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COMORBIDITY AND MORTALITY IN PRADER-WILLI SYNDROME

Hasanain Shukur

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Comorbidity and mortality in Prader-Willi syndrome

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By

Hasanain Shukur.MD

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Principal Supervisor:
Associated Professor Charlotte Höybye
Karolinska Institutet
Department of Molecular Medicine and Surgery

Co-supervisor(s):
Associated Professor Laith Hussain-Alkhateeb
University of Gothenburg, Sahlgrenska Academy
Institute of Medicine
School of Public Health and Community Medicine
Global Health.

Professor Ole Kämpe
Karolinska Institutet
Department of Medicine

Opponent:
Associated Professor Risto Lapatto
University of Helsinki
Pediatrics Department

Examination Board:
Professor Anna-Marie Landtblom
Uppsala University
Department of Neuroscience

Professor Peter Bergsten
Uppsala University
Department of Medical Cell Biology

Associated Professor Michael Alvarsson
Karolinska Institutet
Department of Molecular medicine and Surgery

To my kids

There are things known and there are things unknown, and in between are the doors for perception.

Aldous Huxley

POPULAR SCIENCE SUMMARY OF THE THESIS

Prader-Willi syndrome (PWS), is a rare chromosomal disorder, affecting one in 10.000–30.000 inhabitants. PWS is a multi-symptomatic disease and the main characteristics are hyperphagia (excessive eating) with a high risk of obesity, endocrine deficiencies, muscular hypotonia (muscle weakness), behavioral problems and intellectual disability. Comorbidity and mortality are both high in patients with PWS, mainly caused by morbid obesity and its consequences. So far, there is no cure or effective treatment of this condition, and a restricted controlled diet, regular physical activity, treatment of co-morbidities and replacement of hormone deficiencies are cornerstone treatments. The first description of PWS was almost 70 years ago, and the knowledge about it had increased considerably over time. However, there are still undiscovered areas and unanswered questions, among them contemporary information on comorbidity and mortality.

In this thesis, comorbidity and mortality as well as use of drugs in the patients with PWS living in Sweden were studied using data from National registers. For a further evaluation of comorbidity, long-term exposure to cortisol in adults with PWS was assessed by measuring cortisol in hair, where cortisol is stored. In addition, the impact of growth hormone on sleep apneas and respiratory problems was evaluated.

In 411 patients with PWS, 37658 comorbidity diagnoses were registered, and 365 patients received 83629 different drug prescriptions. The most common causes of comorbidity were mental and behavioral disorders and diabetes and its complications, and in accordance, the medications to treat the mentioned comorbidities were the most frequent prescriptions. The most common causes of death in 144 patients with PWS were unspecific, but among the specific causes, cardiovascular diseases, diabetes, obesity and respiratory causes were most frequent. Hair cortisol in patients with PWS was higher than in control subjects, and it increased with increasing the weight and reported stress. Short- and long-term GH replacement did not negatively affect the parameters of respiration and sleep.

In conclusion, the patterns of comorbidity and mortality and the prescribed medications confirmed the multisymptomatic character of the syndrome. The increased morbidity and mortality were mainly related to diseases secondary to obesity, indicating that more efforts are needed to prevent obesity in this vulnerable group of patients.

ABSTRACT

Background: Prader-Willi syndrome (PWS) is a multisymptomatic, rare, genetic, disorder, due to lack of the expression of paternal genes in the q11-q13 region of chromosome 15. The main characteristics of PWS are muscular hypotonia, hyperphagia, obesity, behavioral problems, cognitive disabilities, and endocrine deficiencies. Comorbidities, are frequent, including sleep apnea. Mortality rate is high, and life expectancy short. The aim of this thesis was to further describe comorbidity, use of medication and mortality in PWS, and to evaluate long-term cortisol exposure and the effects of GH treatment on respiratory parameters.

Methods: Data of comorbidity, mortality and use of drugs were retrieved from Swedish National registers and categorized into groups and subgroups (study 1). Long-term cortisol exposure was assessed in hair in adults with PWS and age and sex matched controls, and related to responses to questionnaires on health and medication (study 2). The effect of GH treatment on polysomnographic measurements were evaluated in 12 months randomized, placebo-controlled GH trial, followed by a two-year open phase GH treatment period (study 3).

Results: In 411 patients, 37658 comorbidity diagnoses were registered, and in 365 patients, 83629 different drug prescriptions. Many codes were unspecific, but mental and behavioral problems and corresponding prescriptions were the most common; followed by diabetes, and antidiabetic treatments. The most common cause of death (n=144, mean age 24,7 years, (SD 17,4) range 0 months to 67 years, 61% males), were unspecific (34%) cardiovascular (23%), and endocrine (diabetes and obesity complications) (13,9%) (study 1). Hair cortisol in patients with PWS (n=29) was higher (12.8±25.4 pg/mg) than in controls (n=105) (3.8±7.3 pg/mg), and increased with BMI and reported stress (study 2). In the GH treatment trial apnea-hypopnea index (AHI) was 1.4 (0.0-13.9). No differences in sleep or respiratory parameters were seen between GH and placebo treated patients. The sleep efficiency improved throughout the study, independent of BMI. AHI inconsistently increased within normal range (study 3).

Conclusion: The pattern of comorbidity, mortality and prescribed drugs confirmed the multi-symptomatic character of the syndrome. The high frequency of unspecific codes for comorbidity and mortality could indicate undetected hypocortisolism, but the long-term cortisol exposure suggested an adequate cortisol response to chronic stress. No clinically significantly negative impact of GH treatment on respiration was seen. The increased morbidity and mortality were mainly related to diseases secondary to obesity, which underscore the need for continued and intensified efforts to prevent obesity in this vulnerable group.

LIST OF SCIENTIFIC PAPERS

I. **Comorbidities, mortality and use of medications in patients with Prader-Willi syndrome – A cross-sectional register study.**

Hasanain Hamid Shukur, Laith Hussain-Alkhateeb, Ann Nordgren, Charlotte Höybye.

Manuscript.

II. **Hair cortisol-a method to detect chronic cortisol levels in patients with Prader-Willi syndrome.**

Hasanain Hamid Shukur, Yolanda B de Rijke, Elisabeth FC van Rossum, Laith Hussain-Alkhateeb, Charlotte Höybye.

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III. **Effect of Growth Hormone treatment on sleep-related parameters in adults with Prader-Willi syndrome**

Hasanain Hamid Shukur, Laith Hussain-Alkhateeb, Stense Farholt, Anders Jorgensen Palmstrøm, Charlotte Höybye.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AHI	Apnea-hypopnea index
ARF	Acute renal failure
BMI	Body mass index
BPH	Benign prostatic hypertrophy
CRF	Chronic renal failure
DVT	Deep vein thrombosis
GH	Growth Hormone
GHD	Growth hormone deficiency
HC	Hair Cortisol
HPA axis	Hypothalamus pituitary adrenal axis
ID	Intellectual disability
MBR	Medical Birth Registry
OSA	Obstructive sleep apnea
PLM	Periodic limb movement
PSG	Polysomnography
PWS	Prader-Willi syndrome
REM	Rapid eye movement
SAO2	Saturated oxygen concentration
SCR	Swedish Cancer Registry
SE	Sleep efficiency
SRBD	Sleep related breathing disorders
UPD	Uniparental disomy
UTI	Urinary tract infection
VTE	Venous thromboembolism

1 INTRODUCTION

1.1 PWS BACKGROUND

In 1867, John Landon Dawn (1828-1896) described a 14 years old girl, with a weight of 89 kg and a hight of 132 cm. She had intellectual disability (ID), small hands and feet and menarche at the age of 25 years. This, probably, was the first report of what was defined, a century later, by Prader, Labhart and Willi as Prader-Willi syndrome (PWS) [1].

PWS (OMIM 176270) is a rare, hypothalamic and complex genetic disease, which affects metabolism, behaviour, the endocrine and the neurologic systems. The main clinical characteristics of PWS include muscular hypotonia, short stature, hypogonadism, intellectual disabilities, behavioural problems and hyperphagia [1].

Globally, the incidence of PWS is one in 10.000–20.000 new-borns, and the prevalence is one in 10.000–30.000 inhabitants. PWS is equally frequent in males and females [2–8]. The rates of morbidity and mortality are higher in adults with ID, and the life span is significantly shorter, also for patients with PWS [9–12]. Aging in individuals with ID is challenging. Like other people, they have significant health needs that appear with aging, and they have further specific needs according to their disability. Health conditions like sensory impairment, epilepsy, obesity, mental health abnormalities and gastro intestinal problems are common, but easily missed and poorly managed [13]. However, the life expectancy in individuals with ID recently has increased markedly [13], due to improvement in nutrition, early interventions, and better control of different illnesses and complications [13].

The knowledge about health issues in aging patients with PWS is poor. Research in PWS has mainly focused on patients with PWS in their childhood or early adulthood. During adulthood, many individuals with PWS have a more independent life, they are studying or doing their own sheltered jobs, having their own money, making friendships, and having more active social life. This is a new challenging situation for the patients, their families and caregivers with less environmental control from the family or caregivers, and some of the individuals with PWS may take inappropriate decisions regarding food (amount and/or quality). Furthermore, ID, behavioural problems, mental illnesses, muscular hypotonia, and many other factors will complicate the situation even more [14]. In some societies, it is difficult to trace adults with PWS by the healthcare system due to different limitations. Adding to that, clinical features become more evident in adulthood, especially if the PWS diagnosis was late and/or proper intervention was not initiated during childhood [15]. The risk of severe obesity is high as well

as the risk for complications due to obesity, such as cardiovascular diseases and diabetes type 2, but the knowledge about adults with PWS is still limited [15].

This chapter starts with a description of the chromosomal abnormality in PWS, then explaining thoroughly PWS from early childhood to early adulthood. Then follows a literature review about the endocrine system functions, sleep related breathing disorders, and morbidity and mortality in PWS.

1.2 CHROMOSOMAL ABNORMALITY

PWS is caused by the lack of expression of genes in the paternally inherited q11-q13 region of chromosome 15. The genetic mechanisms that cause PWS include paternal 15q microdeletion (65-70%), maternal uniparental disomy (UPD) (30-35%), imprinting defects and translocations (1-3%) [16,17]. The specific genetic cause for PWS was characterized in 1981, when a deletion in chromosome 15 in some patients with PWS was reported. Some years later it was shown that deletions always are found on the paternal inherited chromosome, and that PWS could also be due to UPD, imprinting defects and translocations. The paternal copy of chromosome 15 is silent and the maternal is the active form. If the reverse happened, and the defect happened in the maternal copy of chromosome 15, this will lead to another condition called Angelman's syndrome.

Around 20 genes are missing in every patient with PWS, particularly, the genes that transcript small nuclear RNAs (snoRNAs). These genes help in regulating the transcription and translation of other types of RNA molecules. SNORD116 cluster, is the specific group of snoRNA that may play a major role in causing PWS symptoms [18,19].

The genetic PW71 methylation-test has been used for many years, but a fluorescence in situ hybridization (FISH) test is needed for differentiation between the specific lesions. Nowadays, most laboratories use the MLPA analysis, which at the same time detects abnormal methylation caused by deletion as well as UPD. Several different genes in the chromosome 15q11-13 region have been identified, but the clinical implications are not completely known [17].

1.3 PWS IN CHILDREN AND ADOLESCENTS

1.3.1 1.3.1 In infants and young children

PWS is a multisystem neurobehavioral disorder and most of the symptoms will continue throughout life. Therefore, understanding PWS in childhood is fundamental to understand PWS in adulthood. Infants typically have severe muscular hypotonia with poor suckling, failure to thrive, lack of interest in eating, genital hypoplasia, hypogonadism and increased daytime

sleepiness. Some facial characteristics are typical for PWS. They include almond-shaped eyes, narrow frontal diameter, strabismus, thin upper vermilion with downturned corners of the mouth, and enamel hypoplasia. Small hands and feet are also common.

1.3.2 In older children

PWS is clinically characterized by poor growth velocity, short stature, hyperphagia, obesity, hypogonadism, psychomotor delay, and sleep-related breathing disorders (SRBD) [16]. Among the most prominent features of this syndrome is the extreme hyperphagia, which leads to early obesity if left uncontrolled, and PWS is the most frequent genetic cause of morbid obesity in children. Accumulation of excess body fat begins around the age of 2-4 years [16].

1.3.3 Nutritional phases

From birth to adulthood, five nutritional phases have been identified; starting from poor feeding in early infancy to normal feeding with or without obesity and up to an increased interest in food and development of hyperphagia [20]. Increasing weigh and severe obesity are also identified. In the later stage, a continuous and never-ending hunger and/or decreased satiety easily leads to unfavourable habits, like engaging in obsessive food seeking behaviors including stealing and/or hoarding food, foraging, and persistently thinking and talking about meals and snacks. In this stage, if they don't want to eat, this warrants for a serious health problem that must be investigated [21,22]. Imaging and preclinical studies have demonstrated alterations in the brain function in several areas, that might be responsible for the excessive hyperphagia and constant hunger in PWS [23,24].

For individuals with PWS on a low caloric diet and living in a strict environment with limited access to food, will effectively control weight gain. However, the persistent hyperphagic drive and related behavioral problems, represent a drawback that causes a significant negative impact on the quality of life in individuals with PWS [25]. If food intake is uncontrolled, there is also a risk that the stomach will be full with food almost all the time, leading to its distention. Adding to that, emptying of the stomach is slow and vomiting is rare in individuals with PWS, which all might precipitate gastroparesis. Some of the individuals with PWS feel less pain due to increased pain threshold, and their complaints are less. A distended stomach might therefore go unnoticed and end up with gastric necrosis and rupture. Moreover, some of the patients with PWS eat unusual things like hair, which can create an indigestible ball in the stomach. The prevalence of constipation is 40% in PWS [25,26].

1.3.4 Other comorbidities

In addition to the hyperphagia, other specific behavioral characteristics have been described; and temper outbursts, repetitive or ritualistic behaviors and skin picking are very common. In almost all individuals with PWS, a mild to moderate ID is present with an overall delay in motor and language skills [27]. The average of autism spectrum disorders (ASD) in PWS has been reported to range from 12.7% to 40%, or even much higher (78% to 84%) as seen in some recent studies that used more precise diagnostic tools [28].

Among the behavioral problems, the prevalence of temper outbursts is 88% [27]. However, the behaviours during the outbursts differ among individuals. The most common observed symptoms are shouting, crying, screaming and arguing. This might be followed by damaging properties and throwing of objects. These challenging behaviours start in childhood, but increase with age and negatively affect the individuals with PWS, their relatives and caregivers. The patients sometimes need to be hospitalized because their outbursts are too violent to be managed [29].

