Prader-Willi syndrome

Adrenarche, gonadal function, cognition, psychosocial aspects and effects of growth hormone treatment in children

Elbrich P. C. Siemensma

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The studies described in this thesis were supported by an investigator-initiated independent research grant provided by Pfizer Inc., USA.

Publication of this thesis was financially supported by the Dutch Growth Research Foundation and Pfizer bv.

Cover image: illustration designed after a photograph taken by Mariëlle van Eekelen
Layout: Sander van Schaik
Printed by: Uitgeverij BOXPress, ‘s-Hertogenbosch

ISBN/EAN: 978-90-8891-524-6

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Prader-Willi syndroom

Adrenarche, gonadale functie, cognitie, psychosociale aspecten en effecten van groeihormoonbehandeling bij kinderen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. H.G. Schmidt
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 30 november 2012 om 9.30 uur

door

Elbrich Petronella Catharina Siemensma

Geboren te Heinkenszand

Promotor:	Prof. dr. A.C.S. Hokken-Koelega
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Voor alle kinderen met Prader-Willi syndroom
en hun ouders

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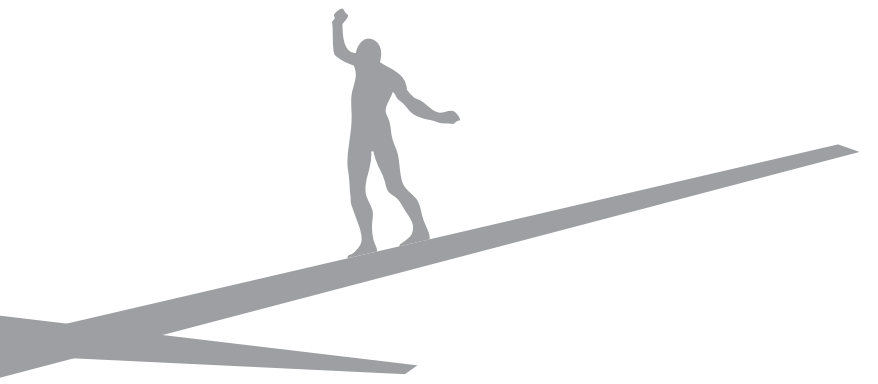
General introduction and aims of the thesis



Introduction

The first patient with Prader-Willi syndrome (PWS), described in 1887 by Langdon-Down[1] (Figure 1), was an adolescent girl with mental impairment, short stature, hypogonadism, and obesity. The first official group of patients with PWS was described by endocrinologists Prader, Labhart, and Willi in 1956[2]. They described an unusual pattern of abnormalities, including neonatal hypotonia resulting in feeding problems in infancy, cryptorchidism, short stature and retarded bone age, small hands and feet, delayed developmental milestones, characteristic faces, cognitive impairment, onset of gross obesity in early childhood due to insatiable hunger, and a tendency to develop diabetes in adolescence and adulthood when weight was not controlled.

Behavioral and psychological problems associated with PWS were not described until the 1980s[3].

Since these first reports, knowledge on different aspects of the syndrome has vastly increased, although a lot of questions still remained and needed to be further investigated.

This chapter describes the genetic basis of PWS, clinical manifestations in different stages of life, the hypothalamus and pituitary, and growth hormone (GH) treatment in children with PWS. It further describes a summary of the results from the Dutch national growth hormone trial for children with Prader-Willi syndrome from 2002 to 2009 and characteristics of PWS within the scope of this thesis. Finally, the objectives of the studies described in the various chapters of this thesis will be presented.

Figure 1.
Two adolescent girls with Prader-Willi syndrome. On the left: the girl described by Langdon Down in 1887. On the right: a girl participating in the Dutch PWS Cohort study, the picture was taken in 2011 (with permission).

1.1 Prader-Willi syndrome

PWS (OMIM 176270) is a complex multisystem disorder that results from a lack of expression of paternally inherited imprinted genes on chromosome 15q11-13. It is not associated with gender, race or social-economic status. Based on epidemiological surveys, the birth incidence is estimated around 1 in 25,000[4-6]. PWS is associated with hypotonia, short stature if not treated with growth hormone, incomplete sexual development[7] and mental retardation with an average intelligence quotient (IQ) of 70[8-10]. Very commonly, these children have a short total hand size, narrow palms and short feet with short toes. Characteristic facial features include narrow temporal distance and nasal bridge, almond-shaped eyes, strabismus, a thin upper lip, and hypopigmentation of hair, eyes, and skin, relative to other family members[11,12]. Children with PWS have sweet and loving personalities, but the syndrome is also characterized by behavioral problems, social difficulties and psychiatric symptoms [13,14]. At birth the infant typically has low birth weight for gestational age and severe hypotonia causing failure to thrive. During childhood, however, mostly between the ages of two and five, there is a change towards the development of hyperphagia which can lead to excessive eating behavior and severe obesity.

1.2 Genetic cause

1.2.1 History

Until 1981, the diagnosis of PWS was based on a combination of symptoms listed in the consensus diagnostic criteria[7], but from the late 1970's, associations between PWS and chromosomal rearrangements on chromosome 15 were made, and in 1981 it was reported that the cause of PWS was an interstitional deletion of the long arm of chromosome 15 at region q11-q13 in the majority of patients[15]. One year later, in 1982 it was discovered that this deletion only affected the paternally inherited chromosome[16]. Another common genetic cause of PWS is a maternal uniparental disomy (mUPD), which was first described in 1989[17]. To date it is known that PWS is due to lack of expression of paternally inherited genes located on chromosome 15, locus 15q11-13. Another term for the locus 15q11-13 on chromosome 15 is the 'Prader-Willi region'. Expression of the genes in the Prader-Willi region is lost due to a deletion, an mUPD, an imprinting center defect (ICD) or a translocation[18-22].

1.2.2 Genomic imprinting

Genes are stretches of deoxyribonucleic acids (DNA) that carry the genetic information used in the development and functioning of all living organisms. Genes are located on chromosomes, of which humans have 23 pairs in the nucleus of each cell in their body. Children inherit 23 chromosomes from their father and 23 chromosomes from their mother, which form

the 23 pairs in each cell. Chromosome 15 is one of these 23 chromosomes.
In healthy subjects, the Prader-Willi region of the maternally inherited chromosome 15 is silenced by a process called imprinting, whereas this region of the paternally derived chromosome is expressed. Gene imprinting is a mechanism by which part of a chromosome is silenced during gametogenesis, which leads to a different expression according to the parent of origin. Abnormal or absent expression of paternally derived genes on the Prader-Willi region causes PWS. PWS is one of the first discoveries of a genomic imprinting disorder in humans[23].

1.2.3 Deletion, maternal uniparental disomy, imprinting center defect and translocation

According to the literature, a paternal deletion is the most common chromosomal defect in PWS and present in 70% of patients[18,20,24]. The defect occurs either as a large type I deletion or as a smaller type II deletion. mUPD is the second most frequent cause of PWS and has been reported in 25% of patients according to the literature[25]. In children with mUPD, the paternally inherited chromosome 15 is absent, while two copies of the maternally inherited chromosome 15 (which are both imprinted) are present. In a small number of cases (less than 5%), PWS results from an ICD[22,26]. These individuals have apparently normal chromosomes 15 of biparental inheritance, but the paternal chromosome carries a maternal imprint. This leads to a complete loss of the paternally expressed genes in the Prader-Willi region. In less than 1% of patients, part of the paternally inherited chromosome is situated on another chromosome, this is called a translocation[18,27,28]. For a schematic overview of the chromosomal defects, see Figure 2.

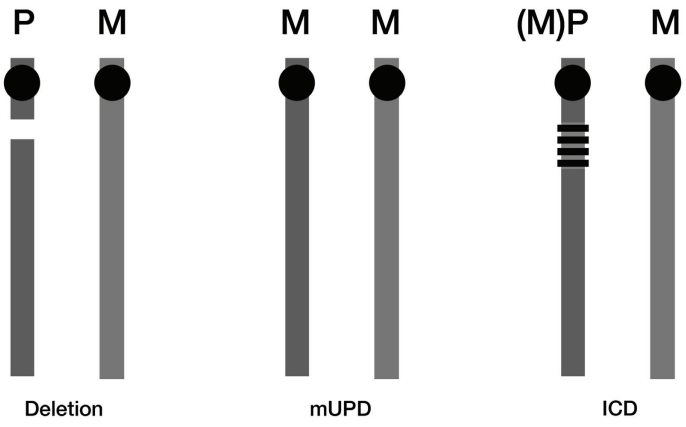


Figure 2.
Schematic overview of the chromosomal defects described in Prader-Willi syndrome
P=paternally derived chromosome, M=maternally derived chromosome, mUPD=maternal uniparental disomy, ICD=imprintings center defect.

1.2.4 Correlations between genotype and phenotype

Since it is known that PWS can be caused by different genotypes, differences between the phenotypes of these subtypes have been studied and a number of differences have been found, especially between the two largest groups, the deletion and the mUPD genotypes. Table 1 shows the main differences that were indicated between the two genotypes in several studies.

Table 1. Differences between patients with a deletion and an mUPD

Trait	Deletion	mUPD
Cognitive functioning[29,30]		
- Performal IQ (PIQ)*	65 (47-86)	62 (42-82)
- Verbal IQ (VIQ)*	61 (43-79)	70 (58-82)
- Visuospational skills	+	-
- Coding ability	+	-
Psychiatric illness[31,32]	+/-	+
Behavioral problems[33-36]		
- Self-injury	+	+/-
- Food stealing	+	+/-
- Compulsive behavior	+	+/-
- Pervasive developmental disorders (PDD)	+/-	+
Postterm deliveries[37]	+/-	+
Hypopigmentation[38]	+	-

*Data presented as mean (CI95%)

1.3 Prader-Willi syndrome in different phases of life

1.3.1 The fetus and neonate

During pregnancy, mothers report lack of foetal activity, which is in most cases also noticed by obstetricians. Furthermore the rate of polyhydramnios is elevated. The frequency of induced labor is high in PWS and often results in caesarian section[39,40]. Both premature and post term deliveries are frequently observed, and there is also a significantly greater risk of premature birth for babies with PWS due to mUPD compared to babies with PWS due to a deletion[41]. Birth weight for all full term PWS babies is generally lower than average. On the other hand, birth length of full term babies with PWS is mostly within the normal range[41].

In infancy, the most consistent clinical feature is marked central hypotonia, which causes decreased movements, a head lag, lethargy with decreased arousal, weak cry, and poor reflexes, including poor suckling leading to feeding difficulties and failure to thrive[12]. Problems with thermoregulation and hypogonadism with genital hypoplasia are evident at birth and throughout life[42].

The uniform presence of hypotonia in infants with PWS has led to the recommendation that all newborns with persistent hypotonia should be tested for PWS[11] (Figure 3).

Figure 3.
Hypotonia in an infant with Prader-Willi syndrome (left), which causes feeding difficulties and the need for tube feeding (right).

1.3.2 The infant

After the neonatal phase, hypotonia becomes gradually less marked, although feeding difficulties remain and poor weight gain is typically noted on standard infant growth charts. Despite the low weight, excessive body fat is found in infants with PWS by skinfold measurements[43], dual energy-x-ray absorptiometry, and double labeled water[44]. Conversely, lean body mass measurements are decreased in PWS infants, correlating with a 30% lower energy expenditure as compared to healthy individuals[44].
Gross motor and language milestones are delayed in infants with PWS. Early milestones are reached on average at double the normal age[45,46].

1.3.3 The child

Obesity begins typically during early childhood, between 1 and 4 years of age. During later childhood, a seemingly insatiable appetite develops[47,48]. Food seeking is common, and if intake is not controlled externally, this will result in extreme obesity. Children with PWS have an abnormal body composition with a relatively high body fat percentage and low lean body mass, which contributes to exercise intolerance. Due to muscle imbalance, children with PWS may have genu valgum and often develop scoliosis[49].
Cognitive disability is evident by school age. The average IQ is 70, but even children with low to normal IQs almost all have learning difficulties[8-10].
A characteristic behavioral pattern begins in early childhood. It is typified by skin-picking, temper tantrums, stubbornness, controlling and manipulative behavior, compulsive-like behaviors and difficulty with changes in routine[13]. Autism spectrum disorder, attention deficit/

hyperactivity symptoms, and insistence on sameness are common and of early onset[34,50]. Individuals with PWS have sleep-disordered breathing, including central and obstructive sleep apnea, abnormal arousal, abnormal circadian rhythms in rapid eye movement (REM) sleep, reduced REM latency, and abnormal response to hypercapnia as well as excessive daytime sleepiness[51-53]. Obesity can worsen the sleep disorder[54].

1.3.4 The adolescent

Pubertal development in patients with PWS remains controversial, because structural studies about this aspect in PWS are limited. Failure of spontaneously completed puberty and late or absent development of beard and body hair in males, and menarche and menses in women with PWS have been described[55]. Early pubarche and precocious puberty, although more rarely, were also found in these patients[42]. Failure of spontaneous puberty has generally been attributed to hypothalamic dysfunction, but might also be caused by a combination of both hypothalamic dysfunction and primary hypogonadism[56]. Spontaneous growth velocity is impaired and the pubertal growth spurt may be lacking. Both conditions contribute to a decreased adult height. Typical adolescent rebelliousness and behavioral problems are common in PWS, and are particularly food related. Psychosis may occur, in particular in adolescents with mUPD[31].

1.3.5 The adult

Characteristic behaviors in adult patients with PWS are temper tantrums, self-injury, impulsiveness, lability of mood, inactivity and repetitive speech[57]. Morbidity in adults with PWS includes marked obesity, metabolic diseases, sleep apnoea and lipolymphoedema. Growth hormone deficiency and decreased levels of insulin-like growth factor I are also common in adult patients[58]. Adults with PWS are generally incapable of living independently.

Median adult height is 145 cm for women and 155 cm for men[3,59]. In adults with compromised pubertal development and absent pubertal onset, secondary sex characteristics are often absent or incomplete[3,60]. There are, however, a few case reports of pregnancy in females with PWS[61,62], but paternity in PWS has never been reported.

The adult PWS population of today had usually been diagnosed relatively late, when obesity was already present. So far no studies are available on the clinical picture of PWS patients who have been diagnosed early in life by genetic testing and who were treated from infancy onwards, with diet, exercise and hormonal substitution. Complications of severe obesity, such as diabetes mellitus type II or respiratory insufficiency frequently occur and may lead to an early death[60]. However, if severe obesity can be avoided, patients with PWS may have a reasonable life expectancy[63]. To date, the majority of patients are diagnosed during the first months of life by genetic testing. An earlier diagnosis should allow earlier introduction of care aiming to reduce morbidity and improve quality of life.

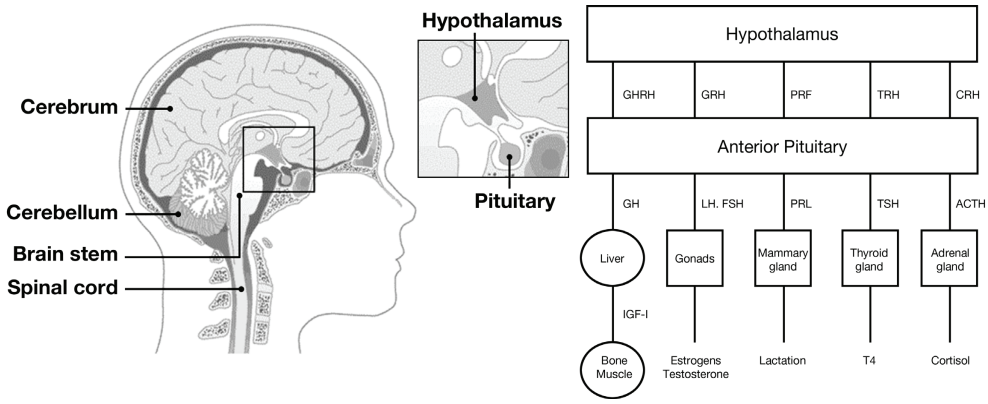


Figure 4.
The hypothalamus regulates the release of hormones from the anterior pituitary. GHRH: Growth hormone releasing hormone; GRH: Gonadotrophin releasing hormone; PRF: Prolactin-releasing factors; TRH: Thyroid releasing hormone; CRH: Corticotropin releasing hormone; GH: Growth hormone; LH: Luteinizing hormone, FSH: Follicle stimulating hormone; PRL: Prolactin; TSH: Thyroid stimulating hormone; ACTH: Corticotropin; IGF-I: Insulin-like growth factor 1; T4: Thyroxin.

1.4 The hypothalamus and pituitary

The hypothalamus is a part of the brain that contains a number of small nuclei with a variety of functions. It is located below the thalamus, just above the brain stem (Figure 4). One of the most important functions of the hypothalamus is to link the nervous system to the endocrine system via the pituitary gland.

The hypothalamus synthesizes and secretes certain neurohormones, which stimulate the anterior pituitary (Figure 4). They are often called hypothalamic-releasing hormones, i.e. thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GRH), growth hormone-releasing hormone (GHRH) and corticotropin-releasing hormone (CRH). These hormones are released into the hypophyseal portal system and stimulate the anterior pituitary to secrete the following hormones: GH, gonadotrophins (LH/FSH), thyrotropin (TSH), prolactin (PRL) and corticotropin (ACTH). The hypothalamus controls body temperature, hunger, thirst, fatigue, sleep, anger, timing of birth and circadian cycles[64].

Dysfunction of various hypothalamic systems may be the basis of a number of symptoms in Prader-Willi syndrome. Symptoms that have been related to hypothalamic dysregulation include abnormal temperature control, excessive daytime sleepiness, sleep-related breathing disorders, abnormalities of sleep architecture, insatiable hunger, decreased activity level and energy expenditure, temper tantrums, hypogonadotropic hypogonadism, cryptorchidism, growth hormone deficiency and stress-related central adrenal insufficiency[42,64-67].

1.5 Growth hormone treatment in children with PWS

In 2002, the Dutch national GH trial for children with Prader-Willi syndrome was started to investigate the effects on growth, body composition, activity level and psychological development. At first children were treated in a GH randomized controlled trial (RCT), lasting 1 year for infants and 2 years for prepubertal children. After the RCT, children were subsequently followed during continuous GH-treatment in a cohort study (for study designs see Appendix 1). To date, infants from 6 months until 3 years of age are still included in the Dutch PWS Cohort study and followed during GH-treatment until they reach adult height.
Several studies, including the Dutch national GH trial show that GH-treatment is an effective and safe treatment in children with PWS on the short and on the long term[25,68-83]. GH-treatment improves height gain, which results in a normal adult height, particularly when GH-treatment is started before onset of puberty. GH-treatment has also been found to improve head-circumference, body mass index, respiratory function, physical performance, resting energy expenditure, bone mineral density and body composition by decreasing body fat percentage and increasing lean body mass[69,73,74,76-79]. Furthermore, GH-treatment improves psychomotor development in the very young and has psychological and behavioral benefits[46,82]. There were no adverse effects of GH-treatment on glucose homeostasis, blood pressure and serum lipids. At the end of 2002, GH-treatment was registered and reimbursed for children with PWS and to date all children with PWS in the Netherlands are treated with GH.

1.6 Results from the Dutch national GH trial in children with Prader-Willi syndrome: 2002-2010

This GH trial generated a lot of information about children with PWS. Not only about the effects of GH-treatment on different aspects of the syndrome, but also about various characteristics of the PWS phenotype before start of GH-treatment. Important studies on the PWS phenotype and effects of GH-treatment were performed on: physical characteristics, i.e. body composition, anthropometrics, bone mineral density, bone maturation and scoliosis; hormone levels, i.e. growth factors, thyroid hormone levels and cortisol; protein levels; glucose homeostasis; cardiovascular risk factors and different psychological aspects. A summery of the results of the Dutch national GH trial is presented in Appendix 2.

1.7 Unresolved issues

Clinical topics that needed to be further investigated were adrenarche and gonadal function in boys and girls with PWS and the effects of GH-treatment. Because almost no data were

available about cognition, quality of life and behavior in children with PWS and the effects of GH-treatment on these psychological aspects, these also needed to be investigated.

1.7.1 Adrenarche and Pubarche

Arenarche is defined as the increased production of the androgen precursors, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), by the adrenal glands, occurring between age 6 and 8 years. Nowadays, there is increasing evidence that adrenarche is a gradual process starting at an early preschool age[86]. Clinical signs of adrenarche are the appearance of adult type body odor, acne and comodones, oily hair and the appearance of pubic and/or axillary hair, which is called pubarche.

Adrenarche is premature if clinical signs occur before the age of 8 years in girls or before 9 years in boys in the presence of increased serum adrenal androgen levels for age[87]. Premature pubarche is the appearance of pubic hair before the above-mentioned age limits.

Androgens are produced by the adrenal glands (Figure 5). The adrenal gland consists of two separate organs: the cortex and the medulla. The adult adrenal cortex contains three layers with distinct histology and function. The innermost layer, the zona reticularis (ZR) produces the adrenal androgens DHEA, DHEAS and androsteendione.

Adrenal androgen production (Figure 6) is elicited by the 17-hydroxylase and 17,20-lyase activities of P450c17, which catalyze the conversion of pregnenolone to 17-hydroxypregneno-

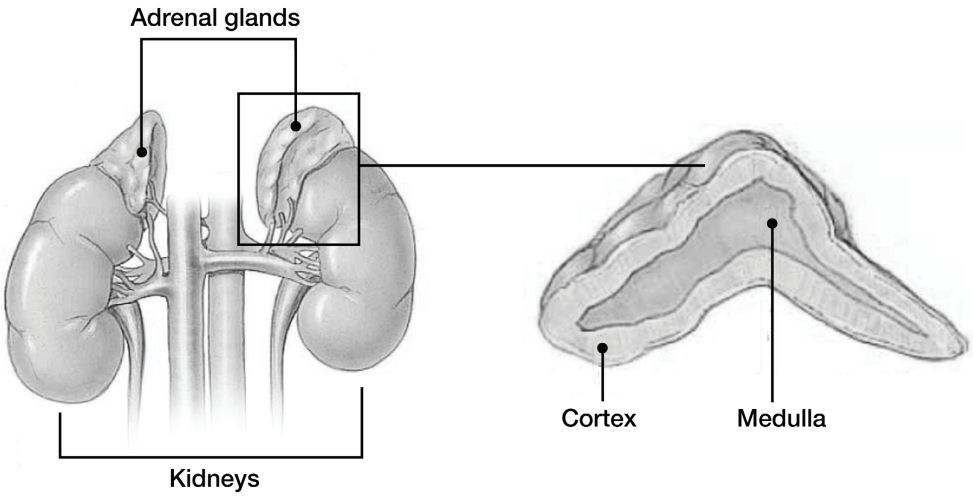


Figure 5.
The adrenal glands are situated on top of the kidney. The adrenal gland consists of two separate organs, the cortex and the medulla.

lone and further to DHEA. Low activity of the enzyme 3β-HSD type 2, theoretically favors androgen production because of higher availability of steroid precursors for DHEA production. High 3β-HSD type 2 levels on the other hand enable the production of cortisol and aldosterone. The last step in the synthesis of DHEAS is the sulfonation of DHEA by the sulfotransferase (SULT2A1) enzyme. The expression of SULT2A1in the ZR increases from 5 years of age onwards[88].

In patients with PWS, premature adrenarche had been described, but often retrospectively, in small groups, or in mixed groups including adults and children. For example, in children and adults with PWS, premature pubarche was reported retrospectively in 14% of females and males[42]. However, these analyses included also girls younger than 8 years and boys younger than 9 years, who normally do not have pubarche yet.

Weight gain and obesity are associated with higher serum DHEAS levels[89]. As premature adrenarche is described in PWS and PWS is associated with obesity, one would expect a higher prevalence of premature adrenarche and higher levels of DHEAS in overweight or obese children with PWS compared to a reference population. However, knowledge about the age at onset and the prevalence of premature adrenarche and serum androgen levels in PWS were scarce and nothing was known about the effect of GH-treatment on androgen levels.

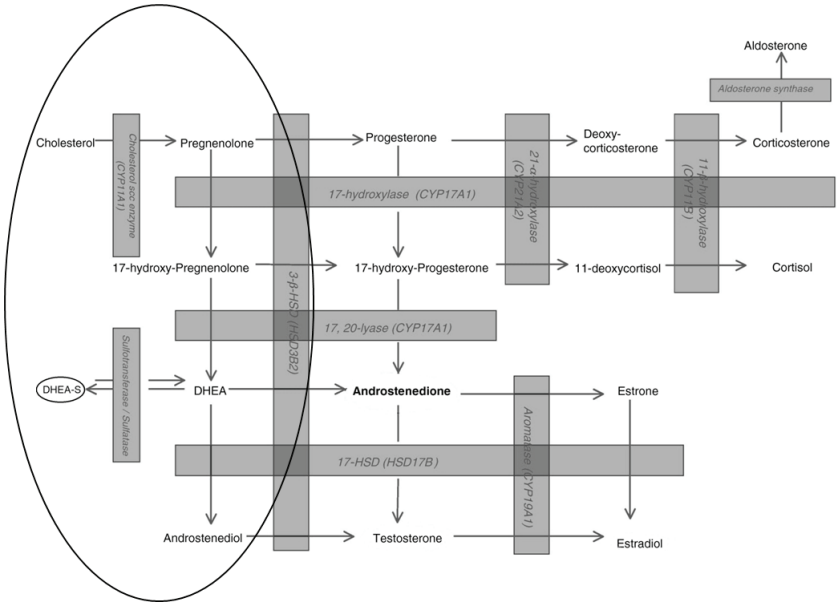


Figure 6.
Steroid genesis. Steps in the synthesis of DHEAS are encircled.

