour problems. For the mUPD group, higher plasma vasopressin levels were related to more behaviour problems.

Conclusion: These findings support existing evidence of a vasopressin system defect in PWS and for the first time identify potential differences in the oxytocin and vasopressin systems across PWS genetic subtypes.

KEYWORDS

Prader-Willi syndrome, oxytocin, vasopressin, behaviour, plasma, saliva

1 Introduction

Prader Willi syndrome (PWS) is a neurodevelopmental disorder that arises from the absence of expression of paternally inherited imprinted genes in the chromosome 15q11-q13 region (1). In 60-70% of cases, this loss of expression is due to paternal deletion of whole or part of the region; in 20–35% of cases, it is due to maternal uniparental disomy (mUPD) of chromosome 15; and in fewer than 5% of cases, it is due to gene translocation or mutation of the imprinting centre (2, 3). Physical characteristics of PWS include hypotonia, hypogonadism, obesity, dysmorphic facial features, short stature, hypopigmentation, and thick saliva (4). Individuals with PWS can exhibit a range of behaviours, known as the PWS behaviour phenotype, that often begins in childhood and can persist into adulthood (5–8). These behaviours include hyperphagia, temper outbursts, repetitive and ritualistic behaviours, skin-picking, rigidity, and social skill difficulties (5, 9, 10). People with PWS also have an increased risk of developing psychosis (more commonly in the mUPD subtype) and/or depression (more commonly in the deletion subtype), which usually present in adolescence or early adulthood (10, 11). PWS behaviour problems, like hyperphagia, temper outbursts, skin-picking, and psychosis, are the primary cause of morbidity for individuals with PWS and their families (12–14). Unfortunately, there are few if any effective treatments for most PWS behaviour problems. So, there is an urgent need to better understand the nature of and mechanisms underlying these behaviours to inform the development of targeted interventions (10, 15).

Oxytocin (OT) and Arginine Vasopressin (AVP) are two neuropeptides thought to be involved in the PWS phenotype (16, 17). Considered sister-neuropeptides as they share evolutionary origins and differ by only two of the nine amino acids, OT and AVP can act as neurotransmitters, neuromodulators, and hormones (18–20). OT and AVP are primarily produced in the paraventricular nucleus and supraoptic nuclei of the hypothalamus and secreted from the posterior pituitary gland. However, AVP-producing neurons are also found in other limbic regions (21, 22) and a smaller amount of OT and AVP are released peripherally in various tissue (23). The OT receptor and AVP receptors (AVPR1A and AVPR1B) are 85% homologous allowing OT and AVP actions to partly overlap (24, 25).

At the genetic level, people with PWS have reduced expression of the OT receptor gene on chromosome 3p25 in RNA (26); hypomethylation of the OT gene and hypermethylation of 12 of 32 genes in the OT pathway (27). A recent study reported lower methylation in the intron 1 region of the OT receptor gene in DNA blood samples of individuals with PWS compared to age-, sex- and body mass index (BMI) matched controls. The authors further found males with PWS and psychosis showed significantly lower methylation of the OXTR exon region 1 than those without psychosis, suggesting that an OT deficiency in PWS might be associated with the higher rate of psychosis found in PWS (28). Regarding brain function, PWS has long been considered a disorder of the hypothalamus. The hypothalamus plays an integral role in controlling body temperature, hunger, thirst, fatigue, sleep, sexual development, and circadian cycles. All of these are disrupted in PWS (29, 30). Post-mortem studies suggest that compared to typically developing controls, people with PWS have smaller than average hypothalamic periventricular nuclei as well as reduced OT-producing neurons (17); OT mRNA and cells immunoreactive for OT in the hypothalamic paraventricular nuclei (31). These findings suggest a potential OT deficiency in PWS, which is supported by preclinical studies; for a recent summary see (32).

Höybye et al. (33, 34) compared plasma OT levels in people with PWS to a 'normal range' level (15 ± 5 pmol/L) that was established in a previous study. Plasma samples from a previous cohort of typically developing people with obesity were also used in this study therefore, the controls were not age- or sex-matched to the PWS participants (33, 34). Höybye et al. (33, 34) found that plasma OT levels in PWS adults were no different from the 'normal range' levels but were significantly lower than plasma OT levels of typically developing people with obesity. The authors suggested that reduced plasma OT levels might be associated with hyperphagia in PWS. In contrast, other studies suggest plasma OT levels (35); and cerebrospinal fluid OT levels (36) are higher in people with PWS compared to typically developing controls. The inconsistency in these findings highlights the need for more research to better understand the nature and potential role of abnormal endogenous OT levels in PWS.

Clinical trials examining the efficacy of exogenous OT on PWS symptomology have also produced mixed findings. Trials that reported positive results suggesting intranasal OT may reduce food-related problematic behaviours (37–39) and improve emotion and behaviour problems and social functioning (37–40) and infant sucking (41). Some trials found that the positive effects of intranasal OT were limited to younger children (38), boys and individuals with the genetic deletion subtype (37). In contrast, two trials found that OT does not produce positive effects on any PWS symptoms (42, 43). One trial found children 12 years of age and older reported significantly more sadness and anger, and less happiness (38) while another found higher doses of OT increased temper tantrums (42), one of the most debilitating behavioural characteristics of PWS (8). Together, these findings suggest that OT may be involved in the PWS behaviour phenotype. However, the inconsistent findings mean the synthetic exogenous OT that has been trialled might not be the most effective treatment and more research is needed to understand the nature of the altered OT system in PWS.

We hypothesised that the increase in temper outbursts observed after the administration of exogenous OT might be explained by the binding of OT to AVP receptors (42). AVP has been shown to play a role in aggressive behaviours (44) and the regulation of emotion and autonomic systems (45). Since people with PWS have reduced OT-producing neurons (17) and their OT receptor gene may be methylated (28) it is possible that they also have a deficit in OT receptors (42). However, even if the OT receptor is not available, exogenous OT might potentially stimulate the AVP receptors, assuming these are still functional in PWS.

Five studies have examined the AVP system in individuals with PWS. The first reported no difference in the number of hypothalamic AVP-producing neurons in people with PWS compared to controls (17). The second found lower CSF AVP

levels (36) in females with PWS compared to female controls. However, this difference did not persist when the two males with PWS were removed; that study did not include male controls (36). The third study found the AVP precursor 7B2 was present in two out of five PWS patients. However, the processed AVP was absent in the supraoptic- and paraventricular- nucleus, which the authors suggested might mean that some people with PWS may have a AVP processing deficit (46). Finally, a recent magnetic resonance imaging study found the signal intensity of the 'bright spot' in the posterior pituitary gland was negatively correlated with hyperphagia and ASD-like behaviours in adolescents and adults with PWS. The signal intensity is thought to reflect the secretion of the AVP precursor (47). Together, these findings support an AVP system defect in PWS.

We are not aware of a published study that has examined the relationships between endogenous OT and AVP levels and PWS behaviours. This information would help determine what behaviours might be related to OT-AVP system defects and could be used as primary outcome variables in future clinical trials.

The specific aims of the present study are to:

1. Compare plasma OT and AVP and saliva OT levels in individuals with PWS to typically developing age-matched controls.
2. Evaluate whether plasma OT and AVP and saliva OT levels differ across sex, genetic subtype, or the presence of psychosis in individuals with PWS.
3. Examine whether plasma OT levels correlate with saliva OT levels and plasma AVP levels in PWS and controls.
4. Examine the relationship between plasma OT and AVP and saliva OT levels and PWS symptoms.

2 Methods

2.1 Participants

The study was reviewed and approved by The University of Sydney and the Royal Children's Hospital Human Research Ethics Committees. Participants were invited into the study *via* a flyer circulated by Australian and international PWS associations and on The University of Sydney website. Participants with PWS were accompanied by a parent or primary caregiver who had known them for at least 12 months. Informed consent was obtained from all participants and the primary caregiver of the person with PWS.

Participants included 30 people with PWS (11 female/19 male) and 30 typically developing age-matched controls (19 female/11 male). Genetic subtype was known for 27 of the 30 participants with PWS: 15 had PWS due to deletion, 11 had PWS due to mUPD, and one had an imprinting centre defect. Six participants with PWS had a history of psychosis, three with mUPD, two with deletion and one with an imprinting centre defect. See Table 1 for a summary of participant characteristics. Of the 30 participants with PWS, 29 were taking at least one medication. The number of medications ranged from 0-10 with an average of two. The most common medications were antipsychotics (n=11), antidepressants (n=10), and growth hormone (n=8). See Table 2 for a summary. None of the

TABLE 1 Participant characteristics, time of blood draw, and neuropeptides measured by group presented as frequency, or mean (SD).

Variables	PWS (n=30)		Controls (n=30)
	Frequency N (%)		
Sex	Male	19 (31.7%)	11 (18.3%)
	Female	11 (18.3%)	19 (31.7%)
Genetic subtype	mUPD	15 (25%)	0
	Deletion	11 (18.3%)	0
	Mean (SD)		**Mean (SD)**
Age	22.57_a (6.17)		22.43_a (3.43)
Height	160.07_a (12.06)		170.13_b (9.10)
Weight	84.66_a (35.18)		64.46_b (12.68)
BMI	32.58_a (12.73)		22.47_b (3.09)
Full IQ	64.14_a (15.50)		116.70_b (12.19)
Time of blood draw	13.26 (1.85)		13.23 (2.16)
Ln plasma OT	5.14 (0.47)		5.20 (0.51)
Ln plasma AVP	3.80 (0.64)		4.26 (0.58)
Ln saliva OT	3.12 (0.26)		3.12 (0.37)

Values highlighted in blue on the same row not sharing the same subscript are significantly different at $p< 0.05$.
Cells with no subscript are not included in the test. Tests assume equal variances.

TABLE 2 Medications for participants with PWS, frequency (%).

Medication	Total (n=30)	Male (n=19)	Female (n=11)
Antipsychotic	11 (37%)	8 (42%)	3 (27%)
SSRI	10 (33%)	6 (32%)	4 (36%)
Levothyroxine	7 (23%)	4 (21%)	3 (27%)
Testosterone	7 (23%)	7 (37%)	–
Growth Hormone	8 (22%)	6 (32%)	2 (18%)
Oestrogen	5 (17%)	–	5 (46%)
Metformin	5 (17%)	4 (21%)	1 (9%)
Gliclazide	4 (13%)	2 (11%)	2 (18%)
Progesterone	2 (7%)	–	2 (19%)

control participants had a health condition, but one female control was using contraception.

2.2 Measures

The intelligence test was completed by people with PWS and typically developing controls. All other measures focused on PWS behaviours and thus were only completed for the PWS cohort by a parent or primary caregiver.

2.2.1 Intelligence

The Wechsler Abbreviated Scale of Intelligence Second Edition (WASI-II) (48) was used to assess intelligence quotient (IQ). People who had a Wechsler IQ test administered within the last two years did not have to do this test if data from the previous test were available.

2.2.2 Emotion and behaviour problems

The Developmental Behaviour Checklist (DBC) Primary Carer (49) and Adult Versions (49) were used to assess emotion and behaviour problems as reported by parents of people with PWS. The DBC is an informant measure that has been successfully used in a number of PWS studies (6, 7, 9, 42, 50). The Total Behaviour Problem Score (TBPS) gives an overall measure of emotion and behaviour disturbance, the subscale scores describe domains of disturbance, and the individual items can give a fine-grained indication of specific problems. The DBC has high inter-rater reliability (ICC =0.80), high internal consistency (0.941) and high concurrent validity and has been tested with other measures of behaviour disturbance. The sensitivity to change of these measures has been documented (51). The DBC-A subscales were used in the present study, subscale item examples are provided in the Supplementary Material to provide an indication of the constructs they measure.

2.2.3 Hyperphagia

The Hyperphagia Food Questionnaire for Clinical Trials (HQ-CT) was used to measure eating behaviours (52). Based on the Dykens Hyperphagia (53); the HQ-CT includes nine items that assess the severity of hyperphagic behaviours specific to PWS on a 5-point Likert scale and are summed for a total score, with higher scores indexing more hyperphagic symptoms.

2.2.4 Emotion recognition

The Ekman Emotion Recognition Task is the most widely used and validated series of photographs in facial expression research (54). This test was used to assess participants' abilities to recognise emotions from faces. This test comprises 60 photographs of faces posed by actors, each photograph depicting one of the basic emotions: happiness, sadness, anger, fear, surprise, and disgust. This measure has previously been used with individuals with PWS (55).

2.2.5 Sleep

The Epworth sleepiness scale (ESS) is a simple 8-item measure widely used in sleep research including in people with intellectual disabilities and PWS (42, 56, 57). It has been shown to have good content validity when used with people with PWS (58). The primary caregiver of the person with PWS completed the ESS.

2.3 Procedure

The study was conducted at The University of Sydney's Brain and Mind Centre and the Royal Children's Hospital in Melbourne.

2.3.1 Plasma collection

Participants underwent a blood draw. Approximately 2000 µl of blood was collected in chilled glass tubes containing disodium EDTA and kept in ice. After collection, the blood sample was centrifuged at 3000-3500 rpm for ten min at 4°C. The plasma (supernatant) was collected (400 µl per aliquot), and aliquoted into two microcentrifuge tubes (1.5 ml Eppendorf tubes) immediately and stored at -80°C (for long term) until assay.

2.3.2 Saliva collection

Participants' saliva samples were collected using a Salivette. Participants were asked to leave the Salivette swab in their mouth for two minutes without chewing. The swab was then sealed, labelled, and stored for analysis. Salivettes were ice-chilled for up

to 1 hour before being centrifuged at 4°C at 1500 × g for 15 minutes. The liquid samples were stored at −80°C.

2.3.3 Plasma and saliva processing

To measure the concentrations of OT and AVP, highly sensitive Enzyme Immunoassay kits (EIA; Arbor Assays LLC., Ann Arbor, Michigan, USA) were used. The EIA has a minimal detection rate of 16.38 pg/mL for OT and 4.096 pg/mL for AVP. The EIA has minimal cross-reactivity for other neuropeptides. To ensure the reliability of the assays, all samples were run at the same time by an investigator blind to the origins of the samples. All but one of each of the plasma coefficients of variance were less than 14.4 (m=6.03) for OT and 18.4 (m=3.5) for AVP in intra-assays, and less than 4.97 for OT and 1.42 for AVP in inter-assays. Removing the two samples that had higher coefficients made no difference to the findings, so they were retained. Similarly, the saliva CV were less than 8.88 for OT in intra-assays and less than 3.88 for OT in inter-assays. The samples were not extracted as previous research has shown that accurate measurement of OT in human blood plasma can be obtained without extraction (59, 60). Additional information on extracted vs. non-extracted plasma OT measurement can be found in the study by Plasencia et al. (61).