The prevalence of skin picking is also very high, between 64% and 78%, compared to 1 – 4% in the typical background population [30]. Skin picking has major short-term and long-term consequences, including scars, infection, wounds and feeling of guilt. Skin picking is usually triggered by anxiety or boredom, and the patients often feel relaxed and happy during the process [31].

Muscular hypotonia is another cardinal feature of PWS. It starts in early infancy, continues throughout childhood, into adulthood life, but improves with age. In early infancy, it affects feeding. Later, it significantly affects body posture, speed of movement, bone mineral density, metabolism and energy expenditure [32].

Scoliosis is very prevalent, 60% to 70%, and is mostly due to the muscular hypotonia. It has a bimodal age distribution with a top before the age of four (23%), and again in the adolescent period. The level of scoliosis increases with age advancement and 15% of PWS children need spine surgery [33].

Temperature regulation is affected in PWS. Some patients can have high body temperature without disease, while others have low body temperature in other situations, for example during a depression. Infection without fever is not rare in patients with PWS increasing the risk of delayed diagnosis. Furthermore, the high pain threshold results in few complaints even in conditions like fractures or abdominal diseases. So, general practitioners, other health care

providers and the patients' families need to be aware of this in order to not overlook serious illnesses [34].

Most of the patients with PWS have a cognitive impairment with an IQ around 60 [35–37]. A complex early life path was described by Griggs et al. [38], showing that a combined effect of reduced activity, hypotonia, hyperphagia and compulsivity, leads to an increased risk of morbid obesity and concomitant comorbidities [35].

Because of the hypothalamic dysfunction, several endocrine deficiencies including hypogonadism, growth hormone (GH) deficiency, hypothyroidism and central adrenal insufficiency (CAI), can be seen. Low GH levels result in impaired growth velocity, short stature, a decrease in lean body mass and an increase in body fat [16]. GH treatment is approved for children with PWS in many countries and has, in multiple studies, been shown to increase height velocity and normalize final height, as well as improving body composition, motor skills and behaviour [16].

Hypogonadism is another major feature in children with PWS, but the severity of this condition and the clinical manifestations are variable between patients and age dependent. Furthermore, the aetiology of hypogonadism in PWS is heterogeneous, ranging from profound gonadotropin deficiency to primary gonadal failure with a marked increase in the levels of gonadotropins. Moreover, most of the boys with PWS have cryptorchidism, which should be treated within the age of one to two years, because of the risk of subsequent malignancy if cryptorchidism is left untreated.

In girls, hypoplasia of labia minora and clitoris are common. As in boys, hypogonadism is frequent and of similar aetiology, menstrual irregularities and amenorrhoea are common [39]. A complete pubertal development is rare in both genders. Precocious puberty has been described. After puberty, both genders show an interest in sex. Sex experiences are common in adolescents with PWS, and hormone replacement might increase libido. Therefore, education in sex and relationship and contraception in some patients, are important [40].

1.4 PWS IN ADULTS

Most of the symptoms seen in children with PWS continue into adulthood. Thus, the clinical problems of hyperphagia, obesity, behaviour, cognitive deficits and endocrine deficiencies remain. The clinical picture and the comorbidities in an adult with PWS depend to a high extent on what happened since birth; the time of diagnosis, the level of care and supervision, the treatment and the health care system, the presence of PWS care facilities, and many other

factors (38). However, adulthood brings with it other challenges, because adults with PWS are more independent, some of them have their own jobs and money, some are studying, making friendships and socializing. Therefore, they will be without much of the supervision and control on food they had in childhood, this might deteriorate pre-existing comorbidities and raise new health problems [41].

The frequently occurring behavioral problems and psychiatric disorders in adults with PWS are the most frequent causes for a reduced quality of life and limited participation in social life in adulthood [27,42]. Together with the hyperphagia these problems are the main limitations preventing adults with PWS from living independently [20,43]. Individuals with PWS tend to have higher susceptibility to psychiatric disorders, especially obsessive-compulsive disorders, autism spectrum disorders, depression, and psychosis. However, it seems that there is a genotype dependent pattern of distribution, for example, patients with maternal UPD show higher rates of psychosis [27,42,44].

1.4.1 Endocrine system in adults with PWS

A complex hypothalamic-pituitary dysregulation is responsible for most of the various endocrine abnormalities [i.e., hypogonadism, growth hormone deficiency (GHD), central hypothyroidism and central adrenal insufficiency] [45,46]

1.4.1.1 Growth Hormone Deficiency (GHD)

GH secretion has been evaluated in adults with PWS using different methods; insulin tolerance test, GHRH-Arginine test, arginine test and L dopa [16]. GHD, defined according to guidelines, has been found in between 0–100% of patients with PWS [47].

In children, GH treatment has shown to improve linear growth, motor skills, body composition, respiratory function, communication and social skills, adaptive function, psychomotor development [19] and it is recommended to start GH treatment as early as possible [19,48].

In studies that investigated the effects of GH treatment in adults with PWS, no changes were noted on BMI, waist circumference, or waist/hip ratio, but an improvement in body composition with an increase in lean body mass and a reduction in fat mass were observed in all studies [16] . Corresponding beneficial effects on physical capacity and quality of life has been shown in some studies, wile no health concerns were noticed in a recent meta-analysis [19,49]. GH secretion decreases gradually with advancement of age and consequently a lower dose of GH is needed in children compared to adults [16,49].

The adverse effects of GH treatment are few and consist of sleep related breathing disorders (SRBD) [51,52] and slipped capital femoral epiphysis (especially in obese children) [51,52], and some metabolic alterations. Adding to that, discontinuation of GH treatment might deteriorate their behavioral problems [53].

In some studies, a slight increase in glucose levels and insulin resistance was seen during GH treatment in adults with PWS [54], but the alteration in glucose metabolism was mainly due to weight gain rather than the GH treatment [55]. In order to reduce the adverse metabolic outcomes, a combined healthy lifestyle and regular exercise at the same time with the GH treatment will be of benefit [56,57].

Another effect of GH treatment is the reduction of the peripheral conversion of cortisone to cortisol. This is happening when insulin growth factor I (IGF-I), in adipose tissue and liver, inhibits the activity of 11 beta hydroxysteroid dehydrogenase 1. Therefore, GH treatment may precipitate adrenal insufficiency in susceptible hypopituitary patients, which might be a concern in clinical practise. However, patients with PWS are theoretically less prone to the decrement in serum cortisol due to GH treatment, because the activity of the enzyme is larger in visceral fat compared to subcutaneous fat and patients with PWS have a higher subcutaneous fat than visceral fat proportion [58].

BMI, IGF-I levels, glucose metabolism, and GH secretion must be monitored before, during and after GH treatment, and the dose of GH should be adjusted accordingly. Ghrelin increases GH levels, drives appetite, promotes fat storage and tells the brain when additional calories are needed. Ghrelin levels are high in PWS but the levels during GH treatment are not known [19,59]. There is some evidence of increased collagen deposition in the heart, impaired regulation of the autonomic and the sympathetic nervous systems, and an impairment in the cardiorespiratory response to hypoxia and increased risk of sleep apnea [60]. In adults with PWS and sleep apnea on GH treatment, cardiovascular monitoring might therefore be considered.

To summarize, the use of GH therapy in adults has overall shown to be beneficial and safe, but effects and side effects, especially during long-term treatment should be carefully monitored [60].

1.4.1.2 Hypogonadism

Potential benefits of testosterone include increasing bone mineral density, preventing osteoporosis and increasing muscle tone and mass. The treatment might have an impact on the

self-image and self-esteem, and other people's attitudes, because it can lead to more mature and age-appropriate appearance. On the other hand, it is a strong belief that testosterone treatment can aggravate aggressive behaviour in PWS adolescents and adults. Although this is not evidenced based, it is an understandable worry. Treatment needs therefore to be monitored not only by regularly measuring testosterone levels, but also by evaluating any possible behavioural changes. However, psychiatric, behavioural and nutritional issues are so problematic in PWS, that parents, care givers and physicians are reluctant to induction of puberty with testosterone. Adding to that, many (if not all) adolescents with PWS have enough androgen production for axillary and pubic hair growth, giving an impression that pubertal development is going on. Nevertheless, complete pubertal development is rarely reached. Another consideration, affecting the decision about treatment with testosterone to adolescents with PWS is that androgen replacement leads to more rapid skeletal maturation, which might limit the possibility to gain normal height, especially in those on GH treatment [40,61]. Due to all these considerations, many adolescents with PWS will not receive testosterone treatment, and in some, testosterone treatment will be discontinued in adulthood.

In adult males with PWS, except for aggressive behaviour, there is no other common contraindications for the androgen replacement and most PWS men are potential candidates for androgen replacement. In addition, most PWS adult men are very interested in receiving androgen replacement, because they want to develop a more mature appearance and improve their body image while others refuse androgen replacement for different reasons, for example that they don't want to become too hairy.

Almost all females have oligomenorrhea or secondary amenorrhea and in comparison to age and BMI matched controls, women with PWS have small ovaries with low antral follicle counts on ultrasound examination. Hormone replacement must be individualized in women with PWS, and the marker for fertility, inhibin B, should be measured in each patient to be able to estimate the fertility, although it is very rare [40,61]. However, in four genetically verified women, four pregnancies were reported [40]. Two of the children were born with normal chromosomes, two with Angelman's syndrome (maternal deletion chromosome 15).

Hypogonadism, in both sexes, contributes to a low bone mass, strength and density. Adults with PWS are at increased risk for osteoporosis, because of the hypotonia, decreased physical activity, increased body fat, GH deficiency and risk of insufficient intake of calcium rich food due to the severe dietary restrictions. Hormone replacement, calcium and vitamin D supplementation, and weigh bearing exercise are routinely recommended as important steps towards osteoporosis prevention [40,61]. As previously mentioned, sexual interest and

experiences are common between adolescents and adults with PWS despite the hypogonadism. Hormone replacement might increase libido, and sex education should be offered [40,61].

1.4.1.3 Hypothyroidism

Although, most of the patient with PWS are euthyroid, hypothyroidism is frequently seen. A prevalence of hypothyroidism of 20-30% has been observed, but one study reported a rate of hypothyroidism in infants to be 72.2% based on low T4, free T4 and normal TSH. Recently, a study investigated the prevalence of thyroid dysfunctions in 339 children and adults with PWS, and hypothyroidism was estimated to be 13.6% [62]. The reason behind this discrepancy might be the age of the patients or the timing of the investigations. Anyhow, TSH, free T3 and free T4 must be monitored regularly, starting at baseline and with monthly intervals until stability is obtained [63]. According to some studies, GH treatment affect the thyroid function, and hypothyroidism unmasked during the treatment with GH. In PWS patients that are already on thyroid medications, and in whom GH treatment is initiated, dose adjustment of T4 might be needed [64].

1.4.1.4 Central adrenal insufficiency

A normal function of the hypothalamus-pituitary-adrenal axis (HPA) in PWS was anticipated until a high number of CAI was found based on an insufficient ACTH response to an overnight single-dose metyrapone test in 15 out of 25 (60%) children [65]. Previous studies have reported findings of small adrenal glands in autopsy in PWS children [66], and small volumes of the hypothalamic paraventricular nuclei with a decreased cell number in adults with PWS [67]. The clinical significance of these findings is unclear, but available data indicate that some degree of CAI may be present in PWS, although clinically relevant adrenal failure in PWS appears to be rare [68–70].

Symptoms of CAI are non-specific, i.e., lethargy, asthenia, loss of appetite, nausea, dizziness and hypoglycemia. Symptoms can be subtle or present with a life-threatening condition with cardiovascular collapse, especially if associated with trauma, severe illness or surgery [71].

Cortisol is secreted in a circadian rhythm with peak values in the morning and nadir values during night. Apart from the circadian rhythm, cortisol increases during psychological and physical stress. Adrenal function is typically monitored by single measurement of morning serum cortisol, while dynamic testing (Synacthen Test and Insulin tolerance test (ITT)) are often needed to diagnose adrenal insufficiency [72].

Traditionally, cortisol is measured in serum, saliva and urine. The use of saliva cortisol is increasing as it is non-invasive, less stressful and can easily be collected in many different situations. New methods for measurement of cortisol have been developed recently. Since a few years, measurements of cortisol in human hair have become an option for a non-invasive method to measure long-term cortisol exposure [73,74]. The measurements have been performed in healthy individuals [75], as well as in specific groups [67–70]. All studies have shown that the content of cortisol in hair is higher in individuals exposed to stress. Several recent studies showed that hair cortisol levels are significantly elevated in obese individuals compared to normal-weight persons [66,76,77]. Dynamic measurements of cortisol are often a challenge in patients with PWS due to their behavioral and psychiatric problems. Hair cortisol analyses is therefore very attractive examinations in this group of patients.

1.4.2 Comorbidities in Adults with PWS:

In PWS, low muscle mass, hypotonia and low resting energy expenditure are present throughout life, whereas the hyperphagia, cognitive deficiency, endocrine deficiencies and behavioural problems continue into adulthood. In general, the clinical profile is quite variable in PWS, and related to age of diagnosis, national treatment guidelines, access to centre-based care by a multidisciplinary team, and the availability and the duration of GH treatment. Most of the symptoms will persist during adult life [78]. A restricted and controlled diet and regular physical activity in addition to treatment of endocrine deficiencies and comorbidity remain the only treatments of PWS.

Much of the knowledge about comorbidities in adults with PWS is based on case reports and few observational studies, but it is well known that many patients with PWS suffer from several comorbidities such as obesity, diabetes, cardiovascular diseases, low bone mineral density, scoliosis, respiratory problems, behavioural problems, psychiatric problems and arthrosis. Like all other adults, BMI in adults with PWS remains the main predictor of longevity, and weight reduction decreases the adverse health outcomes.

The main comorbidities associated with severe obesity in PWS include respiratory problems (pulmonary embolism, respiratory failure and pulmonary hypertension) and obstructive sleep apnoea, cardiovascular diseases (myocardial infarction, heart failure) abnormalities of the digestive system (gallstones and hepatic steatosis), chronic leg oedema and venous thromboembolism. Hyperlipidaemia was reported in about one third of the patients with PWS [39].