1.7.2 Male gonadal function

Male patients with PWS show clinical signs of hypogonadism[42,90]: cryptptorchidism, scrotal hypoplasia, small testicular volume, infertility and delayed or incomplete puberty.

In healthy boys, the onset of puberty is associated with alterations in the circulating concentrations of reproductive hormones, inhibin B, follicle stimulating hormone (FSH), testosterone and luteinizing hormone (LH). FSH and LH, also called gonadotrophins, are produced by the pituitary gland when stimulated by the hypothalamus, while inhibin B and testosterone are produced by the testicles after stimulation by FSH and LH. Production of inhibin B and testosterone results in a negative feedback to the pituitary and hypothalamus. This is the male hypothalamic-pituitary-gonadal axis (Figure 7). Inhibin B is produced by the Sertoli cells of the testis after stimulation by FSH and is a marker for spermatogenesis. Testosterone is produced by the Leydig cells of the testis after stimulation by LH, which is necessary to continue the process of spermatogenesis and is important in the development of male characteristics, including muscle mass and strength, fat distribution, bone mass and sex drive.

Hypogonadism can be central, which is also called hypogonadotropic hypogonadism or peripheral, which is also known as hypergonadotropic hypogonadism. In case of hypogonadotropic hypogonadism, there is a hypothalamic or pituitary dysfunction. If the hypothalamus fails to stimulate the pituitary, the pituitary will not produce and secrete FSH and LH and consequently there will be no testicular production of inhibin B and testosterone. In case of hypergonadotropic hypogonadism there is a testicular dysfunction. In case of testicular dysfunction, the testes cannot produce enough testosterone and thus, the negative-feedback of the release of gonadotrophin releasing hormone (GnRH) by the hypothalamus is lacking. Because there is no negative feedback to the hypothalamus or the pituitary, this will result in high serum levels of FSH and LH.

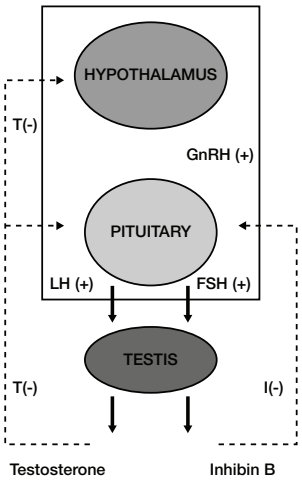


Figure 7.
Male hypothalamic-pituitary-gonadal axis
T: Testosterone; GnRH: Gonadotrophin releasing hormone; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; I: Inhibin B

As most symptoms of PWS are attributed to hypothalamic dysfunction, the hypogonadism in PWS was hypothesized to be hypogonadotropic[64,91]. A few studies described hypogonadotropic hypogonadism in adults with PWS[90,92,93], but there were some results indicating that primary gonadal dysfunction, especially primary damage of the tubular compartment, could be a major contributor to the abnormal pubertal development in boys and men with PWS[56]. Some studies showed low inhibin B and high FSH levels in pubertal and adult patients with PWS, indicating a defect in the tubular compartment of the testes in these patients[56,94,95]. However, also normal Inhibin B levels were described in pre-pubertal children[56], and a normal minipuberty, the rise of gonadotrophin-dependent high sex steroid levels during the first months of life, were found in infants with PWS[56,96]. It remained therefore unclear if there was a gonadal dysfunction in boys and men with PWS and if so, at what age this gonadal dysfunction arised.

These questions could only be answered by a longitudinal study of reproductive hormones in boys and male adolescents with PWS.

1.7.3 Female gonadal function

Female patients with PWS show clinical signs of hypogonadism[42,90]: hypoplasia of labia minora and/or clitoris and pubertal delay or absence of puberty. Results of a cross-sectional study showed variable combinations of a primary ovarian failure and hypothalamic dysfunction in women and girls with PWS[97,98]. Longitudinal information about gonadal function in females with PWS was, however, lacking.

Ovarian function is difficult to evaluate in women because menstrual cycles do not always indicate ovulation, but it is even more difficult in girls and women with PWS, since most of them do not even have a menstrual cycle. Anti-Müllerian hormone (AMH) is a relatively new cycle-independent fertility marker reflecting ovarian oocyte reserve[99]. The granulosa cells of follicles in the primary and pre-antral stages, follicular stages following the primordial stage, secrete AMH. Since serum AMH is exclusively produced by the ovaries, independent of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool[100-104]. FSH and inhibin B are other markers of ovarian function. Both play an important role in later folliculogenesis. Folliculogenesis (Figure 8) is the process of follicle maturation from the primordial follicle to the ovulatory follicle. Follicles are continuously recruited from the dormant primordial follicle pool, the so-called initial recruitment, into the growing follicle pool and start to express AMH and inhibin B. After puberty, at every new cycle, a limited number of follicles is selected from this pool of small, growing follicles under the influence of FSH, the so-called cyclic recruitment. From this smaller cohort of growing follicles, ultimately one follicle, the Graafian follicle, is selected for dominance and ovulation under the influence of LH[105]. Inhibin A is a product of granulosa cells of larger antral follicles, the dominant follicle and the corpus luteum[106]. It has been suggested that inhibin A can be used as a marker of the quality of the mature follicles[107].

One report showed normal AMH and FSH levels combined with low inhibin B levels in girls and women with PWS[98], but there was no information about inhibin A or the development of reproductive hormone levels during long-term follow-up.

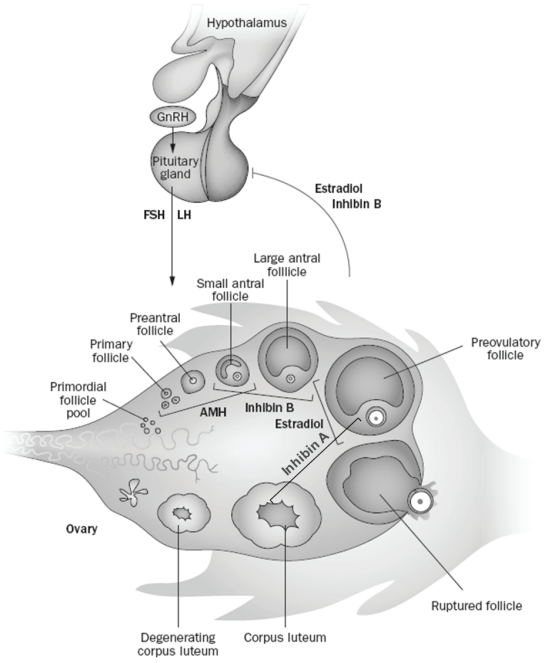


Figure 8.
Folliculogenesis
GnRH: Gonadotrophin releasing hormone; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; AMH: Anti-Müllerian hormone.

1.7.4 Psychological aspects

The psychological aspects of children with PWS deserve attention, as PWS is known for its mental retardation, emotional disturbances, including temper tantrums, and behavioral problems. As nowadays many children with PWS are treated with GH, the effects of this treatment on the psychological aspects required further investigation.

1.7.5 Cognition

Intellectual development in PWS has been the focus of study over the past few years. A population-based study of children and adults reported a near-normal distribution of the Full Scale Intelligence Quotient (FSIQ) around a mean of 60, which is 40 points under the normative population score of 100[10]. Other authors reported similar or somewhat higher mean FSIQ scores[9,33,108,109]. In these studies, a wide variation in intellectual functioning was found, with most PWS patients showing mild to moderate retardation and up to 25% displaying normal or

borderline functioning (FSIQ > 70). Another study reported lower mean FSIQ scores of about 50, with only one patient out of 18 having a score above 70[110]. The degree of intellectual impairment did not seem to depend on sex, age or body mass index (BMI)[8].

Delays in psychomotor, cognitive and emotional development appeared to be present in most cases to variable degrees, leading to learning disabilities and adaptation difficulties. Patients with PWS have poor short-term memories, deficits in sequential processing, perform relatively well on visuo-spatial tasks and have exceptional skills with jigsaw puzzles[8,18]. Scores on 2 WISC subtests (Object assembly and Block design) were lower in subjects with both deletion and mUPD than in a reference group with mental disability for other reasons[8].

Systematic differences between patients with a deletion and mUPD have been found. Mostly, the FSIQ was comparable between the genotypes, but verbal IQ was significantly higher in children with an mUPD compared to children with a deletion and performal IQ was significantly higher in children with a deletion compared to children with an mUPD[33,111]. When a cognitive profile was made, children with a deletion showed a standard cognitive profile, in which verbal skills are comparable to performal skills, while children with an mUPD show a profile with significantly better performance on the verbal IQ compared to the performal IQ[29,111].

There was some information about the effect of GH-treatment on cognitive development in infants with PWS, showing significantly improved mental and motor development during one year of GH-treatment compared to randomized controls[46,112], but information about this effect in children with PWS above 3 years of age, beyond a period of 6 months, was lacking.

1.7.6 Health related quality of life

Health related quality of life (HRQOL) refers to the impact of health and illness on an individual's quality of life, including emotional, social and cognitive domains[113,114]. HRQOL can be measured by generic and disease-specific instruments[114]. Generic HRQOL instruments allow comparison with normative data and across disease populations[114]. Disease-specific measurements include domains that are only valid for a specified condition and maximize content validity and result with greater sensitivity and specificity[114].

To determine a child's HRQOL, the opinion of a child itself is probably most important[115]. However, the use of self-reported questionnaires with children is problematic[116]. The dominance of short-term memory and recent incidents, a different time perspective and probably the lack of necessary language skills may lead to invalid and unreliable results[115,117]. Parents are generally quite able to determine their child's HRQOL. However, parents may over- or underestimate the importance of certain aspects of a child's life[115]. Therefore the parent's opinion cannot be substituted for the opinion of a child. The method of a combination of child reports and parent reports seems to be most useful[115,116,118].

GH-treatment improved the physical and psychological aspects of HRQOL in adults with PWS[119,120]. However, information about HRQOL in children PWS was scarce and nothing was known about the effect of GH-treatment on HRQOL in children with PWS.

1.7.7 Behavior

Many studies have reported behavioral characteristics in children with PWS, such as temper tantrums, impulsivity, mood fluctuations, stubbornness and aggression[121-123] as well as a range of repetitive behaviors such as skin picking, repetitive speech and obsessive and ritualistic behaviors[123,124]. The obsessive behavior of children with PWS becomes evident when trivial alterations in routines occur, as such events may provoke a tantrum. These symptoms are very specific for patients with PWS and could not be explained by their mental disability.

The existence of different genotypes in PWS has prompted several studies of genotype-phenotype correlations and a number of reports concerning phenotypic differences between patients with a deletion or an mUPD. The most consistent finding in terms of behavioral differences appears to be that fewer patients with an mUPD show self-injurious behaviors such as skin picking[125,126]. In addition, Dykens et al.[122] reported that mUPD may be associated with lower levels of obsessive-compulsive symptoms. Patients with an mUPD have also been reported to be more prone to other cognitive and psychiatric disturbances, including severe affective disorder with psychotic features[31,111] and visual perceptual abnormalities[30]. Patients with an mUPD are at higher risk than those with a deletion for autism spectrum disorders, most likely because of duplication and over-expression of maternally expressed genes in the 15q11-q13 region[34].

During 1 year of GH-treatment there was no effect on behavior in children with PWS[69,127]. However, nothing was known about the effects of behavior beyond this period or during long term GH-treatment.

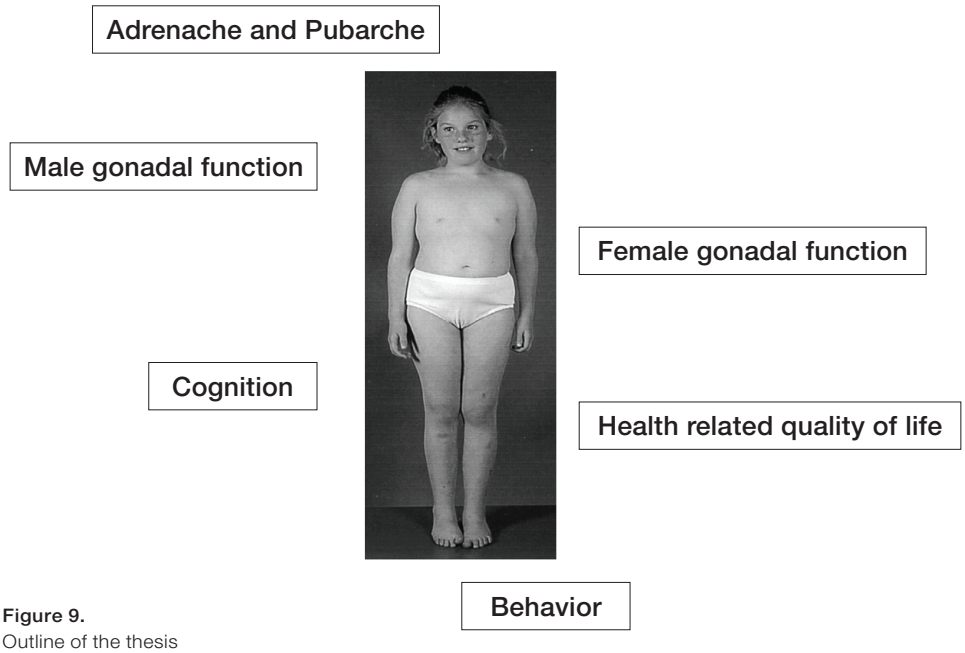


Figure 9.
Outline of the thesis

1.8 Aims of the studies and outline of the thesis

This thesis presents a detailed description of the studies performed to improve the knowledge about, and the care for patients with PWS. The aims of the studies described in this thesis were to evaluate various characteristic aspects in children with PWS during their development to adolescence, such as adrenarche, pubertal development, gonadal function, cognition, health related quality of life and behavior and to describe the effects of GH-treatment on these characteristic aspects (Figure 9). The study populations consisted of children participating in the Dutch national GH RCT or Dutch PWS Cohort study. Each of the characteristic aspects was evaluated at baseline first and the effect of GH-treatment was investigated thereafter during the GH RCT. Long-term effects of GH-treatment were measured during 4 years of GH-treatment in the PWS Cohort study. Study designs are described in Appendix 1.

Chapter 1 gives an introduction in the topics described in this thesis.
Chapter 2 reports DHEAS levels, the age at and progression of pubarche and the prevalence of premature pubarche in children with PWS compared to healthy controls and the effects GH-treatment.
Chapter 3 presents the longitudinal study of serum hormone levels of the pituitary-testicular axis in boys with PWS during childhood, puberty and adolescence and describes gonadal dysfunction in boys and male adolescents with PWS and the effects of GH-treatment.
Chapter 4 presents the longitudinal study of female reproductive hormone levels and evaluates gonadal function of female adolescents with PWS and whether they are fertile and thus need contraceptive treatment. In addition, it describes the effects of GH-treatment on reproductive hormones, gonadal function and fertility.
Chapter 5 describes cognitive functioning and the effects of GH-treatment in children with PWS. Furthermore, cognitive functioning in the same group of children during long-term GH-treatment is presented.
Chapter 6 describes HRQOL in children with PWS and the effect of GH-treatment on HRQOL according to children and their parents. Furthermore, HRQOL in the same group of children during long-term GH-treatment is presented.
Chapter 7 describes behavior in children with PWS and the effect of GH-treatment. Furthermore, behavior in the same group of children during long-term GH-treatment is presented.
Chapter 8 discusses the results and conclusions in the light of the current literature and presents clinical implications of the study results.
Chapter 9 contains an English and a Dutch summary of the results described in this thesis.
Chapter 10 contains lists of abreviations, co-authors and affiliations and publications. It further contains the PhD portfolio, CV and acknowedgements.

Appendix 1: Dutch national PWS studies

Project Coordination

Both the Dutch multicenter randomized controlled GH trial (Dutch GH RCT) and the multicenter follow-up study (the Dutch PWS Cohort study) are coordinated by the Dutch Growth Research Foundation, Rotterdam, the Netherlands. The PWS research team consists of one or two MD-researchers, a research nurse and a psychologist. Three-monthly, 18 hospitals throughout The Netherlands are visited by the MD-researcher and the research nurse, where children are examined, in collaboration with the local pediatrician or pediatric endocrinologist (Figure 1). Standardized measurements take place at the Erasmus University Medical Center - Sophia Children's Hospital Rotterdam, The Netherlands, at start, at 6 and 12 months and subsequently once a year.

Patients

Until 01-01-2012, 143 Dutch children with PWS were included in the RCT GH trial and the Cohort Study (Figure 2). For both studies, children had to meet the following criteria:

Inclusion criteria:

- Genetically confirmed diagnosis of PWS;
- Age between 6 months and 16 years;
- Maximal bone age of less than 14 years in girls, or 16 years in boys.

Exclusion criteria:

- Non-cooperative behavior;
- Extremely low dietary intake of less than minimal required intake according to guidelines set by the World Health Organization;
- Medication to reduce weight (fat);
- In children above 3 years of age: height above 0 SDS, unless weight-for-height is above 2 SDS;
- Previous treatment with GH (not applicable for the Dutch PWS Cohort study)

Design

Infants

The RCT infant group consisted of 61 children aged between 6 months and 3 years at start of study. Stratified for age, they were randomized into either a GH-treated group or a control for the duration of one year (Figure 2). The GH-treated group received somatropin 1 mg/m^2 per day, whereas the control group was not treated with GH. From 12 months of study onwards, all children were treated with somatropin 1 mg/m^2 per day and were prospectively followed in the Dutch PWS Cohort study in collaboration with pediatricians or pediatric endocrinologists throughout The Netherlands.

Figure 1.
Participating centers

Rotterdam:	E.P.C. Siemensma, N.E. Bakker, R.J. Kuppens, Z.C.E. Troeman, R.F.A. Tummers-de Lind van Wijngaarden, D.A.M. Festen, P.M.C.C. van Eekelen, G.C.B. Bindels-de Heus, A.C.S. Hokken-Koelega, Erasmus University Medical Center Rotterdam / Sophia Children's Hospital (in black);
Nijmegen:	A. E. M. van Alfen-van der Velden and B.J. Otten, University Medical Center St. Radboud
Amsterdam:	J. Rotteveel, VU University Medical Center;
Amsterdam:	A.S.P. van Trotsenburg, Amsterdam Medical Center;
Apeldoorn:	L. Lunshof, Gelre Hospitals.
Den Bosch:	P.E. Jira, Jeroen Bosch Medical Center;
Den Haag:	E.C.A.M. Houdijk, Haga Hospitals / Juliana Children's Hospital;
Eindhoven:	R.J.H. Odink, St. Catharina Hospital;
Enschede:	R.C.F.M. Vreuls, Medical Center Twente;
Groningen:	G. Bocca, University Medical Center Groningen / Beatrix Children's Hospital;
Harderwijk:	M. van Leeuwen, St. Jansdal Hospital;
Leeuwarden:	E. van Pinxteren-Nagler, Medical Center Leeuwarden;
Leiden:	D.A.J.P. Haring, Diaconessenhuis;
Leiden:	W. Oostdijk, University Medical Center;
Lelystad:	J.W. Pilon, Ijsselmeer Hospitals;
Nijmegen:	C. Westerlaken, Canisius-Wilhelmina Hospital;
Utrecht:	J.J.G. Hoorweg-Nijman, H. van Wieringen, St. Antonius Hospital;
Zwolle:	E.J. Schroor, Isala Hospitals.

Prepubertal group

The RCT prepubertal group consisted of 47 children; girls aged between 3 and 12 years with Tanner breast stage < 2 and boys between 3 and 14 years with Tanner genital stage < 2 and a testicular volume < 4 ml. Stratified for BMI, children were randomized into either a GH-treated group or a control group for the duration of 2 years (Figure 2). The GH-treated group received somatropin 1 mg/m^2 per day , whereas the control group was not treated with GH. Dietary advice and exercise training were offered to both groups and started three months prior to study in order to minimize a priori between group differences. From 24 months of study onwards, all children were treated with somatropin 1 mg/m^2 per day and were prospectively followed in the Dutch PWS Cohort study in collaboration with pediatricians or pediatric endocrinologists throughout the Netherlands.

Pubertal group

The RCT pubertal group consisted of 7 children; girls > 12 years and boys > 14 years, both with spontaneous or induced puberty. All pubertal children were treated with GH, but were randomized to receive either 1 mg/m^2 per day or 1.5 mg/m^2 per day until adult height (Figure 2). Dietry advice and exercise training were offered to both groups and started three months prior to study in order to minimize a priori between group differences.

Inclusion directly in the PWS Cohort study

Since 2009, 28 infants between 6 months and 3 years were directly included in the PWS Cohort study for follow-up during long term GH-treatment until final height. All children were treated with somatropin 1 mg/m^2 per day and were prospectively followed in the Dutch PWS Cohort study in collaboration with pediatricians or pediatric endocrinologists throughout the Netherlands.

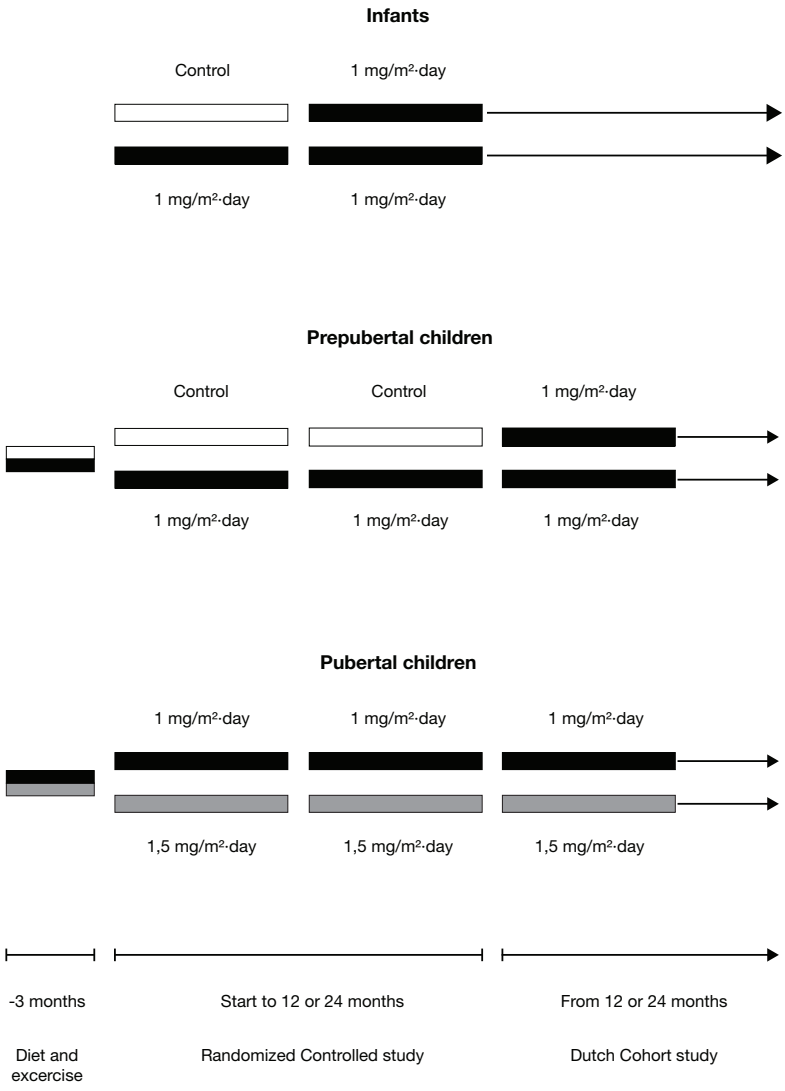


Figure 2.
Design of the multicenter randomized controlled GH trial and the Dutch PWS Cohort study

Appendix 2: Summary of the results from the national GH trial for children with PWS

	Baseline	Effects of GH-treatment
Body composition		
Fat mass	Fat mass is higher compared to healthy peers; In infants the fat% is 28.4; In children the fat% is 36.9, which is higher than in healthy references and above the normal range, fat% is elevated in 95% of the children[128,129]	Fat mass decreases during GH-treatment on the short and on the long term, but remains higher compared to healthy peers[128,129]
LBM	LBM is lower compared to healthy peers[128,129]	LBM increases during GH-treatment, while it further decreases in untreated children on the short term; During long term GH-treatment LBM stabilizes, but does not normalize and remains lower than in healthy references[128,129]
BMI	Infants have a normal BMI and children have a higher BMI compared to healthy peers. BMI is elevated in 24% of children[128,129]	BMI remains stable during GH-treatment in infants, but decreases in children on the short term; During long-term GH-treatment, BMI increases compared to healthy references, but decreases compared to PWS reference values, BMI decreases and remains lower than at baseline during 4 years[128,129]
Anthropometrics		
Height	Height is lower compared to healthy peers[128,129]	Height normalizes during GH-treatment on the short term; During long term GH-treatment height normalizes in most children[128,129]
Head circumference	Head circumference is smaller compared to healthy peers[128,129]	Head circumference normalizes during GH-treatment on the short term; During long term GH-treatment, head circumference increased during the first 2 years of treatment and stabilizes thereafter[128,129]
Growth factors		
IGF-I	IGF-I levels are lower compared to healthy peers[128,129]	IGF-I levels increase rapidly during short term and long term GH-treatment to significantly higher levels than in healthy peers, but stay within the normal range in most; The IGF-I/IGFBP-3 ratio increased during GH-treatment, suggesting that more unbound IGF-I is present in the circulation[128,129]
IGFBP-3	IGFBP-3 levels are similar as in healthy peers[128,129]	IGFBP-3 levels increase during short term and long term GH-treatment but not to the same extend as IGF-I levels[128,129]