2.4 Statistical analyses

Analyses were conducted using IBM SPSS Statistics 28 for Windows. Two-tailed unpaired independent t-tests were conducted to determine the between-group differences (PWS vs. typically developing controls) for participant demographic characteristics. Categorical variables such as sex and genetic subtype are presented as frequencies while continuous variables are presented as mean (SD). Known confounders of endogenous OT levels include sex, time of day, age and menstrual cycle variation (62, 63). We controlled for all but menstruation as only 3/11 females with PWS had a menstrual cycle, which is not uncommon in females with PWS (64).

Comparisons of plasma and saliva neuropeptide levels between categorical variables like group allocations (PWS vs. control), sex (male vs. female) genetic subtype (mUPD vs. deletion), and psychosis (Yes vs. No) were analysed by building multifactorial one-way analysis of covariance (ANCOVA) models using the general linear model (GLM) function in SPSS. The GLM was chosen to ascertain if a combination of the categorical predictor variables explains the variability in neuropeptide levels. Age and time of blood draw were included as covariates to account for possible confounding effects. For significant results from the GLM, pairwise comparisons were made using Bonferroni as a *post hoc* test to determine if a significant difference was present in each group. ANCOVA adjusts for continuous covariates so there is a distinctive assessment of the impacts of discrete predictors. The significance level was set at $p<0.05$ but given the exploratory nature of the study, near-significant trends at $p<0.1$ were highlighted in the results.

Pearson's correlation coefficients were used to correlate neuropeptides against each other in the entire participant cohort followed by correlation by subgroups including PWS only, typically developing control group only, the male and female cohort respectively as seen in Table 3.

Correlations were also conducted for neuropeptides against variables representing emotion and behaviour problems including, TBPS from the DBC, single subscale items from the DBC, Ekman Emotion Recognition Task, ESS, and HQ-CT. Mean item scores were used to combine the TBPS and shared DBC-P and DBC-A subscales. However, some DBC-P and A subscales differ slightly between the two measures. We used the DBC-A subscale, so the subscales that are unique to the DBC-A only included data from adults with PWS and have a slightly smaller sample size.

3 Results

The natural log transformations (Ln) of neuropeptides were used for statistical analyses as tests of normality resulted in non-normally distributed (skewed) outputs. All other respective data assumptions were met for the parametric test conducted, including normality, constant variance, and parallel lines.

3.1 Participant characteristics

Independent samples t-test revealed that age was similar across those with PWS and controls. Weight and BMI were significantly higher in the PWS group while height and IQ were higher in the typically developing group (Table 1). AVP was significantly different (t= -2.93, p= 0.005) between groups, i.e., lower in PWS compared to typically developing

TABLE 3 Independent samples t-test results of neuropeptides and time of blood draw across sex, genetic subtype, and psychosis in PWS group only.

Variables	PWS ONLY					
	Sex		Genetic Subtype		Psychosis	
	Male	Female	Deletion	mUPD	Yes	No
	Mean (SD)					
Ln Plasma OT	5.09 (0.55)	5.22 (0.29)	5.19 (0.39)	5.23 (0.39)	5.10 (0.45)	5.16 (0.49)
Ln Plasma AVP	3.77 (0.66)	3.84 (0.62)	3.89 (0.72)	3.81 (0.58)	4.02 (0.52)	3.75 (0.68)
Ln Saliva OT	3.06 (0.24)	3.21 (0.28)	3.11 (0.25)	3.22 (0.32)	3.33 (0.38)	3.10 (0.24)
Time of Blood Draw	13.10 (1.88)	13.54 (1.86)	13.47 (1.35)	12.82 (1.99)	14.40 (2.41)	12.90 (1.61)

There was no significant difference.

controls (Table 1). The t-test results for neuropeptides and time of blood draw across sexes, genetic subtypes and presence of psychosis showed no significant differences (Table 3). There were 14 participants with PWS taking sex (testosterone, oestrogen and/or progesterone) and/or thyroid medication, which holds the potential to influence neuropeptide levels. Differences in AVP levels between people with PWS and controls remained when these participants were removed. The sample size was too small to conduct comparisons within the PWS participants (sex, genetic subtype, and presence of psychosis) if levels from these 14 were removed.

3.2 Comparing neuropeptides in the full cohort across group allocations and sex with covariates

ANCOVA models were developed to compare the impact of group allocation (PWS vs. control) and sex (male vs female) categories on the neuropeptides i.e., plasma OT, plasma AVP and saliva OT levels while controlling for age and the time of blood draw as covariates. There were no significant differences in plasma OT levels between PWS and controls [F (1,55) = 0.184, p= 0.669] or between males and females [F (1,55) = 0.037, p = 0.848]. Non-significant results were also found for saliva OT levels between sex and group allocation at [F (1,51) = 3.729, p = 0.059] and [F (1,51) = 0.266, p = 0.608], respectively. However, for the plasma AVP neuropeptide, levels were significantly lower [F (1,55) = 6.564, p = 0.013)] in PWS (M = 3.142, SE= 0.064) compared to controls (M = 3.096, SE = 0.060) as depicted in the boxplot (Figure 1). There were no significant differences in AVP between males and females [F (1,55) = 1.058, p = 0.308]. See Table 4.

3.3 Comparing neuropeptides in the PWS group across genetic subtypes and sex with covariates

The same modelling process was carried out to examine whether neuropeptides differed across genetic subtypes and sex within the PWS cohort. There were no differences recorded between plasma OT and AVP levels across both groups (genetic subtype and sex). However, saliva OT was significantly higher [F (1,17) = 6.543, p= 0.020] in females with PWS (M = 3.340, SE = 0.081) compared to males with PWS (M = 3.087, SE = 0.059) (Figure 2). Similarly, significant saliva OT levels F (1,17) = 4.398, p = 0.050] were found in the genetic subtype with higher levels in the mUPD group (M = 3.319, SE = 0.083) compared to deletion (M = 3.107, SE = 0.059) also shown in the boxplot, Figure 3. The effect size was evaluated from the partial ETA squared result of 0.278 indicating that a 27.8% change/variance in saliva OT can be accounted for by sex. Similarly, the effect size from the partial ETA squared result for the genetic subtype was 0.206, meaning a 20.6% change in saliva OT was attributed to the genetic subtype. See Table 4.

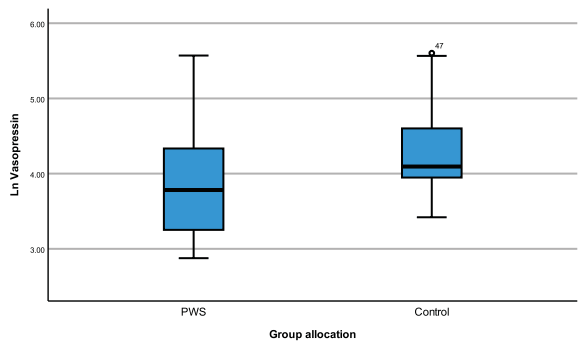

FIGURE 1
Simple boxplot of vasopressin by group of allocation (PWS and control).

3.4 Comparing neuropeptides in the PWS group across psychosis and sex with covariates

An ANCOVA model was generated to examine whether neuropeptides differed between those with and without psychosis and sex categories focusing on the PWS group only and controlling for age and time of blood draw as covariates. Results show no significant differences in plasma OT and plasma AVP levels across both psychosis and sex groups. In saliva OT, there were significant results for sex [F (1,20) = 5.492, p = 0.030] with females (M = 3.340, SE = 0.081) having higher level of saliva OT than males (M = 3.087, SE = 0.059). The effect size was evaluated from the partial ETA squared result of 0.215 indicating that a 21.5% change/variance in saliva OT can be attributed to sex.

3.5 Relationship between OT and AVP levels

As presented in Table 5, plasma OT had a significant positive correlation with plasma AVP and saliva OT levels in the full cohort (r^2 = 0.550, p < 0.001, r^2 = 0.411, p = 0.002), typically developing control group (r^2 = 0.561, p = 0.001, r^2 = 0.518, p = 0.003) and female group (r^2 = 0.485, p = 0.007, r^2 0.483 = 0.008), respectively. Scatterplots of neuropeptides by group allocation and gender are shown in Figures 4, 5. However, there were only significant correlations between plasma OT and plasma AVP for PWS (r^2 = 0.574, p < 0.001) and male-only groups (r^2 = 0.636, p < 0.001). All other correlations across neuropeptides were not significant but there was a positive trend between plasma AVP and saliva OT (r^2 = 0.224, p = 0.097) in the full cohort.

3.6 Relationship between OT and AVP levels and behaviour

Given that differences were found in neuropeptide levels across sex and genetic subtype within the PWS cohort, we examined the association between neuropeptide levels and behaviours separately for males and females and for deletion and mUPD groups, see Table 6. Given the number of analyses (9 behaviours for each sex and genetic subtype) and the small sample size (n=30), these findings must be interpreted with caution.

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TABLE 4 General Linear model/Univariate analyses of covariance of neuropeptides across fixed factor variables controlling for age and time of blood draw as covariates.

Cases	Neuropeptides	Categorical Variables		Statistics	
		Subgroup 1	Subgroup 2	ANCOVA adjusted for Age and Time of Blood Draw	
		Mean ± SE	Mean ± SE	F	p-value
Full Cohort	Ln Plasma OT				
	Group Allocation	PWS	Control		
		5.143 (0.093)	5.201 (0.093)	0.184	0.669
	Sex	Male	Female		
		5.159 (0.094)	5.185 (0.094)	0.037	0.848
	Ln Plasma AVP				
	Group Allocation	PWS	Control		
		3.142 (0.064)	3.096 (0.060)	6.564	0.013
	Sex	Male	Female		
		3.032 (0.063)	3.206 (0.062)	1.058	0.308
	Ln Saliva OT				
	Group Allocation	PWS	Control		
		3.142 (0.064)	3.096 (0.060)	0.266	0.608
	Sex	Male	Female		
		3.032 (0.063)	3.206 (0.062)	3.729	0.059
PWS only	Ln Plasma				
	Genetic Subtype	mUPD	Deletion		
		5.259 (0.133)	5.190 (0.109)	0.163	0.691
	Sex	Male	Female		
		5.182 (0.100)	5.267 (0.140)	0.243	0.627
	Ln Plasma AVP				
	Genetic Subtype	mUPD	Deletion		
		3.880 (0.221)	3.872 (0.182)	0.001	0.977
	Sex	Male	Female		
		3.826 (0.167)	3.926 (0.234)	0.121	0.732
	Ln Saliva OT				
	Genetic Subtype	mUPD	Deletion		
		3.319 (0.083)	3.107 (0.059)	4.398	0.050
	Sex	Male	Female		
		3.087 (0.059)	3.340 (0.081)	6.543	0.020
PWS only	Ln Plasma OT				
	Psychosis	Yes	No		
		4.972 (0.219)	5.218 (0.100)	1.036	0.319
	Sex	Male	Female		
		5.016 (0.134)	5.175 (0.165)	0.765	0.390

(Continued)

TABLE 4 Continued

Cases	Neuropeptides	Categorical Variables		Statistics	
		Subgroup 1	Subgroup 2	ANCOVA adjusted for Age and Time of Blood Draw	
		Mean ± SE	Mean ± SE	F	p-value
	Ln Plasma AVP				
	Psychosis	Yes	No		
		3.912 (0.312)	3.788 (0.143)	0.129	0.722
	Sex	Male	Female		
		3.816 (0.192)	3.884 (0.235)	0.070	0.794
	Ln Saliva OT				
	Psychosis	Yes	No		
		3.319 (0.083)	3.107 (0.059)	2.642	0.120
	Sex	Male	Female		
		3.087 (0.059)	3.340 (0.081)	5.492	0.030

Values highlighted in blue, and green are significant or near significant at $p < 0.05$ and $p < 0.1$, respectively.

3.6.1 PWS cohort

The relationship between OT and AVP levels and behaviour was examined within the PWS group only. For the entire PWS cohort, we found higher levels of plasma OT level were related to lower disruptive behaviours (r^2 = -0.38, p = 0.04) as well as DBC TBPS, although the latter was not statistically significant. Plasma AVP levels positively correlated with daytime sleepiness (r^2 = 0.37, p = 0.05). Saliva OT also positively correlated with depressive symptoms (r^2 = 0.47, p = 0.05).

3.6.2 PWS males

For PWS males, significant correlations were recorded between plasma OT with the strongest negative correlation in the DBC TBPS overall (r^2 = -0.58, p= 0.009) followed by social relating difficulties (r^2 = -0.57, p= 0.01), symptoms of hyperphagia (r^2 = -0.51, p = 0.03) and disruptive behaviour scales (r^2 = -0.46, p= 0.05). This means that increased plasma OT in PWS males was related to decreased behaviour problems, disruptive behaviour, social relating difficulties and hyperphagia.

3.6.3 PWS females

For PWS females, significant correlations among specific PWS symptoms were observed across all three neuropeptides (plasma OT and AVP and saliva OT). Plasma OT was positively correlated with being self-absorbed (r^2 = 0.76, p= 0.007) and negatively related to emotion recognition abilities (r^2 = -0.61, p= 0.02). Plasma AVP positively correlated with daytime sleepiness (r^2 = 0.60, p = 0.05). Finally, saliva OT was positively correlated with social relating difficulties (r^2 = 0.82, p = 0.004).

3.6.4 Deletion subtype

In the deletion group, there was a negative correlation between plasma OT and disruptive behaviour (r^2 = -0.54, p= 0.04). All other correlations in the deletion subtype were negative and trending towards significance between plasma OT and TBPS (Figure 6) as well as saliva OT and self-absorbed and depressive behaviour but did not reach statistical significance.

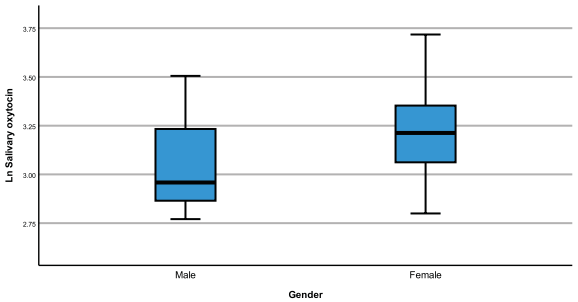

FIGURE 2
Simple boxplot of salivary oxytocin by gender (male and female) in the PWS group.