Patients with PWS have greater amount of fat mass than individuals with non-syndromic obesity, despite having the same degree of weight excess in all ages [79,80]. Excess adipose tissue in patients with PWS is classically subcutaneously distributed at the level of the trunk and proximal extremity of the limbs [81]. So, less visceral adipose tissue and more subcutaneous fat tissue than in BMI matched controls [79,82]. Furthermore, the lean body mass is lower compared to age- and BMI-matched control subjects. Resting energy expenditure (REE) is reduced. Once controlled for fat-free mass, these differences between the PWS patients and matched-controls will disappear [83,84]. Adults with PWS self-select to walk at slower speeds than those without PWS. A faster walk speed was associated with greater bone mineral density and Basal Metabolic Rate (BMR) [32]. Furthermore, wider steps and longer time with both feet on the ground likely reduces the load placed in a single limb [32].

Type 2 diabetes (T2DM), as a consequence of severe obesity, occurs in 7-24% in adults with PWS [85,86], but T2DM has been reported in one study in almost 50% of adults with PWS after the 5th. decade [87]. In addition to obesity, beta cell dysfunction in children with PWS is noted due to impaired vagal parasympathetic tones to the pancreas [88].

Despite severe obesity, it is relatively uncommon to see both hyperinsulinemia and insulin resistance. Most of PWS patients with T2DM are asymptomatic and with few diabetes-related complications. The reasons for high insulin sensitivity are mainly, the lower visceral fat proportion and adiponectin concentrations [89,90], the elevated ghrelin concentrations for the degree of obesity [91], the impaired processing of proinsulin to insulin [92] and the impaired GH secretion [93]. Acylated ghrelin is an orexigenic hormone that has been implied as a potential cause of hyperphagia and weight gain, inducing a positive energy balance and could be involved in the development of diabetes in PWS [92]. GH treatment is not adversely affecting the metabolic control of PWS subjects with diabetes, unless weight gain occurs [39]. Furthermore, adults who received GH treatment during their childhood had lower mean insulin resistance index and lower hemoglobin A1c (HbA1c) in comparison with those never treated before [94].

A recent study from Denmark showed that the occurrence of multiple behavioural and cardiovascular illnesses is higher among the patients with PWS in comparison with the general population [79]. This increment in risks warrants for more attention towards improving disease prevention, screening, diagnosis and treatment.

Respiratory diseases are frequently seen in PWS. Their prevalence is higher than comparable rates in non-PWS obese adults. However, there is a very limited information on SRBD in adults with PWS [48].

Mental health problems and anxiety are common, and some patients need treatment with psychiatric medications. A genotype-phenotype correlation is established, for example, individuals with maternal UPD are reported to have more social deficits, autism spectrum disorders and psychosis [95].

Due to the increment of the levels of stress with advancement in age, the behavioural problems in adults with PWS during adulthood might convert to mental illness [44]. Proper intensive and coordinated management of the different medical and psychological conditions diminish the negative health results and improve quality of life and the lifespan for older individuals with PWS [9]. Treatment given and prescribed medication in PWS is not known in detail and an update on morbidity and use of drugs will lead to a better understanding of PWS and provide information for optimisation on care and treatment of this particular group of patients. Like children with PWS, adults have decreased pain sensation due to the increased pain threshold, and their complaints are few. Therefore, general practitioners and caregivers must be careful while dealing with them and starting new medications with the lowest doses to avoid the side effects (33).

Furthermore, scoliosis, skin picking, mouth and teeth hygiene problems, gastroparesis and frequent constipation and vision problems are many. Special attention must be paid to these comorbidities and proper education to the patients and their care givers is mandatory.

1.4.3 Sleep-related breathing disorders (SRBD) in GH-treated adults with PWS

The presence of SRBD in PWS is well-known [96]. Marked obesity or intercurrent respiratory tract infection can exacerbate obstructive apnoea and may even lead to sudden death [16].

The SBRD are thought to be caused by reduced central chemoreceptor sensitivity to hypoxia and hypercapnia, together with obesity, muscular hypotonia, craniofacial abnormalities and adenotonsillar hypertrophy [3,97,98]. Because GH therapy can theoretically lead to lymphoid tissue growth due to increased IGF-I effects, there have been some concerns about the potential association between GH therapy and unexpected death in children. However, a correlation between GH treatment and increased death rate has not been found [99].

The SBRD include central (CA) and obstructive (OA) apnea and hypopnea. CA is mostly seen in non-obese children with PWS, whereas OA is mostly seen in older, obese patients with PWS (3,9,22,90). A nonsignificant decline in apnea-hypopnea-index (AHI) after 6 months of GH treatment was found in 35 prepubertal children with PWS has been described [100], and a recent review concluded that GH can be safely administered in children, provided that SRBD are monitored and treated appropriately [66]. Recently, Donze et al 2019, found no difference between GH treatment and placebo on AHI, OA-index and CA-index measured by polysomnography. This study was performed on young PWS adult patients on treatment with GH since childhood [101]. However, there is very limited information on SBRD in adults with PWS and no studies have been published in older adults with PWS who did not receive GH treatment during childhood.

1.5 MORTALITY IN PWS

PWS is associated with decreased life expectancy and the annual death rate in PWS has been reported to be high (up to 3%/year) [3]. Until a decade ago, only a very limited proportion of individuals with PWS reached 40 years of age [3]. However, a study from United Kingdom reported declining mortality rates in older adults [9]. Deaths in younger children are mostly related to only mild or moderate upper respiratory tract infections [22,96,102]. Also, case descriptions of young children have reported death by shock and cardiac arrest [75,97,103]. Among the causes of death in older children and adults were respiratory diseases [6,66,99] and complications from obesity and diabetes mellitus [22,41]. Without supervision, these patients may suddenly die due to choking (mainly due to heavy eating) [104] or stomach rupture and necrosis [105]. Another study reported that 38% of deaths were due to respiratory problems and 16% to cardiac diseases [106,107]. However, several reported deaths were unexpected and unexplained. An undiscovered HPA-axis dysfunction could be the aetiology to the sudden and unexplained death in patients with PWS [100]. Moreover, in patients with paternal deletion, premature mortality may be higher, and require additional surveillance and more aggressive interventions to modify the traditional cardiovascular risk factors [41].

2 AIMS OF THE THESIS

The general aim of my thesis was to explore comorbidity, use of drugs and mortality in PWS, and in adults with PWS to examine levels of cortisol and the effect of GH treatment on respiratory function as potential aetiologies to co-morbidity.

The thesis consists of three different studies:

Study 1: Comorbidity, mortality and use of medications in Prader-Willi syndrome – a cross-sectional register study.

This was a project on comorbidity, use of medications and mortality in PWS, to identify the most frequent disease areas for potential early intervention or expanded monitoring. Furthermore, it was analysed if prescribed medications corresponded to registered comorbidities.

Study 2: Hair cortisol-a method to detect chronic cortisol levels in patients with Prader-Willi syndrome.

Measurements of hair cortisol was used as a method to evaluate chronic cortisol exposure and the effect of stress caused by intercurrent comorbidities, mental and behavioural problems and BMI on the long-term cortisol. HC levels was compared to results from population-based controls.

Study 3: Effect of growth hormone treatment on sleep-related parameters in adults with Prader-Willi syndrome.

In this study the short and long-term effects of GH treatment on the sleep and respiration parameters were evaluated.

3 MATERIALS AND METHODS

This chapter describes for each study, the study population, the methods used, the statistical analysis and the ethical considerations.

3.1 STUDY POPULATION

3.1.1 Patient characteristics

The first study was a register cross-sectional study, where data was collected from the Swedish National Patient Register, the Swedish Drug Prescription Register, the Swedish Causes of Death Register and the Swedish Cancer Register.

In study two, patients were recruited between the years 2015 and 2016 and enrolled either through the Swedish PWS association, or at routine visits to the Department of Endocrinology, Karolinska University, Stockholm.

Patients in study three were part of the Scandinavian study (2005 – 2010), which investigated the effect of GH treatment in adults with PWS from Norway, Denmark and Sweden [108–110].

3.1.2 Control subjects

No controls were included in the first study.

In the second study, controls were retrieved from the Lifelines Cohort Study, which is a population-based cohort study, of health and health-related behaviors of individuals living in the north of The Netherlands [111–113]. Data from The Lifelines cohort study includes a large number of data including hair cortisol concentrations that were used as reference values.

In the third study, the 1st year of the study was a double-blind, randomized, placebo-controlled study. The 2nd two-years part of the study was an open phase GH treatment period and the patients were their own controls.

3.2 STUDY POPULATION DETAILS

3.2.1 Study population details for the first study:

Data on patients with PWS were retrieved from a large study of patients with rare genetic diseases, followed from birth, until the end of follow-up (2019, except for the data from the Swedish Cancer Registry which was until 2017), migration or death.

All PWS cases (born in Sweden 1964-2019) were included and diagnosed by standardized criteria, and evaluated at a Swedish hospital, documented in Swedish outpatient journals and

registries with the PWS ICD-10 coding from1997 to 2019 (Q87.1F)**.** Patients who migrated from Sweden were excluded from the first day of migration.

Resources for identification of PWS:

- Swedish National Patient Register based on ICD-10 diagnostic criteria

Resources for identification of outcomes:

- Swedish National Patient Register based on ICD-9 and 10 diagnostic criteria (1997-2019).
- Swedish Causes of Death Register (1952-2019).
- Swedish Cancer Register (1958-2017).
- Swedish Prescribed Drug Register (2005-2019).

3.2.2 Study population details for the second study:

Twenty-nine adults with PWS were enrolled and data from 266 control subjects were retrieved from the Lifelines Cohort Study. In order to make fairly balanced age-sex groups, with approximately 10 years interval for each group, the PWS patients were categorized into four age-groups [(18-25 years), (26-35 years), (36-45 years) and (46-60 years)]. Patients with PWS from each sub-group were matched for age and sex corresponding controls in a ratio of 1:5, total number of controls n=105. However, in the 18-25 age-group, the ratio was 1:2.5 (nine adults with PWS and 23 matched controls). To ensure random selection of subjects, the "seed" command in STATA 15.0 was used for the matching process.

3.2.3 Study population details for the third study:

The Scandinavian study was an investigator-initiated trial with a one-year randomized placebo-controlled period, followed by a 2-year open phase GH treatment period. Forty-two adults with PWS (21 women) , completed the trial period. For the present study, 37 patients were retained, they were GH naïve, and none had received GH treatment for at least one year prior to participation in the study. Five patients were excluded, two of them due to lack of data, one patient left the study after the first visit, one patient dropped out during the study period due to a 10-kg increase in body weight, while one patient was excluded because of continued linear growth.

3.3 STUDY PROCEDURES AND MEASURMENTS

3.3.1 Study procedures and measurements for the first study:

A cross-sectional register study design was used and all patients with the diagnosis of PWS were included. For exploring the comorbidities, we used record linkage between databases and used the data from birth until migration, death or end of follow-up in 2019, except the data from the cancer registry which ended in 2017.

Cardiovascular, endocrine, neuropsychiatric and other comorbidities were identified. In Sweden, patients are diagnosed by standardized criteria, and evaluated at a hospital, documented in the outpatient medical records and in registries with ICD-coding. For cancer outcomes, data were retrieved from the Swedish Cancer Registry, documented with ICD-coding.

The codes of the comorbidity data were organized in different sub-chapters (main groups), according to the first letter and first number in the ICD-10 codes. All different sub-chapters of comorbidity were also rearranged according to the most common frequency (Table 1). The ICD codes were then translated (after looking for the whole code) to the sub-group of the corresponding comorbidity inside each sub-chapter. The frequency and percentage of the different comorbidities in each sub-chapter and subgroup of comorbidity were calculated.

The codes for comorbidity were in 95% ICD-10 codes and 5% ICD-9 codes. The ICD-10 codes contain one letter and numbers, while the ICD-9 codes contain digits only, therefore, the first two digits were considered to identify the sub-chapters and include them manually into the sub-chapters identified before with reference to ICD-10 codes. Then the ICD-9 codes were translated to the corresponding diagnosis within each sub-chapter to form the subgroups. This process was performed in order to use both codes in the calculations.

The use of medication was investigated from data retrieved from the Swedish Prescribed Drug Register. The codes were translated and organized into main sub-chapters and subgroups inside each sub-chapter, and the most common medications were identified within each subgroup (Table 2).

The data on mortality were retrieved from the National Death Register. The codes were translated into diagnoses, which were then divided into groups (Table 3), and then into subgroups.

In the comorbidity and mortality data, contained many unspecific diagnoses. In each comorbidity subgroup, the unspecific causes were gathered together in one group, and the same procedure was used the mortality data (Table 3).

Furthermore, some of the translated causes of comorbidity and mortality were PWS. As this is not a diagnosis of comorbidity nor a cause of mortality, it was excluded from the comorbidity tables. At the same time, they were considered in the total number of comorbidities, in order to give an idea about the burden of the comorbidities on both the patients and the health care system. For the mortality data, they were included in the group of unspecific causes of death.

Finally, for clarifying the results and the discussion more, the sub-chapters and subgroups of mental and behavioral comorbidities, and the prescribed drugs for them were gathered in one table (Table 4), while the endocrine (diabetes and obesity) comorbidities and their corresponding prescribed drugs were collected in table 6. The other remaining comorbidities were gathered in table 5, and other prescribed medications, rather than the two mentioned groups, were gathered in table 7.

3.3.2 Study procedures and measurements in the second study

3.3.2.1 Hair processing and analysis

A hair sample (approximately 100-150 hairs) was cut from the posterior vertex of the scalp, as close to the scalp as possible. Then, the hair sample was stored on a paper inside an envelope until the time of analysis [111]. A proximal three centimeter of hair (10 mg) was cut into 1 cm segments, then washed in isopropanol and left to dray after washing. For extracting cortisol, menthol was used [111], and after purification, the cortisol was quantified using liquid chromatography − tandem mass spectrometry (LC–MS/MS) (Waters, Milford, MA) [114].