Thyroid hormone levels		
FT4	FT4 levels are lower than in healthy peers, but the majority (93.7%) have FT4 levels within the normal range (above -2 SDS), only 6.3% of patients has FT4 below the normal range (-2 SDS), but without abnormalities in TSH and T3 levels[130]	FT4 levels decrease during 1 year of GH-treatment, but stay in the normal range in the majority (91.2%) and are unchanged in untreated children; The change in FT4 over 1 year of GH-treatment is not associated with change in IGF-I or clinical signs[130]
TSH	TSH levels are similar as in healthy peers[130]	TSH levels do not change and are within the normal range during 1 year of GH-treatment [130]
T3	T3 levels are higher than in healthy peers, 5.5% had levels above the normal range (+2 SDS)[130]	T3 levels do not change and are within the normal range during 1 year of GH-treatment[130]
Adipocytokine levels		
Adiponectin	Adiponectin levels were higher compared to healthy references[84]	Adiponectin levels increase during 1 and 2 years of GH-treatment, while they remain similar to baseline in untreated children; This increase in adiponectin levels is related to the decrease in body fat percentage[84]
Acylation stimulating protein (ASP)	ASP levels are elevated in 68% of the infants and in 94% of children and higher compared to healthy references[85]	GH-treatment had no effect on ASP levels[85]
Glucose homeostasis		
Insulin (fasting)	Insulin levels were within the normal range in all infants; children had higher insulin levels than infants, although within the normal range[84,85,129]	Insulin levels remain within the normal range during GH-treatment, while in untreated children, levels increase; During long-term treatment, insulin levels increase with age and GH has no effect on insulin levels after correction for age [84,85,129]
Glucose (fasting)	Glucose levels were within the normal range in most infants[84,85,129]	Glucose levels remain similar to baseline during GH-treatment and are within the normal range; During long-term treatment glucose levels increase with age, GH has no effect on glucose levels after correction for age [84,85,129]

Cardiovascular risk-factors		
	Infants: 63% has at least 1 and 33% has at least 2 of the cardiovascular risk factors as mentioned below; Children : 73% has at least 1 and 49% has at least 2 of the cardiovascular risk factors mentioned below[85]	
Total Cholesterol (fasting)	Total cholesterol levels are elevated in 26% of infants and in 35% of children[85,129]	Total cholesterol levels do not change during GH-treatment[85,129]
LDL-cholesterol (fasting)	LDL cholesterol levels are elevated in 33% of infants and in 46% of children[85,129]	The HDL/LDL ratio improves during GH-treatment; LDL-cholesterol decreases during long-term GH-treatment[85,129]
HDL-cholesterol (fasting)	HDL-cholesterol levels are decreased in 11% of infants and in 20% of children[85,129]	HDL-cholesterol levels do not change during GH-treatment; HDL is positively associated with IGF-I levels[85,129]
Triglycerides (fasting)	Triglycerides are above the normal range in 15% of the children[84]	Triglycerides remain similar to baseline during GH-treatment[84]
Lipoprotein (fasting)	Lipoprotein levels are elevated in 31% of infants and in 11% of children[85]	
Blood pressure	Systolic and diastolic blood pressure are normal in infants; Systolic blood pressure was elevated in 12% of children, diastolic blood pressure was in the normal range in all children[85,129]	There were no changes in systolic and diastolic blood pressure during GH-treatment on the short and on the long term[85,129]
Psychological aspects		
Mental development: infants	*Infants*: mental development was 71.6% of expected development; Mental development was not associated with severity of SRBD; Mental development in infants with OSAS was lower than in those without;	Infants: mental development improves 9.3% during 1 year of GH-treatment[46]
Mental development: children	*Children*: had significantly lower scores compared to peers on 4 subtests of WIPPSI-R or the WISC-R; 60% of children had higher verbal than performance scores, 24% had higher performance than verbal scores and 16% of children had a verbal and performance score within the same range; Sleep efficiency index was associated with better performance on the Picture arrangement /completion Wechsler subscales; There were no associations between SRBD and cognition[45,46,131]	
Motor development	*Infants*: motor development was 56.8% of the expected motor development ;There were no associations between motor development and severity of SRBD[45,46]	In infants motor development improves 11.2% during 1 year of GH-treatment; Infants with lower developmental age show the greatest improvement[46]
Behavior	*Children*: had less anxiety-related problems and more emotional and social related problems when compared to references with a comparable IQ; Neurobehavioral abnormalities are related with daytime sleepiness; There were no associations between SRBD and neurobehavioral abnormalities[46,131]	

Remaining areas		
Sleep related breathing disorders (SRBD)	Mean apnea hypopnoea index (AHI) was 5.1/hr, of which 2.8 were central apneas (normal range: 0-1/hr); 9% of non-obese and 50% of obese children have obstructive sleep apnea syndrome (OSAS)[53]	AHI remains comparable to baseline after 6 months of GH-treatment[53]
Bone mineral density (BMD)	Total body BMD is comparable to healthy references, lumbar spine BMD is lower than in healthy references but within the normal range; Lumbar spine bone mineral apparent density (BMAD) is higher compared to healthy references, but within the normal range; BMI is positively associated with BMD; IGF-I levels were not associated with BMD[132]	Total body BMD does not change during 2 years of GH-treatment; IGF-I is positively associated with total body- and lumbar spine BMD; After correction for BMI, the increase in lumbar spine BMD was higher in GH-treated than in untreated children; There is no effect of GH-treatment on Lumbar spine BMAD[132]
Bone maturation	The BA/CA ratio is lower than in healthy references[129]	BA showed catch-up with CA during GH-treatment; GH-treatment has no effect on the ΔBA/ΔCA ratio[129]
Scoliosis	The total prevalence of scoliosis is 36% (2.7% in the non-PWS population); 50% of children with scoliosis need referral to a orthopedic surgeon; 13% of children need conventional or surgical treatment; Scoliosis is present in 9% of infants, 15% of juveniles and 80% of adolescents; Children with scoliosis have a higher BMI than those without; Children with severe scoliosis have lower IGF-I levels compared to children with less severe scoliosis[133,134]	GH-treatment has no effect on the onset or severity of scoliosis, the curve progression and the start of scoliosis treatment; IGF-I levels have no significant effect on the progression of scoliosis[134]
Central adrenal insufficiency (CAI)	60% of the children have CAI during stress; All children had normal morning cortisol levels and a normal diurnal rhythm of cortisol; Children with stress related CAI had a higher central apnea index; SRBD is worse in children with CAI; A central apnea index of 4.15 per hour and higher is indicative for having CAI[67,135]	

LBM: Lean body mass; ASP: acylation stimulating protein; IGF-I: insulin like growth factor-I; IGF-BP3: IGF-binding protein 3; TSH: Thyroid stimulating hormone; fT4: Free thyroxin; T3: Triiodothyroxin; ASP: Acylation Stimulating Protein; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SRBD: Sleep related breathing disorders; AHI: Apnea hypopnoea index; OSAS: obstructive sleep apnea syndrome; BMD: Bone mineral density; BMAD: Bone mineral apparent density (BMD corrected for height); CAI: Central adrenal insufficiency

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2

Pubarche and serum dehydroepiandrosterone sulfate levels in children with Prader-Willi syndrome

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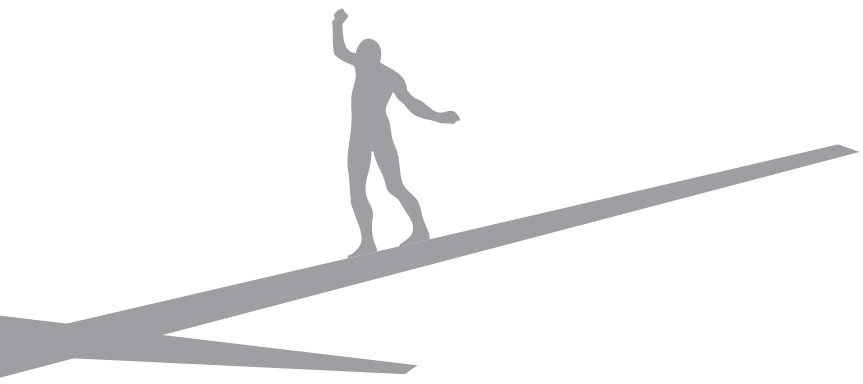
Clinical Endocrinology (2011) 75, 83-89



Abstract

Context: Premature pubarche (PP) is reported in children with Prader-Willi syndrome (PWS). Pubarche is preceded by adrenarche, an increase in serum levels of adrenal androgens, most specifically dehydroepiandrosterone-sulfate (DHEAS).

Objectives: To assess DHEAS levels, the age at and progression of pubarche and the prevalence of PP in children with PWS.

Design/Patients: In the Dutch PWS Cohort Study, 120 children (6 months-17 years) are prospectively followed. Their age at onset of pubarche and various pubic hair stages and prevalence of PP were determined. Serum DHEAS levels were assessed in 97 children.

Results: Median serum DHEAS levels were significantly higher in children with PWS than in healthy age-matched controls at ages 3 to 6 years (girls: $p=0.004$ and boys: $p=0.010$) and 6 to 10 years (girls: $p=0.045$ and boys: $p=0.001$). Age and gender significantly influenced DHEAS levels in children with PWS. The median [P10-P90] age at onset of pubarche in children with PWS was significantly younger than in healthy peers, 9.04[6.75-11.84] years in PWS girls ($p<0.0001$) and 10.31[8.65-12.29] years in PWS boys ($p=0.003$). The prevalence of PP in children with PWS was 30.0% in girls and 16.1% in boys.

Conclusions: Compared to healthy children, children with PWS have significantly higher DHEAS levels from 3 to 10 years of age. They are younger at onset of pubarche and have a higher prevalence of PP. DHEAS levels in PWS are influenced by age and gender. Our findings indicate an earlier maturation of the zona reticularis of the adrenal glands in children with PWS.

Introduction

Prader-Willi syndrome (PWS) is a genetic disorder resulting from the lack of expression of the paternally derived chromosome 15q11-q13, caused by deletion, maternal uniparental disomy (mUPD), imprinting center defect (ICD), or balanced translocation[1,2]. PWS is characterized by a number of signs and symptoms, including hypotonia, psychomotor delay, temper tantrums, short stature, obesity, hyperphagia, and hypogonadism[1-6]. Hypothalamic dysfunction may be responsible for many features of PWS[7, 8]. Knowledge about the age at onset and the prevalence of premature adrenarche in PWS is very scarce. Early adrenarche has been described but often retrospectively, in small groups, or in mixed groups with adults and children. Information about serum androgen levels in children with PWS is also very limited.

Originally, adrenarche was defined as the increased production of the adrenal C_{19} steroids dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) occuring between age 6 and 8 years. Nowadays, there is increasing evidence that adrenarche is a gradual process starting at an early preschool age[9]. Clinical signs of adrenarche, caused by the increase in circulating androgen concentrations, are the appearance of adult type body odor, acne and comodones, oily hair and the appearance of pubic and/or axillary hair. Adrenarche is premature if clinical signs occur before the age of 8 years in a girl or before 9 years in a boy in the presence of increased serum adrenal androgen levels for age[10]. The appearance of pubic hair is called pubarche. Premature pubarche (PP) is the appearance of pubic hair before the above-mentioned age limits.

In the present study, in a large group of children with PWS, we assessed serum DHEAS levels, the age at onset of pubarche, development of pubic hair and the prevalence of PP, in comparison with healthy controls. We hypothesized that children with PWS start adrenarche at a younger age, are younger at onset of pubarche and have a higher prevalence of PP. We assessed DHEAS levels because it arises primarily from the adrenal cortex, has a relatively long half-life in the circulation and therefore does not exhibit a circadian rhythm[11]. For these reasons determination of serum DHEAS levels are appropriate for evaluation of adrenarche. Our choice to describe appearance of pubic hair as clinical sign of adrenarche was based on the fact that it is a clear and objective sign and according to Utriainen et al, the last clinical sign in adrenarche, associated with high levels of serum androgens[12].

Since hyperandrogenism in simple obesity is assumed to arise from hyperinsulinism and increased insulin-like growth factor I (IGF-I) levels, we also assessed BMI SDS, fat mass percentage SDS (Fat%SDS) by DEXA, IGF-I and fasting insulin and glucose for HOMA calculation, in order to investigate the effect of these factors on DHEAS levels in children with PWS.

Methods

Patients

Between April 2002 and July 2009, 120 children were enrolled in a randomized controlled trial investigating the effects of GH-treatment in children with PWS[13]. Four years after inclusion, GH-treatment (Genotropin 1mg/m2/day) was continued in the PWS Cohort Study[14]. Children fulfilled the following inclusion criteria: genetically confirmed diagnosis of PWS by positive methylation test, age between 6 months and 16 years and bone age less than 14 years (girls) or 16 years (boys).

All children visited the Erasmus Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands. The study protocol was approved by the Medical Ethics Committee of Erasmus MC Rotterdam, the Netherlands. Informed consent was obtained from parents and children above 12 years, and assent from children below 12 years.

Design

Blood samples were collected in the morning after a 12h overnight fast, immediately centrifuged and stored at -20ºC until assayed. Levels of serum DHEAS, IGF-I, glucose and insulin were assessed in 97 children with PWS, 75 samples were taken before start of GH-treatment and 85 samples after 1 or 2 years of GH-treatment, 63 samples were taken both before and during GH-treatment. Because of the difficulties in obtaining blood from children with PWS, we could not determine serum DHEAS levels in all 120 children. None of the children received glucocorticoid medication around or immediately preceding blood sampling.

The serum DHEAS levels were compared with those of a control group comprised of 335 healthy age-matched prepubertal children with a normal stature who were referred to the hospital for a minor surgical procedure. Blood was obtained before anesthesia was given. All children were between 6 months and 17 years of age. None of the children had a syndrome or chromosomal abnormality, endocrine or metabolic disorder, or any other illness or use of drugs that might have affected DHEAS levels. All serum DHEAS levels were determined in one central laboratory.

Children were examined three-monthly by the PWS research team of the Dutch Growth Research Foundation in collaboration with local pediatric endocrinologists and pediatricians throughout The Netherlands. At each visit, height and weight were measured and stage of pubic hair and puberty according to Tanner[15,16] were recorded. Pubic hair was present at time of inclusion in our study only in 12 of 120 patients, information about the age at pubarche onset in these patients was obtained from the records of the pediatrician. To determine the median age at onset of pubarche, and at the various pubic hair stages, the data of all 120 PWS patients was analyzed by Kaplan Meier survival estimates[17].

PP was defined as reaching pubic hair stage 2 (P2) before the age of 8 years in girls, and 9 years in boys[18].

Anthropometrics

Standing height was measured with a Harpenden stadiometer and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on an accurate scale (Servo Balance KA-20-150S), and Body Mass Index (BMI, kg/m^2) was calculated. Standard deviation scores (SDS) for weight, height and BMI were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org) according to age- and sex-matched reference values of the Dutch population[19].

Dual Energy X-ray Absorptiometry

In all children fat mass was measured by Dual Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare, Chalfont St. Giles, UK) Quality assurance was performed daily. The coefficients of variation for fat tissue were 0.41% to 0.88%. Fat mass was expressed as percentage of total body mass (fat%). Fat%SDS was calculated according to reference values for age and gender of the Dutch population for children with a height above 87 cm[20, 21].

Assays

Serum DHEAS levels in children with PWS and in healthy controls were measured using chemiluminescence based competitive immunoassays. An Immulite 2000 (Siemens Medical Solutions DPC, Los Angeles, California) was used in children with PWS, and an Immulite 1 (DPC, Los Angeles, California) was used in healthy children. In the Immulite 2000, the interassay coefficient was 7.9% and the limit of detection was 0.41 µmol/l. In the Immulite1 the interassay coefficient was 8% and the limit of detection was 0.2 µmol/l. Linear regression between Immulite 2000 and Immulite 1 showed a correlation coefficient of 0.986, (IML 2000) =1.06 (IML)-0.13 µmol/l. Values below the detection level were expressed as 0.1 µmol/l.

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra- and interassay CVs were 4 and 6%, respectively.

Serum glucose levels were assessed on an Abbott Architect Clinical Chemistry Analyzer (Abbott Laboratories, Irving, TX), with intra- and interassay CVs of 0.7 and 0.8%. Serum insulin levels were measured by immunoradiometric assay (Medgenix, Biosource Europe, Nivelles, Belgium) with intra- and interassay CVs of 2 to 4.7% and 4.2 to 11.3%, respectively.

SDS were calculated for IGF-I according to age- and sex-matched reference values from the Dutch population[22]. Homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the model HOMA-IR =(fasting insulin x fasting glucose)/22.5[23].

Statistics

We used an independent samples t-test to test for differences in baseline characteristics between girls and boys. Baseline characteristics were normally distributed.

Correlation coefficients between age and DHEAS were measured separately for girls and

boys with PWS and healthy girls and boys by regression analysis. For each group the curve that fitted best and its R^2 are shown in the figures.
Serum DHEAS levels are presented as median and interquartile range [iqr]. Children with PWS and healthy controls were divided into various age groups: group I, 0.50-2.99 yr; group II, 3.00–5.99 yr; group III, 6.00–9.99 yr and group IV, > 10 yr. Differences per subgroup of age and gender in serum DHEAS levels between children with PWS and healthy controls were tested by Mann-Whitney U test.
Effect of age, gender (boys coded as 0, girls coded as 1), GH-treatment (before treatment coded as 0, during treatment coded as 1), IGF-I, fat mass, BMI and HOMA on DHEAS levels were measured by multiple regression analysis. Outliers, which were defined as values above 1.5 interquartile range (IQR), were left out this analysis.
Median age at reaching each pubertal stage was estimated for boys and girls with PWS separately, by Kaplan Meier survival estimates[17] based on the data of all 120 children. Differences in age at various pubertal stages between children with PWS and healthy Dutch children were analyzed by the Wilcoxon signed rank test. Differences in age at reaching various pubertal stages between girls and boys with PWS and differences between premature and normal pubarche, regarding age, BMI SDS, height SDS, weight SDS, duration of GH-treatment (in years) and genotype were determined by Mann-Whitney U- and χ2-test.
To assess prevalence of premature pubarche, only children who had completed 8 and 9 years of life, for girls and boys respectively, were included in the analysis.
Statistical analysis was performed with SPSS V 16.0 (SPSS Inc., Chicago, Illinois, USA). P-values < 0.05 were considered statistically significant.

Results

Table 1 shows the baseline characteristics of the 120 children with PWS, 58 girls and 62 boys. Girls had a significantly lower height SDS (p=0.029) and a higher BMI SDS (p=0.045) at start of study than boys. The other baseline characteristics did not significantly differ between girls and boys.

Serum DHEAS levels

Figure 1A and B show the serum DHEAS levels for age in 45 girls and 52 boys with PWS and age-matched controls. For both girls and boys with PWS and healthy girls and boys, a significant correlation was found between serum DHEAS levels and age (R^2= 0.313, P<0.0001 for girls with PWS; R^2=0.557, P<0.0001 for boys with PWS; R^2=0.551, P<0.0001 for healthy girls; and R^2=0.731, P<0.0001 for healthy boys).
Table 2 shows the serum DHEAS levels and age in the various age groups for girls and boys with PWS compared to age-matched controls. The serum DHEAS levels in the age groups II (3.00 to 5.99 years) and III (6.00 to 9.99 years) were significantly higher for girls and boys

with PWS compared to healthy controls. Boys, but not girls with PWS in the age group IV (>10 years) also had significantly higher DHEAS levels compared to healthy controls (Table 2). We found no significant differences in serum DHEAS levels between the four genetic PWS subtypes (Deletion, mUPD, ICD, translocation).

Five girls with PWS had very high DHEAS levels. They are indicated in Figure 1 by numbers 1 through 5. The girls numbered 2 and 4 had the highest DHEAS levels and PP, they reached pubarche at 7.01 and 7.63 years respectively. Two girls, nr 1 and 3, were between 8 and 9 years old at the time they reached P2. One girl, nr 5, reached pubarche at a normal age. Two girls, nr 2 and 5, had a BMI SDS higher than 2.5, the other 3 girls had a normal BMI SDS.

Table 1: Baseline characteristics of the 120 children with PWS before start of GH-treatment

	Total	Girls	Boys
N	120	58	62
Age (years)	4.8 [3.9]	5.3 [3.8]	4.3 [3.9]
Height SDS	-2.2 [1.2]	-2.4 [1.2][1]	-1.9 [1.3]
Weight SDS	-0.9 [1.7]	-0.8 [1.8]	-1.0 [1.6]
BMI SDS	0.6 [1.5]	0.9 [1.4][2]	0.3 [1.6]
Deletion (N)	47	20	27
UPD (N)	43	21	22
ICD (N)	8	5	3
Translocation (N)	2	2	0
Genetic cause unknown (N)	20	10	10

Data expressed as mean [SD], SDS: standard deviation score, UPD: uniparental disomy, ICD: imprinting center defect. [1]Girls vs boys, p=0.029. [2]Girls vs boys, p=0.045

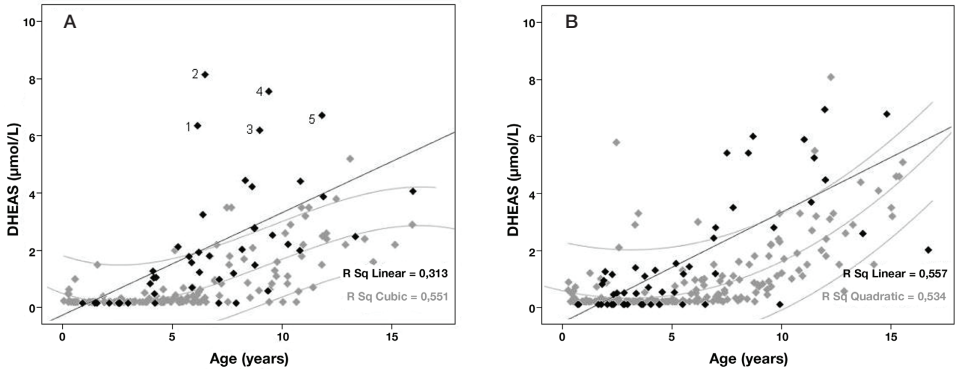


Figure 1A and 1B.

Serum DHEAS levels (µmol/l) for age (years) in girls (Figure A) and boys (Figure B) with PWS (black quadrangles) and healthy controls (gray quadrangles). The linear regression line is shown in black for DHEAS levels at different ages in girls and boys with PWS. The cubic regression line and its 95% confidence interval are shown in gray for DHEAS levels at different ages in healthy girls. High serum DHEAS levels in girls with PWS are indicated by digits 1-5. The quadratic regression line and its 95% confidence interval are shown in gray for DHEAS levels at different ages in healthy boys. To convert serum DHEAS levels in µmol/l to levels in µg/dl, multiply by 36.8.

Effect of GH-treatment, IGF-I, BMI, fat mass and HOMA, on DHEAS levels

Table 3 shows the effect of several variables on DHEAS levels in children with PWS analyzed by multiple regression analysis. The initial analysis with DHEAS as dependent variable included age, gender, GH-treatment, IGF-I SDS, HOMA and BMI SDS (model A). Age and gender significantly influenced serum DHEAS levels in children with PWS (Age: β=0.701, p-value <0.0001; Gender: β=-0.227, p-value=0.002). BMI SDS tended to influence DHEAS levels, but this did not reach significance. In the second model we replaced BMI SDS by Fat%SDS (model B). As in model A, age and gender significantly influenced serum DHEAS levels in children with PWS (Age: β=0.688, p-value <0.0001; Gender: β=-0.245, p-value=0.001). Fat% SDS tended to influence DHEAS levels, but did not reach significance.

Age at onset of the various stages of pubic hair

Table 4 shows the median age at reaching various stages of pubic hair in girls and boys with PWS. The median age [P10-P90] at onset of pubarche (P2) in children with PWS was 9.04 [6.75-11.84] years in girls and 10.31 [8.65-12.29] years in boys. Compared to healthy Dutch children[24], girls and boys with PWS reached P2 and P3 at a significantly earlier age. Girls with PWS reached P4 one year earlier than healthy girls, but this did not reach significance (p=0.069), boys reached this stage at about the same age as their healthy peers. Both boys and girls with PWS tended to reach stage P5 at an older age than healthy boys and girls, but these differences were not significant. The age range at reaching P5 was very large in both girls and boys with PWS, some did not even reach P5.

Figures 2A and B show the age at onset of the different stages of pubic hair in girls and boys with PWS. Not all girls and boys reached P5. Girls with PWS reached P2, P3 and P4 at a significant younger age than boys with PWS (p=0.008, p=0.017 and p=0.018 respectively). No significant age difference between boys and girls with PWS was found for P5.

Premature pubarche

The prevalence of PP was assessed in 71 children with PWS (40 girls who completed at least 8 years and 31 boys who completed at least 9 years of age). Of these children, 12 girls and 5 boys had PP, thus the prevalence of PP was 30% in girls and 16.1% in boys. These percentages were not significantly different between girls and boys.

Children with PP were significantly taller at onset of pubarche than those with pubarche at a normal age (NP) (median [iqr] height SDS at onset of pubarche in children with PP: -0.19 [-1.40 to 0.69] and in children with NP: -1.1[-2.3 to -0.05], p=0.033). There were no differences between children with PP and those with NP with regard to weight SDS and BMI SDS at onset of pubarche, duration of GH-treatment (in years) and genetic subtypes. Notably, none of the 8 children with an imprinting center defect had PP.