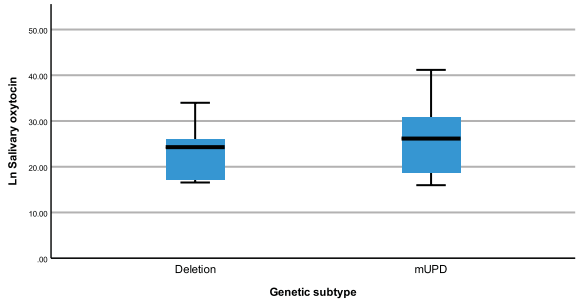

FIGURE 3
Simple boxplot of salivary oxytocin by genetic subtype (deletion and mUPD) in the PWS group.

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TABLE 5 Neuropeptide correlation for all participants, PWS and control separately and males and females separately.

		All COHORT			PWS			CONTROL			MALES			FEMALES		
		Plasma OT	Plasma AVP	Saliva OT	Plasma OT	Plasma AVP	Saliva OT	Plasma OT	Plasma AVP	Saliva OT	Plasma OT	Plasma AVP	Saliva OT	Plasma OT	Plasma AVP	Saliva OT
Plasma OT		1			1			1			1			1		
		60			30			30			30			30		
Plasma AVP		.550**		1		.574**	1		.561**	1		.636**	1		.485**	1
		<.001				<.001			.001			<.001			.007	
		60		60		30	30		30	30		30	30		30	30
Saliva OT		.411**	.224	1	.235	.290	1	.518**	.231	1	.307	-.030	1	.483**	.273	1
		.002	.097		.247	.150		.003	.219		.119	.882		.008	.152	
		56	56	56	26	26	26	30	30	30	27	27	27	29	29	29

LN OT, natural log-transformed plasma OT; LN AVP, natural log-transformed plasma vasopressin; LN OTS, natural log-transformed saliva OT. Each cell provides Pearson r^2, p-value, and the number of participants (n), respectively. Highlighted in blue is a statistically significant trend at <0.05. Cells highlighted in blue with ** are statistically significant at <0.01.

3.6.5 mUPD subtype

In the mUPD genetic subtype, although there were no significant correlations recorded against plasma OT and any PWS symptom, plasma AVP was significantly and positively correlated with five PWS symptoms including TBPS (Figure 7), hyperphagia, social relating difficulties and self-absorbed and depressive behaviours (r^2 = 0.71, p= 0.015; r^2 = 0.79, p= 0.011; r^2 = 0.72, p= 0.013; r^2 = 0.65, p= 0.029; r^2 = 0.71, p= 0.047, respectively). There was also a trend towards significance for a negative correlation between saliva OT and emotion recognition.

4 Discussion

In the present study, we examined differences in plasma OT and plasma AVP and saliva OT levels in people with PWS and age-matched typically developing controls and the relationship between these neuropeptides and PWS behaviours. People with PWS had lower plasma AVP levels than controls. No difference was found in plasma or saliva OT levels between the cohorts. For people with PWS, there was no difference in neuropeptide levels between those with and without psychosis. However, saliva OT levels were lower in people with the deletion genetic subtype compared to the mUPD genetic subtype and in males compared to females.

We found plasma AVP levels were lower in people with PWS compared to age-matched controls. Our findings support the existing literature, which suggests a potential AVP system defect in PWS (36, 46, 47). Studies that have compared AVP levels in individuals with autism spectrum disorder (ASD) to typically developing controls have reported mixed results (65–69). The sparsity of studies and variability in methodology and findings make it difficult to draw conclusions about the role of AVP in ASD (70) However, a randomised-controlled trial (RCT) of intranasal AVP reported improvements in social abilities, anxiety, and some repetitive behaviours in those taking AVP compared to placebo (71). The only other trial of an AVP product in ASD was an RCT of Balovaptan, a selective AVP 1_a receptor antagonist. This large multi-centre study found no improvements (72). AVP has not been trialled in individuals with PWS. However, centrally administered AVP has been shown to "rescue" social deficits in *Magel2-deficient* mice (73). *Magel2* is one of the genes that is reduced in expression in the PWS critical region of chromosome 15.

The findings from two meta-analyses suggest endogenous OT (plasma, urine, or saliva) levels are lower in children with ASD compared to typically developing controls but that this difference is not present in adolescents and adults with ASD (74, 75). Age differences might explain why our findings differed from a study that reported plasma OT levels to be higher in children with PWS (mean age 8.2 years; SD 2) compared to controls (35) but aligned with a study conducted with adults with PWS that reported similar rates of plasma OT with a 'normal' range (33). Our findings also differed from a study that reported higher CSF OT levels in adolescents and adults with PWS compared to typically developing controls (36), which might be due to differences in plasma and CSF. A meta-analysis of 17 studies found that plasma and CSF OT levels did not correlate at baseline but did after

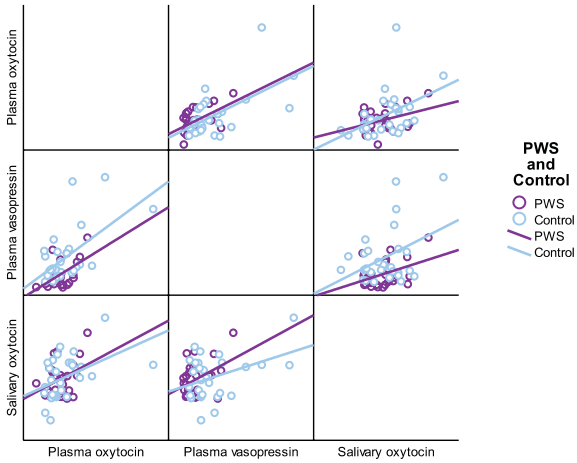

FIGURE 4
Scatterplot matrix of neuropeptides by group allocation (PWS and control).

experimentally inducing stress. However, this meta-analysis did not control for covariates like age and sex and the methodologies varied greatly across studies, including both humans and other species (76).

Recent studies have found stronger correlations in OT levels between CSF and saliva than between CSF and plasma (77, 78), suggesting that saliva might be a better biomarker of central OT levels. Our study is the first to examine saliva OT levels in individuals with PWS, we found no difference to typically developing controls. However, saliva OT levels were lower in people with PWS due to deletion compared to the mUPD genetic subtype and in males compared to females with PWS. These differences were not found in our plasma samples or plasma samples collected from children with PWS (35). We also found that for those with deletion, PWS behaviours positively correlated with plasma and saliva OT but not with plasma AVP. Conversely, for those with mUPD behaviours negatively correlated with plasma AVP but not plasma OT. These findings are discussed in more detail below. Interestingly, the longest clinical trial of intranasal OT conducted in PWS reported more significant improvements in eating and socialising behaviours in children with deletion than mUPD subtype and in males compared to females, which aligns with our findings (37). Individuals with mUPD tend to have more social communication difficulties and are more likely to meet the criteria for ASD than people with PWS due to deletion (79). Based on these behavioural differences and the known decrease in OT in children with ASD (74, 75), one might expect the PWS mUPD cohort to have lower OT levels than those with deletion. It might be that the higher rate of ASD-like behaviours in those with mUPD is associated with AVP system defects rather than OT. Future studies comparing saliva OT levels across genetic subtypes are needed to verify our findings.

Research conducted within the typically developing population has also reported lower OT levels in males compared to females (80, 81). A meta-analysis of studies conducted with people with ASD found that lower OT levels in children with ASD compared to typically developing controls were only present in males (75). However, studies conducted with adult psychiatric populations, such as major depressive disorder and obsessive-compulsive disorder reported no difference in OT levels across sexes (81). It

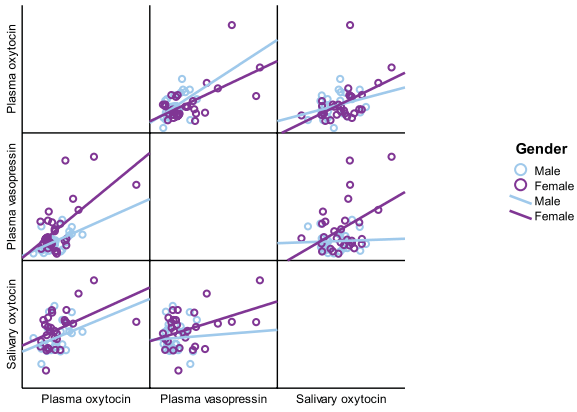

FIGURE 5
Scatterplot matrix of neuropeptides by gender (male and female).

TABLE 6 Relationships between plasma OT, AVP and saliva OT levels and symptoms in the PWS groups.

	ALL PWS			PWS MALES			PWS FEMALES			PWS Deletion			PWS UPD		
	OT	AVP	OTS	OT	AVP	OTS	OT	AVP	OTS	OT	AVP	OTS	OT	AVP	OTS
DBC MIS TBPS	-0.34	0.05	0.00	-0.58**	-0.10	-0.21	0.41	0.34	0.31	-0.47	-0.30	-0.15	0.19	0.71*	0.15
	0.07	0.79	0.99	0.009	0.68	0.43	0.21	0.31	0.38	0.080	0.285	0.601	0.585	0.015	0.717
	30	30	26	19	19	16	11	11	10	15	15	14	11	11	8
DBC MIS Disruptive	-0.38*	-0.03	-0.08	-0.46*	-0.22	-0.03	-0.19	0.32	-0.12	-0.54*	-0.38	0.01	-0.07	0.41	-0.03
	0.04	0.90	0.69	0.05	0.36	0.93	0.58	0.33	0.75	0.040	0.167	0.986	0.847	0.209	0.941
	30	30	26	19	19	16	11	11	10	15	15	14	11	11	8
DBC MIS Communication	-0.17	0.18	-0.01	-0.34	0.13	-0.25	0.49	0.31	0.42	-0.26	0.00	-0.03	0.08	0.59	-0.04
	0.37	0.34	0.98	0.16	0.61	0.34	0.12	0.35	0.22	0.349	0.992	0.925	0.810	0.056	0.922
	30	30	26	19	19	16	11	11	10	15	15	14	11	11	8
DBC MIS Self-absorbed	-0.08	0.20	0.05	-0.27	0.18	-0.21	0.76**	0.30	0.48	-0.07	0.03	-0.47	0.29	0.65*	0.38
	0.67	0.28	0.80	0.27	0.45	0.44	0.007	0.38	0.16	0.800	0.923	0.088	0.389	0.029	0.358
	30	30	26	19	19	16	11	11	10	15	15	14	11	11	8
DBC MIS Depressive	-0.13	0.18	0.47*	-0.38	-0.04	0.12	0.43	0.46	0.78	-0.39	-0.10	0.56	0.33	0.71*	0.37
	0.58	0.44	0.05	0.16	0.89	0.72	0.34	0.30	0.07	0.206	0.749	0.072	0.418	0.047	0.539
	22	22	18	15	15	12	7	7	6	12	12	11	8	8	5
DBC MIS Social relating	-0.21	0.13	0.33	-0.57*	-0.08	-0.34	0.45	0.39	0.82**	-0.38	-0.14	0.31	0.29	0.72*	0.35
	0.26	0.51	0.11	0.01	0.73	0.19	0.17	0.24	0.004	0.167	0.609	0.276	0.386	0.013	0.390
	30	30	26	19	19	16	11	11	10	15	15	14	11	11	8
Epworth daytime sleepiness scale	-0.08	0.37*	0.09	-0.15	0.24	-0.16	0.13	0.60*	0.42	-0.05	0.39	0.21	0.14	0.53	0.04
	0.68	0.05	0.67	0.55	0.33	0.55	0.70	0.05	0.23	0.849	0.151	0.474	0.688	0.093	0.926
	30	30	26	19	19	16.	11	11	10	15	15	14	11	11	8
Hyperphagia questionnaire total score	-0.23	0.16	0.13	-0.51*	-0.12	-0.06	0.14	0.56	0.06	-0.41	-0.09	0.03	0.49	.790*	0.61
	0.24	0.41	0.54	0.03	0.63	0.81	0.71	0.09	0.88	0.124	0.763	0.907	0.184	0.011	0.146
	28	28	25	18	18	16.	10	10	9	15	15	14	9	9	7
Ekman emotion recognition	-0.02	0.01	-0.18	0.18	0.03	-0.14	-.61*	-0.05	-0.37	0.10	-0.04	0.12	-0.38	-0.17	-0.68
	0.92	0.95	0.39	0.49	0.89	0.62	0.02	0.89	0.29	0.715	0.887	0.677	0.314	0.662	0.062
	28	28	26	17	17	16.	11	11	10	15	15	14	9	9	8

Highlighted in green is a nearly statistically significant trend at <0.1; highlighted in blue is statistically significant at <0.05. Each cell provides Pearson r^2, p-value, and the number of participants (n), respectively. Correlation is significant at the 0.01 level (2-tailed)**. Correlation is significant at the 0.05 level (2-tailed)*. Full table is available in the Supplementary Material (Table 3). DBC MIS TBPS, Developmental Behaviour Checklist Mean Item Score Total Behaviour Problem Score.

might be that reduced OT in males with PWS is not related to the syndrome but rather part of typical development.

A recent study reported lower methylation of the OXTR exon region 1 in males with PWS with psychosis compared to those without psychosis (28). We found no difference in OT or AVP levels between people with PWS with and without psychosis, however, only five individuals with PWS in our cohort had psychosis. So, our sample size might have been too small to detect a difference.

We examined the relationship between neuropeptides and PWS symptoms. Given differences were found in saliva OT levels across sex and genetic subtype, we examined the relationship between neuropeptides in males and females and the deletion and mUPD genetic subtype separately. For males, higher plasma OT correlated with fewer behaviour problems, including disruptive behaviour, social relating (ASD-like) difficulties and hyperphagia. For females, higher OT was associated with being self-absorbed and having difficulty with emotion recognition. These findings suggest OT and AVP might be related to different behaviour and social skill difficulties across sexes in PWS and highlight the need to identify sex differences when examining OT-AVP system abnormalities in PWS or trialling related interventions.

In people with the deletion subtype of PWS, higher plasma OT correlated with fewer behaviour problems and higher saliva OT correlated with fewer depressive and self-absorbed difficulties.

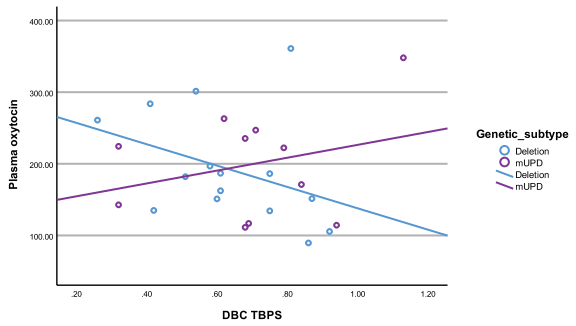

FIGURE 6
Scatterplot of plasma oxytocin and DBC TBPS by genetic subtype (deletion and mUPD).