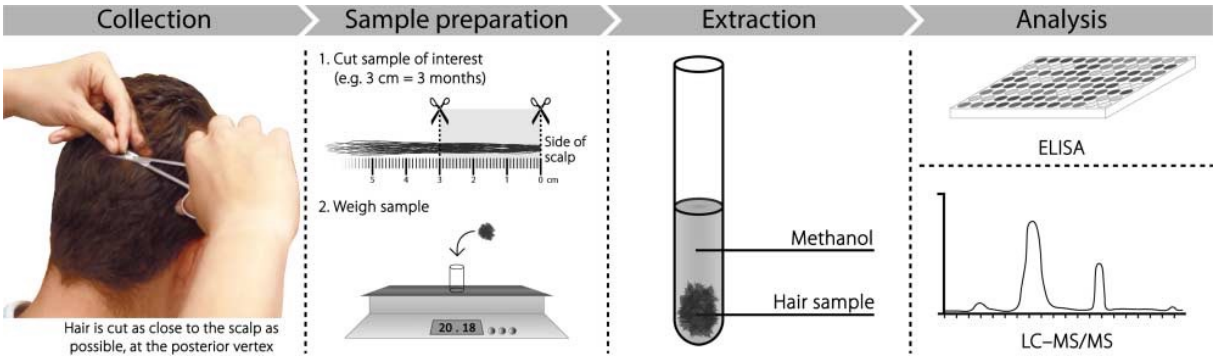

Figure 1. Overview of hair sample collection, work-up and analysis. ELISA, enzyme-linked immunosorbent assay LC–MS/MS, liquid chromatography–tandem mass spectrometry [115].

3.3.2.2 Questionnaires

A questionnaire about age, sex, and anthropometric measurements was filled in by the patients with PWS and/or their caretakers. This questionnaire has been used in multiple studies [113]. For assessment of the level of stress, the participants were asked the question: *Did any stressful events occur during the last 3 months? In case there did, what happened?* Furthermore, the participants were asked in a standardized manner whether they used any products containing corticosteroids in the last three months, and the route of administration (i.e., oral, intravenous, nasal, topical, inhaled, joint injections or others). For the controls, the data extracted from the Lifelines study included information about their age, sex, anthropometric and stressful life events. Dutch version of the List of Threatening Experiences (LTE) was used to evaluate the life events, including occurrence of twelve major life events in the past 12 months [111].

3.3.3 Study procedures and measurements in the third study:

3.3.3.1 The Scandinavian study and PSG measurements

The Scandinavian study which investigated the effect of GH treatment on adults with PWS [108–110], took place between 2005 and 2010. During the first year, the placebo-controlled period, the patients were treated with either GH or placebo. During the first four weeks, the GH dose was titrated in the GH-treated group from 0.3 mg/day or 0.4 mg/day (if body weight was below or above 100 kg) to 0.6 mg/day or 0.8 mg/day, respectively. The dose was maintained fixed for the next 11 months. In the open-phase part of the study, where the GH dose was given to all participants, the GH dose was titrated according to the insulin-like growth factor 1 (IGF1) levels of age-matched controls.

Starting from baseline, the patients repeated the visits every six months thereafter. During each visit (including the baseline visit) data was collected. The data included height, weight, BMI and IGF-I. While the results from Polysomnography (PSG) measurements included: SaO2 %, number of desaturations, apneas and hypopneas, apnea-hypopnea index (AHI), longest apnea, longest hypopnea and periodic limb movement (PLM). Variables on sleep quality included wakefulness duration, total sleep duration, rapid eye movement percentage (REM%), REM latency, sleep efficacy (SE) and delta sleep.

To exclude tonsillar hypertrophy or other significant upper airway obstructions, which would exclude starting in the study, the patients underwent an ear nose and throat (ENT) examination at baseline. The ENT examinations were repeated every 12 months.

A calibrated Harpenden stadiometer was used to measure the standing height, while weight was determined on a calibrated scale and BMI was calculated as weight/squared height.

Polysomnography reports were evaluated by doctors with experience in the international criteria of sleep, also, they performed the analysis and the scoring of sleep [116–119]. Plethysmography strain belts were used to record the chest and abdominal wall movements. Multiple parameters were recorded simultaneously. Pulse oximetry was used to continuously measure SaO2%. Apnea was defined and recorded if the oxygen saturation decreased to 90% or less, for 10 seconds duration or more. Apnea was considered central if the previously mentioned respiratory conditions were associated with absence of respiratory efforts for 10 seconds or more plus an arousal or a decrease in SaO2 of at least 3%. Hypopneas were defined as a decrement in airflow of at least 30% for 10 seconds or more, with a decrease in SaO2% of at least 3% or an arousal. The number of apneas and hypopneas were calculated AHI was calculated per hour of sleep, while the number of apneas and hypopneas were calculated during the total sleep time. REM sleep intervals normally constitute 20-25% of sleep, and occur repeatedly every 90 to 120 minutes throughout sleep. Sleep efficiency (SE) was calculated by dividing the total time in bed (TIB) on the total sleep time (TST), and then multiplied by 100. From 85% and above represent normal SE, more than 90% represents very good SE, while around 100% indicating that the individual did not sleep well. Delta sleep is the deepest form of sleep, it declines with age, it was thought that tissue regeneration and repair occur during this stage. Finally, the severity of apnea was defined as: non-apnea (AHI <5), mild apnea ($5 \leq$ AHI < 15), moderate apnea ($15 \leq$ AHI < 30) and severe apnea (AHI ≥ 30 [117–119].

A time-resolved immunofluorometric in-house assay was used to analyze IGF-1 centrally, with an inter-variation less than 10 percent [108]. Blood lipids, blood pressure and glucose metabolism were monitored throughout the study [109,110,120].

3.4 STISTICAL ANALYSIS

3.4.1 Statistical analysis in study one

Stata 16 was used to perform calculations of frequency, percentage, mean, and standard deviation of the different parameters. Further analysis of the data was not performed.

3.4.2 Statistical analysis in study two

Descriptive statistics of mean ± SD were used to present the results, because it best captures the clinical reality with the large variation in hair cortisol. Student's T-test was used for comparison between the groups. In order to examine the association of crude levels and levels

adjusted for BMI and stress as potential confounders, a generalized linear regression model was used. Furthermore, after using the Akaike Information Criteria (AIC) test, as a tool to confirm the best fit model, both BMI and reported stress were retained in the model and they were predicting the model well. Statistical significance was set at $p<0.05$.

3.4.3 Statistical analysis in study three

Because of the multitude of the data, and to refine the existing dimensions, a principal component analysis (PCA) was performed. Therefore, nine outcomes best representing distinguished dimensions in the data set were identified, these outcomes are: longest apnea, SaO2%, number of desaturations, AHI, PLM, REM%, REM Latency, sleep activity and delta sleep.

Descriptive and analytical statistics were performed and the results were expressed as median and range (min. – max.), because of the non-parametric nature of the outcomes. Mann-Whitney test was used with the baseline data to compare between GH and placebo treated groups. While in the data of the patients treated with GH from baseline to 36 months, and in order to investigate the association between the follow-up interval period (i.e., 6, 12, 18, 24, 30 and 36 months) and each of the nine outcomes (AHI, longest apnea, SaO2%, number of desaturations, REM%, sleep efficiency, PLM, delta sleep and REM Latency), a crude and a BMI-adjusted repeated measures mixed-effect linear regression models were used.

A p-value <0.05 was defined as a statistical significance. All statistical tests were performed using STATA 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: Stata Corp LLC.).

3.5 ETHICAL CONSIDERATIONS

All studies were approved by the ethical committee/ethical authority. (Study one registration no 2016/2378-32, study two registration number 2014/2151-31/1 and study three registration numbers D-nr. 03-448, Aarhus D-nr 20040072 in Stockholm and S-04205 in Oslo.) The Clinical Trial registration number for study three was: NCT00372125.

To conduct research on a group of patients who are intellectually weak and where the patients cannot be expected to fully understand the purpose and examinations of the study is a problem. However, it is a unique group of patients and there is no alternative study model. In the intervention studies accurate and careful information was provided to the patients and their relatives, caregivers and legal guardians and the information was formulated so that the patients could understand it. They were in addition given adequate time to decide whether they wanted to participate or not and in our experience of studies in patients with PWS, they can manage the load that a study entails well. They are generally positive to participate in studies and they are fully capable to very clearly communicate if they want to participate or not. Moreover, it was obligatory for the patients and/or their legal guardians to sign an informed consent before inclusion where it is required.

All data for study one was stored on secure servers. When transferred, the data were in encrypted format on a hard drive with code lock. All data were retrieved and stored following the stipulated rules of confidentiality. Sensitive data, e.g., personal number, date of birth, country of birth etc. were removed, or replaced by proxy codes, or processed to reduce the sensitivity. Finally, all data in the three studies has been discarded after the completion of the analysis.

4 RESULTS

Summary:

In 411 patients, 37658 comorbidity diagnoses were registered, and in 365 patients 83629 different drug prescriptions. Many codes were unspecific, but mental and behavioral problems and corresponding prescriptions were the most common; followed by diabetes, and antidiabetic treatments. The most common causes of death (n=144) were unspecific (34%) cardiovascular (23%), endocrine (diabetes and obesity complications) (13.9%) and respiratory causes (12.5%) (study 1).

Hair cortisol in patients with PWS (n=29) was higher (12.8±25.4 pg/mg) than in controls (n=105) (3.8±7.3 pg/mg) and increased with BMI and reported stress (study 2).

In the GH treatment trial apnea-hypopnea, index (AHI) was 1.4 (0.0-13.9). No differences in sleep or respiratory parameters were seen between GH and placebo treated patients. The sleep efficiency improved throughout the study, independent of BMI. AHI inconsistently increased within normal range (study 3).

4.1 COMORBIDITIES, MORTALITY AND USE OF MEDICATIONS IN PRADER WILLI SYNDROME – A CROSS-SECTIONAL REGISTER STUDY.

The data of comorbidity were investigated in 411 individuals with PWS, average age 17.7 (SD 15.2) years, range 0 to 78 years old. They received 37658 diagnosis codes (27240 after excluding PWS diagnosis codes (n=10481). The number of all prescribed medications in 365 patients with PWS was 83629. During the study period 144 patients with PWS deceased, average age 24.72 (SD 17.4) years, range 0 to 67 years. Of all deaths, 61% (n=88) were males and 38% (n=56) were females, 96% (n=139) were Swedish.

4.1.1 COMORBIDITIES

After excluding the PWS diagnosis code, the mental and behavioral comorbidities group was the most frequently registered diagnoses (table 1). They contributed to 11.4% of the total comorbidity. Within this sub-chapter, pervasive development disorders were the most frequent (24.5%) (Table 4).

Congenital anomalies were the second frequent documented comorbidity (9.8%) (table 1). Congenital malformations of the genitalia were the most frequent anomalies, especially undescended testis (29.6%) (Table 5). Endocrine and metabolic diseases were the third frequent category of comorbidities (8.2%) (table 1). Within this group diabetes contributed with 40%,

thyroid problems (12%), pituitary and gonadotrophic deficiency 37% and metabolic diseases 11.2% (Table 6).

Different symptoms, signs and ill-defined conditions were listed, from the 1881 codes which represented 6,9% from the total comorbidity, symptoms and signs from abdomen and digestion were the most common, representing 27.8% in this category, followed by symptoms of circulatory and respiratory problems (18.1%), general symptoms and signs of illness (like fever, seizure and headache or pain) (14.5%) and lower leg edema (14.9%) (Table 5).

The number of diagnoses for respiratory comorbidity was 1498, representing 5.5% of the total comorbidities. The most common comorbidities under this category were acute upper respiratory tract infection (22,6%), asthma (18,0%), acute and chronic respiratory failure (15.3%), and other diseases of the upper respiratory tract (like hypertrophy of tonsils and/or adenoid or tonsillitis (16,6%) (Table 5).

Musculoskeletal and connective tissue comorbidities represented 5.5% of the total comorbidities, and different types of scoliosis was the most common specific comorbidity (59,1%) with 883 diagnosis codes (Table 5). Different injuries represented 4.8% of the total comorbidity and mostly affected the lower limbs. There were also diagnoses of sexual abuse and complications from surgical procedures and medical care (Table 5).

Diseases of the eye and adnexia were present in 4.3% of the comorbidities. Visual disturbances and blindness contributed to 71% of the total comorbidity under this category (Table 5).

Cardiovascular comorbidities contributed to 3.8% of the total comorbidity. Different cardiopulmonary comorbidities represented in total 48% of this category, followed by hypertensive diseases (21.8%), while other different vascular comorbidities contributed to 24% in total (Table 5).

The comorbidities of the nervous system contributed to 3.8% of the total comorbidity, the episodic and paroxysmal diseases, like migraine, epilepsy and sleep disorders contributed to 66% of this category, epilepsy represented 33.4% from this sub-group, and sleep disorders 29% (Table 5).

Diseases of the ear and mastoid contributed to 3.4% of the total comorbidities, the diseases of the outer ear, ear canal, and middle ear caused 70% of the comorbidities, while different forms of otitis media contributed to 40%. The other different comorbidities were different types of hearing loss (15.5%), inflammation of the mastoid committee and related diseases (13.6%) (Table 5).

The comorbidities of the digestive system affected the patients with PWS with 3.4% from the total comorbidities, almost one third of the cases under this category were due to non-infectious inflammation of the small and large intestine, irritable bowel syndrome, vascular disorders and others (33.47%), whereas constipation represented 10%. While the other common comorbidities were hernia (13.4%), diseases of the esophagus, stomach and duodenum (12%), and diseases of the gallbladder, bile ducts and pancreas (8%) (Table 5).

The most common comorbidities of the genitourinary system, which represented 2.9% of the total comorbidity, were tubulo-interstitial kidney diseases, kidney failure (36.7%), diseases of the urinary tract (32.2%) and non-inflammatory diseases of the female genitalia (12.6%) (Table 5).

Other less common comorbidities were skin diseases (2.5%), infectious diseases (1.8%), benign and malignant neoplasms (1.2%) mostly lymphoma, and diseases of the blood and blood-forming organ (0.9%) (Table 5).

4.1.2 USE OF MEDICATIONS

Among 83629 prescribed medications, the most common were anxiolytics, antidepressants, insomnia treatment, with 19551 prescriptions representing 23.4% of the total prescriptions (Table 2). The most common prescribed antipsychotic medications are mentioned in table 4.

Anti-psychotics are the second most common prescribed medications in patients with PWS with 13901 prescriptions representing 16.6% (Table 2). The most common prescribed medications under this category are listed in table 4.

Somatotropin represented 11% of the total prescribed medications with 9199 documented prescriptions (Table 2 and 6).

Different vitamins and minerals were prescribed 7859 times to the patients with PWS (9.4%) (Table 2). Some of the codes of prescriptions were unspecific, but the most common prescribed vitamins and minerals are different formulations of vitamin D and calcium (Table 7).

Antiepileptics represented 8,5% of the prescribed medications with 7152 prescriptions in total (Table 2), the most common prescribed anti ecliptics were, valproic acid and carbamazepine (Table 7).