Table 2. Serum DHEAS levels per age group in 97 children with PWS and 335 healthy Dutch children

		Children with PWS			Healthy Dutch children			
	Age group	N	Age (years)	DHEAS (µmol/l)	N	Age (years)	DHEAS (µmol/l)	P-value
Girls	I	8	2.3 [1.5-2.6]	0.1[0.1-0.1]	38	1.6[0.6-2.4]	0.1[0.1-0.2]	0.108
	II	10	4.2 [4.1-5.8]	1.0[0.6-1.6]	35	4.8[4.0-5.4]	0.1[0.2-0.4]	<0.0001*
	III	20	8.0 [6.5-8.8]	2.2[1.2-4.3]	30	7.5[6.5-8.7]	0.8[0.4-1.6]	0.002*
	IV	7	11.8 [10.8-13.3]	3.8[2.2-4.4]	24	11.3[10.7-12.8]	2.3[1.2-2.9]	0.054
Boys	I	18	2.3 [1.8-2.6]	0.1[0.1-0.6]	41	1.6[0.8-2.0]	0.1[0.1-0.5]	0.941
	II	16	4.4 [3.7-5.2]	0.5[0.1-1.3]	36	4.2[3.5-5.2]	0.1[0.1-0.3]	0.021*
	III	10	7.7 [7.0-9.0]	2.8[0.9-5.5]	53	7.7[6.8-9.0]	0.7[0.3-1.1]	0.006*
	IV	8	12.1 [11.5-14.6]	4.9[2.9-6.6]	29	12.3[11.1-14.0]	2.7[1.5-3.8]	0.012*

Data expressed as median [interquartile range]. *Significant difference in DHEAS levels between children with PWS and controls. Age groups: I: 0.50 to 2.99 years; II: 3.00 to 5.99 years; III: 6.00 to 9.99 years; IV > 10.00 years. To convert serum DHEAS levels in µmol/l to levels in µg/dl, multiply by 36.8.

Table 3. Multiple regression for serum DHEAS levels in children with PWS

	DHEAS (µmol/l)			
	Model A		Model B	
Variables	β	**p-value**	β	**p-value**
Age (yrs)	0.701	<0.0001	0.688	<0.0001
Gender	-0.227	0.002	-0.245	0.001
GH-treatment	-0.144	0.308	-0.090	0.551
IGF-I SDS	0.020	0.887	0.010	0.947
HOMA	0.085	0.252	0.085	0.256
BMI SDS	0.133	0.058		
Fat% SDS			0.138	0.091
Overall		<0.0001		<0.0001
R2		0.560		0.555
R2 adjusted		0.535		0.530

Significant p-values are presented in bold.
Gender: male=0, female=1.
GH-treatment: before treatment=0, during treatment=1

Table 4. Age at reaching pubertal stages in children with PWS compared to healthy Dutch children.

	Pubic hair stage*	N	Children with PWS Age (years)	Healthy Dutch children Age (years)	P-value
Girls	P2	38	9.04 [6.75-11.84]	11.01 [9.35-12.47]	<0.0001
	P3	35	9.82 [7.71-13.91]	11.89 [10.61-13.22]	<0.0001
	P4	29	11.59 [9.45-17.09]	12.68 [11.40-14.29]	0.069
	P5	16	14.85 [11.34-19.16]	13.76 [12.14-17.74]	0.532
Boys	P2	29	10.31 [8.65-12.29]	11.73 [9.19-13.35]	0.003
	P3	19	12.36 [9.48-15.40]	12.90 [11.58-14.49]	0.018
	P4	11	13.33 [11.73-15.64]	13.76 [12.52-15.21]	0.959
	P5	7	16.80 [13.48- -]	14.97 [13.33-17.37]	0.917

Age expressed as median [10th percentile- 90th percentile].
Significant p-values are bold indicated
*According to Tanner.

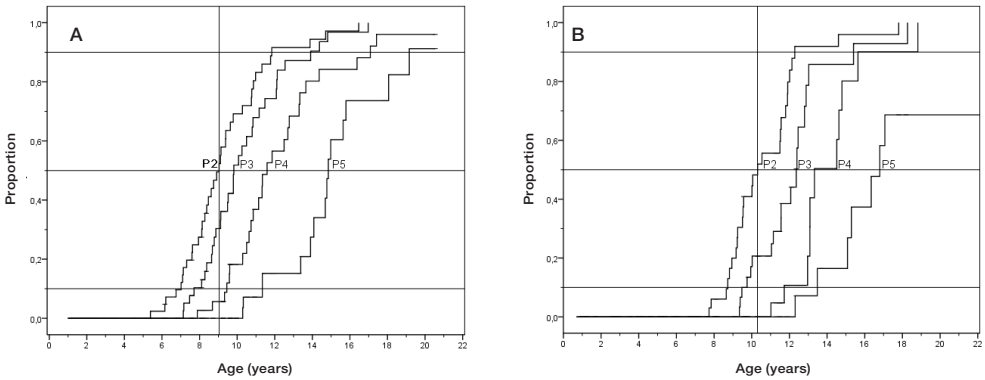


Figure 2A and 2B.
Kaplan Meier survival estimates for reaching the pubic hair stages in girls (Figure A) and boys (Figure B) with PWS. Horizontal lines represent P10, P50 and P90. Vertical line represents age at time of reaching pubic hair stage 2 (P2). P2, P3, P4 and P5: pubic hair stages according to Tanner.

Discussion

Our study showed that compared to healthy children, adrenarche starts at a younger age in children with PWS and children with PWS are younger at onset of pubarche and have a higher prevalence of PP. Age and gender are the most important factors influencing serum DHEAS levels in children with PWS.

Our study is the first to demonstrate that children with PWS have significantly increased serum DHEAS levels between the age of 3 to 10 years. Higher DHEAS levels in children with PWS have been reported[25,26], but it was unknown at which age levels were higher. Our study is also the first to describe the age at pubarche and the development of pubic hair in a large group of children with PWS. The age at pubarche was significantly younger compared to healthy Dutch children. The development of pubic hair after reaching P2 occurred at a normal pace up to P4 and slowed down thereafter. The latter was less evident in girls than in boys. Some children did not even reach P5, and as in patients with hypogonadotropic hypogonadism[27], none of the children with PWS reached P6. Thus DHEAS levels rise earlier and pubarche starts at a younger age in children with PWS. As most children with PWS have hypothalamic dysfunction, our data indicate that the increase of DHEAS levels can occur with less, or even without stimulation via the pituitary-adrenal axis. This indication is supported by our finding that DHEAS levels in 13 children previously diagnosed with central adrenal deficiency (CAI)[28] ranged from 0.5 to 6.8 µmol/l (age at time of blood sampling between 2.5 and 14.8 years old). The observation that almost no progression of pubarche occurred after stage P4, might indicate that a normal pituitary-gonadal axis is required to achieve an adult stage of pubic hair.

Two recent studies investigated sexual development and reproductive hormones in a group of children and adults with PWS (age range 6 weeks to 32 years). They concluded that DHEAS levels were normal in most females and males[29,30]. However, 9 of the 17 girls and 7 of the 14 boys between the age of 5 and 15 years (the age range of the children in our study), had DHEAS levels in the high normal range or above. This shows that compared to healthy controls, serum DHEAS levels are higher in children with PWS between 5 and 15 years, but not in adolescents and adults with PWS. Our observation that children with PWS reached P2 and P3, but not P4 and P5 at a significantly earlier age, is in line with these findings.

The prevalence of PP was 30.0% in girls and 16.1% in boys. Other studies also reported higher percentages of PP in patients with PWS[25,26,31]. In a group of children and adults with PWS, aged from 2.1 to 35.4 years, PP was reported retrospectively in 14% of females and males[32]. However, these analyses included girls younger than 8 years and boys younger than 9 years, who normally do not have pubarche yet. If these children had been left out, the percentages would have been comparable to ours. As in healthy children, the prevalence of PP in children with PWS was higher in girls than in boys. However, this difference did not reach significance. In healthy children, the female to male ratio for premature pubarche is approximately 10:1[33]. The incidence of PP in a population of normal white girls was 2.8%[34], implicating that it would

have been 0.28% in healthy boys. That means that the prevalence of PP in children with PWS was higher in both girls and boys. Age and gender significantly influenced DHEAS levels in children with PWS. These results are comparable to healthy children in whom DHEAS levels increase with age and boys have higher androgen levels than girls[35]. We found no significant influence of GH-treatment on DHEAS levels and no significant difference in duration of GH-treatment between children with PP and NP in children with PWS. In our study 3 children (1 boy and 2 girls, age at pubarche 8.73, 7.63 and 7.59 respectively) showed PP before start of GH-treatment. One other study reported an increase in DHEAS levels in children with PWS during GH-treatment in a small group of children (11 girls, 12 boys), but after correction for age, the increase was no longer significant[26]. In addition, in a large group of short children born small for gestational age, serum DHEAS levels did not significantly change during 1 year of GH-treatment[36]. We conclude that GH-treatment has no significant effect on DHEAS levels and PP in children with PWS.

There were no significant differences in serum DHEAS levels and prevalence of PP between children with different genetic subtypes. Remarkably, none of the 8 children with an ICD (5 girls and 3 boys) had PP.

In general, weight gain and obesity are associated with higher serum DHEAS levels[37]. In simple obesity hyperandrogenism is assumed to arise from hyperinsulinism and increased IGF-I levels. We found no significant effect of IGF-I SDS, BMI SDS, Fat% SDS and HOMA on DHEAS levels in children with PWS. These results are in line with the only other study evaluating the effect of obesity parameters on serum adrenal steroid levels in children with PWS[26]. The authors found no significant correlation between DHEAS levels and IGF-I, BMI, fat mass and HOMA in 23 GH-treated children with PWS with a mean age of 5.6 years. Thus it seems that in children with PWS high androgen levels and PP are not associated with obesity.

In conclusion, our study shows that compared to healthy children, children with PWS have significantly higher serum DHEAS levels from 3 to 10 years of age. Despite the known hypothalamic dysfunction, children with PWS are significantly younger than healthy Dutch children at onset of pubarche, and have a higher prevalence of PP. GH-treatment, BMI and fat mass have no effect on DHEAS levels in children with PWS. These findings indicate that the maturation of the zona reticularis of the adrenal glands starts at an earlier age in children with PWS.

Acknowledgements

We express our gratitude to all children and parents for their enthusiastic participation in this study and acknowledge P. M. C. C. van Eekelen, research nurse. We thank R. van der Wal for his laboratory activities. We also acknowledge Pfizer Inc. for the independent research grant for the trial investigating the effects of GH-treatment in children with PWS.

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Testicular failure in boys with Prader-Willi syndrome: longitudinal study of reproductive hormones

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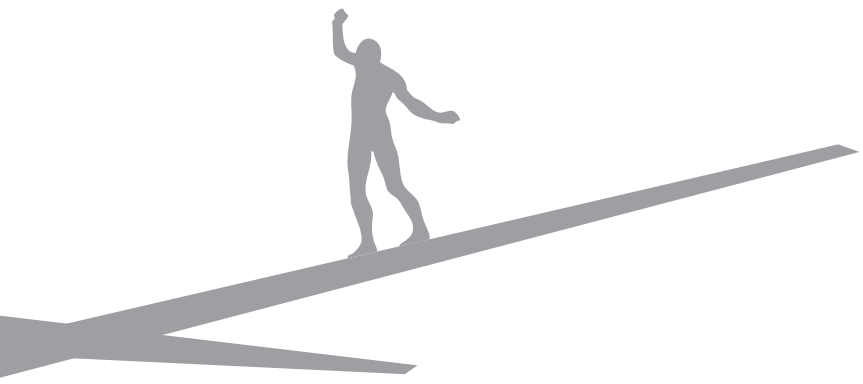
Journal of Clinical Endocrinology & Metabolism
March 2012, 97(3):E452-E459



Abstract

Context: The pathophysiology of hypogonadism in boys with Prader-Willi Syndrome (PWS) remains uncertain. Several reports described hypogonadotropic hypogonadism, some reported primary gonadal failure and others a combination of both.

Objectives: To evaluate gonadal function over time in boys with PWS and the effect of GH-treatment.

Measurements: Longitudinal assessment of inhibin B, FSH, testosterone and LH levels in prepubertal boys and male adolescents with PWS.

Patients and Methods: Sixty-eight boys participating in the Dutch PWS Cohort study. Serum inhibin B, FSH, LH and testosterone levels were compared with reference values.

Results: Boys with PWS had normal inhibin B levels between 6 months and 10 years of age, but after onset of puberty, inhibin B levels declined to less than the 5th percentile (<P5) and FSH levels increased to >P95. Two years after the onset of puberty and in young adults, inhibin B levels were significantly lower ($p=0.008$ and $p<0.0001$) and FSH levels significantly higher ($p= 0.034$ and $p<0.0001$) than at onset of puberty. Testosterone levels increased, but remained <P5 and LH levels increased, but not >P95. Age showed a significant correlation with inhibin B levels ($r=-0.31$, $p=0.001$) after 9 years of age. GH-treatment had no significant effect on inhibin B levels.

Conclusion: Our study indicates that the majority of male patients with PWS have primary testicular failure which becomes apparent after onset of puberty. Hypogonadotropic hypogonadism did not appear to be the main reason of hypogonadism in most boys.

Introduction

Prader-Willi syndrome (PWS) is a genetic disorder resulting from the lack of expression of the paternally derived chromosome 15q11-q13, caused by a deletion, maternal uniparental disomy (mUPD), imprinting center defect (ICD), or balanced translocation[1,2]. PWS is characterized by a number of signs and symptoms, including hypotonia, psychomotor delay, temper tantrums, short stature, obesity, hyperphagia, and hypogonadism[1-6].

Boys and men with PWS show clinical signs of hypogonadism, such as cryptorchidism, scrotal hypoplasia, small testicular volume, delayed or incomplete pubertal development and infertility. As most symptoms of PWS are considered to result from hypothalamic dysfunction, hypogonadism in PWS was hypothesized to be hypogonadotropic[7,8]. A few studies described hypogonadotropic hypogonadism in adults with PWS[9-11], but nowadays more and more evidence becomes available indicating that primary gonadal dysfunction, especially primary damage of the tubular department, is a major contributor to the abnormal pubertal development in PWS. However, it remains unclear at what age the gonadal dysfunction arises, because no longitudinal data in a large group of boys and adolescents with PWS were available. Three studies showed low inhibin B and high FSH levels in pubertal and adult patients with PWS, indicating a defect in the Sertoli cells of the testes in these patients[12-14]. However, normal Inhibin B levels have been described in pre-pubertal children[12], as has normal minipuberty (the gonadotrophin-dependent high sex steroid levels during the first months of life) in infants with PWS with and without cryptorchidsim[12,15]. In the present study, we aimed to longitudinally analyze the hormone levels of the pituitary-testicular axis in boys with PWS during childhood, puberty and adolescence, to find at what age the gonadal dysfunction arises. We therefore longitudinally assessed serum levels of inhibin B, FSH, testosterone and LH in prepubertal boys and male adolescents with PWS.

Based on previous findings we expected normal inhibin B, testosterone and gonadotropin levels compared to references during infancy and childhood and low inhibin B and testosterone levels in combination with high gonadotropin levels in adolescence and adulthood.

In addition, we assessed the effect of GH-treatment on gonadal function in boys with PWS, by measuring serum inhibin B levels before and after 1 or 2 years of GH-treatment.

Methods

Patients

Between April 2002 and February 2010, 68 boys were enrolled in a large randomized controlled trial investigating the effects of GH-treatment in children with PWS[16]. Four years after inclusion, GH-treatment, Genotropin 1mg/m2/day, was continued in the PWS Cohort Study[17]. Boys fulfilled the following inclusion criteria: genetically confirmed diagnosis of PWS by posi-

tive methylation test, age between 6 months and 16 years and bone age less than 16 years. Children were followed every three months by the PWS research team of the Dutch Growth Research Foundation. All children visited the Erasmus Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands at least once a year. The study protocol was approved by the Medical Ethics Committee of Erasmus MC Rotterdam, the Netherlands. Informed consent was obtained from parents and boys above 12 years, and additional assent from boys below 12 years.

Design

Blood samples were collected during yearly visits in the morning after a 12h overnight fast. Samples were immediately centrifuged and stored at -20ºC until assayed. Location of testes and pubertal stage according to Tanner[18] were determined by the PWS research team of the Dutch Growth Research Foundation at time of blood sampling and during three monthly visits. Data regarding cryptorchidism and age at orchiopexy were retrieved from medical records.

Reproductive hormones

Serum inhibin B levels were determined at least once in 66, and serum FSH, testosterone and LH levels in 56 patients with PWS, aged 6 months to 25 years. All levels were compared to reference levels[19,20]. Inhibin B could not be determined in 2 boys, both were infants below 3 years of age, and FSH, testosterone and LH could not be determined in 12 boys below 8 years of age, in all because of difficulties in obtaining blood.

Reproductive hormones in relation to pubertal stage

Twenty patients spontaneously reached puberty (G2 according to Tanner) during our study period and we studied their reproductive hormones in relation to their pubertal stage. Serum inhibin B, FSH, testosterone and LH were determined before and at onset of spontaneous puberty, after 1 and 2 years of puberty onset and after 18 years of age. Six boys started testosterone replacement therapy during our study, their data are presented separately (Table 2). Three of them, aged 14 years and older, started testosterone replacement therapy to induce puberty. The other 3 boys reached G2 spontaneously, but started testosterone replacement therapy because of low bone density, they were included in our analysis until they started the replacement therapy.

Effect of GH-treatment

Inhibin B levels were determined before and after a period of 1 or 2 years of GH-treatment in 40 boys with PWS to determine the effect of GH-treatment on gonadal function.

Hormone Assays

All reproductive hormone measurements were determined in one central laboratory. Serum inhibin B levels were measured using an enzyme-immunometric assay (Serotec, Oxford, UK)[21]. The intra- and inter assay coefficients of variance were below 9 and 15%, respectively. Serum FSH and LH were determined using an automated luminescence-based immunometric assay (Immulite 2000, Siemens DPC, Los Angeles, CA, USA). The intra- and inter assay coefficients of variance were below 5 and 12% for LH and below 3 and 8% for FSH. Total serum testosterone was determined by coated tube RIA (Siemens DPC). The intra-and inter assay coefficients of variance were below 6 and 9%, respectively. Lowest detectable levels were 0.1 U/l for FSH and LH, 10 ng/l for inhibin B and 0.1 nmol/l for testosterone. These levels were all higher than those calculated from blank values (+ 3 SD of the blank).

Statistics

Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL).
The correlations between age, inhibin B, FSH, testosterone and LH were measured in three different age groups. Infants aged below 3 years of age, boys between 3 and 9 years of age and boys aged above 9 years of age. Correlations between inhibin B, age at orchiopexy, pubertal stage, testicular size, position of testes and genetic cause of PWS were determined in the group of boys above 9 years of age only. The first inhibin B measurement of each child in each age-group was used. Because we expected that the testis in the most favorable position would be responsible for higher or more normal inhibin B levels, the most favorable position of one of the testes was used. Correlations were measured by Spearman's rho, because of the size of the groups.
Longitudinal data considering levels of reproductive hormones in relation to pubertal stage were analyzed by linear mixed models analyses for repeated measurements[22]. Differences in inhibin B levels before and during GH-treatment were assessed by logistic regression with correction for age (levels before start of GH-treatment were coded as 0, during GH-treatment as 1).
P-values less than 0.05 were considered statistically significant.

Results

Table 1 shows the baseline characteristics of all 68 boys and adolescents in our study. Cryptorchidism at birth was present in 63 (92.7%) boys, cryptorchism was bilateral in 56 (82.4%) and unilateral in 7 (10.3%) boys. Only 5 boys (7.3%) had no cryptorchidism at birth. In most boys with cryptorchidism (77.8%), orchiopexy was performed. Median (interquartile range) age at orchipexy was 2.6 (1.8-4.7) years. At time of first blood sampling, 58% of boys, had both testes or at least one testis in scrotal position and in 22% both testes were in inguinal position or not palpable.

Table 1. Baseline characteristics

N	68
Age [years]	3.4 (2.0-6.7)[1]
Cryptorchidism at birth (N(%))	
- Bilateral	56 (82.4)
- Unilateral	7 (10.3)
- None	5 (7.3)
Orchiopexy (N(%))[2]	
- Yes	49 (77.8)
- No	14 (22.2)
Age at orchiopexy [years]	2.6 (1.8-4.7)[1]
Testes Position at first blood sampling (N(%))	
Scrotal	
- Both	29 (43)
- One	10 (15)
High in scrotum	
- Both	9 (13)
- One	4 (6)
Inguinal	
- Both	10 (15)
- One	6 (9)
Not palpable	
- Both	5 (7)
- One	9 (13)
Pubertal stage (N(%)) [3]	
- 1	51 (75.0)
- 2	7 (10.3)
- 3	4 (5.9)
- 4	6 (8.8)
- 5	0
Genetic cause of PWS (N(%))	
- Deletion	31 (45.6)
- mUPD	27 (39.7)
- ICD	3 (4.4)
- Translocation	0 (-)
- Not known	7 (10.3)

[1] age at first determination of inhibin B in median (interquartile range)
[2] percentage is based on number of patients with cryptorchidism (N=63)
[3] G stadium according to Tanner[18]
mUPD: maternal uniparental disomy, ICD: imprintingscenter defect

Longitudinal serum levels of reproductive hormones between 6 months and 25 years

Figure 1 depicts longitudinal data of serum inhibin B, FSH, testosterone and LH levels in boys with PWS between 6 months and 25 years of age compared to healthy references[19].

Inhibin B levels were mostly within the normal range in boys between 6 months and 10 years of age. Between 10 and 15 years of age, most boys showed declining inhibin B levels to below the 5th percentile (<P5) while FSH levels increased above reference levels in almost all boys. Testosterone levels increased after 10 years of age, but did not reach the lowest reference level in most after the age of 13 years. LH levels remained within the normal range in most boys, but increased above reference levels in some. Both FSH and LH levels had a wide variation after the age of 10 years but very low levels <P5, did not occur after 16 years of age.

Serum Inhibin B levels in boys with PWS showed a pattern comparable to the reference population, with high levels in early infancy, which decreased thereafter, were stable during childhood and started to increase again between 5 and 10 years of age. However, between 10 and 15 years, levels declined in boys with PWS, while they further increased and stabilized in the reference population. Inhibin B levels started to increase at variable ages, between 4 and 13 years in boys with PWS. Also, the age at which inhibin B levels started to decrease was variable and took place between 10 and 15 years in most boys. Two boys had inhibin B levels above the 95th percentile at an early age, i.e. at 4.9 and 6.8 years of age, respectively. The first boy had an inhibin B level of 115 ng/l, the second had an inhibin B level of 127 ng/l. Both had low or undetectable levels of FSH, testosterone and LH at that age. Remarkably, both boys had precocious pubarche, the first boy had Tanner P2 at 7.8 years and the second at 5.3 years.

Correlations between inhibin B, other reproductive hormones and clinical characteristics

Below 3 years of age we found significant correlations between age and inhibin B ($r=-0.72$, $p=0.008$) and LH ($r= -0.61$, $p=0.04$). There was also a negative correlation between age and FSH, but this did not reach significance ($r=-0.50$, $p=0.10$). Between 3 and 9 years of age there was only a significant correlation between age and testosterone ($r=0.47$, $p=0.001$), but after 9 years of age we found significant correlations between age and inhibin B ($r=-0.31$, $p=0.001$), FSH (0.82, $p<0.0001$), testosterone ($r=0.77$, $p<0.0001$) and LH ($r=0.76$, $p<0.0001$) and between inhibin B and FSH ($r=-0.26$, $p=0.007$). We found no significant correlations between inhibin B levels and age at orchiopexy, present pubertal stage, present testicular size or location and genetic cause of PWS. Among the boys with PWS with an age above 9 years, 4 boys had no cryptorchidism at birth (present age 9.8-15.9 years). Their inhibin B levels varied from 222 to 32 ng/l.