There was no relationship with AVP. For people with the mUPD subtype the reverse was true, higher AVP correlated with higher behaviour problems, including depressive, communication, social relating, and self-absorbed difficulties as well as with higher rates of hyperphagia. There was no relationship with plasma OT and only a nearly significant negative correlation with saliva OT and emotion recognition. These findings suggest that for PWS, some behaviour problems might relate to OT system abnormalities for those with deletion and AVP system abnormalities for those with mUPD. Future studies are needed to validate our findings.

There are several study limitations worth mentioning. First, peripheral neuropeptide levels can differ from those of central levels (63, 76). While some studies suggest saliva OT might be a better biomarker of central OT than plasma (77, 78), more research is needed to confirm this hypothesis. Second, 14 participants with PWS in the current study were taking sex (testosterone, oestrogen and/or progesterone) and/or thyroid medication that could alter neuropeptide levels. Differences in AVP levels between people with PWS and controls remained significant when these participants were removed. The sample size was too small to conduct comparisons within the PWS participants if levels from these 14 were removed. While children with PWS are given growth hormones from a young age, sex hormones are typically prescribed post-puberty, so studies conducted with children could help control for this confound. Third, some authors have recommended sample extraction prior to assay for measuring OT; as unextracted samples provide higher levels than those that are extracted (82). As explained elsewhere, OT has unique properties that make this molecule both biologically active and difficult to measure (60, 83). However, recent research suggests that this discrepancy in estimates of OT may result from the removal of bound OT during the extraction step (60, 84). Whether the bound or unbound OT is more biologically active is unclear. However, several recent studies comparing functional relationships between extracted versus unextracted samples have suggested that stronger relationships between OT and behaviour are detected in measurements of unextracted plasma samples (85, 86).

Finally, this was an exploratory study with several analyses and relatively small sample size, so our findings, particularly the correlation analyses between the neuropeptides and behaviour, must be interpreted with caution.

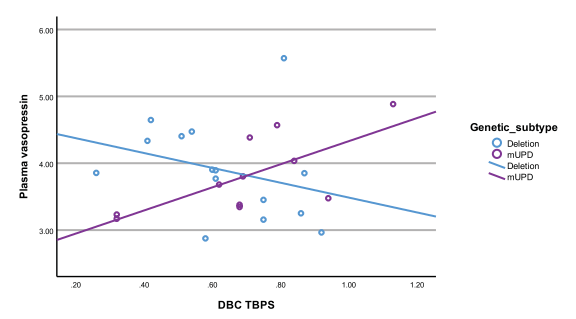

FIGURE 7
Scatterplot of plasma vasopressin and DBC TBPS by genetic subtype (deletion and mUPD).

5 Conclusions

This is the first study to examine saliva OT levels in PWS and to examine the relationship between endogenous OT and AVP and PWS behaviour. We found plasma AVP was significantly lower in individuals with PWS compared to age-matched controls. Within the PWS cohort, saliva OT levels were lower in males compared to females and individuals with the deletion vs mUPD genetic subtype. We also found that the neuropeptides correlated with different PWS behaviours for males and females and for genetic subtypes. For the deletion group, higher plasma and saliva OT levels were related to fewer behaviour problems and for mUPD higher plasma AVP levels were related to more PWS behaviours. Our findings highlight the need to consider sex and genetic subtype differences in future investigations of these neuropeptides.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The University of Sydney and the Royal Children's Hospital Human Research Ethics Committees. Written informed consent to participate in the study was provided by the participants and the legal guardian/next of kin for participants with PWS.

Author contributions

LR, SE, CC, and JH designed the research and obtained financial support. LR obtained ethics and governance approval, recruited participants, and collected data. HPN analysed the plasma and saliva samples. LR and JA analysed the data. LR, HN, and JA wrote

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1183525/full#supplementary-material

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Kidney disease in adults with Prader-Willi syndrome: international cohort study and systematic literature review

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Background: Prader-Willi syndrome (PWS) is a rare, complex, genetic disorder characterized by hyperphagia, hypotonia, delayed psychomotor development, low muscle mass and hypothalamic dysfunction. Adults with PWS often have obesity, hypertension and type 2 diabetes mellitus (DM2), known risk factors for cardiovascular disease (CVD) and chronic kidney disease (CKD). Early symptoms of CVD and CKD may be masked by intellectual disability and inability to express physical complaints. Furthermore, kidney diseases are often asymptomatic. Therefore, renal and cardiovascular disease might be missed in patients with PWS. Microalbuminuria is an early sign of microvascular damage in the kidneys

and other vascular beds. Therefore, we screened our adult PWS cohort for the presence of elevated urinary albumin and (micro)albuminuria.

Methods: We retrospectively collected anthropometric measurements, blood pressure, medical history, medication use, urine dipstick and biochemical measurements form electronic patient files. In addition, we performed a systematic literature review on kidney disease in PWS.

Results: We included 162 adults with genetically confirmed PWS (56% male, median age 28 years), of whom 44 (27%) had DM2. None had known CVD. All subjects had normal estimated glomerular filtration rate (eGFR) according to non-PWS reference intervals. Elevated urinary albumin or (micro)albuminuria was present in 28 (18%); 19 out of 75 (25%) had an increased urinary albumin-to-creatinine ratio (UACR) and 10 out of 57 (18%) had an increased urinary protein-to-creatinine ratio. Elevated urinary albumin was present at a young age (median age 26 (IQR 24-32) years) and was associated with an significantly higher BMI and LDL-cholesterol levels and higher prevalence of DM2, hypertension and dyslipidemia than those with normal UACR (p=0.027, p=0.019, p<0.001, p<0.001, p=0.011 and respectively).

Conclusion: Upon screening, one in every five adults with PWS had increased urinary albumin or (micro)albuminuria, early signs of microvascular disease. All had normal eGFR, according to non-PWS reference intervals, and none had a formal diagnosis of CVD. As muscle mass is low in PWS, creatinine levels and eGFR may be spuriously normal. Urinalysis in this patient group can be used as a screening tool for microvascular (kidney) disease. We propose an algorithm for the detection and management of microvascular disease in adults with PWS.

KEYWORDS

Prader-Willi Syndrome, kidney function tests, proteinuria, urine tract infections, cardiovascular disease, kidney disease

Introduction

Prader-Willi syndrome (PWS) is a complex, genetic, neurodevelopmental disorder caused by loss of expression of a cluster of paternally expressed genes on chromosome 15q11-13 (1). In most cases, this syndrome is caused by a paternal deletion (60-70%) or a maternal uniparental disomy (mUPD, 25-35%). Less common genetic findings include imprinting center defects (ICD, 1-3%), balanced translocations (0.1%) and gene mutations (<0.1%) (1, 2).

During early infancy, PWS is characterized by hypotonia and failure to thrive due to a poor sucking reflex and lethargy (1, 3, 4). In childhood, motor development is delayed and adults often show muscle weakness and decreased muscle mass (5–7).In both children and adults, hypothalamic dysfunction often results in pituitary hormone deficiencies, such as hypogonadism (estimated in 98% in males and 94% in females), growth hormone deficiency and hypothyroidism (estimated prevalence of 14-17%) (1, 8–14). Especially in patients without growth hormone treatment (GHt) (1, 15–19), basal metabolic rate (BMR) is low due to low lean body mass and reduced physical activity. The combination of low BMR and hyperphagia leads to an increased risk of obesity (42% of adults with PWS) (20). This high prevalence of obesity, in turn, leads to an increase in the prevalence of DM2 (17%), hypertension (18%) and hypercholesterolemia (19%) (20). These are known risk factors for cardiovascular disease (CVD) (20) and chronic kidney disease (CKD) (21–23).

With improved healthcare for patients with PWS, life expectancy has increased. As patients with PWS become older, age-related diseases like CVD and CKD become increasingly relevant. Early detection and treatment are crucial to prevent complications, loss of quality of life and early mortality (24, 25).

As symptoms are often atypical, non-specific or even absent (26–29), the detection of CKD can be challenging. In patients with PWS, the detection of CVD and CKD might be even more complicated due to the presence of intellectual disability (mild in the majority of patients) and reduced ability to express physical complaints (30, 31). Furthermore, patients with PWS have a high pain threshold that might mask the sudden thoracic pain or headache that may indicate cardiac or cerebrovascular events. The

lack of fever and pain in people with PWS can also mask other risk factors for CKD, including chronic urinary tract infections (UTIs) (1, 8, 32, 33) that may further increase the risk of CKD (34). Although never investigated, CKD and CVD might be more difficult to detect due to the combination of physical, behavioral, and neurocognitive potentially increasing morbidity and mortality in adults with PWS.

CKD can be diagnosed based on a decreased (estimated) glomerular filtration rate (eGFR) and the presence of (micro) albuminuria (35). (Micro)albuminuria can be defined as an increased urinary albumin-to-creatinine ratio (UACR) or urinary protein-to-creatinine ratio (UPCR) and is associated with an increased mortality independent of the eGFR (36, 37). Preferably, the UACR should be assessed in first morning urine samples as it corresponds more closely with a 24-hour urine collection, which is the golden standard (35, 38, 39). Microalbuminuria can indicate microvascular injury and is therefore a risk factor for CKD and CVD (40–42). In patients with diabetes mellitus, microalbuminuria is predictive of early mortality (43). Besides (micro)albuminuria, an increase in the exertion of low molecular weight (LMW) proteins such as cystatine-C, ubiquitin, retinol binding protein or alpha1-microglobulin, might also indicate kidney disease (44–46).

Little is known about CKD and CVD in the general population of adults with PWS, as these co-morbidities have only been studied in specific 'subtypes' of patients with PWS. In hospitalized patients with PWS, the prevalence of CKD was estimated to be 6.5% (47). In PWS patients with DM, microalbuminuria was found in 6.9-55.6% and proteinuria in 3.4-11.1% of (48–50), which is similar to the prevalence in the general population with DM (51). Apart from these studies in PWS 'subpopulations' of hospitalized and/or diabetic PWS patients, microalbuminuria and proteinuria have not been systematically assessed in the broader adult PWS population. This lack of information is relevant, as both microalbuminuria and proteinuria are associated with an increased risk of CKD and cardiovascular morbidity and mortality (40–42, 52–56). Also, to our knowledge, systematic screening for kidney disease (by both urine and serum analysis) has never been performed in adults with PWS. As a result, there are no guidelines for screening and treatment of CKD in adults with PWS.

To fill this knowledge gap, we have systematically screened urine and serum samples for signs of CKD in a cohort of adults with PWS. We also assessed risk factor for CKD and performed a systematic literature review on kidney and urinary tract disease in PWS. Based on our cohort study and systematic review, we provide practical recommendations for the screening and treatment of (micro)albuminuria and CKD in patients with PWS.

Method

This international cross-sectional study was performed at the Center for Adults with Complex Rare Genetic Syndromes (CRGS) at the Erasmus University Medical Center, Rotterdam, the Netherlands, the Rare Disease Center of reference 'Prader-Willi Syndrome and obesity with eating disorders', in Paris, France and the Division of Auxology in Piancavallo, Italy. Only adults with genetically confirmed PWS who visited the outpatient clinic of one of these reference center between January 2020 and December 2022 were included. Urinalysis was part of a routine systematic health screening in all included PWS patients. Ethical approval and/or individual informed consent was obtained by the Medical Ethics Committee of participating centers according to local rules and regulations.

As previously described (20), the systematic screening generally consists of a structured interview, an extensive physical examination, a medical questionnaire (depending on local guidelines), a review of medical records and biochemical measurements. The systematic screening took place during the first or follow-up visit to the outpatient clinic. If measurements could not be done during the first visit (due to behavioral or logistic issues), the next available measurement was used for the statistical analysis, provided that the time interval between first and follow up visit was less than 12 months.

Height and weight measurements were collected from medical records. When multiple measurements were available, we included the measurement the was closest (in time) to the biochemical and urinalysis. A Body Mass Index (BMI, in kg/m^2) between 18.5 and 24.9 kg/m^2 was considered lean, between 25.0 and 29.9 kg/m^2 overweight, 30.0 or more kg/m^2 obese and a BMI of 40.0 or more grade III obesity according to the World Health Organization Criteria (57).

Resting blood pressure was measured in all patients during their routine visit to the outpatient clinic. In the Dutch cohort, blood pressure was measured using a Mindray sphygmomanometer with trueBP^{TM} technology. If the blood pressure was above 140/90 mmHg (58), the measurement was repeated. If the blood pressure was still elevated, a 30-minute automated blood pressure measurement was carried out. The most recent blood pressure was included in the analysis. As patients with PWS in the Dutch reference center were not evaluated yearly for substance abuse (smoking, alcohol), most recent data were included. However, in some cases, this was more than twelve months prior to blood and urine sample collection.

Biochemical analysis

Blood and midstream urine samples were collected for general medical screening. As the outpatient clinic was often in the afternoon and fasting can be problematic in PWS due to hyperphagia, non-fasting blood samples were used. We evaluated kidney function (urea, creatinine, eGFR using Chronic Kidney Disease Epidemiology Collaboration (CKD-epi) calculation) and lipid profiles ((non-fasting) low density lipoprotein (LDL)-cholesterol, high density (HDL)-cholesterol, triglycerides, total cholesterol), glucose metabolism (fasting or random glucose, hemoglobin A1c (HbA1c)) in blood. In random spot urine samples, we measured microalbumin, urinary albumin-to-creatinine ratio (UACR), total protein and/or urinary protein-to-creatinine ratio (UPCR). As the outpatient clinic was in the afternoon in some centers, first morning urine samples were not

available. If microalbuminuria was found, the patients were referred to the general practitioner to confirm microalbuminuria in a second urine sample. Second samples were not available for this study. A urine dipstick was used to evaluate the presence of glucose, protein, leukocytes, and nitrite. If urine and blood samples could not be collected on the same day, the blood samples with the shortest time to urinalysis were used. If urine and blood samples were collected more than 12 months apart, the blood sample was excluded from analysis.