Glucose lowering medications were prescribed 6876 times (8.2%) (Table 2), mainly metformin (Table 6). Insulin was prescribed 2103 times representing 2.5% of the total prescriptions (Table

2). The most common preparation was insulin aspart and protamine either together or separate, followed by insulin detemir and glargine (Table 6).

Thyroxine was prescribed 3997 times (4,8%) and antithyroid medications were prescribed 41 times (0.05%) (Table 2 and 6).

Different antibiotics were prescribed for patients with PWS (3479 in total) representing 4.16% of the total prescriptions (Table 2 and 7).

Estrogens and progestins were represented 2.7% of the total prescriptions (Table 2), with 2270 different preparation (Table 6), while testosterone was prescribed 1318 times, representing 1.6% of the total prescriptions (Table 2 and 6).

Medication for treatment of ADHD were prescribed 1398 times (1.7% of the total prescriptions), (Table 2 and 6).

Corticosteroids were prescribed 1476 times, representing 1.8% of the total medications (Table 2 and 6).

Different medications were prescribed for the treatment of cigarette, alcohol and drugs addictions, 497 prescriptions represented 0.5% of the total prescribed medications (Table 2 and 7).

Other less frequently prescribed medications were desmopressin (0.5%), alendronic acid (0.5%), cinacalcet (0.2%) (Table 2), antifungal (0.1%), chemotherapy (pazopanib) (0.08%), hormonal therapy (0.07%), antiviral medications (0.05%), pancreatic enzymes (0.01%), all are summarized in table 2 and 7.

4.1.3 MORTALITY

The causes of death in 144 deceased individuals with PWS were stratified in table 3. One third of the causes were unspecific. Cardiovascular causes represented 23.6% of the total causes of mortality, in this subgroup, the cardiac causes alone contributed to 70%. Endocrine and metabolic causes represented 13.9%, within this group the main contributors were obesity complications (55%) and diabetes (35%). Respiratory causes of death contributed to 12.5% of the total number and other causes of death contributed with 14.8% of the total mortality etiologies (Table 3).

Table 1. The main groups (sub-chapters) of comorbidities, in 411 patients with PWS in Sweden (*n*=27240) between 1964-2019.

The total number (*n*=37658)	**freq. (percentage)**
Mental and behavioral disorders	3113 (11.43)
Congenital anomalies	2665 (9.8)
Endocrine, nutritional and metabolic diseases	2221 (8.2)
Symptoms, signs and ill-defined conditions	1881 (6.9)
Disease of the respiratory system	1498 (5.5)
musculoskeletal and connective tissue diseases	1494 (5.5)
Injuries and poisoning	1318 (4.8)
Eye and adnexia	1171 (4.3)
Disease of the circulatory system	1042 (3.8)
Nervous system	1023 (3.8)
Ear and mastoid	928 (3.4)
Digestive system	920 (3.4)
Genitourinary system diseases	799 (2.9)
Skin diseases	694 (2.5)
Infectious disease	490 (1.8)
Benign and Malignant neoplasms	328 (1.2)
Blood and Blood-forming organ	250 (0.9)

Table 2. The main groups (sub-chapters) of prescribed medications (*n*= 83629) among 365 PWS patients in Sweden for the period of 2005-2019.

ATC subcategory	**Frequency (percentage) Total 83629 (100%)**
Anxiolytics, Antidepressants, Insomnia treatment	19551 (23.4)
Anti-psychotics	13901 (16.6)
Somatotropin	9199 (11.0)
Vitamins and minerals	7859 (9.4)
Anti-epileptics	7152 (8.55)
Glucose lowering medications	6876 (8.2)
Thyroxine	3997 (4.78)
Antibiotics	3479 (4.16)
Estrogen, progestin	2270 (2.7)
Insulin	2103 (2.51)
Corticosteroids	1476 (1.76)
ADHD	1398 (1.7)
Testosterone	1318 (1.6)
Treatment of cigarette, alcohol and drugs addictions	497 (0.51)
Desmopressin	426 (0.51)
Osteoporosis medications	419 (0.50)
Cinacalcete	143 (0.17)
Antifungal	100 (0.12)
Chemotherapy	69 (0.08)
Cyproterone	67 (0.08)

Hormonal therapy for prostate and breast CA and other different uses	59 (0.07)
Antithyroid medications	41 (0.05)
Antiviral	39 (0.05)
Pancreatic enzymes (amylase, lipase, protease)	9 (0.01)

Table 3. Matching mortality results of two previous studies with our study results.

Causes of death	Butler *et al.*2017 (*n*=486)	Pacoricona *et al.* 2019 (*n*=113)	Our study (*n*=144)
Infections and their squealy	Sepsis 29 (9,0)	4 (3.5) Sepsis 2 (1.8)	Total 6 (4.2) Sepsis 3 (0.7)
malignancies	4 (2.0)		3 (2.1)
Endocrine and metabolic	Obesity 22 (7.0)		Total 20 (13.9) Obesity 11(7.6) Diabetes mellites 7 (4.9) Thyroid 1 (0.7) Metabolic 1 (0.7)
Neurological	6 (2.0)		Epilepsy 1 (0.7)
Cardiovascular	Total 69 (23.0) Cardiac 50 (16.0) Pulmonary embolism 19 (7.0)	Total 15 (13.3) Cardiac 8 (7.1) Pulmonary embolism 4 (3.5)	Total 34 (23.6) Cardiac 24 (16.7)
Respiratory	Respiratory failure 94 (31.0)	Total 55 (48.7) Respiratory failure 42 (37.2) Respiratory infections 13 (11.5)	Total 18 (12.5)

Surgical, abdomen	30 (10.0)	4 (3.5) Occlusion 3 (2.7)	5 (3.5)
Renal failure	7 (2.0)		4 (2.8)
Injuries and accidents	Total 41 (8.4) Choking 18 (6.0) Accidents 17 (3.5) Hypothermia 3 (1.0) Drug reaction 3 (1.0)		Total 4 (2.8) Choking 2 (1.4) Fall 1 (1.4) Other 1 (1.4)
Other unspecific causes		Total 36 (31.9) Sudden death 18 (60.0) Other 8 (7.1)	49 (34.0)

Table. 4: Description of mental and behavioral comorbidities, and the related prescribed medication use by main and sub-groups.

Mental and behavioral comorbidity subgroups 3113 (11.43)	**Frequency (percentage)**	**ATC subcategory Frequency (percentage**	**The most common medications Frequency (percentage)**
Mental disorders	**1250 (40,15)**	Anxiolytics, Antidepressants Insomnia treatment. 19551 (23.4)	Citalopram 4102 (21.0) Sertraline 3177 (16.25) Fluoxetine 3034 (15.5) Oxazepam 1420 (7.3) Hydroxyzine 1344 (6.9) Zopiclone 1312 (6.7) Propiomazin 861 (4.4) Melatonin 595 (3.0) Escitalopram 552 (2.8)
Pervasive development disorders, unspecific and specific and other developmental disorders	761 (24.45)		
Specific motor development disorders	56 (1.8)		
Unspecific disorders of speech and language	37 (1.19)		
Combined vocal and multiple motor tics	23 (0.74)		
Aggression and lack of social adjustment	18 (0.58)		
Unspecific anxiety disorders	64 (2.1)	Anti-psychotics 13901 (16,6)	Risperidone 6673 (48.0) Olanzapine 2040 (14.7) Aripiprazole 1029 (7.4) Quetiapine 941 (6.8) Haloperidol 799 (5.75) Levomepromazin 690 (5.0) Zuklopentixol 649 (4.7)
Unspecific obsessive-compulsive disorders	57 (1.83)		
Mixed anxiety and depression state	47 (1.51)		
Unspecific physiological malfunction	35 (1.12)		
Obsessive-compulsive disorders	28 (0.9)		
Impulse control disorders	20 (0.64)		

Adaptation disorders	17 (0.55)		
Acute stress	14 (0.45)	ADHD 1398 (1.7)	Methylphenidate 1147 (82.0)
Neurasthenia	14 (0.45)		
Behavioral disorders	**1706 (54.8)**	Treatment of cigarette, alcohol and drugs addiction 497 (0.51)	Nicotine 233 (46.9)
Depression episode, unspecific	54 (1.73)		Methadone 134 (27.0)
Bipolar disorders, without symptoms	50 (1.6)		Naltrexone 113 (22.74)
Psychosis, different	101 (3.24)		
Neurosis, personality disorders	79 (2.54)		
Behavioral disorders associated with physiological and physical factors	93 (3.0)		
	45 (1.45)		
Other, different	99 (3.2)		
Other not specified	**207 (6.65)**		
sleep disorders	20 (0.64)		
Non-organic enuresis	21 (0.67)		

Table. 5: Description of other comorbidities (rather than the first common two), by main and sub-groups, among 411 PWS patients in Sweden (*n*=27240) between 1964-2019.

Main Comorbidity Frequency (percentage) Total=27240 (100)	Comorbidity subgroups	Frequency (percentage)
Congenital anomalies 2665 (9.8)	**Congenital malformations of the genitals, undescended testis**	**789 (6.03)**
Symptoms, signs and ill- de-Fined conditions 1881 (6.9)	**Symptoms and signs of abdomen and indigestion**	**523 (27.8)**
	symptoms of circulatory and respiratory problems	**341 (18.13)**
	Lower leg edema, unspecific	**273 (14.51)**
	Urinary tract symptoms	**280 (14.9)**
	Symptoms and signs of skin and subcutaneous tissue	**106 (5.64)**
	symptoms of nervous system and musculoskeletal disorders	**44 (2.34)**
	Abnormal findings on blood test without diagnosis	**48 (2.6)**
Respiratory system 1498 (5.5)	**Other diseases of the alveoli and other respiratory diseases**	**297 (19.83)**
	Chronic diseases of the lower respiratory tract, like asthma	**303 (20.23)**
	Acute upper and lower, infl. And pneumonia, chronic lower and others	**289 (19.29)**
	acute upper respiratory tract infection, unspecific	338 (22.6)
	Other diseases of the upper respiratory tract, tonsilitis and adenoid	**249 (16.62)**
	Acute upper respiratory tract infections, pneumonia	**199 (13.3)**
	Other acute lower respiratory tract infections	**107 (7.41)**
Musculoskeletal and connective tissue 1494 (5.5)	**Deformal vertebral diseases, scoliosis**	**940 (62.92)**
	Arthrosis	**48 (3.21)**

Injuries and poisoning 1318 (4.8)	**Injury to knees and lower legs** **Complications for surgical procedures and medical care not elsewhere classified** **Head injuries** **Injuries to wrist and hand** **Damage to shoulder and upper arm** **Injuries to ankle and foot** **sexual abuse** **Injuries to the abdomen, lower back, lumbar spine and pelvis** **Burns** **toxic effect with substances of no medical use, other effects of decrease and increase temperature, and light and radiation**	**266 (20.2)** **182 (13.81)** **142 (10.77)** **106 (8.04)** **183 (13.88)** **93 (7.1)** **18 (1.4)** **41 (3.11)** **35 (2.7)** **10 (0.8)**
Eye and adnexia 1171 (4.3)	**Visual disturbances and blindness, refractive errors, strabismus** **Diseases of the choroid and retina, diabetes retinopathy**	**833 (71.14)** **71 (6.1)**
Cardiovascular system 1042 (3.8)	**Cardiac,** cardiomyopathy, arrhythmias **Hypertensive diseases, e**ssential hypertension **Diseases of veins, lymphatic vessels and lymph nodes not elsewhere classified** **Heart failure and other heart diseases** **IHD and diseases of the pulmonary circulation** **Other and unspecified diseases of the circulatory system and hypotension** **Diseases of the veins and lymphatic vessels as well as other diseases in the circulatory system** **Diseases of the arteries, arterioles (small arteries) and capillaries**	**269 (25.82)** **227 (21.79)** **179 (17.18)** **166 (15.93)** **72 (6.91)** **41 (3.93)** **16 (1.53)** **14 (1.34)**
Nervous system 1023 (3.8)	**Episodic and paroxysmal diseases, like migraine, epilepsy sleep disorders and others** Sleep disorders Epilepsy, different Narcoplexy and cataplexy	**677 (66.2)** 298 (29.13) 342 (33.43) 8 (0.8)
Ear and mastoid 928 (3.4)	**Diseases of the outer ear, ear canal, and middle ear, otitis media.** **Hearing loss, different types**	**647 (69.72)**

	Inflammation of the mastoid committee and related diseases	**144 (15.52)**
		126 (13.6)
Digestive system	**Non-infectious inflammation of the small and large intestine,**	**307 (33.37)**
920 (3.4)	**functional disorders IBS, vascular disorders and others**	
	Constipation	98 (10.65)
	Diseases of the esophagus, stomach and duodenum	**111 (12.1)**
	Hernia	**123 (13.37)**
	Diseases of the gallbladder, bile ducts and pancreas	**72 (7.83)**
Genitourinary system	**kidney failure, CRF, nephritis ARF**	**293 (36.7)**
799 (2.9)	**Other diseases of the urinary tract**	**257 (32.17)**
	Urinary tract infection	136 (17.02)
	kidney infection, UTI, stone, cyst, fistula and others	87 (10.9)
	Non-inflammatory diseases of the female genitalia, Frequent/	**101 (12.6)**
	irregular menstruation	
	Diseases of the male genitalia, Phimosis and Foreskin	**55 (6.9)**
	diseases, BPH	
Skin	**Atrophic, hypertrophic, granulomatous, SLE and others**	**165 (23.78)**
694 (2.5)	Skin ulcer, different	90 (13.0)
	Acne, rosacea, sweating diseases, cysts and others	**239 (34.44)**
Infectious disease	**Viral diseases**	**284 (58.00)**
490 (1.8)	**Bacterial diseases, rose fever**	**139 (68.1)**
	Sexually transmitted infections and other spirochete diseases	**8 (1.63)**
	Infectious diseases from the gastrointestinal tract,	**96 (19.59)**
	Gastroenteritis or colitis	
	Late effects of infectious and parasitic diseases	**138 (28.2)**
Benign and Malignant	**Malignant tumors**	**269 (82.01)**
neoplasms	Different types of lymphoma	158 (48.2)
328 (1.2)		
Blood and Blood-forming	**Nutrition and hemolytic anemia**	**22 (8.8)**
organ	**Aplastic and coagulation disorders**	**95 (38.0)**
250 (0.9)	**Other not specified**	**180 (72.0)**
	Familial hypogammaglobulinemia	65 (26.0)

Table 6: Description of endocrine, nutritional and metabolic comorbidities, and the related prescribed medication use by main and sub-groups.