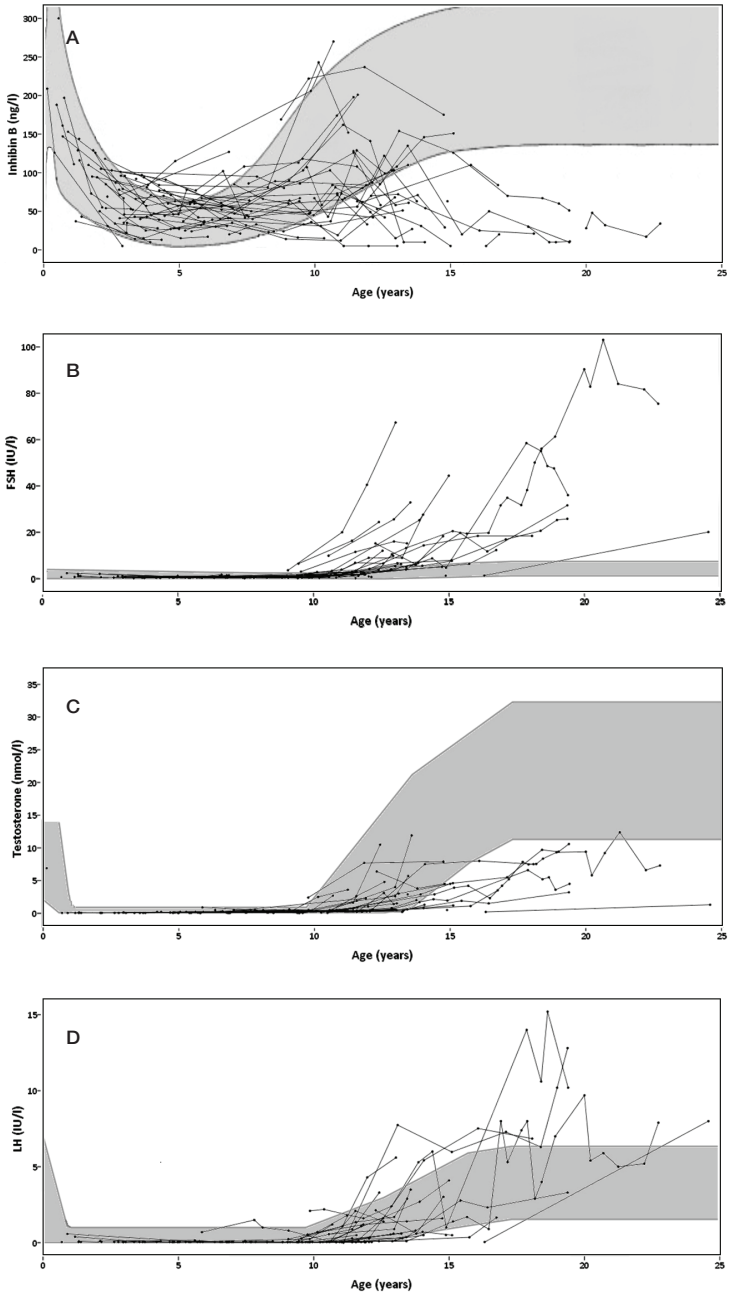
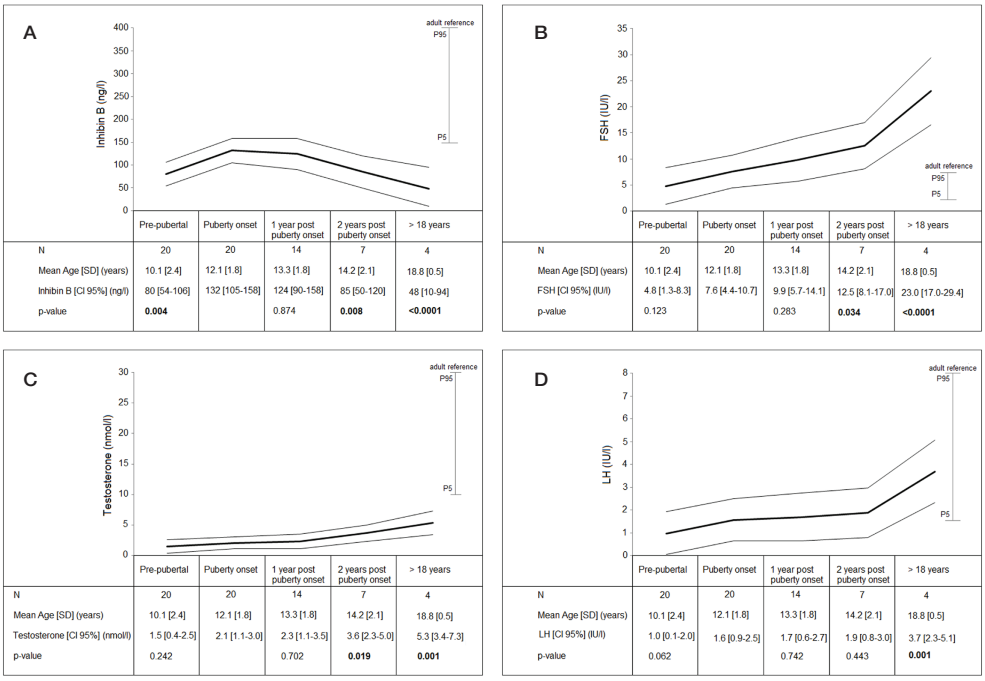


Figure 1A, 1B, 1C and 1C.
This figure presents longitudinal serum levels of inhibin B (figure A), FSH (figure B), testosterone (figure C) and LH (figure D) in individual patients with PWS. Each line represents a patient and the black dots represent the measurements in each patient. The gray area indicates the normal values, between the 5th and 95th percentile of the reference population.



A	Pre-pubertal	Puberty onset	1 year post puberty onset	2 years post puberty onset	> 18 years
N	20	20	14	7	4
Mean Age [SD] (years)	10.1 [2.4]	12.1 [1.8]	13.3 [1.8]	14.2 [2.1]	18.8 [0.5]
Inhibin B [CI 95%] (ng/l)	80 [54-106]	132 [105-158]	124 [90-158]	85 [50-120]	48 [10-94]
p-value	**0.004**		0.874	**0.008**	**<0.0001**

B	Pre-pubertal	Puberty onset	1 year post puberty onset	2 years post puberty onset	> 18 years
N	20	20	14	7	4
Mean Age [SD] (years)	10.1 [2.4]	12.1 [1.8]	13.3 [1.8]	14.2 [2.1]	18.8 [0.5]
FSH [CI 95%] (IU/l)	4.8 [1.3-8.3]	7.6 [4.4-10.7]	9.9 [5.7-14.1]	12.5 [8.1-17.0]	23.0 [17.0-29.4]
p-value	0.123		0.283	**0.034**	**<0.0001**

C	Pre-pubertal	Puberty onset	1 year post puberty onset	2 years post puberty onset	> 18 years
N	20	20	14	7	4
Mean Age [SD] (years)	10.1 [2.4]	12.1 [1.8]	13.3 [1.8]	14.2 [2.1]	18.8 [0.5]
Testosterone [CI 95%] (nmol/l)	1.5 [0.4-2.5]	2.1 [1.1-3.0]	2.3 [1.1-3.5]	3.6 [2.3-5.0]	5.3 [3.4-7.3]
p-value	0.242		0.702	**0.019**	**0.001**

D	Pre-pubertal	Puberty onset	1 year post puberty onset	2 years post puberty onset	> 18 years
N	20	20	14	7	4
Mean Age [SD] (years)	10.1 [2.4]	12.1 [1.8]	13.3 [1.8]	14.2 [2.1]	18.8 [0.5]
LH [CI 95%] (IU/l)	1.0 [0.1-2.0]	1.6 [0.9-2.5]	1.7 [0.6-2.7]	1.9 [0.8-3.0]	3.7 [2.3-5.1]
p-value	0.062		0.742	0.443	**0.001**

Figure 2A, 2B, 2C, 2D.
This figure presents longitudinal changes in serum levels of inhibin B (figure A), FSH (figure B), testosterone (figure C) and LH (figure D) in boys with PWS before, at and after onset of puberty. The p-values represent the significance of differences of inhibin B, FSH, LH and testosterone levels at puberty onset compared to pre-pubertal levels, levels at 1 and 2 years after puberty onset and levels in adolescents aged 18 years or higher. Significant p-values are indicated in bold. P5-P95 of adult reference values for inhibin B, FSH, testosterone and LH used in our lab in are indicated in the figure.

Reproductive hormones in relation to pubertal stage

Figure 2 shows serum inhibin B, FSH, testosterone and LH levels in relation to pubertal stage in boys and adolescents with PWS. From onset of puberty, inhibin B levels declined and FSH levels increased significantly to <P5 and >P95 of adult reference values, respectively. Two years after the onset of puberty and in young adults, inhibin B levels were significantly lower ($p=0.008$ and $p<0.0001$) and FSH levels significantly higher ($p= 0.034$ and $p<0.0001$) than at puberty onset.

Testosterone and LH levels also increased after puberty onset. Testosterone levels were significantly higher at 2 years after onset of puberty than at puberty onset ($p=0.019$). Both Testosterone and LH levels were significantly higher in young adults compared to levels at puberty onset ($p=0.001$ for both). However, testosterone levels were still <P5 of adult reference levels and LH levels remained in the normal range.

Six boys started testosterone replacement therapy during our study, their data are presented separately (Table 2). Three of them (no.1-3), aged 14 years and older, started testosterone

replacement therapy to induce puberty. The other 3 boys reached G2 spontaneously (no.4-6), but started testosterone replacement therapy because of low bone density. One boy (no. 3) was diagnosed as having hypogonadotropic hypogonadism at 17.8 years of age. Remarkably, however, 6 months after stop of the replacement therapy at 24.0 years of age, the same patient showed high gonadotropin levels (above upper reference levels), and would rather be diagnosed having delayed puberty and hypergonadotropic hypogonadism.

Table 2. Characteristics of boys and adolescents who started testosterone replacement therapy

	At start of testosterone replacement therapy						6 months after stop of testosterone replacement therapy			
No	**Age (yr)**	**Tanner stage**	**Inhibin B (ng/ml)**	**FSH (U/l)**	**T (nmol/l)**	**LH (U/l)**	**Age (yr)**	**FSH (U/l)**	**T (nmol/l)**	**LH (U/l)**
1	14.0	A2G1P3	49	0.8	0.4	<0.1				
2	14.1	A3G1P4	42	<0.1	1.4	<0.1				
3	17.8	A3G1P2	<10	1.2	0.2	<0.1	24.0	20.0	1.3	8.0
4	15.1	A2G2P3	63	1.2	0.5	0.5				
5	16.3	A3G3P4	29	18.2	4.5	3.0				
6	17.0	A3G3P4	84	12.2	3.5	1.7				

FSH: Follicle Stimulating Hormone, T: Testosterone, LH: Luteinizing Hormone

Effect of GH-treatment

Inhibin B levels were determined in 40 boys with PWS before and during GH-treatment. After correction for age, we found no significant effect of GH-treatment on inhibin B levels (β=-0.011, p=0.127).

Discussion

Our study is the first to present longitudinal data of reproductive hormones in a large group of prepubertal boys and adolescent males with PWS. It shows primary testicular failure which becomes apparent after the onset of puberty. Hypogonadotropic hypogonadism did not appear to be the main reason of hypogonadism in most boys.

Our results show that most infants and boys with PWS between 6 months and 10 years of age had similar inhibin B levels compared to a reference population. Between 10 and 15 years, however, inhibin B levels declined to below the 5th percentile in most adolescents, while at the same time, FSH levels increased to above the 95th percentile. We also showed that the decline of inhibin B levels and the increase in FSH, testosterone and LH levels were related

with the pubertal stage. In childhood inhibin B is produced by immature Sertoli cells, but post-pubertal, after maturation of Sertoli cells, inhibin B production is dependent on normal spermatogenesis[23]. Our findings suggest a normal function of Sertoli cells during childhood, with normal inhibin B levels during this period, and a failure of the Sertoli cells, spermatogenic cells and/or the interaction between these cells after onset of puberty, resulting in inhibin B levels below the 5th percentile in male adolescents and adults with PWS. Testosterone levels increased but remained below the 5th percentile in almost all boys, while LH levels increased to levels within the normal range in most and above the 95th percentile in some boys, indicating moderate Leydig cell dysfunction that also becomes apparent after onset of puberty. The decline in inhibin B and the increase of FSH and LH levels indicate that hypogonadism is mainly caused by failure of the Sertoli cells, spermatogenic cells and/or their interaction, and not due to hypogonadotropic hypogonadism in most boys with PWS.

Testicular histology in patients with PWS is in line with our findings. Vogels et al.[24] found significantly decreased or absent spermatogonia in testicular biopsies of 6 out of 8 boys with PWS, 5 prepubertal boys and 1 pubertal boy (age 1 to 14 years), and Sertoli cell only (SCO) testes in 1 adult (26 years). After onset of puberty, when Sertoli cells mature, inhibin B production becomes dependent on normal spermatogenesis. In SCO testes, inhibin B is normal during childhood, but adolescents and adults with SCO testes do not express inhibin B normally because there is no spermatogenesis[23]. Decreased or absent spermatogenic cells in the testes or even SCO testes could therefore very well underlie hypogonadism in male patients with PWS.

After 9 years of age, inhibin B decreased significantly with age in adolescents with PWS. This suggests that adolescents with PWS have defective spermatogenesis which will result in infertility in adult PWS patients. This could be investigated by semen analysis, but there are ethical constraints to perform this investigation in adolescents with PWS. However, as far as we know, no men with PWS have fathered a child. The gonadal dysfunction in male PWS patients could be caused by a genetic defect. Expression of the gene C15orf2, that is located in the PWS region on chromosome 15, has been observed in normal adult human testis samples and might thus be involved in primate spermatogenesis[25]. Whether men with PWS have a different expression of the gene has not yet been studied in testis samples of PWS patients, but this may be worthwhile to investigate.

Our results are in line with smaller or cross-sectional studies[12,13]. A recent cross-sectional study[14] however, showed significantly lower inhibin B levels in 10 prepubertal boys with PWS. Their inhibin B levels were compared to those of age-matched healthy controls between 5.1 and 13.5 years of age of whom the pubertal status was not described. The significant difference in inhibin B levels between the two groups might well be explained by the more advanced pubertal stage of the healthy controls compared with the prepubertal boys with PWS. Patients with Klinefelter's syndrome (KS), the most common form of male hypogonadism, have inhibin B and FSH levels comparable to levels found in our study. A retrospective ob-

servational study of 166 males with KS, aged 0.3 to 80.3 years showed normal inhibin B concentrations during childhood and a dramatic decline after onset of puberty. Serum levels were undetectably low in the majority of adult patients. FSH levels increased at the same time to above +2 SD of levels of healthy males, with a maximum of 70 IU/l[26]. Testosterone and LH levels in patients with KS are, however, higher than those in our PWS patients. Compared to reference levels, testosterone levels in the 166 males with KS were within normal limits during childhood and remained in the lower half of the normal range after puberty, and at the same time, LH levels were above + 2 SD in the majority of patients. Thus it seems that Leydig cell function is less impaired in patients with KS than in patients with PWS and that the hypothalamic –pituitary axis in PWS reacts less sufficiently than in patients with KS. However, as in our boys with PWS, the increase of LH levels in boys with KS was also less marked than the increase of FSH levels[27]. Furthermore, the increase in both FSH and LH levels in boys with PWS occurred gradually between 10 and 15 years of age and even after that age in one adolescent, while in the boys with KS, these levels increased sharply after the age of 10 years. This might be caused by the difference in timing of puberty onset between PWS and KS and suggests that eventually we might find LH levels in patients with PWS comparable to those in patients with KS. Primary gonadal failure is also found in other syndromes like Turner and Noonan syndrome, indicating that the gonadal dysfunction in boys with PWS is related to the syndrome.

Boys with PWS had variable gonadotrophin levels and the question may arise whether FSH and LH levels showed an adequate response to the gonadal dysfunction in all boys. Boys with inadequate gonadotrophin levels may have lower function of the hypothalamic-pituitary-gonadal axis. This variation is in line with the variation in hypothalamic dysfunction in the ACTH stress response in children with PWS. In 60% of children with PWS tested by a metyrapone test, central adrenal insufficiency (CAI) was found, the rest showed an adequate ACTH response[28]. However, the very low levels of FSH and LH in patients with isolated hypogonadotropic hypogonadism (IHH) contrast clearly with our findings of high levels of FSH and normal to high levels of LH in boys with PWS. In addition, patients with IHH show no testosterone rise during infancy[29], while normal mini puberty has been described in boys with PWS[15]. One of our patients with PWS was diagnosed with hypogonadotropic hypogonadism at 17.8 years of age, but demonstrated high gonadotrophin levels at 24 years of age (patient no. 3 in Table 2). In this patient, extremely late puberty was misinterpreted as hypogonadotropic hypogonadism. As delayed pubertal development is a common feature in boys with PWS, this might explain why some studies found hypogonadotropic hypogonadism as the main cause of hypogonadism in PWS patients. Reversal of IHH after discontinuation of GnRH therapy has been described in 10% of men with characteristics of IHH[30]. The authors suggested that the reversal might be due to plasticity of the GnRH producing neurons in adulthood. Whether this mechanism might also occur in men with PWS is yet unknown.

In healthy boys, age at orchiopexy is an important factor in fertility outcome: the younger the

age at orchiopexy, the better the fertility outcome[31]. In our study, however, we found no significant effect of age at orchiopexy on inhibin B levels in boys with PWS. This suggests that gonadal dysfunction in boys with PWS is not only caused by cryptorchidism but is particularly related to PWS. Similar results were found in 3 adults with Noonan syndrome with normal testicular descent. All 3 had Sertoli cell dysfunction and the authors concluded that bilateral cryptorchidism was not the main contributing factor to impairment of testicular function in men with Noonan syndrome[32].

GH-treatment did not affect testicular function in boys with PWS, as inhibin B levels were similar before and during GH-treatment. These results are in line with findings in prepubertal boys and young men born small for gestational age[33].

In conclusion, our study shows that hypogonadotropic hypogonadism is not the main reason of hypogonadism in most boys with PWS. Some boys might have a combination of gonadal dysfunction and decreased functioning of the hypothalamic- pituitary-gonadal axis. In the majority of male adolescents with PWS, primary testicular dysfunction, especially in the seminiferous epithelium, and moderate Leydig cell dysfunction underlie hypogonadism.

Acknowledgements

We express our gratitude to all children and parents for their enthusiastic participation in our Dutch PWS Cohort study and acknowledge the work of P. M. C. C. van Eekelen, research nurse. We thank all pediatricians and pediatric endocrinologists for their collaboration in the care for children with PWS. We thank R. van der Wal for his laboratory activities. We also acknowledge Pfizer Inc. for the independent research grant for the investigator initiated trial investigating the effects of GH-treatment in children with PWS.

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4

Ovarian function and reproductive hormone levels in girls with Prader-Willi syndrome

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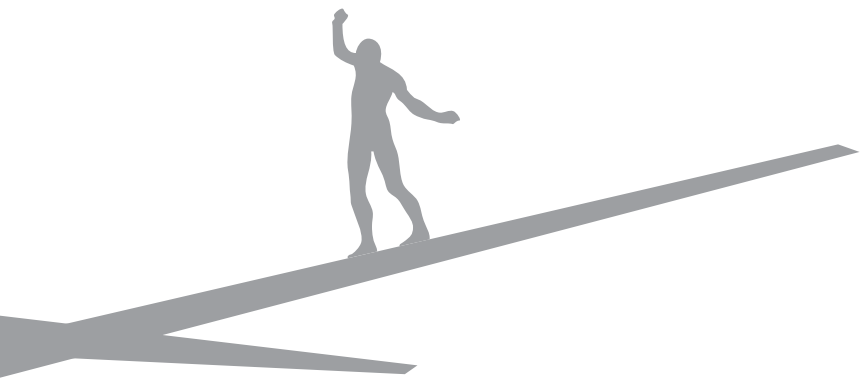
Journal of Clinical Endocrinology & Metabolism
Sept 2012, 97(9):E1766-E1773



Abstract

Context: The etiology of hypogonadism in girls with Prader-Willi Syndrome (PWS) remains uncertain.

Objectives: To evaluate gonadal function longitudinally in girls and female adolescents with PWS.

Measurements: Longitudinal assessment of anti-Müllerian hormone (AMH), gonadotrophins, estradiol (E_2), inhibin B and A and pubertal development in girls and female adolescents with PWS.

Patients and Methods: Sixty-one girls participating in the Dutch PWS Cohort study. Serum AMH, gonadotrophins, E_2 and inhibin B and A levels were compared with reference values.

Results: AMH levels in girls and female adolescents with PWS were comparable to reference levels between 6 months and 22 years of age. From 10 years of age, FSH and LH levels increased to above the 5th percentile compared to reference levels. E_2 and inhibin B levels were in the low normal range in the majority, and inhibin A levels were low, but detectable in almost half the female adolescents with PWS. The median age at puberty onset was comparable, but the median ages at attaining Tanner M3 ($p=0.05$) and M4 ($p<0.0001$) were significantly higher in girls with PWS than in healthy references.

Conclusion: Our study shows that the primordial follicle pool and number of small antral follicles are conserved in girls and female adolescents with PWS. We found no classical hypogonadotropic hypogonadism. However, maturation of follicles and progression of pubertal development are impaired, which might be due to dysregulation of LH secretion. As these impairments are not absolute, ovulation and thus conception cannot be ruled out in individual female adolescents with PWS.

Introduction

Prader-Willi syndrome (PWS) is a rare disorder, characterized by hypotonia, short stature, hyperphagia, hypogonadism, scoliosis, psychomotor delay, and temper tantrums[1-3]. PWS results from the lack of expression of genes in the q11-q13 region of the paternally derived chromosome 15, caused by a deletion, maternal uniparental disomy (mUPD), imprinting center defect (ICD), or a unbalanced translocation[3, 4].

Clinical signs of hypogonadism, such as hypoplasia of labia minora and/or clitoris, are reported in girls with PWS. As most symptoms of PWS are considered to result from hypothalamic dysfunction, hypogonadism in PWS was hypothesized to be hypogonadotropic. A few studies in female and male adults with PWS support this hypothesis[5-7]. However, in a recent longitudinal study, we found that most boys with PWS have failure of the Sertoli cells, spermatogenic cells and/or their interaction, and no classical hypogonadotropic hypogonadism[8].

In girls and females with PWS, longitudinal information about gonadal function is lacking. A cross-sectional study showed variable combinations of a primary ovarian failure and hypothalamic dysfunction in 15 women (ages 17-32 years) and 30 girls (0.1- 16 years)[9, 10].

The granulosa cells of follicles in the primary and pre-antral stages, follicular stages following the primordial stage, secrete anti-Müllerian hormone (AMH). AMH is involved in the regulation of the folliculogenesis[11]. Since serum AMH is exclusively produced by the ovaries, independent of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool[12-16].

Inhibin A and inhibin B regulate FSH secretion through negative feedback[17]. Inhibin B is mainly produced by granulosa cells of small antral follicles (2-7 mm) whereas inhibin A is a product of granulosa cells of larger antral follicles (>7 mm), the dominant follicle and the corpus luteum[18]. It has been suggested that inhibin A can be used as a marker of the quality of the mature follicles[19].

In the present study, we aimed to evaluate gonadal function in girls and female adolescents with PWS by longitudinally analyzing serum levels of AMH, LH, FSH, estradiol (E_2), inhibin B and A, and compared these to reference levels. Our second aim was to evaluate whether female adolescents with PWS might be fertile and thus need contraceptive treatment in case circumstances would request this.

Based on findings in boys with PWS[8, 20-22], we hypothesize that hypogonadism in girls and female adolescents is mainly caused by a primary ovarian failure. Furthermore, as spontaneous menarche, precocious puberty and even a few pregnancies in women with PWS are reported[23-25], we expect that the gonadal function in girls and female adolescents with PWS will be less abnormal when compared with healthy peers than in boys and male adolescents, and that some female adolescents with PWS might be fertile.

Methods

Patients

Between April 2002 and August 2011, a total of 77 girls and female adolescents, aged 6 months to 16 years, were enrolled in the Dutch PWS study. The diagnosis of PWS was genetically confirmed by positive methylation test in all girls. Fifty-eight girls were originally included in the randomized controlled trial investigating the effects of GH-treatment in children with PWS and continued treatment in the PWS Cohort study, while 19 girls were directly included in the PWS Cohort study [26, 27]. In the PWS Cohort study, all girls were treated with Genotropin 1mg/m2/day. Girls were followed every three months by the PWS research team of the Dutch Growth Research Foundation. All children visited the Erasmus Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands, at least once a year. The study protocol was approved by the Medical Ethics Committee of Erasmus MC Rotterdam, the Netherlands. Written informed consent was obtained from all parents and girls above 12 years, and assent from girls below 12 years.

For the present study, 61 of the 77 girls and female adolescents were eligible, as it was impossible to take blood samples in 14 infants and 2 pubertal girls. All girls were treated with GH during the study period. Mean (SD) duration of follow-up in the study was 5.9 (1.8) years.

Design

Blood samples were collected during yearly visits, in the morning after a 12h overnight fast. Samples were immediately centrifuged and stored at -20ºC until assayed. Pubertal stage according to Tanner[28] was determined by the PWS research team of the Dutch Growth Research Foundation at time of blood sampling and during three monthly visits. Age at menarche and characteristics of menses, when present, and anthropometric measurements were recorded every 3-months. Body Mass Index (BMI) and BMI SDS were calculated by growth analyzer version 4.0 (www.growthanalyser.org).

Reproductive hormones

As serum AMH levels in healthy females are measurable from birth to adulthood[16], we determined serum AMH levels at least once in all 61 girls and female adolescents with PWS between 6 months to 22 years of age. Because serum LH, FSH and Estradiol (E_2), levels in healthy girls start to rise before puberty onset and serum inhibin B and A from 10 years of age[29], we determined E_2, LH and FSH at least once in all girls and female adolescents, aged 5 years and older and inhibin A and B in all girls aged 9 years and older (one year before the possible rise of inhibin A and B). All levels were compared to age- appropriate reference levels[16, 29].

Eight girls started 17β-estradiol treatment during the study, their data are presented separately (Table 3). Three of them started replacement therapy to induce puberty, the other 5 reached M2 spontaneously, but started 17β-estradiol replacement therapy because of low bone density. Their data were included in our analyses until start of the replacement therapy.

Assays

Serum AMH, FSH, LH, E_2 and inhibin B and A, were determined in the same laboratory. Serum AMH levels were assessed using an in-house double antibody ELISA[30]. The limit of detection was 0.1 ug/l. The intra- and inter-assay variation coefficients were less than 5% and 10%. Dimeric inhibin A and B levels were assessed using an immuno-enzymometric assay obtained from Serotec (Oxford, Oxon, UK). The detection limit of the assay, defined as the amount of inhibin equivalent to the signal of the blank +3 s.d. of this signal, was 3.4 ng/l for both inhibin A and B. Intra- and interassay coefficients of variation were less than 8% and 15% for inhibin A and less than 8% and 14% for inhibin B respectively. Serum levels of LH and FSH were measured using luminescence based immunoassays (Immulite, Diagnostic Products Corp., Los Angeles, CA, USA). Serum E_2 levels were measured using coated tube radioimmuno-assays provided by the same supplier. Sensitivities of the assays were 0.1 U/l for FSH and LH and 10 pmol/l for E_2. Intra- and interassay coefficients of variation were less than 5% and 6% for LH, less than 5% and 7% for FSH and less than 5% and 7% for E_2.