Cut-off levels

DM2 was defined as a fasting glucose of 7.0 mmol/L or higher (if available) or a repeated non-fasting glucose above 11.1 mmol/L. As fasting glucose were only available in a few patients, non-fasting glucoses were used in further analysis. Impaired glucose tolerance was tested using an oral glucose tolerance test and diagnosed if measured serum glucoses was between 7.8 to 11.1 mmol/L after two hours according to the American Diabetes Association classification (59). HbA1c was not used as a diagnostic criterium for DM2, but was used to monitor glycemic control. Furthermore, patients with a previous diagnosis of DM2 were considered as having DM2. Hypercholesterolemia was defined as a non-fasting LDL-cholesterol above 4.1 mmol/L and dyslipidemia was defined as a non-fasting LDL-cholesterol above 4.1 mmol/L or a (non-fasting or fasting) triglyceride above 2.0 mmol/L (60). The CKD-epi formula was used to calculate the estimated glomerular filtration rate (eGFR) values (61). An eGFR >90 mL/min was defined as normal kidney function. Microalbumin was considered elevated when >0.02 g/L (measured in one random spot urine sample). Microalbuminuria was defined as an UACR between 3 and 30 mg/mmol creatinine and proteinuria as an UACR >30 mg/mmol creatinine according to the Kidney Disease: Improving Global Outcomes (KDIGO) guideline (35). Proteinuria was defined as an UPCR of >20 mg/mmol creatinine (0.2 mg/mg) and nephrotic proteinuria as an UPCR of >300 mg/mmol creatinine (3.0 mg/mg) (62). Urinary total protein levels >0.15 g/L in a24 hour urine collection were considered elevated. An albumin excretion rate of 30-300mg/24 hours was considered increased and >300 mg/24 hours was considered severely increased or nephrotic proteinuria (35). When leukocytes and/or nitrite was positive in urine dipstick analysis, patients were referred to their general practitioner for urine culture and, if positive, (antibiotic) treatment.

Systematic literature review

We performed a literature search in June 2020 (updated in March 2022) using Embase, Medline, the Web of Science Core Collection, Cochrane Central Register of Controlled Trials and Google Scholar (Table S1). Studies were assessed for eligibility using the PRISMA 2020 (63) guideline and were included if they reported on both 1) (the prevalence of) kidney disease, urological abnormalities, treatment for kidney disease or markers for kidney function and 2) PWS, Prader-Willi-like syndrome (PWLS) with an abnormality in 15q11.2-13 or one of the genes on the PWS critical region. We also scanned the references of included articles. Studies reporting on cases of kidney or urological cancer, combinations of PWS with other genetic syndromes, PWLS with a deletion extending beyond the PWS critical region and articles only describing the expression of a gene in the kidney were excluded. Meeting reports, workshop summaries, reviews, conference abstracts, guidelines, articles without full-text availability and articles that were not available in English were also excluded.

Screening based on title and abstract was performed independently by three reviewers (either NN and KP or DA and KP). Full-text screening was performed independently by DA and KP. Disagreements were resolved in a meeting with a third reviewer (LG). Data was extracted by DA and NN.

Data analysis

Data was analyzed using IBM SPSS version 28.0. Continuous variables are presented as median [interquartile range (IQR)], dichotomous variables as number and percentage of patients n (%). To investigate the association between microalbuminuria, macroalbuminuria and the presence of risk factors (e.g. smoking, alcohol usage, DM2, blood pressure/hypertension and hypercholesterolemia), a Chi-squared test was used for dichotomous variables and a Mann-Whitney U test for continuous variables. A p-value of <0.05 was considered statistically significant. To adjust for BMI as possible confounder, a logistic regression analysis was performed.

Results

We included 162 adults with PWS. Baseline characteristics are displayed in Table 1. Median age at urinalysis was 28 [IQR 22-38] years. Ninety out of 162 patients were male (56%). Most patients had a deletion as the underlying genotype (n=102 (63%)), followed by mUPD (n=52 (32%)) and ICD in 1%. In six patients, PWS was genetically confirmed, but the genotype was unknown or unspecified. Sixty-seven percent of patients had received GHt, 65% received GHt during childhood and 56% were currently using GHt. The median BMI was 33.0 [IQR 26.8-41.0] kg/m^2 and 60% of subjects had obesity. Grade III obesity was present in 47 of 97 (48.5%) of patients with obesity. Forty-four (27%) patients were diagnosed with DM2 and 17out of 162 (11%) had IGT. Hypertension and hypercholesterolemia were present in 29 (18%) and 23 patients (14%) respectively. In the French cohort, dyslipidemia was present in 37%. None of the patients were diagnosed with cardiovascular events (myocardial infarction or CVA).

In eight Dutch and all French patients, urine samples were present without recent blood samples (i.e. <12 months between urine and blood sample collection). Of the patients with available recent blood samples, all had normal eGFR (CKD-EPI eGFR >90 mL/min/1.73m^2). Median eGFR (CDK-epi) was 125 [IQR 114-132] mL/min/1.73m^2. In males, median creatinine was 61 [IQR 54-69]

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TABLE 1 Baseline characteristics of included adults with PWS.

	Dutch cohort *n=68*	Italian cohort *n=51*	French cohort *n=43*	Total *n=162*
Age at urine sample, years	29 [23-38]	34 [23-43]	21 [19-32]	28 [22-38]
Male gender	40 (59)	24 (47)	26 (61)	90 (56)
Genotype				
Deletion	40 (59)	40 (78)	22 (51)	102 (63)
Type 1	8 (12)			8 (5)
Type 2	18 (27)			18 (11)
Atypical	6 (9)			6 (4)
Unknown	8 (12)			70 (43)
mUPD	24 (35)	11 (22)	17 (39)	52 (32)
ICD	2 (3)	0	0	2 (1)
Other	2 (3)	0	2 (5)	4 (3)
Unspecified	0	0	2 (5)	2 (1)
BMI (kg/m^2)	28.2 [24.0-34.7]	35.0 [27.9-41.8]	40.4 [32.7-47.0]	33.0 [26.8-41.0]
Obesity[a]	30 (44)	31 (61)	36 (84)	97 (60)
Alcohol usage	12 (27), *n=44*	1 (2), *n=50*	1 (9), *n=11*	14 (13), *n=106*
Units per week	1.2 [0.98-1.96]	7	NA	1.5 [1.0-2.0]
Smoking[b]	6 (12), *n=51*	3 (6), *n=50*	1 (3), *n=30*	10 (8), *n=131*
Cigarettes per day	9 [5.3-10]	12 [10-15]	NA	10 [7.5-11.5]
Growth hormone treatment				
Ever	45 (66), *n=68*	36 (71), *n=51*	28 (65), *n=43*	109 (67), *n=162*
During childhood	41 (64), *n=64*	28 (65), *n=43*	NA	69 (65), *n=107*
During adulthood	37 (62), *n=60*	20 (57), *n=35*	NA	57 (60), *n=95*
Current	31 (57), *n=54*	17 (53), *n=32*	NA	48 (56), *n=86*
Diabetes Mellitus				
Type 1	0	0	0	0
Type 2	11 (16)	13 (26)	20 (47)	44 (27)
IGT	7 (10)	10 (20)	0	17 (11)
DM in the past	3 (4)	0	0	3 (2)
Diabetes treatment				
No medication	1 (9), *n=11*	1 (8), *n=13*	0, *n=20*	2 (5), *n=44*
Metformin	4 (36), *n=11*	3 (23), *n=13*	1 (5), *n=20*	8 (18), *n=44*
GLP-1	0, *n=11*	0, *n=13*	3 (15), *n=20*	3 (7), *n=44*
Insulin only	1 (9), *n=11*	0, *n=13*	0, *n=20*	1 (2), *n=44*
Combination without insulin	5 (46), *n=11*	3 (23), *n=13*	5 (25), *n=20*	13 (30), *n=44*
Combination with insulin	0, *n=11*	6 (46), *n=13*	11 (55), *n=20*	17 (38), *n=44*
Pre-existing hypertension	8 (12)	14 (28)	7 (16)	29 (18)
Hypertension treatment	7 (88), *n=8*	13 (93), *n=14*	7 (100), *n=7*	27 (93), *n=29*
ACE inhibitor	3 (38)	3 (23)	2 (29)	8 (30)
ATII receptor antagonist	1 (13)	0	1 (14)	2 (7)
Ca antagonist	2 (25)	0	1 (14)	3 (11)
Diuretic	0	2 (15)	0	2 (7)
Combination	1 (13)	8 (62)	3 (43)	12 (45)
Hypercholesterolemia	4 (6)	19 (37)	NA	23 (14)
Dyslipidemia	NA	NA	16 (37)	16 (10)
Statin treatment	2 (50)	4 (21)	8 (50)	14 (9)
Cardiovascular event[c]	0, *n=65*	0, *n=51*	0, *n=43*	0, *n=162*
Laboratory results			NA[d]	
Non fasting glucose (mmol/L)	5.2 [4.8-6.2], *n=58*	5.0 [4.4-5.8], *n=51*		5.2 [4.6-6.0], *n=109*
HbA1c (mmol/mol)	38 [35-40], *n=44*	38 [33-46], *n=51*		38 [34-43], *n=95*
Creatinine (µmol/L)	62 [54-70], *n=60*	55 [47-60], *n=51*		58 [52-65], *n=111*
Normal eGFR (mL/min)	60 (100), *n=60*	51 (100), *n=51*		111 (100), *n=111*
eGFR (mL/min/1.73m^2)[e]	124 [113-131], *n=60*	126 [114-133], *n=51*		125 [114-132], *n=111*
LDL-cholesterol (mmol/L)	2.73 [2.27-3.15], *n=58*	2.94 [2.50-3.46], *n=51*		2.84 [2.33-3.18], *n=109*

Dichotomous data is displayed as number (%), continuous variables are displayed as median [IQR]. angiotensin-converting enzyme (ACE), angiotensin-II (ATII), body mass index (BMI), calcium (Ca), cholesterol (chol), diabetes mellitus (DM), glucagon-like peptide 1 agonist (GLP-1), hemoglobin A1c (HbA1c), impaired glucose tolerance (IGT), imprinting center defects (ICD), interquartile range (IQR), low-density lipoprotein (LDL), high-density lipoprotein (HDL), maternal uniparental disomy (mUPD), not available (NA). [a] Obesity was specified as BMI ≥30 kg/m^2. [b] Three patients used to smoke in the past, but stopped at time of data collection. [c] Cardiovascular events included coronary disease, myocardial infarction, transient ischemic attack and stroke. [e] eGFR was calculated using CDK-epi. [d] Not calculated as dates of blood samples were unavailable.

μmol/L (reference value 65-115 μmol/L) and eGFR was 127 [IQR 118-135] mL/min/1.73m^2. In females, median creatinine levels were 55 [IQR 47-61] μmol/L (reference value 55-90 μmol/L) and eGFR was 120 [IQR 111-128] mL/min/1.73m^2. All patients were classified as CKD stage G1 (35). When applying previously suggested alternative reference values for eGFR for adults with PWS (>98 ml/min/1.73m^2 in males and >93 ml/min/1.73m^2 in females) (20) renal function of only two males and two females were below the reference value (94 and 98 ml/min/1.73m^2 in males and 90 and 92 ml/min/1.73m^2 in females respectively).

Urinalysis

Microalbuminuria and proteinuria

Twenty-eight out of 160 patients (18%) had elevated urine albumin, microalbuminuria or proteinuria (Table 2). Two patients were excluded from this analysis as data on (micro)albuminuria, proteinuria, UACR and UPCR were missing. Of the two males and two females with impaired renal function according to alternative reference values for eGFR for adults with PWS, only one female had microalbuminuria (Case 8, Table 3) (20).

Urine albumin was elevated in 24 of 157 subjects (15%). The UACR was calculated in 75 patients, as urine creatinine was not systematically assessed in all patients, and was abnormal in 19 of 75 patients (25%), of whom 15 had microalbuminuria and four had macroalbuminuria. UPCR was available for 57 patients, of whom ten (18%) had proteinuria and one nephrotic proteinuria (i.e. 460 mg/mmol).

We did not find any association between elevated urine microalbumin or (micro)albuminuria and age, gender, genotype, GH treatment, alcohol consumption or smoking (Table 2). Three of 28 patients with normal BMI, three of 37 who were overweight and 22 of 95 with obesity had elevated urine microalbumin or (micro) albuminuria. Patients with elevated urine microalbumin or (micro) albuminuria had significantly higher BMI (38.7 vs 32.4 kg/m^2,

TABLE 2 Comparison of adults with PWS and normal versus (micro)albuminuria[a].

	Number of observations	Normal *n=132*	Number of observations	(micro)albuminuria *n=28*	p-value
Age at urine sample (years)	132	20 [21-38]	28	25 [23-33]	0.65
Male gender	132	76 (58)	28	14 (50)	0.46
BMI (kg/m^2)	132	32.4 [26.4-40.4]	28	38.7 [31.1-43.8]	**0.027**
Obesity		73 (55)		22 (79)	**0.023**
Genotype	132		28		0.62[b]
Del		84 (64)		16 (57)	
mUPD		42 (32)		10 (36)	
ICD		2 (1)		0	
Other		4 (3)		2 (7)	
Alcohol usage	89	10 (11)	16	3 (19)	0.40
Units/week	9	2 [1-5]	2	NA[c]	NA[c]
Smoking	105	8 (8)	25	2 (8)	0.69
Cigarettes/day	6	10 [8-13]	2	NA[c]	NA[c]
GH treatment					
Ever	132	92 (70)	28	17 (61)	0.35
Childhood vs never	92	62 (67)	13	7 (54)	0.34
Adulthood vs never	82	52 (63)	11	5 (46)	0.25
Ongoing vs never	73	43 (59)	11	5 (46)	0.40
Diabetes Mellitus type 2	132	28 (21)	28	16 (57)	**<0.001[d]**
Pre-existing hypertension	132	17 (13)	28	11 (39)	**<0.001[e]**
Blood pressure	128		26		0.49
Systolic		125 [119-133]		124 [117-141]	0.65
Diastolic		80 [70-83]		80 [70-88]	
Hypercholesterolemia	103	20 (19)	14	3 (21)	0.86
Dyslipidemia	29	7 (24)	14	9 (64)	**0.011[f]**
Laboratory results					
Glucose (mmol/L)	97	5.1 [4.6-6.0]	12	5.2 [4.7-6.0]	0.58
HbA1c (mmol/mol)	84	38 [34-42]	11	41 [36-56]	0.20
Creatinine (umol/L)	98	58 [53-65]	12	58 [42-66]	0.44
eGFR (mL/min/1.73m^2)	98	125 [113-131]	12	127 [118-134]	0.34
LDL cholesterol (mmol/L)	97	2.81 [2.31-3.14]	12	3.35 [2.68-4.06]	**0.019**

Data are presented as median [IQR] for continuous variables and as n (%) for nominal data. body mass index (BMI), deletion (del), estimated glomerular filtration rate (eGFR), growth hormone (GH), hemoglobin A1c (HbA1c), imprinting center defect (ICD), low-density lipoprotein (LDL), maternal uniparental disomy (mUPD), not available (NA). [a] Data presented for n=160 as in two Dutch patients, only urine dipstick was available. [b] del vs mUPD. [c] not available because of small numbers. [d] After adjusting for BMI, p=0.002. [e] p=0.013 after adjusting for BMI. [f] After adjusting for BMI, p=0.032.

Bold values mean a statistically significant difference.