Endocrine, nutritional and metabolic comorbidities 2221 (8.2)	**Frequency (percentage)**	**ATC subcategory Frequency (percentage**	**The most common medications Frequency (percentage)**
Diabetes	**889 (40.03)**	Somatotropin 9199 (11.0)	Somatotropin 9199 (100)
T2DM without complications	444 (20.0)		
T1DM without complications	188 (8.46)	Glucose lowering medications 6876 (8.2)	Metformin 4869 (70.8) Glipizide 343 (5.0) Pioglitazone 263 (3.82) Sitagliptin 191 (2.8) Rosiglitazone 125 (1.82) Glimipride 113 (1.64) Unspecific 670 (9.74)
T1DM with ophthalmopathy	43 (1.94)		
T2DM with multiple complications	41 (1.85)		
T2DM with eye complications	33 (1.49)		
T2DM with unspecific complications	29 (1.31)		
Unspecific diabetes without complications	52 (2.34)		
T1DM with multiple complications	21 (0.95)	Thyroxine 3997 (4.78)	Thyroxine 3997 (100)
T2DM with renal complications	21 (0.95)		
Diabetes	13 (0.59)	Estrogen, progestin 2270 (2.7)	Medroxyprogesterone 867 (38.2) Estradiol 495 (21.8) estradiol, noretisteron 180 (7.9) medroxiprogesteron 112 (5.0) unspecific 103 (4.5)
Diabetes with ketoacidosis	4 (0.2)		
Thyroid	**266 (12.0)**		
Unspecific hypothyroidism	225 (10.13)		
Different thyrotoxicosis	20 (1.0)		
Congenital hypothyroidism without goiter	8 (0.4)		
Other, different	15 (0.7)	Insulin 2103 (2.51)	Insulin aspart and insulin aspart protamine 979 (46.6) Protamine insulin 402 (19.1) Insulin detemir 196 (9.3) Insulin glargine 192 (9.1) Insulin lispro 139 (6.6)
Other endocrine glands	**835 (37.6)**		
Hypopituitarism	439 (19.8)		
Idiopathic GH deficiency	176 (7.92)		
Gonadotropin deficiency	34 (1.53)		
Testicular hypofunction	26 (1.17)		
Other different including diabetes	83 (3.74)	Corticosteroids 1476 (1.76)	Prednisolone 1001 (67.8) Betamethasone 288 (19.5) Hydrocortisone 124 (8.4)
Metabolic diseases	**231 (11.21)**		

		Testosterone 1318 (1.6)	Testosterone 1318 (100)
		Desmopressin 426 (0.51)	Desmopressin 426 (100)
		Osteoporosis medications 419 (0.50)	Alendronic acid 403 (96.2)
		Cinacalcete 143 (0.17)	Cinacalcete 143 (100)
		Cyproterone 67 (0.08)	Cyproterone 67 (100)
		Hormonal therapy for prostate and breast CA and other different uses 59 (0.07)	Letrozole 18 (30.5) Triptorelin 13 (22.0) Buserelin 11 (18.6)
		Antithyroid medications 41 (0.05)	Thiamazole 30 (73.2) Propylthiouracil 11 (26.8)
		Pancreatic enzymes (amylase, lipase, protease) 9 (0.01)	Pancreatic enzymes (Amylase, lipase, protease) 9 (100)

Table 7: Description of other prescribed medication use (*n*= 83629) among 365 PWS patients in Sweden for the period of 2005-2019.

ATC subcategory	frequency (percentage) Total 83629	The most common medications Frequency (percentage)
Vitamins and minerals	7859 (9.4)	Vit.D and Calcium 1709 (21.75) Potassium 1355 (17.2) Iron 1116 (14.2) Cyanocobalamin 1048 (13.3) Calcium 844 (10.7) Vit.D 732 (9.3) Folic acid 196 (2.5) Unspecific, including calcium and Vit.D 375 (4.7)
Anti-epileptics	7152 (8.55)	Carbamazepine 1835 (25.7) valproic acid 2502 (35.0) lamotrigine 819 (11.5) levetiracetam 516 (7.2)
Antibiotics	3479 (4.16)	Phenoxy methyl penicillin 797 (22.9) Flucloxacillin 602 (17.3) Clindamycin 234 (6.7) Sulfamethoxazole, trimethoprim 228 (8.6) Amoxicillin 211 (6.1)
Treatment of cigarette, alcohol and drugs addictions	497 (0.51)	Nicotine 233 (86.9) Methadone 134 (27.0) Naltrexone 113 (22.7)
Antifungal	100 (0.12)	Antifungal 100 (100)
Chemotherapy	69 (0.08)	Pazopanib 68 (100)
Antiviral	39 (0.05)	Antiviral 39 (100)

4.2 HAIR CORTISOL

Twenty-nine adults participated in this study (15 men and 14 women). The PWS diagnosis was confirmed genetically in all. The mean (SD) for their age was 33.4 (12.7) years, while the mean (SD) age for the control group was 42.1 (11.6) years. After matching for age and sex between the two groups, there were no significant differences between the patients with PWS and the controls.

In the lifelines Cohort study, the median BMI was 26 kg/m2 (42% overweight and 19% obese). The use of steroids in the last three months was reported in 12% while 58% reported at least one stressful life event in the last 12 months.

According to the individual hair cortisol levels displayed in table 8, six patients with PWS had hair cortisol levels more than 10 pg/mg, four of them reported the occurrence of stressful situation.

Twenty-three other adults with PWS had hair cortisol levels of ten or below, 13 of them did not report any stress. While the remaining ten adults reported different kinds of stress that did not result in high levels of hair cortisol. Furthermore, six patients with hair cortisol below 1.3 pg/mg did not report any stress or use of glucocorticoids.

There was a huge variation in the levels of hair cortisol between the adults with PWS. In all patients with PWS, the mean (SD) was 12.8 ± 25.4 pg/mg compared to 3.8 ± 7.3 pg/mg in the controls ($p = 0.001$). After excluding the two high readings, the mean hair cortisol in the PWS group was 6.43 ± 9.66 pg/mg.

Through the crude regression model analysis, the hair cortisol levels in patients with PWS was significantly higher than the controls ($\beta = 1.21$; 95% CI [0.44–1.98]) ($p = 0.002$), and the association remained higher even after adjustment for BMI and reported stress ($\beta = 0.84$; 95% CI [0.11–1.56], $p = 0.023$) (Table 9).

By using the same linear regression model, in patients with PWS, hair cortisol increased with BMI ($\beta = 0.05$; 95% CI [0.02–0.09], $p = 0.002$, which remained after adjustment for stress ($\beta = 0.04$; 95% CI [0.01–0.08], $p = 0.012$) (Table 10). Likewise, hair cortisol increased with stress ($\beta = 1.16$; 95% CI [0.41–1.91], remained after adjustment for BMI ($\beta = 0.90$ (95% CI [0.19–1.60], $p = 0.014$) (Table 10).

Table 8. Characterizations of 29 patients with Prader-Willi syndrome, sorted according to the patients' codes.

Females	Age (year)	BMI	Hair cortisol pg/mg	Steroid use	Reported stress	Males	Age (year)	BMI	Hair cortisol pg/mg	Steroid use	Reported stress
No 1	45	32	36.1	Yes, cream	No	**No 2**	28	33	<1.3	No	No
No 4	19	32	1.9	No	Yes unspecified	**No 3**	30	48	2.0		No
No 6	22	20	8.5	Yes, cream	Yes unspecified	**No 5**	22	20	7.7	Yes, cream	Yes unspecified
No 8	21	23	2.7	Yes, cream	No	**No 7**	23	26	12.5	no	Yes unspecified
No 13	28	28	3.2	Yes, cream	Yes unspecified	**No 9**	45	24	1.4	no	Yes unspecified
No 14	27	22	11.4	No	No	**No 10**	21	35	10.0	no	No
No 15	33	37	<1.3	No	No	**No 11**	19	24	2.1	no	No
No 19	58	23	2.1	No	Yes unspecified	**No 12**	58	26	89.7	no	Yes, chronic pain
No 22	40	39	<1.3	No	No	**No 16**	29	25	<1.3	no	No
No 23	29	23	1.8	No	Social stress	**No 17**	20	18	<1.3	no	No
No 25	18	32	1.9	No	Yes	**No 18**	49	28	39.4	Yes, inhalation	New job
No 26	59	40	105.6	no	Surgery	**No 20**	28	27	3.3	no	No
No 27	36	39	3.0	Yes, nasal	Social stress	**No 21**	51	27	5.6	no	No
No 28	43	45	7.6	No	No	**No 24**	30	31	<1.3	no	No
						No 29	37	24	1.5	no	Moving

Table 9. Crude and adjusted regression Coefficients and 95% Confidence intervals (CI) using a linear regression model for HC in patients with Prader-Willi syndrome (PWS) and matched-controls

	n	Mean (SD)	**Crude Regression Coefficients** (95% CI)	P-value	**Adjusted** Regression Coefficients** (95% CI)	P-value
Controls **PWS**	29 105	12.8 (25.4) * 3.8 (7.3)	Reference 1.21 (0.44-1.98)	0.002	Reference 0.84 (0.11-1.56)	0.023

*p-=0.001 **adjusted for BMI and stress.

Table 10. Crude and adjusted regression Coefficients and 95% Confidence intervals (CI) using a linear regression model for hair cortisol in 29 patients with Prader-Willi syndrome (PWS) adjusted for BMI or stress and adjusted for both BMI and stress.

	Adjusted for BMI or stress		**Adjusted for BMI and stress**	
	Regression Coefficients (95% CI)	P-value	**Regression Coefficients** (95% CI)	P-value
PWS **BMI** kg/m^2 *****	0.05 (0.02-0.09)	0.002	0.04 (0.01-0.08)	0.012
PWS **Stress** **Yes**	1.16 (0.41-1.91)	0.002	0.90 (0.19-1.60)	0.014

*BMI (Body Mass Index). **adjusted for BMI and stress.

4.3 EFFECT OF GROWTH HORMONE TREATMENT ON SLEEP RELATED PARAMETERS IN ADULTS WITH PWS.

4.3.1 Baseline characteristics

In this study, the median age of the patients with PWS was 29.5 (16.0 – 41.6) years. The participants were overweight with median BMI 27.1 (17.9-44.8) kg/m^2). Their IGF1 was low (115 (61.0-185.0) µg/L). Both SE and AHI were normal; 89.0 % (41.0 – 99.0) and (1.4 (0.0-13.9) respectively. From the total group of patients, nineteen (51%) (7 males and 12 females) patients were randomized to GH treatment, and 18 (49%) (8 males and 10 females) to placebo treatment. There were no differences between the two groups (Table 11).

At baseline, most of the patients (34 patients) had no apnea (AHI below 5), and only three patients had mild apnea (AHI from 5 to less than 15), while no patients suffered from moderate (AHI from 15 to less than 30) or sever apneas (AHI>30) at the baseline.

4.3.2 Comparison between the placebo and the GH treated group at one year

IGF-I had increased at year one to 165 (69-257) µg/L in the GH treated group, while it remained unchanged in the placebo group (p=0.013). At the end of the year, no difference in the respiration and sleep parameters were seen between the GH and placebo groups (Table 11).

4.3.3 Effect of long-term GH treatment

In patients who were randomly assigned for GH treatment since the beginning to 36 months, IGF-I was elevated from 104 (66-186) to 178 (113-295) µg/L). Median SE at the end of year three was increased to 91% (57-100), p-value 0.001, however a trend towards an increase in AHI was also observed (2.4 (0.0-52.9), p-value 0.105). No other changes on the clinical characteristics or the sleep or respiration parameters were seen.

After performing the mixed-effect regression model, an increase with AHI was seen over time compared with baseline, but with intermittent significant association (Table 3). After adjusting for BMI, the results didn't change. The number of desaturations also inconsistently increased, even after adjustment for BMI, while the length of the longest apnea was significantly increased at the last visit and after adjustment for BMI.

SE significantly increased through the study period, starting from the 6-months visit, the increment remained significant even after adjustment for BMI, while the other parameters did not change (Table 11). No one of the participants died during the entire study period.

4.3.4 CPAP and ENT examinations

At the baseline, five patients were treated for sleep apnea with CPAP, two of them remained on CPAP until the end of the study, they were assigned for GH treatment from baseline, while the other three patients, who were assigned for the placebo treatment at baseline, discontinued CPAP treatment after six months or one year. One patient from the placebo group started up CPAP treatment for a short period of time for treating an already existing sleep apnea.

ENT examination for all the patients at the baseline revealed that five patients had tonsillar hypertrophy (four patients in the placebo group and one in the intervention group). Two of them underwent tonsillectomy. Furthermore, a cleft palate operation was performed in the first year of the study in one patient in the GH-treated group, while no other ENT abnormality was seen in any patient at the second or the third year of follow-up. The above ENT abnormalities did not cause a significantly effect on the PSG measurements (data not shown).