Statistics

Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL).

We cross-sectionally assessed correlations between AMH levels and age, genetic subtype, BMI-SDS and age at puberty onset and between BMI-SDS and age at puberty onset using Pearson's correlation coefficient. We used the firstly determined AMH level for each patient. AMH levels were log transformed because of a skewed distribution. When correction for age was needed we used linear regression analysis.

Differences in FSH, LH and E_2 levels and age at various pubertal stages between girls with PWS and healthy references were analyzed by the Wilcoxon signed rank test. Median age at reaching each pubertal M stage was estimated by Kaplan Meier survival estimates[31].

To assess the difference in AMH levels between girls aged 15 years or above who had and those who had not yet had their menarche, we used the Mann-Whitney test. P-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics

Table 1 shows the baseline characteristics of 61 girls and adolescents with PWS at first blood sampling. Girls had a median age (range) of 6.2 (0.6-17.2) years and a median (interquartile range) BMI SDS of 1.2 (0.6-1.9). Nineteen (31.1%) girls had a deletion, 32 (52.5%) girls an mUPD, 5 (8.2%) an ICD and 1 (1.6%) an unbalanced translocation.

Longitudinal Serum AMH levels between 6 months and 22 years of age

Figure 1A shows the longitudinal serum AMH levels in 61 girls with PWS between 6 months and 22 years of age. Levels were in the normal range for almost all girls with PWS compared to reference levels, indicating a normal size of the primordial follicle pool in most of them. AMH levels significantly increased with age in girls between 1 and 8 years of age, (r=0.40, p=0.01) and stabilized between 8 and 22 years, when the correlation between AMH and age was no longer significant. There was no significant correlation between AMH levels and genetic subtype, BMI-SDS or age at puberty onset (Tanner M2 stage).

Longitudinal Gonadotropin and E_2 levels between 5 and 22 years of age

Figure 1B, C and D show serum FSH, LH, and E_2 levels, respectively. FSH levels were in the normal range in all girls with PWS and showed a similar pattern compared to reference levels. LH and E_2 levels were in the low normal range in the majority of girls with PWS. From 10 years of age, both FSH and LH levels increased to above the 5th percentile compared to reference levels, indicating no classical hypogonadotropic hypogonadism in the majority of girls and female adolescents with PWS, but relatively low LH levels in combination with the low E_2 levels. A few adolescents occasionally showed higher levels of FSH and LH in combination with higher E_2 levels, resembling mid cycle peak values. Two girls had LH levels below the reference range, but their FSH levels in the same samples were within the reference range.
We also evaluated FSH, LH and E_2 levels in 18 girls at approximately 15 years of age (median (iqr)14.8 (14.5-15.4) years) and with Tanner breast stage M3 (median (iqr) (3.0 (2.8-4.0)). Compared to healthy girls with a similar pubertal stage[29] all levels were in the normal range, but FSH levels in girls with PWS were significantly higher, LH levels were lower but not significantly, and E_2 levels were significantly lower (Table 2).

Longitudinal inhibin B and A levels between 9 and 22 years of age

Figures 2A and B show inhibin B and A levels compared to healthy references. Inhibin B levels were in the low normal range in all female adolescents with PWS and Inhibin A levels were low, but detectable in 46.3% (19 out of 41). This indicates that most females with PWS have a normal number of small antral follicles, but an impaired, although not completely absent, further maturation of the follicles.

Girls with AMH levels below or above reference levels

Four girls had AMH levels below the reference range. Three of them were infants (age below 3.0 years), but one was an adolescent girl of 16.8 years of age. Her serum AMH level was 0.2 µg/l. She had reached Tanner stages M3 and P4, had no menarche yet and was the only girl with a translocation. She started 17β-estradiol replacement therapy at 20.3 years of age because of low E_2 levels and low bone density (Table 3, patient no. 8).

Table 1. Baseline characteristics [1] of 61 girls with PWS

N		61
Age (years)[2]	6.2	[0.6-17.2]
BMI SDS[3]	1.2	[0.6-1.9]
Tanner M (N(%))		
- prepubertal	20	(32.8)
- M2	7	(11.5)
- M3	15	(24.6)
- M4	14	(22.9)
- M5	5	(8.2)
Genetic cause of PWS (N (%))		
- Deletion	19	(31.1)
- UPD	32	(52.5)
- ICD	5	(8.2)
- Translocation	1	(1.6)
- unknown	4	(5.5)

[1] Characteristics at first blood sampling. [2] Age is presented as median [range]. [3] BMI SDS is presented as median [inter quartile range]

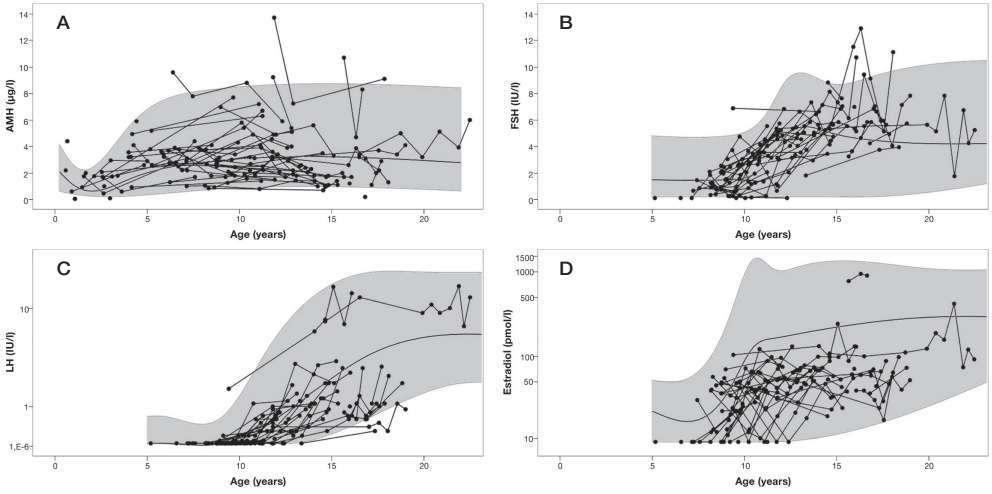


Figure 1A, 1B, 1C and 1D.
This figure presents longitudinal serum levels of AMH (Figure A), FSH (Figure B), LH (Figure C) and E_2 (Figure D) in girls and female adolescents with PWS from 6 months (AMH) and 5 years (FSH, LH and E_2) of age to 22 years of age. The y-axes of LH and E_2 levels are log transformed.
Each line represents a patient and the black dots represent the measurements in each patient. The gray area indicates the normal values of the reference population between the 5th and the 95th percentile. The thick black line represents the median levels of the reference population.

Table 2. Gonadotrophin and E_2 levels in 18 girls with PWS, aged 15 years compared to healthy girls with similar Tanner stage

	PWS[1]	References[2]	p-value
FSH (IU/l)	5.6 (4.6-6.8)	4.6 (2.9-8.8)	0.02
LH (IU/l)	1.1 (0.9-2.2)	2.2 (0.61-9.8)	0.11
E_2 (pmol/l)	64 (36.8-90.5)	128.5 (<18-1379)	0.03

[1] Values are presented as median (inter quartile range). [2] Values are presented as median (2.5 to 97.5 percentile)

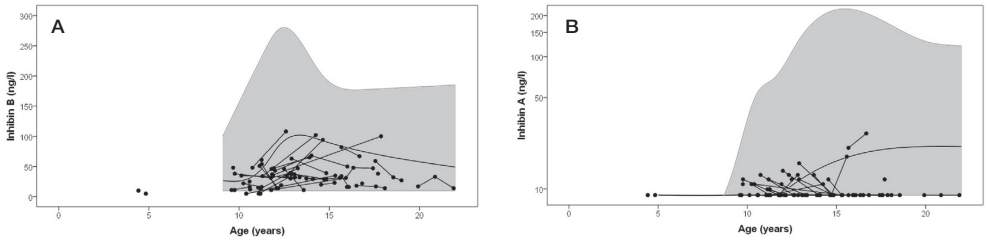


Figure 2A and 2B.
This figure presents serum inhibin B (Figure A) and A (Figure B) levels in adolescents with PWS from 10 to 22 years of age. The y-axis of Inhibin A levels is log transformed.
Each line represents a patient and the black dots represent the measurements in each patient. The gray area indicates the normal values of the reference population between the 5th and the 95th percentile. The thick black line represents the median levels of the reference population.

AMH levels were above the reference range in 5 girls; their characteristics are displayed in Table 3. One of them was an infant. None of them older than 10 years had their menarche yet. Treatment with 17β-estradiol was started in one girl (no. 3) at 13 years of age because of low bone density. Girl no.5 had reached an adult pubertal stage. Her E_2 levels were comparable to pre-ovulatory levels in all blood samples and all other reproductive hormone levels were in the normal range at several time points. Abdominal ultrasonography showed small ovaries with more than 12 follicles per ovary and a small uterus. Although she had the highest inhibin A levels of all girls, she had no menarche yet.

Pubertal development, spontaneous menarche and puberty induction in girls and female adolescents with PWS

The median (P_{10}-P_{90}) age at attaining each of the pubertal M stages (M2-M5) in girls and female adolescents with PWS is presented in Figure 3. Compared to healthy references[32], the median age at attaining M2 in girls with PWS was similar, while the median ages at attaining M3 ($p=0.05$) and M4 ($p<0.0001$) were significantly older in female adolescents with PWS. It was not possible to calculate the median age at attaining M5, because only 5 adolescents with PWS reached that stage. Thus girls and female adolescents with PWS started puberty

Table 3. Characteristics of 8 girls with PWS at start of 17β-estradiol replacement therapy and of 5 girls with AMH levels above the 95th percentile

Patient	Age (yr)	AMH (µg/l)	FSH (IU/l)	E_2 (pmol/l)	LH (IU/l)	Inhibin B (ng/l)	Inhibin A (ng/l)	Tanner stage	Genetic cause	BMI SDS
8 girls at start of 17β-estradiol replacement therapy										
1	12.0	1.0[1]	7.1	73	3.1	102	11	M1P3	Deletion	0.7
2	14.7							M1P3	ICD	1.7
3	15.1	1.0[1]	4.1	88	2.0	94	<10	M2P5	Deletion	1.6
4	16.0	1.2[1]	7.0	71	1.0	16	<10	M1P1	mUPD	1.9
5	17.0	3.1[1]	3.1	32	0.4	32	<10	M4P5	Deletion	1.0
6	18.0	9.1[1]	4.9	47	1.1	100	<10	M3P5	mUPD	-1.3
7	19.0	2.6[1]	3.9	98	0.3	32	<10	M4P5	mUPD	1.3
8	20.3	0.2[1]	5.7	73	2.0	22	<10	M4P5	Translocation	1.5
5 girls with AMH levels above the 95th percentile										
9	0.7	4.4[2]						M1P1	mUPD	3
10	6.4	9.6[1]						M1P1	unknown	1.6
	10.4	8.8[1]	0.1	66	0.1			M3P4		0.5
11 (2)	11.8	9.2[1]						M1P2	ICD	2.6
12 (6)	11.9	13.7[1]	3.0	54	0.1	36	<10	M1P2	mUPD	0.1
	18.0	9.1[1]	4.9	47	1.1	100	<10	M3P5		-1.3
13	15.7	10.7[1]	3.2	770	0.8	82	21	M5P5	ICD	-0.9

AMH reference levels (P 2.5-P 97.5): [1]0.7-8.4 µg/l between 8 and 25 years of age and [2]0.1-2.6 µg/l for 12 months of age. [3]Not possible to calculate BMI because of young age. Patients no 2 and 11 in this are the same patient; Patients no 6 and 12 are the same patient. Empty cells present missing values.

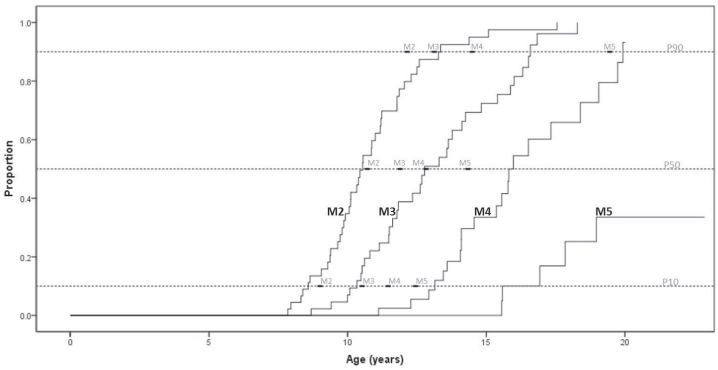


Figure 3.
This figure presents pubertal development in 61 girls and female adolescents with PWS. Solid lines present the proportion of girls and female adolescents with PWS that reached a particular Tanner M stage at a certain age and the dashed horizontal lines represent the P10, P50 and P90. The median (P10-P90) ages at each Tanner M stage for healthy references[32] are indicated by horizontal black bars.

at a similar age as healthy Dutch peers, but their progression of puberty was significantly delayed or incomplete. We found no significant differences in age at puberty onset between the genetic subtypes in girls aged 12.1 years and older and no significant correlation between BMI-SDS and age at onset of puberty.
Five adolescents with PWS reported spontaneous menarche at a median (range) age of 14.9 (12.7-16.7) years. At time of menarche, all had reached M3 or higher. After menarche, one of them developed regular menses, 2 had oligomenorrhea and 2 secondary amenorrhea after a limited number of cycles. We found no significant differences in serum AMH levels between those with or without menarche. Among the girls who had spontaneous menarche, 3 had an mUPD, one a deletion genotype and one an imprinting center defect. Because only few girls had menarche, we could not test the relation between genetic subtype and spontaneous menarche.

Discussion

Our study is the first to show longitudinal levels of reproductive hormones in prepubertal girls and female adolescents with PWS. Our findings indicate that girls with PWS have a normal size of the primordial and antral follicle pool, which remained similar to healthy references throughout puberty and adolescence. Inhibin B levels were in the normal range, indicating that folliculogenesis from primordial to antral follicles occurred in most girls. Inhibin A levels were low, indicating impaired maturation of antral follicles into the dominant follicle. However, as inhibin A was detectable in nearly half the female adolescents, occasional development of a dominant follicle, and thus ovulation and the possibility of conception, cannot be ruled out. Gonadotrophin and E_2 levels were in the normal range, but the median FSH level in girls with PWS was significantly higher than in healthy references, while the median LH and E_2 levels were respectively non-significantly and significantly lower compared to healthy references. Puberty onset occurred at the same age as in healthy girls, but there was a progressive delay in pubertal development beyond stage M2. Only 5 adolescents above the age of 12 years had menarche, of which only one developed regular menses. It is possible that the adolescents with PWS in our study will have menarche and develop menses at an older age, it is therefore important that this be further investigated.
Although we did not find classical hypogonadotropic hypogonadism in female adolescents with PWS, LH levels were relatively low considering the low E_2 levels, indicating dysregulation of LH secretion. Inadequate LH levels might indicate lower function of the hypothalamic-pituitary-gonadal axis. In the present study, only one female adolescent had a clearly inadequate gonadotrophin response to a very low AMH level (Table 3, patient no 8). Her gonadotrophin levels should have been similar to those of girls with Turner Syndrome[33]. This variation in individual gonadotrophin response is in line with variations in other hormonal axes in children

with PWS, for example in the HPA-axis[34].

Between 1 and 8 years of age, AMH levels correlated significantly with age in girls with PWS, and remained at the same level between 8 and 22 years of age. These findings are in line with those in 926 healthy girls and females, in whom AMH levels increased from 4 years to 8 years of age and stabilized thereafter (16). Because inhibitory effects of obesity on reproductive hormones are described in overweight women[35], we investigated the effect of BMI SDS on AMH levels and age at onset of puberty in girls with PWS. Although there was a large variation in BMI SDS, we did not find a significant effect of BMI SDS on AMH levels or age at onset of puberty in girls and female adolescents with PWS.

A recent cross-sectional study[9] found low normal AMH levels in 20 girls (age 0-16 years) and 15 female adolescents (age 17-32 years) with PWS and undetectable inhibin B levels in 10 of 15 female adolescents, while we found normal or even high AMH levels and low normal inhibin B levels. One explanation for the difference might be that our data are longitudinal. Some girls in our study had indeed low or undetectable levels at the first blood sampling but a normal level on a later occasion. Another explanation could be that in our study all girls were treated with GH, while in the cross-sectional study, the majority was untreated. GH and IGF-I might play a role in follicular maturation, as GH and IGF-I receptors are present in the ovary[36].

The normal AMH, FSH and inhibin B levels, but low normal LH and E_2 levels in most, and undetectable inhibin A levels in more than half the female adolescents with PWS suggest lack of progression from small antral follicles to a mature follicles in female patients with PWS. Insufficient maturation of follicles will result in an inadequate production of E_2, which in turn will lead to lack of progression of puberty and amenorrhea in female patients with PWS. Indeed we found that, although puberty started at the same age as in healthy girls, the progression of puberty to stages M3 and M4 occurred at a significantly later age, indicating an impaired progression of puberty in most of them.

A recent report showed that loss of the Magel2 gene could very well play a role in the reduced gonadal function and abnormal progression of puberty we found in most females with PWS. This gene is located in the PWS critical region and mutation of this gene alters reproduction in female mice[37]. In mice, Magel2 is highly expressed in the suprachiasmatic nucleus of the hypothalamus and is found to regulate a normal circadian output, as mice deficient for this gene have blunted circadian rhythms[38]. A mutation in this circadian rhythm gene affects female fertility, as Magel2-null female mice showed significant smaller litter size than controls, indicating fewer ovulations than control mice. Also, ovaries collected from Magel2-null mice showed absence of corpora lutea despite normal numbers of developing and mature follicles, indicating normal folliculogenesis with missed ovulations. Furthermore, female Magel2-null mice showed a slight but significant delay in initiation of puberty and a significant delay in progression of puberty with abnormal and extended estrous cycles. This is comparable to what we found in our study. The effect of loss of Magel2 on gonadotrophin levels has only been studied in male mice. Magel2 null males have similar FSH levels, non-significantly lower

LH levels and significantly lower testosterone levels as controls. The level and timing of the LH peak plays a crucial role in ovulation and thus fertility. In case the LH peak is too small or incorrectly timed, ovulation might not take place and hence, conception is not possible. Even in healthy women, timing and level of the LH peak is sometimes inadequate, resulting in an anovulatory cycle from time to time. Furthermore, the number of anovulatory cycles rises in case of increasing variation in duration of cycles. As Magel2 is involved in circadian rhythms, loss of Magel2 in PWS might result in dysregulation of LH secretion and disturb the levels and the timing of LH peaks. This in turn might contribute to the impaired maturation of follicles and ovulation and result in irregular or absent menstrual cycles and delayed and incomplete pubertal development as found in female adolescents with PWS.

The present findings in girls and female adolescents with PWS are in line with our previous results in boys and male adolescents with PWS[8]. In fact, both female and male adolescents with PWS have a defect in the gametogenesis after onset of puberty. While the development of primary germ cells in prepubertal girls and boys with PWS was similar as in healthy girls and boys, further maturation to a mature ovum or spermatozoa was impaired. However, as menarche, menses and even pregnancy occurs in females with PWS, and, as far as we know, no men with PWS have fathered a child, this defect appears to be less severe in female than in male patients with PWS. One explanation could be the fact that in women the size of the primordial follicle pool is already determined before birth, while in men the development of primary germ cells occurs during childhood.

In conclusion, findings in our study indicate that the primordial follicle pool is conserved in girls with PWS throughout puberty and adolescence. We did not find classical hypogonadotropic hypogonadism. Our findings further indicate defects in the maturation of follicles and in the progression of pubertal development in female adolescents with PWS, which might be related to dysregulation of LH secretion. However, as these defects are not absolute, ovulation and thus conception cannot be ruled out in an individual female adolescent with PWS. Therefore, contraceptive therapy might be considered if clinical and laboratory findings and circumstances request this.

Acknowledgements

We express our gratitude to all children and parents for their enthusiastic participation in our Dutch PWS Cohort study and acknowledge the work of P. M. C. C. van Eekelen, research nurse. We thank Pfizer Inc. for the independent research grant for the investigator initiated trial investigating the effects of GH-treatment in children with PWS.

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5

Beneficial effects of growth hormone treatment on cognition in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study

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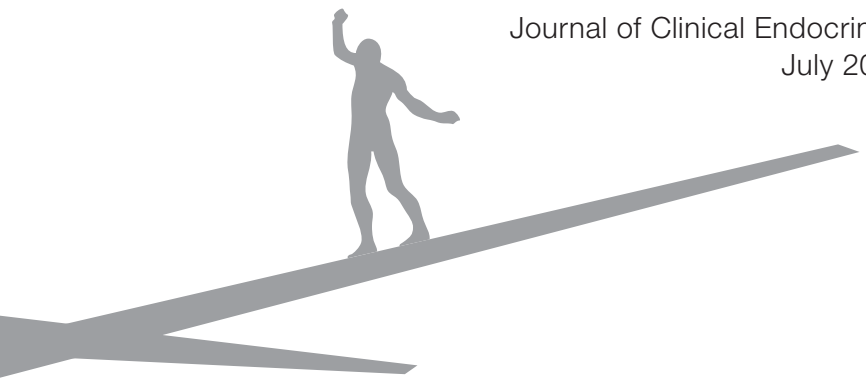
Journal of Clinical Endocrinology & Metabolism
July 2012 97(7): 2307-2314



Abstract

Background: Knowledge about the effects of GH-treatment on cognitive functioning in children with PWS is limited.

Methods: Fifty pre-pubertal children, aged 3.5 to 14 years were studied in a randomized controlled GH trial during 2 years, followed by a longitudinal study during 4 years of GH-treatment. Cognitive functioning was measured biennially by short forms of the WPPSI-R or WISC-R, depending on age. Total IQ (TIQ) score was estimated based on 2 subtest scores.

Results: During the RCT, mean SD-scores of all subtests and mean TIQ score remained similar compared to baseline in GH-treated children with PWS, while in untreated controls mean subtest SD-scores and mean TIQ score decreased and became lower compared to baseline. This decline was significant for the Similarities ($p=0.04$) and Vocabulary ($p=0.03$) subtests. After 4 years of GH-treatment, mean SD-scores on the Similarities and Block design subtests were significantly higher than at baseline ($p=0.01$ and $p=0.03$, respectively) and scores on Vocabulary and TIQ scores remained similar compared to baseline. At baseline, children with a maternal uniparental disomy had a significantly lower score on the Block design subtest ($p=0.01$), but a larger increment on this subtest during 4 years of GH-treatment than children with a deletion. Lower baseline scores correlated significantly with higher increase in Similarities ($p=0.04$) and Block design ($p<0.0001$) SD-scores.

Conclusions: Our study shows that GH-treatment prevents deterioration of certain cognitive skills in children with PWS on the short term and significantly improves abstract reasoning and visuospational skills, during four years of GH-treatment. Furthermore, children with a greater deficit had more benefit from GH-treatment.

Introduction

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from the absence of expression of paternally expressed genes, located on chromosome 15 at the locus q11-q13 caused by paternal deletion, maternal uniparental disomy (mUPD), imprinting errors, or by paternal chromosomal translocation[1]. PWS is characterized by a number of signs and symptoms, including muscular hypotonia, hypogonadism, short stature, obesity, psychomotor delay, neurobehavioral abnormalities, and cognitive impairment[2].

Long term continuous growth hormone (GH) treatment is an effective and safe treatment for children with PWS[3,4]. Previously we showed that one year of GH-treatment significantly improved mental and motor development in infants with PWS, compared to randomized controls[5]. There is little information about the effect of GH-treatment on cognitive development in infants with PWS[5,6] and no information about this effect in children with PWS above 3 years of age beyond a period of 6 months. Studies in children born small for gestational age (SGA) showed a significant increase in total IQ-score during 9 years of GH therapy, mainly due to increased scores in the performance area, compared to a Dutch reference population[7,8]. Recently, a study in adolescents with Down syndrome demonstrated a positive, although not significant, effect of GH-treatment on cognitive function in 12 GH-treated patients compared to 10 controls, 15 years after its discontinuation. GH-treatment was started at 7 years of age and continued for 3 years[9].

In our randomized controlled GH trial, we investigated the effect of GH-treatment on cognitive functioning in children with PWS. Furthermore, we studied cognitive functioning during 4 years of continuous GH-treatment in the PWS Cohort study and the effects of age at start of GH-treatment, serum IGF-I level, head circumference and genotype on cognitive functioning. We hypothesized that long term GH-treatment has a positive effect on total IQ score and especially on performance, i.e. non-verbal, abilities.

Patients and Methods

Patients

Fifty prepubertal children with PWS were included. All participants fulfilled the following inclusion criteria: (i) genetically confirmed diagnosis of PWS; (ii) age between 3 and 12 years (girls) or 14 years (boys) at start of study; (iii) bone age < 14 years (girls) or 16 years (boys); (iv) prepubertal at start of study, defined as Tanner breast stage ≤ 2 for girls and testicular volume < 4 ml for boys[10]. Children were regularly seen by a physiotherapist and speech therapist. The activity level of all children was standardized at 3 months prior to start of study. Compliance to exercise was evaluated by the research nurse in close collaboration with the physiotherapist and speech therapist.