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TABLE 3 Clinical characteristics and laboratory results of nine Dutch adults with PWS and microalbuminuria or elevated UACR.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
CKD stage (35)	G?A2[a]	G?A2[a]	G1A1	G1A2	G1A2	G1A3	G1A2	G1A2	G1A2
Age at urine sample, years	29	47	25	36	25	26	27	52	25
Gender	Female	Female	Female	Male	Male	Female	Female	Female	Male
Genotype	Del, atypical	mUPD	Del type 2	Del, atypical	mUPD	Del type 1	Other	Del type 2	Del, atypical
BMI (kg/m^2)	28.6	31.8	40.8	23.8	28.1	37.4	23.3	38.5	21.8
Alcohol usage/week **Smoking**/day	0 0	NA NA	0 0	NA NA	1 0	NA NA	1.5 0	NA 0	0.8 7
GH treatment	Never	Never	Only as child	Only as child	Started as child, ongoing	Only as child	Started as child, ongoing	Never	Started as child, ongoing
Comorbidities Diabetes Pre-existing hypertension Hypertension treatment Blood pressure systolic/ diastolic (mmHg) Hypercholesterolemia Hypothyroidism Hypotthyroidism treatment	No No NA 141/87 No No No	No Yes None 190/95 No Subclinical No	No Yes ATII NA No No No	No No NA NA No No No	IGT Yes CA 156/105 No No No	No No ACE-I 145/106[b] No Yes Yes	IGT No NA 140/80 No Yes Yes	No Yes ACE-I 137/79 In the past Yes Yes	No No NA 122/66 No No No
Serology Serum creatinine (µmol/L) eGFR (mL/min/1.73m^2) LDL cholesterol (mmol/L)	 NA NA NA	 NA NA NA	 47 132 3.2	 67 117 2.1	 74 122 2.8	 65 113 4.1	 56 123 3.5	 66 92[c] 4.0	 59 134 2.1
Urine Protein Leukocytosis Nitrite Total protein (g/L) UPCR (mg/mmol) Microalbuminuria (mg/L) UACR (mg/mmol)	 Neg Pos Pos <0.06 NA 6.0 3.8	 Trace Pos Neg 0.15 30.1 61.3 12.3	 Trace 2+ Pos 0.08 0.07 20.6 1.9	 + 1+ Neg 0.2 NA 26 NA	 Trace Neg NA 0.22 11.3 80.7 4.1	 + Neg Neg 0.77 74.0 570 54.8	 Neg 4+ Neg 0.12 43.0 65 23.2	 Neg Pos Neg NA NA 37.4 10.7	 NA NA NA 0.05 11.0 25 5.8
Persistent microalbuminuria	NA	NA	NA	NA	Yes	Yes	No	NA	No

Angiotensin-converting enzyme inhibitor (ACE-I), angiotensin-II receptor antagonist (ATII), body mass index (BMI), calcium antagonist (CA), chronic kidney disease (CKD), deletion (del), estimated glomerular filtration rate (eGFR), growth hormone (GH), impaired glucose tolerance (IGT), low-density lipoprotein (LDL), maternal uniparental disomy (mUPD), not available (NA), urinary albumin-to-creatinine ratio (UACR), urinary protein-to-creatinine ratio (UPCR). [a] No recent eGFR available, based on previous laboratory results, CDK stage for both case 1 and 2 would be G1A2. [b] 30 minute blood pressure evaluation was performed as hypertension was not previously diagnosed, 24 hour urine sample showed evident proteinuria and patient was treated with an ACE-inhibitor. [c] When applying PWS adjusted reference values, eGFR is mildly decreased (<93 mL/min/1.73^2 in females).

p=0.027) and obesity was more prevalent (22 out of 28 (79%) vs 73 of 132 (55%), p=0.023). DM2 and hypertension were more prevalent in those with elevated urine microalbumin or (micro) albuminuria than without (57% vs 21%, p<0.001 and 39% vs 13%, p<0.001 respectively). After adjusting for BMI as possible confounder, these variables remained significantly associated with elevated urine microalbumin or (micro)albuminuria (p=0.02 and p=0.013 respectively). Laboratory results on glucose, HbA1c, creatinine, eGFR did not differ significantly between the patients with and without elevated urine microalbumin or (micro) albuminuria. However, LDL cholesterol was significantly higher in those with elevated urine microalbumin or (micro)albuminuria than those without (3.35 [2.68-4.06] vs 2.81 [2.31-3.14], p=0.019).

Characteristics, comorbidities, and laboratory results of the Dutch patients with elevated urine microalbumin or UACR are shown in Table 3. In the Dutch cohort, six out of nine (67%) patients with elevated UACR or UPCR also had leukocyturia and in two subjects, nitrite was also positive. Patients with both leukocyturia and nitrite in the urine sample were advised to have their urine checked for urinary tract infection at the general practitioner. Two patients had transient microalbuminuria or proteinuria (Table 3), one of them had leukocytosis at dipstick analysis. Neither had hypertension. Two other Dutch patients had microalbuminuria/proteinuria that persisted after repeating the urine sample. In one case (case 6) a 24-hour urine sample was collected that showed an elevated total protein of 0.56g/24h). A 30-minute blood pressure measurement at home was carried out, which showed systolic and diastolic hypertension. During analysis of hypertension, psychosocial stress turned out to be a strong contributing factor. This patient was referred to our

neuropsychologist for psychosocial support and stress management. In addition, an angiotensin-converting enzyme (ACE) inhibitor was prescribed.

Leukocyturia in the Dutch cohort

Urine dipstick in the Dutch cohort was positive for leukocytes in 18 of 55 (33%) patients and nitrite in two of 54 (4%). In males, leukocyturia was seen in four of 33 patients (12%, and there was 1+ protein in two, 2+ protein in one and amount unknown in one patient). In females, 14 out of 22 (64%) had leukocyturia with 1+ protein in two, 2+ protein in four, 3+ protein in one, 4+ protein in two and amount unknown in five patients. As in the non-PWS population, leukocyturia was more prevalent in females than in males ($p<0.001$).

Glucosuria in the Dutch cohort

Urinary glucose was present in three out of 51 (6%) patients, all of whom had DM2 and were already on a treatment regime. Non-fasting (random) blood glucose levels of these patients ranged from 11.9 to 17.4 mmol/L and HbA1c from 62 to 70 mmol/mol, indicating suboptimal glucose management. None of them had proteinuria.

Systematic literature review

Forty-nine publications were included in the literature search (Figure 1). Multiple case reports have been published on patients with PWS and kidney disease. We found eight case reports describing proteinuria (64–71). There were three case reports in which albuminuria was reported in patients with PWS and DM2 (72–74). One article described a patient with stage 3 CKD (75). Five cases were found describing acute kidney injury (76–80) due to several different causes: pneumoperitoneum with abdominal compartment syndrome (76), secondary rhabdomyolysis caused by hyperpyrexia (77), nephrotoxic medication (78), hyperosmolar hyperglycemic state (79) and respiratory failure due to obesity hypoventilation syndrome (80). We found two case reports describing adults with PWS who needed hemodialysis due to end stage kidney disease, one patient from intrinsic nephrotic disease, the other case from uncontrolled diabetes mellitus (81, 82). Congenital renal or urological abnormalities, such as a horseshoe kidney, bilateral non-communicating paraurethral meatus, and absence of one kidney were also reported (83–85). Multiple UTI were reported in one case report (86) and a congenital disorder (megacystic-microcolon-intestinal hypoperistalsis syndrome) in

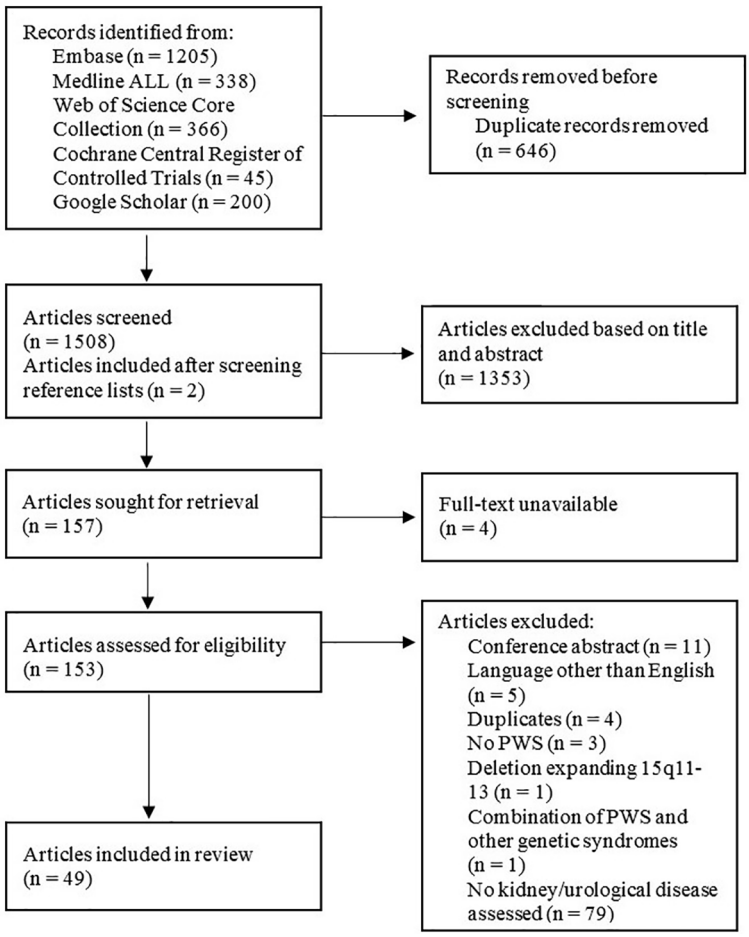

FIGURE 1
Systematic literature review (63).

another (87). Two articles reported on potential underlying genetic components: i) the *NDN* gene which is on the PWS region and ii) *NIPA2*, a gene not imprinted in patients with PWS that influences magnesium transportation, and that possibly reflects an association between kidney homeostasis or disease and PWS (88, 89). Three studies reported on the use of specific medications, such as ACE-inhibitors and (loop)diuretics, that could influence kidney function (90–92). One article mentioned the use of these drugs, without reporting on kidney function (90). In two other articles about these drugs, kidney function was normal (91, 92).

Kidney disease

We found 16 studies reporting on kidney disease in patients with PWS (Table 4). Sinnema et al. (17) reported a prevalence of urinary tract problems or kidney disorders in 6% (six of 102 adults) with a confirmed diagnosis of PWS. In these patients, blood and urine analysis were performed based on a clinical suspicion of urine tract or kidney problems. Systematic screening of urine or blood samples was not performed in these patients.

In patients with PWS and diabetes, three studies reported microalbuminuria (7-56%) or proteinuria (3-11%) (48–50). In 480 patients with PWS, Pemmasani et al. (47) reported a prevalence of CKD of 6.5% (31 patients), that increased with age. Renal insufficiency was reported by Höybye et al. (93) in one out of ten patients with PWS who were treated with GHt. Koizumi et al. studied body composition in seven patients with PWS, one of whom was diagnosed with proteinuria and was taking an ACE-inhibitor (94). Van Nieuwpoort et al. reported a median [IQR] serum creatinine in 15 patients of 69.0 [10.0] µmol/L with no significant difference between men and women. According to Manzardo et al. (96) renal dysfunction was more prevalent in patients with PWS who had been diagnosed with thromboembolism than those without (6 out of 34 (17%) vs 14 out of 1013 (1.4%), $p<0.001$).

Multiple studies have investigated causes of death in PWS. Cohen et al. (97) described autopsy results in three children with PWS in whom urinalysis was negative for proteinuria. The kidneys had normal weight but showed glomerular enlargement and dilatation of capillaries as well as increased cellularity compared to controls, suggesting hyperfiltration. Nagai et al. (98) compared 20 patients with PWS with (age range 0.7 to 15 years) and without (never received, age range 9 months to 34 years) GHt. In the group that did not receive GHt, two patients died from renal and cardiac failure, versus none in the GHt group. Two other studies investigating causes of death in PWS both found that 2% died with or because of CKD (24, 99).

Urological disorders, diseases and congenital anomalies of kidney and urinary tract

Results of studies reporting urological disorders in PWS are shown in Table 5. Torrado et al. (104) reported that five out of 180 (3%) patients with PWS had Congenital Anomalies of Kidney and Urinary Tract (CAKUT), which was significantly higher than in the general population (0.1-0.5%) ($p<0.05$). Malformations found included renal hypoplasia, ureteral duplication, bifid renal pelvis, vesicoureteral reflux, pelvicalyceal dilatation and ureteral valves (104). No association between genotype and reno-ureteral malformations was found ($p=1.00$). Sinnema et al. (101) found CAKUT (bilateral duplication of kidney and ureter system) in one of 12 patients and Pacilli et al. (105) found a horseshoe kidney in one of 33 patients.

Nocturnal enuresis was described in 14-22% (100, 102). Two studies found a decrease in nocturnal enuresis with increasing age (100, 102). Daytime urinary incontinence was present in 3-12% (100, 102). Urgency symptoms, possibly related to overactive bladder, were reported in 26-60% of whom 22-32% had a history of UTI's (100, 102).

Chao et al. (34) reported that urodynamic tests were abnormal in 17 out of 34 patients with PWS, with abnormal flowmetry in 12 out of 34 and elevated residual bladder volume in 10 out of 20. In patients with lower urinary tract symptoms (LUTS), peak flow rate, voided volume, and bladder capacity were significantly decreased compared to patients without LUTS.

Discussion

Our study in 162 adults with PWS showed that one in every five (young) adults had early signs of microvascular disease, measured by elevated urine microalbumin or (micro)albuminuria, while eGFR was normal. However, as muscle mass is low in PWS (6), creatinine levels and eGFR may be falsely 'normal'. Our findings suggest that pre-symptomatic kidney injury may be missed when only blood measurements are performed. Therefore, in this patient group, urinalysis is essential for timely screening of microvascular (kidney) disease. We propose an algorithm for the detection and management of microvascular disease in adults with PWS.

Twenty-eight out of 160 (18%) patients had elevated urine albumin, (micro) albuminuria or proteinuria; eleven of whom were diagnosed with hypertension and sixteen with DM2. No age difference was found between patients with normal and elevated UACR.

In our literature review, we found an overall prevalence of microalbuminuria of 7-56% and macroalbuminuria of 3-11% in patients with PWS and diabetes (48–50). We did not find any studies on microalbuminuria or proteinuria in patients with PWS without DM2. Our study is the first to analyze the presence of (micro)albuminuria in patients with PWS with and without DM2. In the general population, around 25% of patients with longstanding (>10 years) DM2 have microalbuminuria and 5-20% proteinuria (51). Before antihypertensive treatment, patients with essential hypertension (in the general population) developed proteinuria in 35-65% and renal insufficiency in 33%, depending on severity of hypertension, DM2, medication, and age (106). In our cohort, 16 of 44 (36%) of patients with DM2 had (micro) albuminuria. Notably, the prevalence of albuminuria in our PWS patients *without* DM2 was similar to that in non-PWS patients *with* long-term DM2. The albuminuria in our PWS patients suggests microvascular damage is already present at a young age (23-33 years).