Table. 11: Baseline and 12-months placebo – intervention, characteristics for respiratory and sleep parameters for 37 adults with Prader-Willi syndrome (PWS) presented in median (min–max), and mixed-effect regression model assessing the association of eight outcomes by 36 months treatment with growth hormone (GH), crude and BMI-adjusted for (n=37)

	Baseline			**Placebo intervention**			**Regression analysis**				
	Placebo (n=18)	GH (*n*=19)	P-value **	Placebo (n=18)	GH (*n*=19)	P-value **	Follow up periods (months)	Crude		BMI adjusted [a]	
								Coef. (95% CI)	P value	Coef. (95 % CI)	P value
AHI	0.9 (0.0-54.1)	2.0 (0.0-28.0)	0.994	0.9 (0.0-54.1)	2.0 (0.0-28.0)	0.994	Baseline	Ref.		Ref.	
							6 m	-0.004 (-2.38 - 2.37)	0.997	-0.38 (-2.70 - 1.95)	0.751
							12 m	1.56 (-0.82 - 3.93)	0.200	1.003 (-1.34 - 3.34)	0.400
							18 m	3.03 (0.65 - 5.41)	0.048	2.77 (0.42 - 5.12)	0.021
							24 m	2.94 (0.57 - 5.32)	0.015	2.36 (0.31 - 4.96)	0.026
							30 m	2.55 (0.09 - 5.01)	0.042	2.55 (0.14 - 4.96)	0.038
							36 m	2.19 (-0.33 - 4.77)	0.097	2.12 (-0.44 - 4.68)	0.105
Longest apnea (seconds)	20.9 (0.0-65.0)	20.0 (0.0-68.9)	0.726	20.9 (0.0-65.0)	20.0 (0.0-68.9)	0.726	Baseline	Ref.		Ref.	
							6 m	0.67 (-8.67 to 10.01)	0.888	0.74 (-8.64 – 10.122)	0.877
							12 m	-2.38 (-11.64 to 6.78)	0.614	-2.29 (-11.60 – 7.03)	0.630
							18 m	5.10 (-4.07 – 14.28)	0.276	5.23 (-4.05 – 14.52)	0.270
							24 m	6.74 (-2.51 – 16.00)	0.153	6.79 (-2.50 – 16.08)	0.152

							30 m	8.32 (-1.22 – 17.85)	0.087	8.35 (-1.21 – 17.92)	0.087
							36 m	11.58 (1.43 – 21.71)	0.825	11.62 (1.45 – 21.79)	0.025
PLM	29.5 (2.0-286.0)	32.0 (2.0-250.0)	0.646	29.5 (2.0-286.0)	32.0 (2.0-250.0)	0.646	Baseline	Ref.		Ref.	
							6 m	-9.71 (-34.70 – 15.29)	0.447	-9.52 (-34.62 – 15.58)	0.475
							12 m	-4.85 (-29.69 – 20.00)	0.702	4.43 (-29.39 – 20.53)	0.728
							18 m	11.84 (-12.81 – 36.48)	0.347	12.24 (-12.69 -37.19)	0.336
							24 m	12.26 (-12.41 – 36.92)	0.330	12.38 (-12.39-37.14)	0.327
							30 m	25.89 (0.43 – 51.35)	0.046	25.85 (0.28-51.41)	0.048
							36 m	20.60 (-5.92 – 47.13)	0.128	21.81 (-5.13-48.75)	0.113
SAT (%)	96 (86-99)	96 (83-196)	0.804	96 (86-99)	96 (83-196)	0.804	Baseline	Ref.		Ref.	
							6 m	2.82 (-0.58 – 6.16)	0.097	2.91 (-0.44 – 6.25)	0.089
							12 m	-0.54 (-3.88 – 2.79)	0.749	-0.43 (-3.78 – 2.92)	0.803
							18 m	-1.72 (-5.04 – 1.61)	0.312	-1.73 (-5.11 – 1.64)	0.314
							24 m	-0.16 (-3.49 – 3.17)	0.926	-0.09 (-3.44 – 3.25)	0.956
							30 m	-0.03 (-3.50 – 3.44)	0.985	0.03 (-3.46 – 3.52)	0.987
							36 m	0.62 (-2.96 – 4.19)	0.736	0.71 (-2.92 – 4.34)	0.701
IGF-I (µg/l)	116.5 (46-198)	164.5 (69-257)	0.013	116.5 (46-198)	164.5 (69-257)	0.013	Baseline	Ref.		Ref.	
							6 m	2.19 (-1.35 – 5.73)	0.225	2.27 (-1.30 – 5.84)	0.213
							12 m	1.66 (-1.92 – 5.24)	0.362	1.75 (-1.85 –5.36)	0.340
							18 m	3.90 (0.32 – 7.48)	0.033	3.96 (0.32 – 7.60)	0.033
							24 m	5.33 (1.81 – 8.85)	0.003	5.37 (1.83 – 8.92)	0.003
							30 m	3.80 (-0.07 – 7.65)	0.054	3.81 (-0.07 – 7.7)	0.054
							36 m	3.15 (-0.7 – 6.1)	0.109	3.29 (-0.64 – 7.21)	0.101
No. of desaturations (total)	16.0 (0.0-525.0)	24.5 (1.0-525.0)	0.657	16.0 (0.0-525.0)	24.5 (1.0-525.0)	0.657	Baseline	Ref.		Ref.	
							6 m	0.22 (-3.84 – 4.28)	0.916	0.19 (-3.89 – 4.27)	0.926
							12 m	-3.30 (-7.45 – 0.84)	0.118	-3.27 (-7.43 – 0.89)	0.124
							18 m	-0.39 (-4.17 - 3.37)	0.836	-0.49 (-4.29 – 3.29)	0.798
							24 m	0.79 (-2.93 - 4.53)	0.674	1.06 (-2.69 – 4.820	0.578
							30 m	-0.33 (-4.29 - 3.61)	0.866	-0.17 (-4.13 – 3.79)	0.933
							36 m	-1.36 (-5.35 - 2.62)	0.502	-1.07 (-5.13 – 2.97)	0.602

REM (%)	17.3 (0.0-45.0)	17.5 (7.5-42.4)	0.614	17.3 (0.0-45.0)	17.5 (7.5-42.4)	0.614	Baseline 6 m 12 m 18 m 24 m 30 m 36 m	Ref. 11.1 (-23.80 – 46.00) 26.31 (-8.20 – 60.82) 8.98 (-25.51 – 43.48) -18.56 (-52.68 – 15.56) 4.56 (-31.82 – 40.93) 11.66 (-23.97 – 47.30)	 0.533 0.135 0.610 0.286 0.806 0.521	Ref. 10.15 (-25.03 –45.34) 24.94 (-9.88 – 59.76) 9.69 (-25.32 – 44.70) -19.66 (-54.05- 14.73) 3.65 (-33.00 – 40.31) 9.70 (-26.52 – 45.92)	 0.572 0.160 0.587 0.263 0.845 0.600
REM latency (minutes)	75.3 (0.0-473.0)	64.8 (0.0-184.0)	0.493	75.3 (0.0-473.0)	64.8 (0.0-184.0)	0.493	Baseline 6 m 12 m 18 m 24 m 30 m 36 m	Ref. 2.80 (0.75 – 6.35) 4.97 (1.44 – 8.49) 6.24 (2.66 – 9.83) 4.81 (1.22 – 8.40) 7.04 (3.18 – 10.91) 6.64 (2.86 – 10.41)	 0.122 0.006 0.001 0.009 0.000 0.001	Ref. 2.66 (-0.91 – 6.22) 4.79 (1.25 – 8.33) 6.12 (2.49 – 9.76) 4.79 (1.2 – 8.39) 7.1 (3.20 – 10.96) 6.52 (2.69 – 10.36)	 0.144 0.008 0.001 0.009 0.000 0.001
Sleep efficiency (CE) (%)	88.0 (48.6-100.0)	86.7 (58.5-99.9)	0.562	88.0 (48.6-100.0)	86.7 (58.5-99.9)	0.562	Baseline 6 m 12 m 18 m 24 m 30 m 36 m	Ref. -2.12 (-5.02 – 0.78) 3.57 (0.62 – 6.52) 1.40 (-1.55 – 4.36) 2.21 (-0.69 – 5.12) 0.52 (-2.63 – 3.67) 0.60 (-2.57 – 3.78)	 0.152 0.018 0.352 0.135 0.745 0.710	Ref. -2.06 (-4.99 – 0.87) 3.63 (0.65 – 0.87) 1.46 (-1.55 – 4.48) 2.23 (-0.69 – 5.16) 0.52 (-2.65 – 3.70) 0.68 (-2.57 – 3.92)	 0.167 0.017 0.340 0.135 0.746 0.682
Delta sleep	22.5 (1.7-52.1)	21.6 (1-38.4)	0.386	22.5 (1.7-52.1)	21.6 (1-38.4)	0.386	Baseline 12 m 24 m 36 m	Ref. 24.65 (11.27 – 38.03) 64.78 (51.51 – 78.03) 63.07 (49.57 – 76.58)	 0.001 0.001 0.001	Ref. 25.11 (11.78 – 38.46) 64.99 (51.79 – 78.20) 63.44 (49.78 – 77.01)	 0.001 0.001 0.001

*AHI: apnea hypopnea index REM: rapid eye movement PLM: periodic limb movement. SAT: oxygen saturation. IGF1: insulin like growth factor 1 *Mann-whiteny test **Mann-Whitney test. a: adjusted for BMI*

5 DISCUSSION

PWS is a rare, genetic, neurodevelopmental multisystem disease including hypothalamic dysfunction, behavioral problems intellectual disability and specific dysmorphisms [16]. During the last decades the knowledge of several aspects of the syndrome has increased considerably, but there are still many unanswered questions and remaining gaps. While treatment with GH completely has changed the phenotype of children and young adults the complex mixture of reduced activity muscular hypotonia, lack of motivation, compulsivity and hyperphagia leads to a continuously increased risk of morbid obesity and its complications [78].

This PhD research project, focused on comorbidities and mortality in PWS with the aim to provide a cross-sectional summary of existing comorbidities and mortality and their relation to use of drugs. It furthermore examined the effect of comorbidities on long-term exposure to cortisol and the effect of GH on respiration.

5.1 AN OVERVIEW OF COMORBIDITIES, DRUG USAGE AND MORTALITY IN PWS

Patients with PWS can experience increased risks of cardiovascular diseases [121,122], OSA [123,124], and death due to VTE [6,96,125–128], and the incidence of all-cause mortality, DVT and PE events are significantly higher in PWS population than in matched controls as reported by previous studies [78]. Furthermore, mental health and anxiety issues are very common [129,130].

Data from 365 patients on use of medication showed that they received 83629 prescriptions from outpatient clinics [131], which might indicate a risk of polypharmacy. Polypharmacy is a frequent problem in other patient groups with intellectual disabilities [132]. Nevertheless, a few numbers of patients could have used several medications, but it was not possible to further evaluate this question from the data available for the present study.

The pain threshold is generally decreased in patients with PWS, the temperature is not well-regulated [34], and gastric emptying and the vomiting reflex are delayed [133,134] Patients with PWS also have a lower proportion of visceral fat and more subcutaneous fat [134], and a difference in the activity of cytochrome P450 drug metabolizing enzyme have been reported between individual patients with PWS [135]. All these factors put patients with PWS at a high risk for developing side effects. It can also be speculated if an elevated number of adverse effects and the interaction between different medications might augment the development of

obesity, diabetes and metabolic comorbidities, which were in fact the second most common group of comorbidities in the present study.

Medications prescribed to treat mental and behavioral disorders represented 40% of the total prescriptions. In contrast, the frequency of documenting mental and behavioral disorders was 11,4% of the total comorbidity in patients with PWS. A possible explanation is that diagnose codes for congenital malformations and PWS in many cases were used to register the visits, and could include mental and behavioral disorders, interpreted as part of PWS. Hence the frequency of the behavioral and mental comorbidities was probably higher than 11.4% of the total comorbidity seen in table 1. Furthermore, patients with PWS might receive multiple medication to treat a mental and behavioral disorder, which further explains the difference between the frequency of diagnosis of mental and behavioral comorbidities and the high frequency of the medications prescribed to manage the same comorbidities. According to our data of prescribed medications, there were 91 prescriptions per patients to treat their mental and behavioral comorbidities. This rate of prescription could have a significant implication on the medical approach to improve follow up and control, which in patients with PWS is crucial.

Methylphenidate was prescribed 1147 times, and at the same time, pervasive development disorders were reported 761 times. This is going with published data showing the high prevalence of different ASD and other pervasive development disorders [28]. Interestingly, patients with PWS also received prescribed medications to treat addictions especially for treatment of cigarette and alcohol addiction (Table 4). This likely to be associated with psychiatric problems in patients with PWS [136]. Among the 411 PWS patients, diabetes ICD diagnosis appeared as comorbidity 899 times, and in the prescribed drugs data, 365 patients received 6876 prescriptions of oral anti-diabetics, and 2103 different forms of insulin. The relatively high number of prescriptions for treatment of diabetes (n=7775) was not surprising, as the prevalence of diabetes is high in PWS [39,133]. For the oral antidiabetics, the pattern of the prescriptions followed guidelines. Thus, Metformin was the most prescribed, followed by sulphonyl urea and thiazolidinediones. As of today, the guidelines have changed and sulphonyl urea is used to a lesser extent.

Somatotropin – GH – was the most common prescribed single medication. This is not surprising, as GH treatment is a well-established treatment in PWS unless contraindicated [16].

Hypopituitarism and GH insufficiency both represented around 10% of the endocrine, nutritional and metabolic comorbidities in our data, which constitutes around 0,5% of the total comorbidities. This again raises the issue of unspecific coding for comorbidity, probably

because GH deficiency was seen as part of PWS, and because PWS is a registered diagnosis of GH deficiency in children. Therefore, the diagnosis of hypopituitarism might not have been specifically registered.

Hypogonadism is present in almost all patients with PWS [57,90]. Different ICD codes for gonadotropin deficiency, testicular hypofunction and hypopituitarism were used. Estrogen and progesterone were prescribed, but not as frequent as would be expected considering that more than 90% of patients with PWS have hypogonadism [40,137]. Perhaps the common perception that the estrogen produced in the fat tissues is sufficient, the risk of side effects, especially for thromboembolism, which is already elevated in patients with PWS in comparison with the general population or problems with menstrual bleedings decreased the use of estrogen [78]. Similarly, testosterone was prescribed to a lesser degree than would be expected, probably because of fear for adverse effects [57,90]. Interestingly, the frequency of prescribing testosterone was less than the frequency of prescribing estrogen and progesterone which contrasts with clinical experience. In the literature conflicting data on prevalence of hypothyroidism are reported [62,63]. Different forms of hypothyroidism were documented in the comorbidity data. In accordance with clinical experience, the patients received around a high number (n=4000) prescriptions of thyroxine, while only a few records were registered for thyrotoxicosis. If hypothyroidism is present, thyroxine treatment is important to optimize metabolism.

Vitamin D and Calcium were prescribed either in combination, or separately. They were the most common vitamins and minerals prescribed to patients with PWS according to our data. Alendronate for the treatment of osteoporosis was prescribed 403 times, but osteoporosis was not specifically represented in the data of comorbidity. However, osteoporosis and low bone mineral density are well-known conditions in patients with PWS [48] and we believe the numbers in our data are reflecting this. Desmopressin was prescribed 426 times, representing 0,51% of the total medications prescribed for treating the endocrine comorbidities, but diabetes insipidus was not mentioned as a specific code. Desmopressin is often used in children to treat enuresis which could be an explanation. However, hypopituitarism was a frequently used code (n=439).

Use of corticosteroid was documented in the data of prescriptions, probably for topical use. Different antiepileptics were prescribed, and different ICD codes of epilepsy were documented, which is in line with the literature showing that epilepsy is not uncommon in patients with PWS, especially in children [138]. Antibiotics were frequently prescribed to treat different conditions. In the comorbidity, different codes for infections like diseases of the urinary tract,

bacterial diseases, infectious diseases from the gastrointestinal tract, acne, rosacea, cysts and skin ulcers, otitis media, pneumonia and upper respiratory tract infections.