Design

Randomized Controlled Trial

In April 2002, a multi center, Randomized Controlled Trial (RCT) was started in 50 children with PWS, investigating the effects of GH-treatment versus no GH-treatment on growth, body composition, activity level, and psychosocial development. After stratification for age and body mass index (BMI) children were randomly assigned to either the GH-treatment group or control group for 2 years.

Follow-up during 4 years of continuous GH-treatment

After the RCT, all children were treated with GH and followed in the Dutch PWS Cohort study. To investigate the effect of long term GH-treatment on cognition, we analyzed their data from start of GH-treatment until after 4 years of GH-treatment. Children who had been in the control group of the RCT were on average 2 years older at start of GH-treatment, than those who had been in the treatment group of the RCT. Two children dropped out of the Cohort study. One during the first year of GH-treatment because of family problems, and the other during the third year of GH-treatment, because of very high IGF-I levels, even with a low GH dose. The data of these children were included in our analysis until they dropped out.

Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY), dose 1.0 mg/m2/day, was administered sc once daily at bedtime in children of the treatment group during the RCT and in all children during the Cohort study. All children were naïve to GH-treatment at start of the RCT. During the entire study period, children were seen three-monthly for antropometric measurements by the PWS research team of the Dutch Growth Research Foundation, in collaboration with Dutch pediatric endocrinologists and pediatricians.
Cognitive functioning was measured biennially during the RCT and the follow-up during 4 years of GH-treatment. All cognitive measurements described in this study were performed in the Children's Hospital Erasmus MC-Sophia, by one psychologist experienced in testing children with PWS. The psychologist was blinded for the randomization. Missing values occurred because children were tested by their school psychologist during the same period, this happened in a maximum of 14% of children at each time point.
The study protocol was approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands. Written informed consent was obtained from the parents and from children older than 12 years and assent in children younger than 12 years of age.

Anthropometry

At baseline, and at 3-monthly intervals, anthropometric measurements were performed. Height was obtained using a Harpenden stadiometer. Weight was measured on an accurate scale. Height, weight, BMI, and head circumference were expressed as standard deviation

scores (SDS), adjusting for age and gender[11,12]. BMI and SDS of BMI, height, weight and head circumference were calculated with Growthanalyser, version 3.0, www.growthanalyser.org. A detailed description of anthropometric measurements was previously published[4].

Cognitive functioning

To assess intelligence, a short form of 4 subtests: Vocabulary, Similarities (verbal IQ subtests), Block design and Picture arrangement (performance IQ subtests) of the Wechsler Intelligence Scale for Children-Revised, Dutch version (WISC-R), was used in children over 7 years of age[13]. A short form of 4 subtests: Vocabulary, Similarities (verbal IQ subtests), Block design and Picture completion (performance IQ subtests) of the Wechsler Preschool and Primary Scale of Intelligence-Revised, Dutch version (WPPSI-R), was used for children with age below 7 years[14,15]. We used short forms because of the short attention span in children with PWS.
Good correlations have been found between the short-form IQ and the full-scale IQ both for the WISC-R the WIPPSI-R[16-18]. Wechsler (1974) showed that WPPSI IQ and WISC IQ are comparable in 6 years old children[19]. Three subtests: Vocabulary, Similarities and Block design were the same in the WISC-R and the WPPSI-R short forms. We therefore combined the results of these subtests in order to increase the sample size, and corrected for different type of test in our analyses.
Results of the Picture subtest of both WISC-R and WPPSI-R short forms, had to be analyzed separately for each short form, because this subtest differed between the WISC-R and the WPPSI-R (Picture arrangement in the WISC-R and Picture completion in the WPPSI-R short form). This resulted in too small numbers of patients per test and we could therefore not analyze the scores on this subtest.
The scores on all subtests were expressed as standard deviation scores, based on normalized standard scores (s-scores) with a mean of 10, ranging from 1 (-3 SDS) to 19 (+3 SDS), based on Dutch population data for the same age[13,15]. Total IQ (TIQ) score was calculated according to an equation based on Dutch outpatient population reference (TIQ = 45.3 + 2.91 x Vocabulary s-score + 2.50 x Block design s-score), as has been used in other studies[7,8].

Assay

Serum IGF-I levels were measured in one central laboratory using a immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California). The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age and gender dependency, IGF-I levels were transformed into SDS[20].

Data analysis

Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL).
Independent samples t-tests were used to compare the baseline characteristics between the GH-treated and the untreated controls.

To analyze the effect of GH-treatment during the RCT and the longitudinal study, Linear Mixed Models[21] was used with GH-treatment and time as factors (GH-treatment coded as: 1=GH-treatment group; 0=control group; time coded as 1=baseline; 2=after 2 years of study) in the RCT and time (time coded as: 1=baseline, 2=after 2 years of GH-treatment, 3= after 4 years of GH-treatment) as factor in the longitudinal study.
All subtest scores and TIQ scores were corrected for test type (WPPSI-R or WISC-R) age, gender and genotype. The effects of age at start of GH-treatment, gender, genotype, serum IGF-I level, head circumference and baseline scores on cognitive functioning during GH-treatment were determined by using these variables as factors (in case of nominal or ordinal variables) or covariates (in case of scale variables) in the model.

Results

Randomized Controlled Trial

Baseline characteristics

Fifty prepubertal children with PWS (21 boys, 29 girls) were included (Table 1). At start of the RCT, the mean (SD) age was 7.4 (2.5) years in the treatment group and 6.4 (2.2) years in the control group (p=0.2). Children had a baseline height and head circumference significantly below 0 SDS (P< 0.0001 for both) and low IGF-I levels. Twenty children (40%) had a deletion of chromosome 15q11-q13, 19 (38%) a maternal uniparental disomy, and 5 (10 %) an imprinting center defect. Positive methylation test was demonstrated in the remaining 6 (12%) patients, but the underlying genetic defect was not identified. There were no significant differences between the treated and untreated controls at baseline.

Table 1. Baseline characteristics at start of the RCT

	GH-treated	Untreated Controls	p-value
N	29	21	
Age (years)	7.4 [2.5]	6.4 [2.2]	0.165
Height SDS	-2.2 [1.4]	-2.4 [1.1]	0.540
Weight for height SDS	1.4 [1.3]	1.6 [1.0]	0.624
BMI SDS	1.2 [1.1]	1.4 [0.8]	0.439
Head circumference SDS	-0.7 [1.0]	-0.6 [0.8]	0.742
IGF-I SDS	-1.7 [1.1]	-1.9 [1.0]	0.504
Genetic cause			
- Deletion	13	7	
- UPD	9	10	
- ICD	1	1	
- unknown	3	3	

Data are expressed as mean [SD], SDS= standard deviation score,
UPD= uniparental disomy, ICD= imprintingscenter defect

Effect of GH-treatment versus no treatment on cognitive functioning

Figure 1 shows the mean subtest scores and mean TIQ score at baseline and after 2 years of study of GH-treated versus randomized controls with PWS. At baseline, there were no significant differences in subtest SD-scores and TIQ scores between the treatment group and the controls.

After 2 years of study mean SD-scores on all subtests and mean TIQ score remained similar compared to baseline in GH-treated children with PWS, indicating that the development of cognitive functioning and TIQ score of GH-treated children with PWS, measured by Similarities, Block design and Vocabulary subtests, took place at a similar pace as in healthy references. In untreated controls, however, mean subtest SD-scores and TIQ score were lower compared to baseline after 2 years of study. This decrease was significant for the Similarities and Vocabulary subtests (mean difference (CI 95%) between baseline and after 2 years of study -0.7 (-1.3 to 0.03) SDS, p=0.04 for Similarities and -0.7 (-1.3 to 0.07) SDS, p=0.03 for Vocabulary). Thus in untreated controls, there was a significant deterioration of certain cognitive skills and a non-significant deterioration of TIQ compared to healthy references.

After 2 years of study, we found no significant differences between the subtest scores and TIQ scores of the GH-treated children and the control group, probably due to the large variation in subtest scores and TIQ scores within each group.

Long term GH-treatment

Fifty pre-pubertal children, originally included in the RCT, were followed during 4 years of continuous growth hormone treatment. Their mean (SD) age at start of GH-treatment was 7.8 (2.4) years.

Cognitive functioning during long term GH-treatment

Figure 2 shows the longitudinal data during 4 years of GH-treatment. After 4 years of GH-treatment, mean SD-scores on the Similarities and Block design subtests were significantly higher than at baseline (mean difference (CI 95%) between baseline and after 4 years of GH-treatment +0.4 (-0.1 to 0.7) SDS, p=0.01 for Similarities and +0.3 (0.07 to 0.6) SDS, p=0.01, for Block design), indicating that long term GH-treatment had significantly improved abstract verbal reasoning (Similarities subtest) and visuospatial skills (Block design subtest) and had reduced the gap between children with PWS and healthy controls on these skills.

Mean SD-scores on the Vocabulary subtest remained unchanged. Thus, during long term GH-treatment, children with PWS developed their vocabulary at the same pace as healthy references. Mean estimated TIQ score improved 4 points during 4 years of GH-treatment. This improvement did not reach significance (p=0.2), probably due to the large variation in TIQ scores in children with PWS.

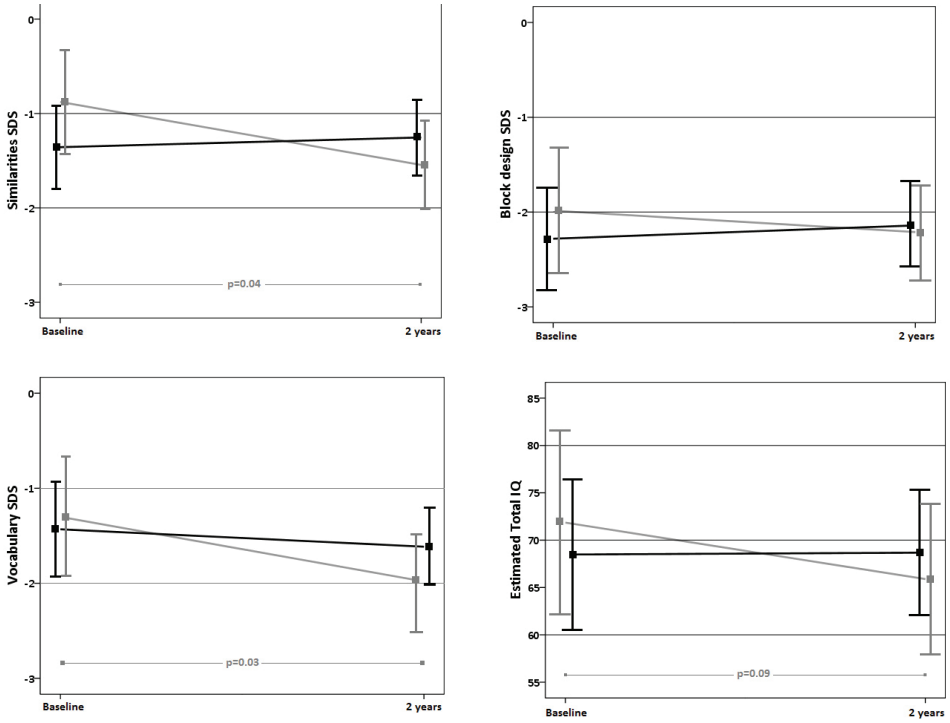


Figure 1.
Subtest scores and TIQ score during RCT in GH-treated and untreated children with PWS. This figure shows the mean SDS on the subtests Similarities, Block design and Vocabulary and the total IQ score and their CI95% at baseline and after 2 years of study in GH-treated children with PWS (black lines) and untreated controls (grey lines). P-values of differences between baseline and after 2 years of study in the untreated controls are indicated in grey in the figure.

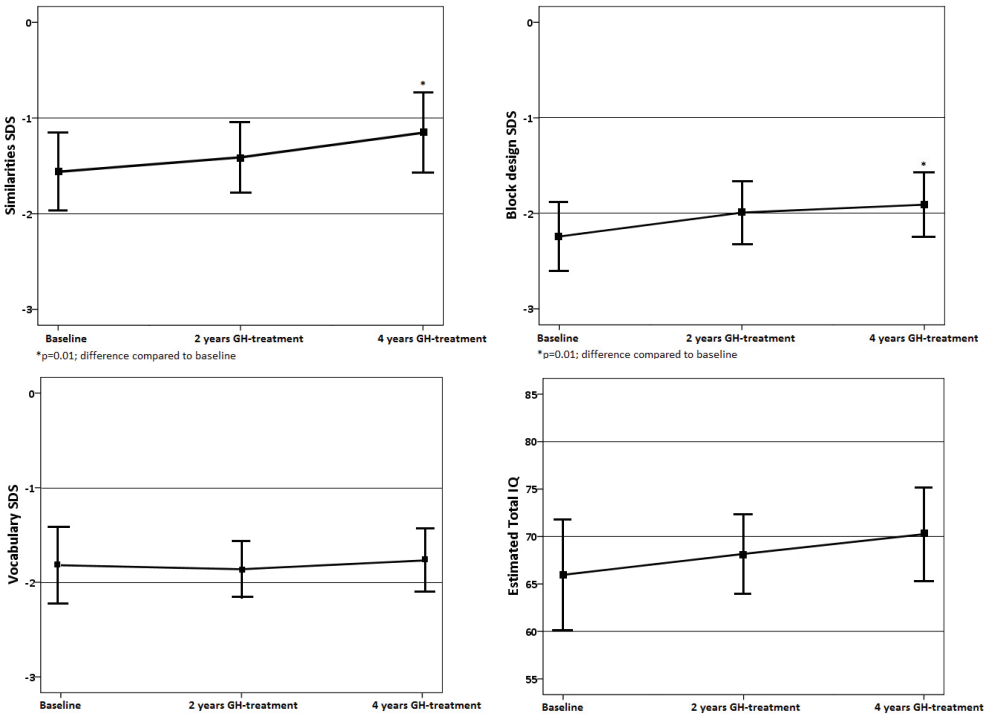


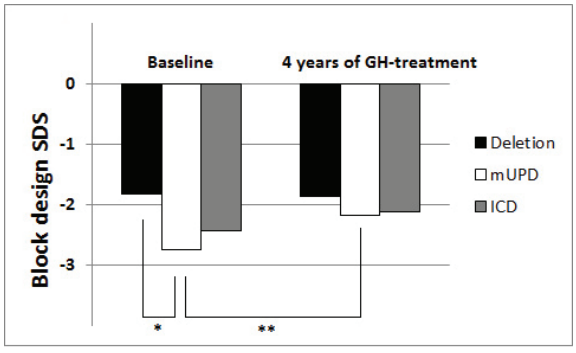
Figure 2.
Cognitive development during 4 years of continuous GH-treatment. This figure shows the mean SDS on the subtests Similarities, Block design and Vocabulary and the total IQ score and their CI95% during 4 years of continuous GH-treatment in children with PWS. Significant p-values of differences between baseline and after 4 years of GH-treatment are indicated in the figure. The scores on the subtests and the total IQ score at start of the longitudinal study is the mean total IQ score of all children at start of GH-treatment. Thus for children in the treatment group of the RCT, this is the total IQ score at start of the RCT, and for children in the untreated control group this is the total IQ score at the end of the RCT.

Influence of clinical and genetic characteristics on cognitive functioning

At baseline, we found significant effects of age at start of GH-treatment and head circumference SDS on Block design and Vocabulary SDS and estimated TIQ score. The younger the children were at baseline, the higher they scored on these subtests and the higher their TIQ scores (p=0.006, p=0.02 and p=0.005 for Block design SDS, Vocabulary SDS and TIQ scores, respectively). We found a comparable effect of age on Similarities SDS, but this did not reach significance (p=0.08). Children with a smaller head circumference SDS scored significantly lower on Block design and Vocabulary subtests and they had a significantly lower estimated TIQ scores than children who had a head circumference in the normal range compared to Dutch references (p=0.03, p=0.04, p=0.02 for Block design, Vocabulary and TIQ scores respectively). After 4 years of GH-treatment, the associations of age and head circumference SDS with cognitive outcomes were no longer significant.

Genotype had a significant effect on Block design SDS at baseline, also after correction for age at start of GH-treatment and head circumference SDS. Scores were significantly lower in children with an mUPD than in children with a deletion genotype (p=0.01). During 4 years of GH-treatment, children with mUPD showed a significant catch-up on the Block design subtest score compared to baseline (p=0.05) and after 4 years, the difference between deletion and mUPD genotype was no longer significant (Figure 3). Children with an ICD genotype showed a comparable pattern as children with mUPD, but the differences were not significant, due to the small number of children with an ICD. We found no effects of genotype on the other subtests or TIQ score.

There were no significant effects of serum IGF-I levels, height, weight, BMI and gender on any subtest scores or TIQ score, neither at baseline nor after 4 years of GH-treatment.



* p=0.01
** p=0.05

Figure 3.
Block design SDS per genotype at baseline and after 4 years of GH-treatment. This figure shows the mean SDS at baseline and after 4 years of continuous GH-treatment on the Block design subtest for children with deletion, mUPD and ICD separately. The significant p-values of the differences between the scores of children with different genotypes and between scores at baseline and after 4 years of GH-treatment are indicated in the figure.

Influence of baseline scores on cognitive functioning

We found a significant effect of baseline SD-scores on the changes in Similarities and Block design subtests scores from baseline to 4 years of GH-treatment. Children with the lowest scores at baseline, showed the highest catch-up in SD-scores (p=0·04 and p<0·0001 for Similarities and Block design respectively).

After correction for baseline scores, we found no effect of age at start of GH-treatment, gender, genotype, Δ head circumference (0-4 years) and Δ IGF-I (0-4 years) on the change in subtest and TIQ scores.

Discussion

Our study is the first to describe the effect of GH-treatment on cognitive functioning in children with PWS during a 2 year randomized controlled trial and during GH-treatment for 4 years. Our results demonstrate that GH-treatment prevents deterioration of certain cognitive skills on the short term and significantly improves abstract verbal reasoning and visuospatial skills during 4 years of GH-treatment. Children with an mUPD started off with significantly lower visuospatial skills, but showed a larger improvement on these skills after 4 years of GH-treatment than children with a deletion genotype. Furthermore, in children with lower cognitive functioning at baseline, GH-treatment had a greater effect on abstract verbal reasoning and visuospatial skills.

There is only one other study reporting the effect of GH-treatment on cognition in children (age > 3 years) with PWS[22]. The authors could not find an effect, but this might be due to their small patient number (n=12) and short period of GH-treatment (6 months). Studies in infants and adults with PWS did show an effect of GH-treatment on cognition[5,6,23], as did studies in children with growth hormone deficiency (GHD)[24], children born SGA[7,8], and, children with Down syndrome[9].

Our findings show that GH-treatment improves abstract verbal reasoning and visuospatial skills in children with PWS. This is in line with other studies showing that GH-treatment can influence spatial skills. In GH deficient adults, GH-treatment prevented spatial memory impairment[25] and in hypophysectomised rats, spatial performance was significantly better in GH-treated than in untreated animals[26].

It is known that GH receptors are located throughout the brain and that GH and IGF-I affect the genesis of neurons, astrocytes, endothelial cells and oligodendrocytes[27]. Recently, GH-treatment has been shown to induce cell genesis in the adult brain[28]. Furthermore, GH increases Connexin-43 expression (an ubiquitous biochemical marker for gap-junction formation in the brain) in the cerebral cortex and the hypothalamus, thereby enhancing cell to cell communication in the CNS[29]. We found no relation between cognitive functioning and IGF-I levels, and as far as we know, such a relationship has not been found in other studies

regarding the effect of GH-treatment on cognitive functioning in children. This suggests that the effect of GH on cognitive functioning in PWS might be paracrine in the brain. It has indeed been shown that GH has many effects in the central nervous system which are independent of serum IGF-I levels[30-32]. The effects of GH-treatment we demonstrated in our RCT and long term study in children with PWS in combination with the findings listed above, indicate plasticity of the human brain and the local activity of GH and IGF-I. Another explanation for the improved cognitive skills during GH-treatment could be that there is a relation with sleep related breathing disorders in children with PWS. A few years ago we studied sleep related breathing during GH-treatment in children with PWS[33] and found a non-significant decrease of the Apnea Hypopnea index after 6 months of GH-treatment. However, as we did not find any significant relation between cognition and the central or obstructive apnea index in untreated children with PWS in another study[34], it seems unlikely that the improved cognitive performance is the result of less sleep-related breathing disorders.

Before start of GH-treatment, older age had a significant negative effect on cognition. Also, untreated controls showed a deterioration of cognitive functioning. These findings indicate that cognitive functioning of untreated children with PWS deteriorates over time compared to healthy children. Our study shows that GH-treatment prevents this deterioration. As a result, the relation between age and cognition was no longer significant after 4 years of GH-treatment. In addition, we found that baseline scores had a significant effect on the change in scores on the Similarities and Block design subtests during GH-treatment. It appeared that GH-treatment was most beneficial for children with the lowest scores. Baseline scores were even more important for the degree of catch-up in cognitive skills than age at start of GH-treatment, gender, genotype, Δ head circumference or Δ IGF-I. Our findings might suggest that GH should best be administered at an early age to prevent deterioration of cognitive functioning in children with PWS, but that GH-treatment also induces a catch-up in cognitive skills in children with PWS who lag behind, even when they start at an older age.

Before start of GH-treatment, children with a deletion genotype scored better on the Block design subtest than children with mUPD. Comparable differences between genetic subtypes of PWS have been noted in other studies. The deletion genotype is associated with better performance IQ scores and the mUPD genotype with better verbal IQ scores[35]. A recent study investigated dorsal and ventral stream mediated visual processing in PWS[36]. They found that children with a deletion genotype, but not children with an mUPD genotype, had a relative strength in visual processing in the ventral stream. This might explain the difference in baseline Block design test scores between the children with a deletion and those with mUPD.

In most studies, patients with PWS are described as having a mild-to-moderate learning disability with a TIQ score below 70[23,35]. At baseline, the majority of the children in our study had indeed a TIQ score below 70, comparable to what is found in other studies. During long term GH-treatment, the mean TIQ score increased, although not significantly, from 66 at baseline to 70 after 4 years of GH-treatment, meaning that at this point only half of the children with

PWS in our study will be diagnosed mentally retarded according to the DSM-IV[37]. In another study investigating the effects of GH-treatment on cognition in children born small for gestational age after 2 and 8 years, authors found a significant increase of 0.7 SDS on the Block Design subtest and of 7 points on total IQ score over a period of 8 years of GH-treatment[8]. After 2 years, there was already a positive but non-significant effect of GH-treatment on cognition, which increased in the subsequent 6 years to the significant improvement at 8 years after start of GH-treatment.

These and our data might suggest that the improvement of cognitive functioning during GH-treatment becomes larger over time. As a result the clinical effects become clearer with longer duration of GH-treatment, especially if one keeps in mind that untreated children with PWS have a deterioration of cognitive functioning as shown in the RCT. The effects of GH-treatment on cognitive skills in children with PWS would probably have been more significant if we could have studied a much larger group. However, PWS is a rare disorder, and in the present study we evaluated a relatively large group of children with PWS.

Next to the increase in TIQ score, parents did not report an increase in behavioral problems or food seeking behavior during GH-treatment. They rather reported a decrease in problem behavior, but this needs further investigation.

Mean subtest scores on all subtests, except Block design, were in the normal range compared to healthy children (higher than -2 SDS) during the entire long term study. The mean score on Block design was below -2 SDS at baseline, but after 4 years of GH-treatment, the mean score on Block design SDS was also in the normal range. This points out that the increase in TIQ score was mostly due to the increase on Block design score, a performance test.

Our study shows that GH-treatment prevents deterioration of certain cognitive skills in children with PWS on the short term and significantly improves abstract verbal reasoning and visuospatial skills during 4 years of GH-treatment compared to a reference population. The more children lag behind, the more they benefit from GH-treatment. Based on our results, we conclude that GH-treatment in children with PWS is not merely an effective treatment for normalizing height and improving body composition, but has also a beneficial effect on their cognitive functioning.

Acknowledgements

We express our gratitude to all children and parents for their enthusiastic participation in our Dutch PWS Cohort study and acknowledge the work of P. M. C. C. van Eekelen, research nurse. We thank Pfizer Inc. for the independent research grant for the investigator initiated trial investigating the effects of GH-treatment in children with PWS.

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6

Beneficial effect of growth hormone treatment on health related quality of life in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study

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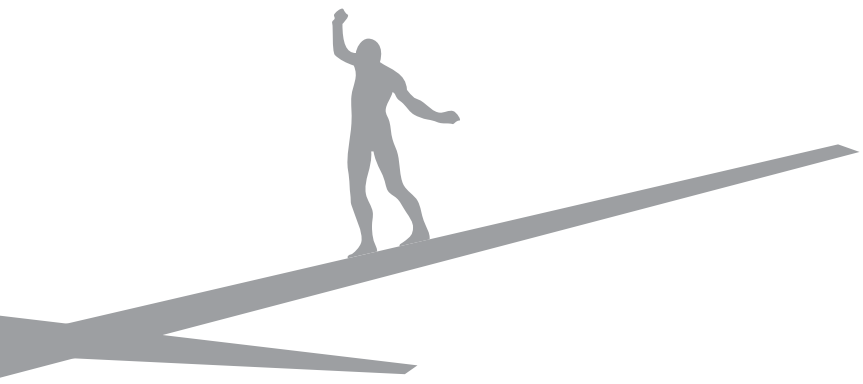
Submitted



Abstract

Objectives: Evaluate health-related-quality-of-life (HRQOL) in children with PWS and investigate the effect of GH-treatment.