In this study, as in the normal population, albuminuria was associated with hypertension, DM2 and obesity. Obesity is

TABLE 4 Results of studies reporting on PWS and kidney diseases with more than one patients.

Author (year)	Study design	Method	Baseline characteristics:	Results	Limitations/ remarks
Kidney disease					
Sinnema et al. (2011) (17)	Cross sectional study	Semi-structured interview with patient and main caregivers and review of medical files.	N=102 Age: mean 36.2 (range 18-66) years BMI: mean 32.2 (SD ±7.9, range 18.6-51.9) kg/m^2 Gender: 49M, 53F Genotype: 55 del, 44 mUPD, 3 ICD	Urinary tract/kidney problems were present in 6 (6%) of patients. No association with genotype (del vs mUPD) was found.	No distinction between urinary tract and kidney problems.
Tsuchiya et al. (2011) (48)	Cross-sectional study	Data collected on medical history, patient characteristics, blood samples and urine samples. Proteinuria was defined as UACR of ≥300 mg/gram creatinine and microalbuminuria as UACR of 30-300 mg/gram creatinine. Confirmed by at least two urine samples.	N=65, N=17 with DM Age: median 19 (range 10-53) years BMI: range 27.6-68.2 kg/m^2 Gender: 43M, 22F Genotype: 52 del, 13 mUPD	Proteinuria was present in 1 out of 17 (6%) and micro-albuminuria in 4 out of 17 (24%) of patients with both PWS and DM. All patients with diabetic nephropathy had a deletion genotype and all but one subject with microalbuminuria were male. Duration of diabetes ranged from 3 to 18 years.	Proteinuria not confirmed by collecting 24-hour urine. Diabetes subtype not specified.
Schmidt et al. (2012) (49)	Retrospective cohort study	Data on patient characteristics, diagnosis laboratory measurements collected from 309 treatment centers in Germany and Austria on patients with DM.	N=23, all with DM Age: mean 16.39 (SD ±3.03) years BMI: mean 37.9 (SD ±11.04) kg/m^2 Gender: 8M, 15F Genotype: NA	13 out of 23 (56%) were diagnosed with microalbuminuria and three out of 23 (11%) with macroalbuminuria in patients with PWS with DM.	Definition of micro- and macroalbuminuria unknown. Diabetes subtype not available for all patients.
Höybye et al. (2015) (93)	Cross-sectional study	Data collected on medical records, physical examination, blood samples. Comparison between GHt started during childhood and adulthood.	N=10 Age: - childhood group mean 16 (SD ±4) years - adulthood group mean 44 (SD ±4) years BMI: - childhood group mean 32.3 (SD ±10.3) kg/m^2 - adulthood group 28.9 (SD ±4.6) kg/m^2 Gender: 10M, 0F Genotype: N=10 methylation positive GHt: N=5 started in childhood, N=5 started as adults, all>5 years treated	One out of 10 patients (10%) was diagnosed with renal insufficiency. Four out of 10 patients (40%) were diagnosed with diabetes mellitus.	No information on cause and severity of renal insufficiency.
Yang et al. (2017) (50)	Retrospective cohort study	Data collected from medical records and screening for DM complication.	N=84, N=29 with DM2 Age: mean 17.4 (SD ±5.1, range 10.3-35.8) years BMI: mean 30.8 (SD ±9.6) kg/m^2 Gender: 52M, 32F Genotype: 59 del, 25 not specified Ethnicity: Asian	Seven of 29 patients with DM2 (24%) had microvascular complications of whom two (7%) microalbuminuria and one (3%) proteinuria. All three had deletion genotype age between 22.5 – 27.0 years at the onset of microvascular renal complication. Microvascular complications (including albuminuria, retinopathy and peripheral neuropathy) were associated with increased age (r=0.393, p=0.047).	Patients with pre-existing chronic kidney disease were excluded from analysis

(Continued)

TABLE 4 Continued

Author (year)	Study design	Method	Baseline characteristics:	Results	Limitations/ remarks
Koizumi et al. (2018) (94)	Retrospective cohort study	Data collected from medical records on patient characteristics, body composition, laboratory results and CT analysis to assess VAT on t=6 and 12 months after cessation of GHt.	N=7 Age: at end of GHt mean 18.9 (SD ±1.8) years BMI: mean 24.2 (SD ±6.5) kg/m^2 Gender: 3M, 4F Genotype: all del	One patient (14%) was diagnosed with proteinuria and was taking an ACE- inhibitor before the onset of the study.	No data available on cause, severity or progression of proteinuria/CKD after cessation of GHt.
Van Nieuwpoort et al. (2018) (95)	Cross-sectional cohort study	Data collected on patient characteristics laboratory results including blood and urine samples, bone metabolism and bone mineral density. Data was compared with n=14 healthy siblings.	N=15 Age: median 22.2 (range 19.2-42.9) years BMI: median 27.5 (IQR [16.7]) kg/m^2 Gender: 4M, 11F Genotype: 14 del, 1 mUPD	The serum creatinine (median, [IQR]) in the whole PWS group was 69.0 [10.0] µmol/L. No significant difference was found in males 71.0 [28.0] µmol/L compared to females 69.0 [10.0] µmol/L, p>0.05. Median [IQR] urine creatinine in the total cohort 1.74 [1.47] mmol/24 h, no significant difference was found in males 3.27 [2.86] mmol/24 h compared to females 1.70 [0.69] mmol/24 h.	(micro) albuminuria was not assessed in urine samples.
Manzardo et al. (2019) (96)	Retrospective cohort study	Survey filled in by parents or caregivers.	N=1067 Age: mean 21.0 (SD ±14, range 0-63) years BMI: mean 28.9 (SD ±12, range 3.6-104) kg/m^2 Gender: 513M, 554F Genotype: 527 del, 325 mUPD, 23 ICD	20 patients (2%) had renal dysfunction of whom 6 out of 38 (17%) had suffered from a thromboembolism vs 14 out of 1013 (1%) with no thromboembolism, p<0.0001. Kidney failure increases the risk for thromboembolism (OR 14.9, 95% CI 5.3 – 41.9).	Kidney dysfunction not specified. Severity of kidney failure unknown.
Pemmasani et al. (2021) (47)	Retrospective cohort study	Data collected from the Healthcare Cost and Utilization Project Nationwide Readmissions Database year 2014 on comorbidities of hospitalized patients.	N=480 Age: mean 27 (SD ±19) years Gender: 242M, 238F BMI: NA Genotype: NA	31 patients (7%) were diagnosed with chronic kidney disease. - Ages 0-12 years: <10 out of 132 - Ages 13-25 years: <10 out of 108 - Ages 26-39 years: <10 out of 112 - Ages ≥40 years: 14 out of 128 (11%)	
Cause of death or post mortal analysis					
Cohen et al. (1975) (97)	Case series/ retrospective cohort study	Autopsy reports of kidneys of patients with PWS compared to kidney of two age-matched control. Kidneys were both macro- and microscopically analyzed.	N=3 Age: range 3.6– 22 years BMI: NA Gender: 3M Genotype NA CHF: 1	Urinalysis was negative for protein in two PWS patients and not done in one. One patient died of aspiration pneumonia, one of massive pulmonary embolism and the last after infectious complications. Combined weight of the kidneys in all patients were not greater than expected. In the patients with PWS, smooth capsular surfaces, widened cortices and absence of scarring was observed on the kidneys. The mean area of Bowman's capsule and glomerular tuft were increased in all three PWS patients compared to the controls. Glomerular enlargement was seen, as well as mild dilatation of capillaries and increased cellularity (mainly mesangial origin). No changes consisted with diabetic nephropathy were seen in all patients.	PWS not genetically confirmed.
Nagai et al. (2005) (98)	Retrospective cohort study	Data on cause of death of patients without GHt collected from Japanese patient support societies (group A). Data collected on cause of death from patients with GHt from medical literature (group B).	Group A, no GHt: N=13 Age: range 9 months – 34 years BMI: range 12 – 45.7 kg/m^2 Gender: 7M, 6F Genotype: 11 del, 1 mUPD, 1	Cause of death of two patients (15%, aged 28 and 34 years) in group A was for one patient renal and cardiac failure due to DM and the other a pulmonary embolism, renal and cardiac failures. No patient with GHt died from renal failure.	GHt group consisted of only children (age <15 years old).

(Continued)

TABLE 4 Continued

Author (year)	Study design	Method	Baseline characteristics:	Results	Limitations/ remarks
			unknown Group B, GHt: N=7 Age: range 0.7-15 years BMI: NA Gender: 7M, 0F Genotype: 3 del, 4 unknown		
Butler et al. (2017) (24)	Retrospective database study	Data collected on cause of death from survey filled in by family/ caregivers and medical reports of deceased PWS patients.	N=486 Age at death: mean 29.5 (SD ±16, range 2 months-67 years) years BMI (N=132): mean 49.3 (SD ±23, range 14-122) kg/m^2 Gender: 263M, 217F Genotype: NA	Seven out of 312 (2%, mean age 34.2 (SD ±11 years)) of patients died from renal failure all of whom were >18 years old. Cause of death due to obesity was reported in 22 out of 312 (7%) of patients, all but one patient were >18 years old. Death due to obesity-related factors (CVD, cardiovascular failure, renal failure), appeared in childhood and increased in adolescence and adulthood.	Cause of death available in only 312 out of 486 included patients. Autopsy performed in only 8%, might lead to underestimation of kidney diseases diagnosis.
Pacoricona et al. (2019) (99)	Retrospective observational study	Data collected on cause of death from the French Epidemiological Center for the Medicale Causes of Death Registry and French Reference Center for PWS database from 2004 to 2014 Survey filled in by physician based on medical history and physical examination and a survey filled in by family	N=104 Age at death: median 30 (range 0.1-58) years BMI: NA Gender: 56M, 48F Genotype: 25 del, 9 mUPD, 4 ICD, 66 unknown	One out of 104 (1%) died from sepsis of unknown origin, previously diagnosed with CKD, hypertrophy of the left ventricle with arrhythmia and diabetes. One out of 104 (1%) died suddenly from end-stage renal failure.	Genetic information missing in 63% of patients.

Angiotensin-converting enzyme (ACE), body mass index (BMI), cardiovascular disease (CVD), chronic heart failure (CHF), chronic kidney disease (CKD), computed tomography (CT), confidence interval (CI), deletion (del), diabetes mellitus (DM), dual-energy X-ray absorptiometry (DXA), female (F), glomerular filtration rate (GFR), growth hormone treatment (GHt), imprinting center defect (ICD), interquartile range (IQR), males (M), maternal uniparental disomy (mUPD), not available (NA), Prader-Willi syndrome (PWS), standard deviation (SD), urinary albumin-to-creatinine ratio (UACR), visceral adipose tissue (VAT).

associated with chronic kidney disease and glomerulosclerosis. A possible mechanism is hyperfiltration, which leads to albuminuria, regardless of the presence or absence of diabetes (107–110). Furthermore, obesity has been associated with increased progression of CKD (111).

In the general population, microalbuminuria is an indicator for microvascular injury, and a risk factor for both CKD and CVD (40–42, 53, 54). Although none of our patients was formally diagnosed with CVD, the presence of microalbuminuria might indicate latent CVD. Young adults with PWS often have cardiovascular risk factors including obesity (60%), DM2 (27%), hypertension (18%), smoking (8%) and hypercholesterolemia (14%) or dyslipidemia (10%). Timely and adequate follow-up of microalbuminuria and treatment of these risk factors might decrease cardiovascular and renal comorbidities and complications.

Clinical recommendations

Initial visit

During the initial visit, the physician should ask about previous UTIs and use of cigarettes and/or alcohol. In case of multiple UTIs, one should consider a kidney ultrasound to detect malformations, assess the post-void residual urine volume and referral to a urologist. In case of substance use, we advise to encourage cessation of smoking or alcohol usage. Furthermore, weight and blood pressure should be measured yearly. In case of hypertension, one should consider analysis to secondary causes such as stress, left ventricle hypertrophy or renal causes and manage both obesity and hypertension according to general guidelines.

Monitoring of eGFR

In our study, we showed that median creatinine levels were at the lower limit of the reference interval of the general (non-PWS) population in both male and female adults with PWS. This finding could be related to low muscle mass, which is decreased by 25 to 37% in patients with PWS (6), resulting in an overestimation of kidney function when measured by eGFR. The eGFR (calculated using the CKD-EPI) might therefore not be the optimal test to estimate kidney function in adults with PWS. We previously proposed alternative reference values of eGFR for adults with PWS (>98 ml/min/1.73m^2 in males and >93 ml/min/1.73m^2 in females) (20) to avoid underdiagnosis of CKD. Another method of assessing kidney injury is by measuring the excretion of LMW

TABLE 5 Results of studies reporting on PWS and urological diseases, symptoms or congenital anomalies with more than one patients.