Eight previous studies investigated the mortality in PWS [6,78,96,125–128], and the Prader-Willi Syndrome Association created a database in 1973 for registration of causes of death [106]. Data for the recent version was provided by the parents of 316 deceased individuals with PWS and the results were published in 2013, which showed that 23% had cardiovascular, and 7% diabetes as causes of death [107]. In another mortality study, respiratory causes were the most frequent [139].

The most frequent specific cause of death in our study was cardiovascular causes, the second most frequent diagnosis endocrine and metabolic causes and the third most common respiratory causes. The possible explanations for this pattern are first of all that the average age of our deceased patients was significantly higher, and the rate of cardiovascular comorbidities increases with advancement in age. Furthermore, the reason for the 34% unspecific ICD codes might be that the patients were not diagnosed with any serious disease before death and a life-threatening disease could not be found after death, but misclassification or underestimation of a disease could not be ruled out. However, there is a similarity in the frequency of cardiovascular and obesity as causes of death between our results and the study from USA [106,107], and a few patients died from diabetes in our study.

The frequent use of unspecific ICD codes tends to be one of the limitations of our study. Furthermore, many of the patients during their visits to the health care facilities received the diagnosis for PWS and not the specific comorbidity, which could be due to the symptoms were viewed as part of the syndrome [140]. Albeit being a register-based study, background information for the use of drugs and comorbidity on individual level were not available and lacked comparison with matched controls but in future studies such information would be of great value to include.

5.2 THE HPA-AXIS IN PWS

Unspecific comorbidity and unspecific causes of death can be caused by undetected hypocortisolism or increased respiratory problems during GH treatment as suggested by published reports [66,75,97,102,103]. The symptoms of CAI are non-specific and can be subtle or present with life-threatening condition and cardiovascular collapse, especially if associated with trauma, surgery, or sever illness [71]. PWS is characterized by impaired hypothalamic function [45,46]. Previous studies have found small adrenal glands at autopsy in PWS children [66], and small volume and decreased cell number of the paraventricular nuclei in adults with

PWS [67]. Hypothalamus is an important area for regulation of appetite and satiety, thirst, sleep, pain threshold and several endocrine systems [45,46]. In a 2008 study using an overnight single-dose metyrapone test, it was discovered that 60% of 25 children had some degree of CAI [65] , but following studies using several different dynamic tests found low prevalence of CAI [68–70]. We evaluated long-term cortisol exposure using HC. Cortisol is accumulated in hair, and because hair is growing at a stable rate (one centimeter/month), three-centimeter hair correspond to cortisol levels during the last three months[73,74]. In our study, the HC levels in the PWS group was higher than in the controls (non-PWS), and remained high after adjustment for BMI and stress. Adding to that, BMI and stress increased the HC levels. This indicates that the HPA axis responded well to chronic stress [141] and our results did not support a high prevalence of CAI in adults with PWS. This is an important finding, because the elevation in HC levels has been shown to be linked to different cardiovascular and metabolic comorbidities [115] and our data on comorbidities and mortality (Study 1) showed that these diseases were frequent in PWS [78].

Chronic stress in PWS can be due to many factors such as the obsession in food, which is considered a lifelong source of stress in individuals with PWS. The negative impact of obesity and chronic stress in PWS is increasing with age [44]. Diabetes prevalence in adults with PWS is also increasing with older age by around 50% in the 5th decade of life, and diabetes is associated with increased HC [87], but the etiology of obesity, diabetes and chronic stress are multifactorial.

Recently, a study investigated HC levels in children and adolescents with PWS [142], the HC level was lower in PWS children than non-PWS controls. The children and adolescents had a much lower BMI than the patients in our adult study, and HC correlates to BMI [76,77,143]. Furthermore, it was shown the daily cortisol production in children is lower than in adults [65,108]. Adding to that, the level of stress was shown to be lower in children with PWS than adults, and stress also correlates to HC [74,115,143]. Age is an important contributor to elevated HC [72,115], as with age advancement, the prevalence of different comorbidities will increase and cause chronic stress [39]. In our comorbidity and mortality study, as mentioned earlier, it was obvious that different comorbidities like diabetes, mental and behavioral problems and the medications used to treat them were very frequent and all these factors contribute to elevating the levels of chronic stress and HC in patients with PWS.

Patients with PWS suffer from ID and behavioral problems, and using any invasive or painful procedure or hospitalization is cumbersome to the PWS subjects. Therefore, HC is sensible because it is simple and non-invasive approach [115]. In the future, HC might be used to guide

the the multidisciplinary teams in estimating the level of stress in this vulnerable group of patients. In our study, PWS patients were comfortable with the procedure, and HC has the potential to offer a careful monitoring of the stress level in patients with PWS.

5.3 GROWTH HORMONE TREATMENT AND SLEEP-RELATED PROBLEMS IN ADULTS WITH PWS

It is well known that SRBD are frequent in children and adults with PWS [96]. The prevalence of OSA in children is has been reported to be 80% [144], while the prevalence of moderate to severe OSA was 22% in adults with PWS [145]. This is due to many factors like; craniopharyngeal abnormalities, reduced central chemoreceptor, sensitivity to hypoxia and hypercapnia, and adenotonsillar hypertrophy and muscular hypotonia [3,97,98]. Obesity and recurrent respiratory tract infections can even worsen the situation and lead to sudden death [19]. In addition, there might be an alteration in the central ventilatory regulation during stress [146]. Excessive day time sleepiness (EDS) is one of the consequences of OSA, however EDS can have other etiologies, and may continue even after OSA is treated.

GH treatment is well-established in children and adults with PWS [16], and there are many accumulating evidences about the benefits of GH treatment in both children and adults [100]. However, there are some concerns that GH treatment might negatively impact the sleep of prepubertal children with PWS [99] and thereby precipitate sudden death [3,9,16,22,96–98,102]. Theoretically, GH treatment might lead to increasing the size of the tonsils, which might decrease the upper airways and precipitate SRBD [16,145]. The estimated risk of death has been reported to be as high as 3% per year, and this is across all age groups, including cases of sudden death, and the respiratory diseases are frequent causes of sudden death [3,9,22,96,97,99,102].

The Federal Drug Administration (FDA) was notified in 2003 that at least seven children with PWS had died within a few months after initiating GH treatment [103]. This was attributed to the association between GH treatment and sudden death [102,103]. It was suspected that the cause of death was respiratory failure, but no causal relationship between GH treatment and respiratory failure was seen. It is unknown also whether these causes of death increased the mortality rate. Sodium and water retention, or stimulation of fibroblast growth leading to soft tissue swelling could be possible mechanisms. Furthermore, children could be more susceptible to airway obstruction due to the smaller diameter of the airways. However, the children received high dose of GH, which was not used in the present study [147].

From the numerous published studies, it is well-known that GH treatment is safe in children with PWS and recent guidelines recommend GH treatment for all children with PWS but also to include continuous monitoring for signs of SRBD during GH treatment [16,66,98,146].

In adults with PWS, the guidelines also favor GH treatment [16]. Few studies investigated the effect of GH treatment on sleep in adults with PWS, of which two studies discussed GH treatment and its effect on respiratory parameters [101,148]. One of the studies is an old intervention one year follow up study and a few of the participants were adults, while the other study is recently published and the investigators followed the participants for one year and it was a placebo-controlled study on adolescents and adults who were already on GH treatment in their childhood. In both studies, no harmful effects on AHI, OSI, CSI and O2 saturation were seen.

No study monitored the effect of GH treatment on sleep in adults with PWS for more than one year. We investigated this in a larger group of adults with PWS compared to previous studies, and additional sleep and respiration parameters were evaluated.

In 37 adults with PWS, the first part of the study (the placebo-controlled part), didn't show any negative effect of GH treatment on sleep. By virtue of the study design nature, only 17 participants received GH treatment for three continuous years (36 months), in this group of patients sleep efficiency (SE) increased even after adjustment for BMI, the improvement started after six months from the start, and continued throughout the study period, this is might be explained by the positive impact of the GH on the respiratory muscles in the patients with PWS. Median AHI increased inconsistently during the study period, but the increase was mild and of no clinical significance. The length of the longest apnea increased during the study, and no other effects of GH treatment on the other sleep and respiration parameters where seen.

In adults with PWS, frequent hypersomnolence and excessive daytime sleepiness with moderate to severe obstructive sleep apnea were reported [98,149]. The improvement in SE, which was observed in our study was important as it can indicate for a better sleep, improves the daytime sleepiness, will decrease the emotional disturbance and the low attention span that is frequently seen in adults with PWS [149].

Our study is part of the Scandinavian study, where the lean body mass improved 2.5 kg in the first year, and 2.8 kg after the end of the study [59,109,110,116]. It is well known that GH treatment improving the body composition and the lean body mass, and this improves respiration [16]. Furthermore, GH treatment increases the muscle strength and improves the respiratory muscle’s function, leaving a positive impact on sleep [16]. Although, the GH

treatment did not lead to REM and delta sleep improvement in our study, it might however explain the improvement of SE. Interesting, the improvement in body composition was seen although only a few patients fulfilled the criteria of adult GHD, suggesting that all adults with PWS can benefit from GH treatment not just those with documented GHD. Probably, different interpretation scenarios of the results of the stimulation tests are needed.

The length of the longest apnea significantly increased during the last visit, i.e., after three years of receiving GH treatment, only after adjustment for BMI, which might be due to the improvement in the quality of sleep. In theory, patients might get used to the PSG examination, and this adaptation might also lead to better sleep patterns during PSG sessions. While the AHI and the number of desaturations were inconsistently mildly increased, the clinical significance is unclear, especially since PSG is affected by many factors such as age, BMI, drugs, mood, behavior and compliance to examination that might appear and disappear over time [101,148]. This could explain the variations observed in the results. However, during this study, small changes in the body weight was observed, but are not expected to affect the study results [150,151]. Furthermore, the ENT examination did not reveal any increase in the tonsillar size. Thus, due to the multifactorial etiology of SRBD, PSG should be performed free-handed in adults with PWS with or without GH treatment.

It is important to treat SRBD in patients with PWS, because if left untreated, the OSA might lead to many adverse health consequences, like cardiovascular complications including hypertension, Cor pulmonale (cardio-pulmonary disease) and stroke, of which are the long-term health consequences [152]. While the short-term health consequences are the excessive daytime sleepiness, decreased cognition, executive functions, and worsening of memory. These complications are primarily due to sleep fragmentation and hypoxemia [145].

Reducing obesity is helpful to decrease the risk of SRBD, this will also help improve the hypertension and the metabolic parameters. OSA is treated by CPAP and surgery, tonsillectomy if tonsillar hypertrophy is the cause of OSA [145].

It is important to carefully monitor the sleep of adults with PWS, because they are often suffering from sleepiness and different forms of hypersomnolence disorders [149].

The design of this study with a double-blinded placebo-controlled period of one year, followed by an open phase period of GH treatment for 2 years, is a strength, because it allowed for evaluating GH effects on respiratory and sleep parameters during short and long terms. The numbers of participants were small, and there were no control subjects at the 24 and 36 months, which decreased the possibility to perform detailed sub-group analyses.

6 CONCLUSIONS

The high number of comorbidities, use of drugs and mortality among the PWS subjects are indicators of a great burden for the patients and the health care systems. Our studies confirmed that comorbidities were primarily caused by mental or behavioral diseases and diabetes, and demonstrated a linkage to their prescribed medications prescriptions. Furthermore, the main cause of death was unspecific, which warranted the use of more specific codes to improve the knowledge of the syndrome.

The function of the HPA axis in patients with PWS is an ongoing issue. In this PWS context, HC was higher in patients with PWS than in controls and both obesity and stress were main contributors to the elevated long-term. Our results suggest an adequate response of the HPA axis to chronic stress and obesity. and therefore, adrenal insufficiency might not be an explanation to the high number of unspecific comorbidity or mortality in patients with PWS.

SRBD are frequent in patients with PWS, the etiology is multifactorial, and frequent monitoring is mandatory whether the patients are on GH treatment or not. GH treatment is well established in PWS, with several documented benefits but there were some concerns about the impact of GH treatment on the sleep of the patients derived from reports of deaths in children with PWS. Our study could not support such effect in adults with PWS – in the short and long terms of treatment with GH, there were no clinically significant negative impact on the sleep and respiration parameters, and no deaths were reported. The SE is improved, but the length of the longest apnea and the number of desaturations both are inconsistently increased, these results need to be further examined.

The frequent use of codes representing unspecific causes of comorbidity and mortality warrants for increasing attention towards the methods of documentation and code classification to avoid underestimating comorbidity and mortality. HC was higher in patients with PWS compared with the controls and indicated an adequate HPA-axis response to chronic stress. Therefore, our results did not suggest hypocortisolism as a major comorbidity. In addition, GH treatment in the short and long term did not negatively impact the respiration in patients with PWS, and there was an improvement in the SE. Thus, diseases related to obesity were the main contributors to the comorbidity and mortality and more attention must be paid to continue and intensify the efforts of preventing obesity and its complications. There are several ongoing studies for the treatment of hyperphagia and hopefully they will in the future be a positive contribution to the prevention of obesity in PWS.

7 POINTS OF PERSPECTIVE

In order to maintain the work around this patient groups, it is important to establish a specific register for patients with PWS. From such a register, numerous important information of importance for clinicians could be retrieved. A systematic collection of biological material would be a valuable addition. A longitudinal cohort study to monitor HC in patients with PWS would help to better consolidating the role of HC in the management and follow up of patients with PWS. New technologies for different measurements are already developed, which can be used in this context. Measurements of cortisol's ultradian rhythm is now possible with the Ultradian technology and such measurements would be of great interest for a careful evaluation of the HPA axis in PWS. Furthermore, adding information about leptin and ghrelin hormones to the HC, might give clearer picture about the association between the different hormones and the patients conditions and comorbidities.

The effect of replacement of sex hormones on the metabolic comorbidities in adults with PWS is another important project. Diabetes prevalence is increasing with age in adults with PWS and the use of new antidiabetics (SGLT-2 inhibitors and GLP-1 analogs) should be evaluated systematically. Finally, there are several ongoing studies on the treatment of hyperphagia in PWS and the results of these studies are awaited. If the hyperphagia can successfully be treated and the prevalence of obesity reduced, this would be of a huge importance for this group of patients.

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