Author (year)	Study design	Method	Baseline characteristics:	Results	Limitations/ remarks
Von Gontard et al. (2010) (100)	Cohort study	Questionnaire filled in by parents/caregivers	N=118 Age: mean 20.5 (SD ±11.5, range 5-45) years BMI: NA Gender: NA Genotype: 32 del, 27 mUPD, 3 ICD, 56 other/unknown	16 out of 118 patients (14%) had nocturnal enuresis of whom nine (56%) between the age of 5-12. Only four adults (7%) experienced nocturnal enuresis. A total of five out of 118 patients (4%) had additional daytime urine incontinence. The mean age in this group was 21.4 (SD ±14.8) years. Urgency symptoms were seen in nine out of 16 patients (60%) with nocturnal enuresis. Five out of 16 patients (32%) had a history of UTIs and only 2 (13%) a history of UTIs with fever.	
Sinnema et al. (2012) (101)	Retrospective cohort study	Semi-structured interviews with patient and main caregivers, questionnaire filled in by parents/caregivers.	N=12 Age: mean 57.8 (SD ±6.2, range 50-66) years BMI: 31.5 (SD ±5.,0 range 23.4-37.1) kg/m^2 Gender: 5M, 7F Genotype: 4 del, 8 mUPD	Kidney malformations were present in only one out of 12 (8%) patients, which was a bilateral duplication of the kidney and ureter system.	Study population overlapping with Sinnema et al., 2011 (17).
Equit et al. (2013) (102)	Cross-sectional study	Questionnaires filled in by parents/caregivers of self-help groups. Comparison with patients with fragile X-syndrome.	N=191 Age: mean 20.0 (SD ±10.5) years BMI: NA Gender: 104M, 87F Genotype: NA	56 out of 191 patients (29%) had at least one elimination disorder. - 42 out of 191 (22%) suffered from nocturnal enuresis and 23 patients (12%) from daytime urinary incontinence. Daytime urinary incontinence was more prevalent in patients with fragile X-syndrome than PWS (p<0001). The prevalence of elimination (NE, DIU or FI) disorders decreased with increasing age. - At age 4-12 had 18 out of 54 (33%) at least one elimination disorder, at age 13-17, this was 16 out of 47 (34%), at age 18-30 15 out of 60 (25%) and 30 years or older six out of 29 (21%) Urgency symptoms were present in 49 out of 191 (26%) of patients with PWS. 42 out of 191 (22%) were previously diagnosed with UTIs. More patients with PWS had have previous UTI's compared to fragile X syndrome (42 out of 191 (22%) versus 18 out of 166 (11%), p=0.005).	Return rate of 48.9% for patients with PWS might have led to selection bias.
Meinhardt et al. (2013) (103)	Retrospective observational study	Data collected on anthropometric measurements, laboratory results and DXA scans at baseline, after one year and at last observation.	N=41 Age: mean 3.8 (SD ±3.0, range 0.4-12.2), years BMI: mean 0.6 (SDS ±1.9) Gender: 22M, 19F Genotype: NA GHt: all, none treated before study	One patient (2%) suffered from a severe UTI and convulsion during GHt after which treatment was stopped after 2.4 years and patient fully recovered.	
Torrado et al. (2013) (104)	Retrospective cohort study	Data collected from medial reports of PWS compared. Prevalences of birth defects in PWS compared to general population by collecting data from multiple population registries.	N=180 Age at diagnosis 1.2 (range 0.01-17.25) years BMI: NA Gender: 93M, 87F Genotype: 109 del, 68 mUPD, 3 ICD	Five out of 180 patients (3%, one female and four males) had congenital reno-ureteral malformation; left renal hypoplasia, bilateral ureteral duplication, left bifid renal pelvis, vesicoureteral reflux, left pelvicalyceal dilatation and bilateral vesicoureteral reflux caused by ureteral valves. The prevalence of reno-ureteral malformations was significantly higher than in the general population in national and international registries (p<0.05). No association between genotype and congenital malformations was found (three del vs two non del, p=1.00).	

(Continued)

TABLE 5 Continued

Author (year)	Study design	Method	Baseline characteristics:	Results	Limitations/ remarks
Pacilli et al. (2018) (105)	Cohort study	Data collected from patient records from the Victorian PWS Register.	N=33 Age: range 6 months – 17 years BMI: NA Gender: all male Genotype: NA	One out of 33 (3%) patients that underwent orchidopexy was diagnosed with a horseshoe kidney.	
Chao et al. (2021) (34)	Retrospective observational study	Data collection on patient characteristics and presence of LUTS. Assessments of LUTD by uroflowmetry, postvoid residual urine (PVR) by abdominal ultrasound. LUTD defined as abnormal uroflow, low peak flow rate (Q_{max}) or elevated PVR. Videourodynamic studies were performed in some cases.	N=37 Age: mean 17.7 (SD ±7.8, range 5-34) years BMI: mean 28.5 (SD ±11.2) kg/m^2 Gender: 15M, 22F Genotype: 16 del, 3 mUPD, 18 unknown	Ten out of 37 patients (27%) had LUTS. The urodynamic tests were abnormal in 17 out of 34 (50%) of patients. Abnormal uroflowmetry pattern (nonbell shaped) was significantly more prevalent in those with LUTS than without (6 out of 8 (75%) versus 6 out of 20 (23%) respectively, p=0.0049). In patients with LUTS (n=10), 4 (40%) had daytime UI, 2 (20%) both daytime and nighttime UI, one (10%) had nocturnal enuresis and three (30%) LUTS without UI. In 10 out of 20 patients (50%) PVR was increased for their age. Q_{max}, voided volume and bladder capacity were significantly higher in those without LUTS (24.0 (9.0) vs 14.4 (13.5) ml/s, p=0.0046; 240.6 (124.0) vs 126.4 (91.8) ml, p=0.0142 and 247.7 (123.2) vs 143.5 (121.1) ml, p=0.0151 respectively). In three LUTS patients in whom videourodynamic studies were performed, all showed detrusor sphincter dyssynergia.	Low compliance to PVR (54%).

Body mass index (BMI), congenital anomalies of the kidney and urinary tract (CAKUT), daytime urinary incontinence (DIU), deletion (del), dual-energy X-ray absorptiometry (DXA), fecal incontinence (FI), female (F), growth hormone treatment (GHt), imprinting center defect (ICD), lower urinary tract dysfunction (LUTD), lower urinary tract symptoms (LUTS), male (M), maternal uniparental disomy (mUPD), nocturnal enuresis (NE), not available (NA), Prader-Willi syndrome (PWS), postvoid residual urine (PVR), standard deviation (SD), urinary incontinence (UI), urinary tract infection (UTI).

proteins, such as cystatine-C, retinol binding protein and alpha1-microglobulin. LMW proteins are filtered and reabsorbed by the kidneys. In kidney injury and (diabetic) proteinuria, an increased excretion of these proteins might be found in urine samples (44–46). Therefore, a more accurate methods of assessing kidney function in patients with PWS might include cystatin-C clearance adjusted for BMI (112, 113) or 24-hour urine creatinine measurement. However, multiple factors such as thyroid dysfunction and corticosteroid use might influence cystatin-C levels (114, 115). We therefore recommend yearly evaluation of creatinine and eGFR according to PWS-specific cut-off values and yearly urinalysis in all adults with PWS (see Figure 2) (20).

Laboratory measurements

We advise yearly evaluation of kidney function, glucose levels and lipids. If an impaired renal function is detected, additional investigation using a 24-hour urine and kidney ultrasound should be performed to identify the cause. In addition, a nephrologist could be consulted. A new diagnosis of diabetes or hypercholesterolemia should be managed according to non-PWS guidelines.

Detection of UTIs

Urgency symptoms (sudden need to pass urine with or without urine incontinence) and UTIs are common in adults with PWS, according to our literature review and previous study in our cohort (26-60% and 22-32%, respectively) (100, 102, 119). UTIs might be prevalent in adults with PWS related to obesity (120). Health care providers should be aware of the increased pain threshold and inability to mount a fever (due to disturbed temperature regulation) in patients with PWS (8, 32). This combination of features might lead to an atypical presentation of UTI (e.g. only changes in behavior), causing underdiagnosis of UTIs in this vulnerable patient population. If leukocytes and/or nitrite are positive, a urine culture should be performed and the patient should be treated with antibiotics if indicated.

Urinalysis for the detection of micro- or macroalbuminuria

We recommend performing urinalysis (midstream urinalysis using urine dipstick and measurement of UACR and UPCR) yearly in all adults with PWS. First morning urine samples are preferred over random urine samples to decrease the influence of orthostatic proteinuria (35). In those diagnosed with hypertension and DM2, we advise stringent control of blood pressure and glucose levels. In addition, we recommend performing urinalysis yearly, as per guidelines for DM2 and hypertension in the general population (116, 117).

In case of abnormalities, a second sample urinalysis should be performed. When microalbuminuria or proteinuria persists, 24-hour urine should be collected to calculate the 24 hour creatinine- and protein excretion. However, in people with of intellectual disability, collecting 24 hour urine might be challenging. In those with normal eGFR but elevated UACR or UPCR without a UTI, an renal ultrasound should be performed.

When proteinuria is confirmed, patients should be screened for underlying diseases and any risk factors should be treated.

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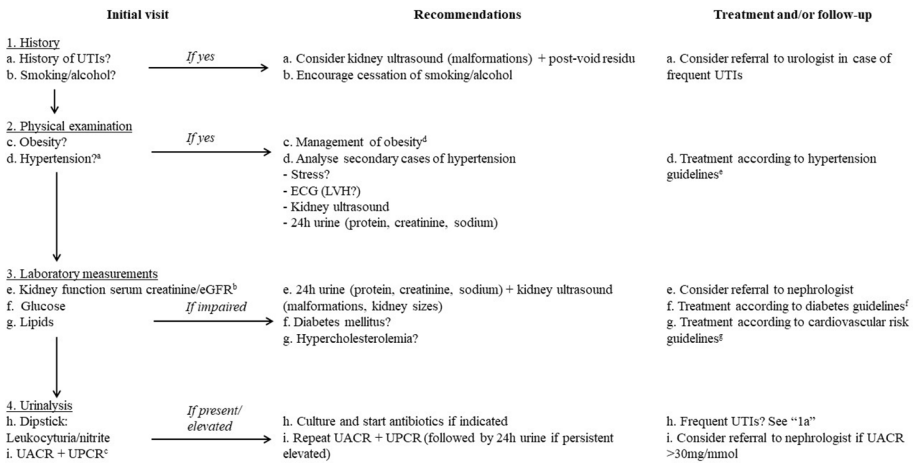

FIGURE 2
Recommendations for screening and the management for (micro)albuminuria and its risk factors in adults with PWS. angiotensin-converting enzyme inhibitor (ACE-I), angiotensin-II receptor antagonist (ATII) electrocardiogram (ECG), estimated glomerular filtration rate (eGFR), Left ventricular hypertrophy (LVH), minutes (min), urinary tract infection (UTI), urinary albumin-to-creatinine ratio (UACR), urinary protein-to-creatinine ratio (UPCR). a. Diagnosed by repeated measurements or 30-minute measurement. b. preferably in a first morning urine sample. c. Consider using adjusted reference values for adults with PWS (e.g. >98 ml/min/1.73m2 in males and >93 ml/min/1.73m2 in females) (20). d. Obesity in adults with PWS should be aggressively management with for example a hypocaloric diet, structured regular exercise and restriction of access to food. e. For example the 2018 ESC/ESH guidelines for the management of arterial hypertension (116). f. For example the 2019 ESC guidelines on diabetes (117). g. For example the ESC 2021 guideline (118) on cardiovascular disease prevention.

Cardiovascular status should be optimized and cardiovascular risk should be assessed. Referral to (or consultation with) a nephrologist should be considered. Those with progressive proteinuria should be referred to a nephrologist. In adults with PWS with albuminuria and hypertension, an ACE inhibitor or angiotensin-II receptor antagonist should be the first line of treatment due to their renoprotective effects (121). If obesity is present, a weight reducing regime should be started.

Strengths and limitations

As all studies, our study has strengths and limitations. This study is, to our knowledge, the first cohort study to systematically assess kidney function and urinalysis in adults with PWS. However, there are several limitations. Blood and urine samples were not always collected on the same day as the blood samples [and in eight Dutch and all French patients, blood results were not included in the statistical analysis due to the prolonged time interval between blood and urine samples (>12 months)]. As random spot urine samples were collected instead of first morning urine samples, the high prevalence of (micro)albuminuria might partly be caused by sample contamination, orthostatic proteinuria or physical exertion. Furthermore, microalbuminuria was not confirmed in a second sample as repeated samples were only available in four patients. In addition, as muscle mass might be low in patients with PWS, leading to a low serum creatinine, defining microalbuminuria by the UACR or UPCR might have led to an overestimation (6). Smoking and alcohol usage were not assessed yearly in some patients, and data could therefore have been out of date. Patients with leukocyturia or nitrites were first referred to their general practitioner for urine culture and UTI treatment if positive for infection, after which urinalysis was repeated. Therefore, the results of urine cultures were not available at our center. Urinalysis was not available in the entire cohort, which might have led to selection bias and a 24-hour urinalysis was only available in one patient. Additionally, patients were not routinely screened for CVD (e.g. by an ECG or cardiac ultrasound). Furthermore, this cohort consists of relatively young adults without cardiovascular events, though risk factors for CVD are present, which justifies urinalysis even in young patients with PWS. Prospective studies in a larger cohort are needed to overcome these limitations.

Conclusion

Upon screening, one in every five adults with PWS had elevated urine albumin or (micro)albuminuria which was already present at young age. All had normal eGFR according to non-PWS reference intervals. However, as muscle mass is low in PWS, normal creatinine levels and eGFR may overestimate kidney function in people with this syndrome. As kidney function may be overestimated when only measured by serum creatinine, renal problems may be missed when urinalysis is omitted. Routine screening for microalbuminuria may allow early intervention to avoid CVD and CKD. Health care providers should be aware of the increased risk for CKD and CVD in adults with PWS and should optimize treatment to reduce these risk factors. To prevent long-term complications and impaired quality of life, we provide an

algorithm for the screening and management of micro-albuminuria and proteinuria in adults with PWS (Figure 2).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors on request, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Commission of the Erasmus Medical Center, Rotterdam, the Netherlands. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Conceptualization, KP, FH and LG. Methodology, KP and LG. Formal analysis, DA. Investigation, DA, NN. Resources, LG. Data curation, DA, NN, GG, CP. Writing—original draft preparation, DA. Writing—review and editing, all authors. Visualization: DA. Supervision, KP, LG and AL. Project administration, DA, KP and LG. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1168648/full#supplementary-material

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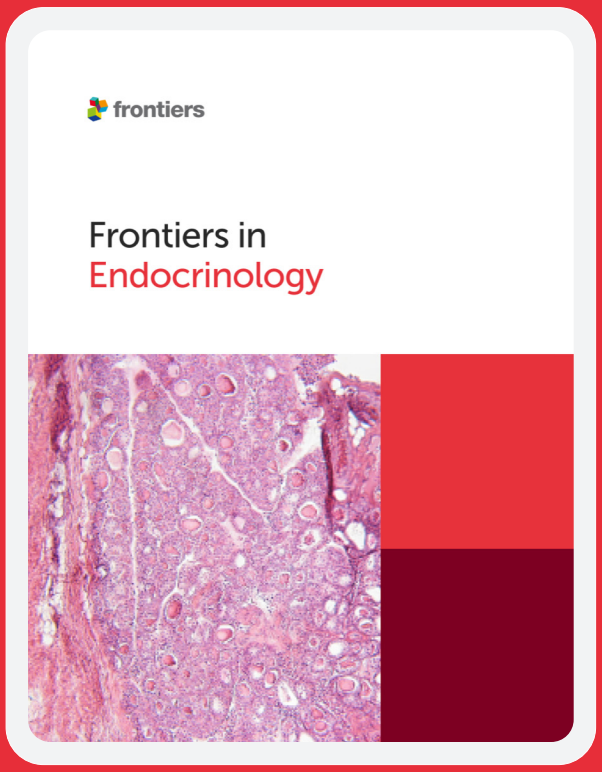

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