Patients and Methods: Twenty-five pre-pubertal children, aged 6 to 14 years were studied in a randomized controlled GH trial (RCT) during 2 years, followed by a longitudinal study during 4 years of GH-treatment. HRQOL was measured biennially by self and parental reports on a generic questionnaire (DUX25), that contained four subdomains (Physical, Home, Social and Emotional) and a PWS-specific questionnaire (DUXPW).

Results: Children with PWS scored significantly higher than their parents on the Physical subdomain ($p<0.0001$) and on the DUXPW ($p=0.01$) and they rated their HRQOL higher than healthy, chronically ill, obese and growth impaired children, especially on the Physical subdomain ($p<0.05$ for all). During the RCT, HRQOL increased significantly compared to baseline according to GH-treated children on the Physical ($p=0.03$) and Home ($p=0.04$) subdomains and on the DUXPW total score ($p=0.002$) and according to parents on the DUX25 total score ($p=0.05$) and the Physical subdomain ($p=0.03$). Scores in untreated children with PWS decreased or remained similar to baseline. During 4 years of GH-treatment HRQOL increased significantly compared to baseline according to children on the DUXPW ($p=0.04$) and on the Home subdomain ($p=0.03$) and according to parents on the DUX25 ($p=0.02$) and Physical subdomain ($p=0.001$).

Conclusion: Children with PWS report a normal HRQOL. According to children and parents HRQOL increases during GH-treatment, in contrast to untreated children with PWS. This effect sustains during long-term treatment.

Introduction

Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder that occurs in approximately 1:25 000 live births[1,2] it results from the absence of expression of paternally expressed genes, located on chromosome 15 at the locus q11-q13 caused by paternal deletion, maternal uniparental disomy (mUPD), imprinting errors (ICD), or by paternal chromosomal translocation[3]. PWS is characterized by a number of signs and symptoms, including muscular hypotonia, hypogonadism, short stature, obesity, psychomotor delay, neurobehavioral abnormalities, and cognitive impairment[4].

Long-term continuous GH-treatment is an effective and safe treatment for children with PWS[5,6] and has beneficial effects on cognitive functioning in children with PWS[7].

Two studies showed that GH-treatment can improve the physical and psychological aspects of health related quality of life (HRQOL) in adults with PWS[8,9], but information about HRQOL or the effect of GH-treatment on HRQOL in children with PWS is scarce. HRQOL refers to the impact of health and illness on an individual's quality of life[10,11]. HRQOL can be measured by generic and disease-specific instruments[11]. Generic HRQOL instruments allow comparison with normative data and across disease populations. Disease-specific measurements include domains that are only valid for a specified condition, which maximizes content validity and result in a greater sensitivity and specificity.

To determine a child's HRQOL, the opinion of a child itself is probably most important[12]. But, as parents are generally quite able to determine their child's HRQOL, the method of a combination of child reports and parent reports seems to be most useful[12-14].

We hypothesized that children with PWS and their parents report lower HRQOL than a healthy reference population, and that HRQOL in GH-treated children with PWS will be higher compared to untreated controls and that this effect sustains during long-term GH-treatment. The first aim of this study was to describe HRQOL in untreated children with PWS. Because nowadays all children with PWS are treated with GH, our second aim was to investigate the effect of GH-treatment on HRQOL of children with PWS according to children and their parents.

Methods

Design

In April 2002, a multi-center, Randomized Controlled Trial (RCT) was started, investigating the effects of GH-treatment versus no GH-treatment on growth, body composition, activity level, and psychosocial development. After stratification for age and body mass index (BMI), children were randomly assigned to either the GH-treatment group or control group for 2 years.

Forty-nine prepubertal children were included in the RCT. All participants fulfilled the following inclusion criteria: (i) genetically confirmed diagnosis of PWS; (ii) age between 3 and 12 years (girls) or 14 years (boys) at start of study; (iii) bone age < 14 years (girls) or 16 years (boys); (iv) prepubertal at start of study, defined as Tanner breast stage < 2 for girls and testicular volume < 4 ml for boys(10). After the RCT, all children were treated with GH and followed in the Dutch PWS Cohort study. HRQOL was measured biannually in children and their parents during the RCT and during 4 years in the Cohort study.

Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY), 1.0 mg/m2/day, was administered sc once daily at bedtime in children of the treatment group during the RCT and in all children during the Cohort study. All children were naïve to GH-treatment at start of the RCT. During the entire study period, children were seen three-monthly for anthropometric measurements by the PWS team of the Dutch Growth Research Foundation, in collaboration with Dutch pediatric endocrinologists and pediatricians.

The study protocol was approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands. Written informed consent was obtained from the parents and children older than 12 years and assent in children younger than 12 years of age.

Measurements

Health related quality of life

HRQOL was measured by two questionnaires, the Dutch Children AZL/TNO Questionnaire Quality of Life short form (DUX25) and a PWS disease specific questionnaire, the DUX Prader Willi (DUXPW)[15]. These questionnaires were constructed for children aged 6 years and older. Therefore, 25 pre-pubertal children with PWS and their parents could be included in the present study (Figure 1). Children filled out their questionnaire separately from their parents, under supervision of a psychologist experienced with testing children with PWS. The psychologist was blinded for the randomization.

DUX25. This generic 25-item questionnaire measures different aspects of daily functioning of children and adolescents. The DUX25 total score is calculated by combining scores on all 25 items and contains four subdomains; Physical, Emotional, Social and Home functioning. Subdomain scores can be calculated for each subdomain. The scoring of the items is done on a 5-point Likert Scale by abstract faces with varying expressions (smiley's), ranging from very happy to sad. The DUX25 is available in a child and parent form[16-19].

DUXPW. Based on the generic DUX25, a PWS disease specific 18-item questionnaire, the DUXPW, was developed[20]. A DUXPW total score was calculated by combining the scores of all 18 questions. The scoring of the items was done in a similar way as in the generic DUX25.

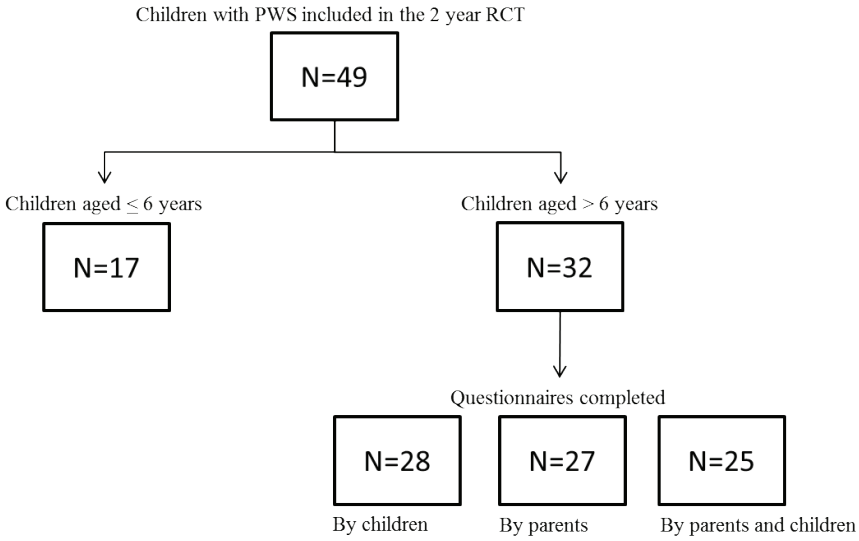


Figure 1.
This flow-chart presents the eligible patients for the present study. RCT: Randomized Controlled Trial.

Anthropometry and cognition

Height was assessed by a Harpenden stadiometer and weight was assessed on an accurate scale. BMI (kg/m2) was calculated and converted into SDS, according to Dutch references for age[21,22]. Head circumference was measured 3 times and the mean was used for analysis. Growth Analyser Version 3.0 was used to calculate BMI, BMI SDS and head circumference SDS (www.growthanalyser.org).

Cognition was measured by WPPSI-R or WISC-R depending on age, and a total intelligence quotient (IQ) was calculated as described before[7].

Assay

Serum IGF-I levels were measured in one central laboratory using a immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California). The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age and sex dependency, IGF-I levels were transformed into SDS[23].

Data analysis

Statistical analyses were performed with SPSS 17.0 (SPSS INC., Chicago, IL). Cronbach's α was calculated to determine internal consistency in the generic DUX25 and the DUXPW,

both tests were proven to be internally consistent (Cronbach's α >0.70)[15,24]. All scores were recoded into a scale from 1 (very bad) to 100 (very good), with the highest scores indicating a better quality of life. When one or two items per subdomain were missing, the value was replaced by the mean of the remaining items. Differences between children with PWS in the GH-treatment group and the control group, between children and parents and between children with PWS and various reference groups at baseline were measured by Mann Whitney U tests.

To analyze the effect of GH-treatment during the RCT and the longitudinal study, Linear Mixed Models for repeated measurements[25] was used with GH-treatment and time as factors (GH-treatment coded as: 1=GH-treatment group; 0=control group; time coded as 1=baseline; 2=after 2 years of study) in the RCT and time (time coded as: 0 = baseline, and 2 and 4 = after 2 and 4 years of GH-treatment, respectively) as factor in the longitudinal study.

The effects of age, gender, genotype, serum IGF-I level, anthropometric measurements and total IQ score on HRQOL during GH-treatment were determined by using these variables as factors (in case of nominal or ordinal variables) or covariates (in case of scale variables) in the model.

P-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics

Table 1 shows the baseline characteristics of the 25 children with PWS. Median (interquartile range, iqr) age was 7.9 (6.8 to 11.4) years. Eleven children had a deletion (44%), 9 an mUPD (36%) and 4 an ICD (16%). Positive methylation test was demonstrated in 1 child, but the underlying genetic defect was not identified. Height SDS and IGF-I levels were significantly below 0 SDS ($p<0.05$). Median (iqr) IQ was 69 (59 to 82), which corresponds to mild to moderate mental retardation. There were no significant differences between the children in the treatment and control group at baseline.

Baseline HRQOL

Children and parents

Table 2 shows baseline HRQOL according to the children with PWS and their parents. Children with PWS scored significantly higher than their parents on the Physical subdomain ($p<0.0001$) and on the DUXPW ($p=0.01$). Scores on the Physical subdomain were significantly correlated between children and parents ($r=0.46$, $p=0.03$). Scores on the DUX25 and the other subdomains did not significantly differ between children with PWS and their parents and there were no significant correlations between children and parents on the total score and the other subdomains of the DUX25 and the DUXPW.

Table 1. Baseline characteristics

	Total group	Treatment group	Control group	p-value[1]
N	25	15	10	
Age*	7.9 [6.8 to 11.4]	9.8 [7.3 to 11.6]	7.4 [6.6 to 9.0]	0.14
Gender (M/F)	10/15	7/8	3/7	
Genetic subtype				
-Deletion	11	7	4	
-mUPD	9	4	5	
-ICD	4	3	1	
-Unknown	2	1	0	
BMI SDS*	1.5 [0.6 to 2.6]	1.5 [1.1 to 1.9]	1.9 [0.1 to 2.6]	0.93
Head circumference SDS*	-0.6 [-1.3 to 0.0]	-0.7 [-1.4 to 0.1]	-0.6 [-1.2 to 0.4]	0.97
Height SDS*	-1.9 [-2.5 to -1.5]	-2.0 [-2.5 to -1.6]	-1.9 [-2.8 to -0.9]	0.75
IGF-I SDS*	-2.1 [-2.9 to -1.0]	-2.1 [-3.1 to -1.7]	-1.9 [-2.7 to -0.5]	0.48
IQ*[2]	69 [59 to 82]	65 [58 to 76]	76 [64 to 85]	0.40

* Data is presented as median [interquartile range]. [1]Difference between treatment and control group, [2]for details see reference[7]

Table 2. Baseline HRQOL in children with PWS according to the children and their parents

	Children	Parents	p-value
DUX25			
-Total	81 [72-92]	72 [67-84]	0.2
-Physical	88 [67-100]	58 [42-71]	<0.0001*
-Home	85 [70-100]	80 [75-90]	1.0
-Emotional	79 [71-93]	79 [68-88]	0.7
-Social	82 [75-96]	77 [65-88]	0.2
DUXPW	72 [64-83]	63 [59-73]	0.01*

*Significant difference between children and parents. Data is presented as median [interquartile range]

Children with PWS compared to reference groups

Table 3 shows HRQOL in children with PWS compared to healthy, chronically ill, obese and growth impaired children. Children with PWS scored significantly higher on the Physical subdomain compared to all groups ($p<0.05$ for all), on the Emotional subdomain compared to healthy, chronically ill and obese children ($p<0.01$ for all) and on the DUX25 total score and the Social subdomain compared to obese children ($p<0.0001$ for both).

Table 3. Baseline HRQOL in children with PWS at baseline compared to healthy children, children with a chronic illness and obese children.

	PWS	Healthy	Chronic illness	Obese	Growth impairment
N	25	1430	76	23	79
DUX25					
-Total	81 (13)	77 (13)	81 (13)	63 (10)[4]	77 (13)
-Physical	83 (17)	75 (18)[1]	79 (16)[3]	53 (20)[4]	74 (17)[3]
-Home	79 (17)	84 (15)	88 (13)	80 (15)	82 (16)
-Emotional	83 (16)	73 (16)[2]	78 (15)[3]	53 (15)[4]	73 (16)
-Social	79 (16)	77 (13)	80 (15)	68 (09)[4]	77 (15)

Data is presented as mean(SD). [1]$p<0.05$, [2]$P=0.002$, [3]$P=0.01$, [4]$p<0.0001$: significant differences between children with PWS and healthy children, children with congenital chronic illness, obese children and children with a growth impairment.

HRQOL during the Randomized Controlled GH Trial

According to children

Figure 2A shows scores on the DUX25 and its subdomains and on the DUXPW reported by the children in GH-treated compared to untreated controls with PWS during the 2-year RCT.

At baseline, scores on the DUX25 and its subdomains were not significantly different between the two groups. However, scores on the DUXPW were significantly lower in the GH-treatment group than children in the control group (mean difference (CI 95%) was 24 (14-34), $p<0.0001$).

After 2 years, scores on the Physical subdomain were significantly higher in the GH-treatment group compared to the control group (mean difference (CI 95%) 19 (3-35), $p=0.03$), while the lowest scores in children with PWS in the control group decreased below the lower limit of normal. There was a trend towards a higher score in GH-treated children compared to controls on the DUXPW (mean (CI 95%) difference between GH-treated children and untreated controls 7 (3-16), $p=0.18$).

Compared to baseline, GH-treated children with PWS scored significantly higher on the Home subdomain and the DUXPW after 2 years of study (mean (CI 95%) difference 9 (0-18), $p=0.04$ for the Home subdomain and 13 (5-20), $p=0.002$ for the DUXPW) and there were trends towards significantly higher scores in GH-treated children after 2 years compared to baseline on the DUX25 and the Emotional subdomain (mean (CI 95%) difference 6 (-1 to 12), $p=0.09$ and 7 (-4 to 17), $p=0.19$ for the DUX25 and the Emotional subdomain, respectively). In

untreated controls with PWS, scores on the DUXPW had significantly declined during 2 years of study (mean (CI 95%) difference between baseline and after 2 years -18 (-28 to 7), p=0.002) and there was a trend towards a significant decline on the Physical subdomain (mean (CI 95%) difference -18 (-38 to 2), p=0.08).
There were no differences between GH-treated and untreated children with PWS between baseline and after 2 years in scores on the Social subdomain.

According to parents
Figure 2B shows scores on the DUX25 and its subdomains and on the DUXPW reported by parents in GH-treated compared to untreated controls with PWS during the 2-year RCT.
At baseline, there were no significant differences between the two groups.
According to parents, GH-treated children showed a significant increase in HRQOL during 2 years of GH on the DUX25 and the Physical subdomain (mean (CI 95%) difference 9 (0-18), p=0.05 and 13 (1-25), p=0.03 for the DUX25 and Physical subdomain, respectively), while the scores on the Physical subdomain decreased to below the lower limit of normal in most of the untreated children, although this did not reach significance (mean (CI 95%) difference -10 (-24 to 5), p=0.19). There was a trend towards a significant increase on the DUXPW in GH-treated children (mean (CI 95%) difference 4 (-2 to 11), p=0.16). According to parents, there were no significant differences between GH-treated children and untreated controls or between baseline and after 2 years on the Home, Emotional, and Social subdomains.

HRQOL during 4 years of continuous GH-treatment

According to children
Figure 3A shows HRQOL in children with PWS according to children during 4 years of continuous GH-treatment.
Scores on the Physical subdomain increased significantly during 2 years of GH-treatment (mean (CI 95%) difference compared to baseline 9 (0-17), p=0.04) and stabilized thereafter.
After 4 years of GH-treatment, scores on the Home subdomain and DUXPW had significantly increased compared to baseline (mean (CI 95%) difference 9 (0-17), p=0.03 and 7 (0-14), p=0.04 for the Home subdomain and the DUXPW, respectively). Scores on the DUX25 and the Emotional and Social subdomains remained comparable to baseline.
During 4 years of GH-treatment, IQ had a significant effect on the DUX25 total score (β=0.51, p=0.001) and on the Physical (β=0.40, p=0.03), Home (β=0.43, p=0.01), Emotional (β=0.61, p=0.01) and Social (β=0.57, p=0.01) subdomains. IGF-I SDS had a significant effect on the Social subdomain (β=7.8, p=0.003). There were no significant effects of age, BMI SDS or head circumference SDS on the DUX25 total score and scores on the subdomains and no significant differences between boys and girls or genotypes. None of the parameters had a significant effect on the DUXPW total score.

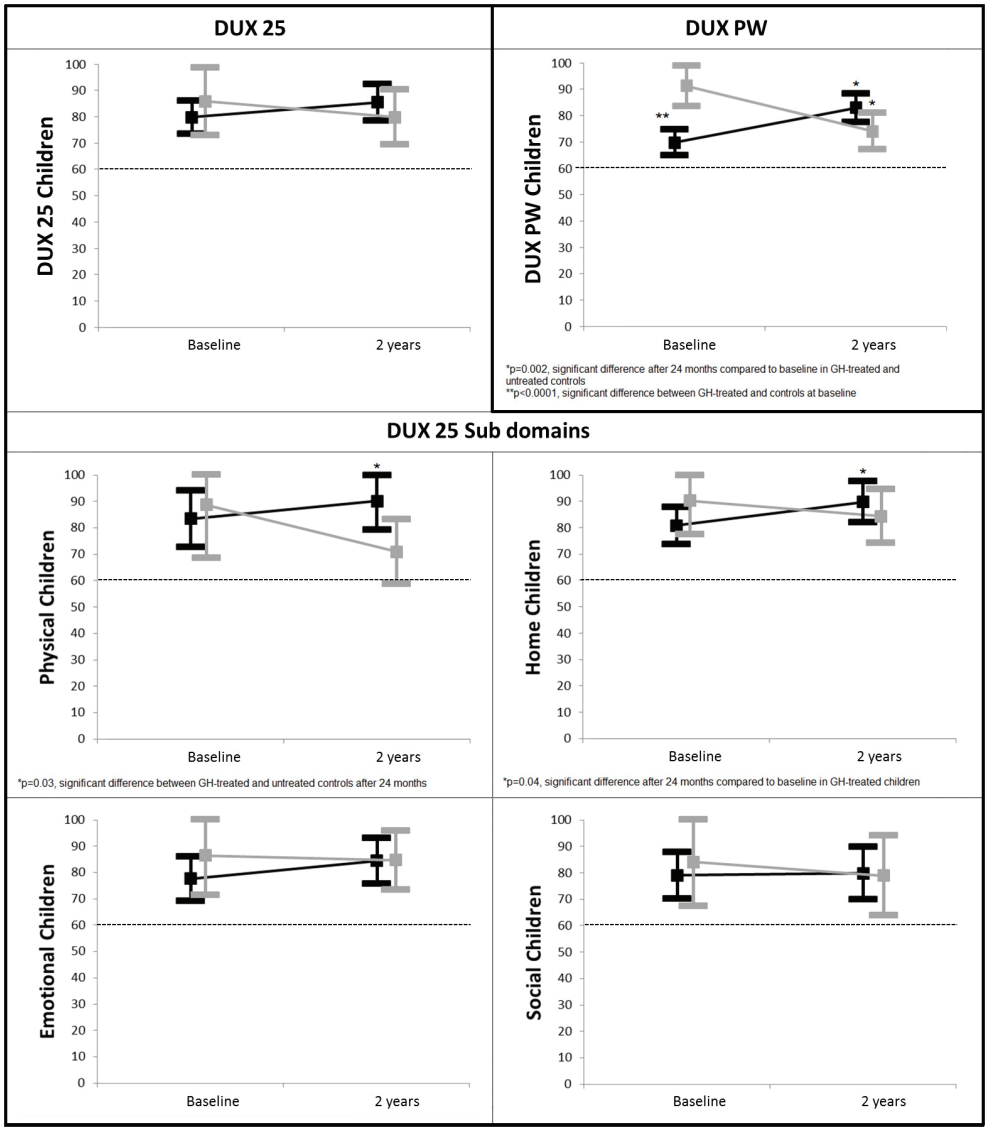

DUX 25
DUX PW
DUX 25 Children
DUX PW Children
Baseline
2 years
*p=0.002, significant difference after 24 months compared to baseline in GH-treated and untreated controls
**p<0.0001, significant difference between GH-treated and controls at baseline
DUX 25 Sub domains
Physical Children
Home Children
*p=0.03, significant difference between GH-treated and untreated controls after 24 months
*p=0.04, significant difference after 24 months compared to baseline in GH-treated children
Emotional Children
Social Children

Figure 2A.

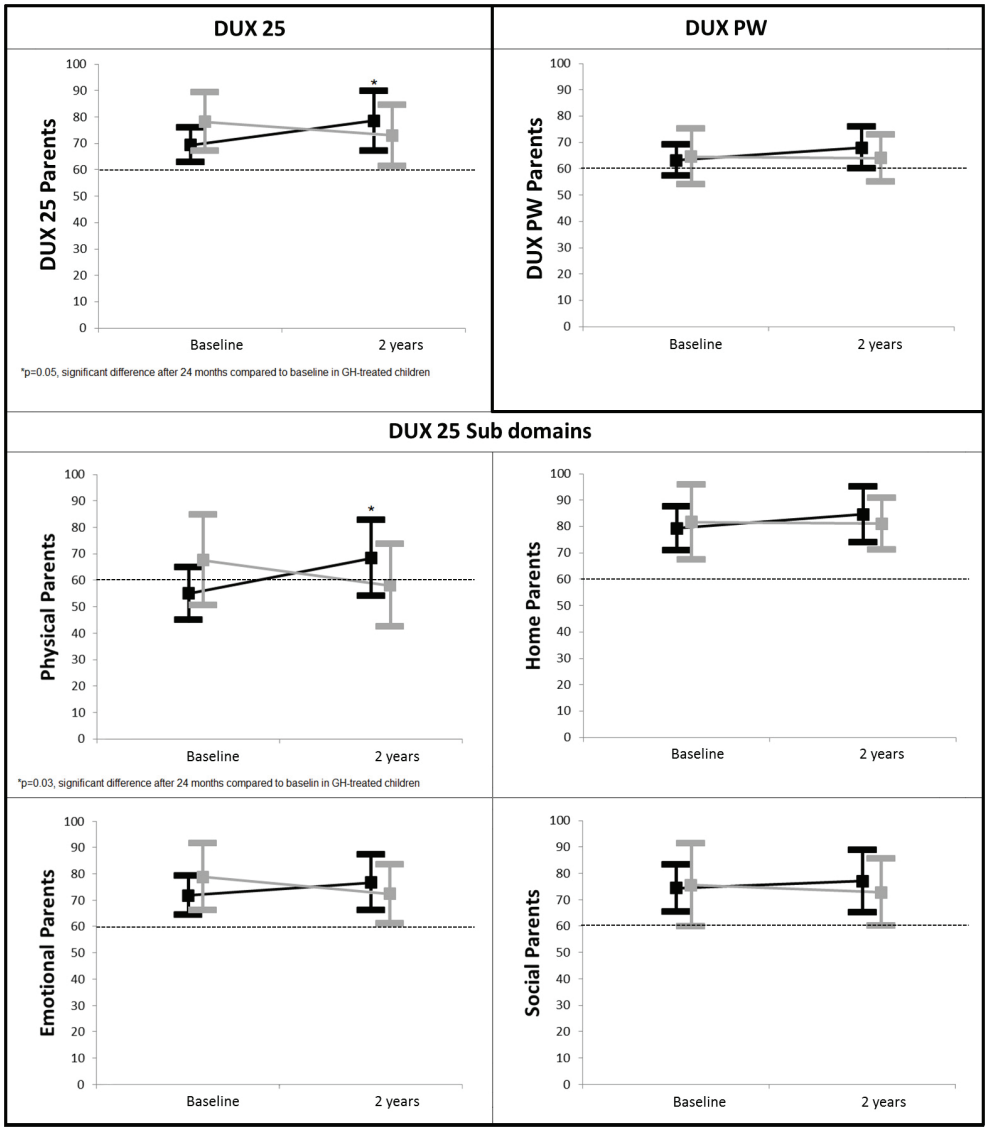


Figure 2B.

Figure 2A and 2B.
This figure presents mean scores and their CI 95% on the DUX25, subdomains of the DUX25, and DUXPWS according to children (Figure 2A) and their parents (Figure 2B) in GH-treated (in black) compared to untreated (in gray) children with PWS at baseline and after 2 years of study during the RCT. Scores on the DUX25, the DUXPW and all subdomains are corrected for age. Significant differences between GH-treated and untreated children and between baseline and after 2 years of study are indicated in the figure. The lower limit of normal is indicated by a dashed line.

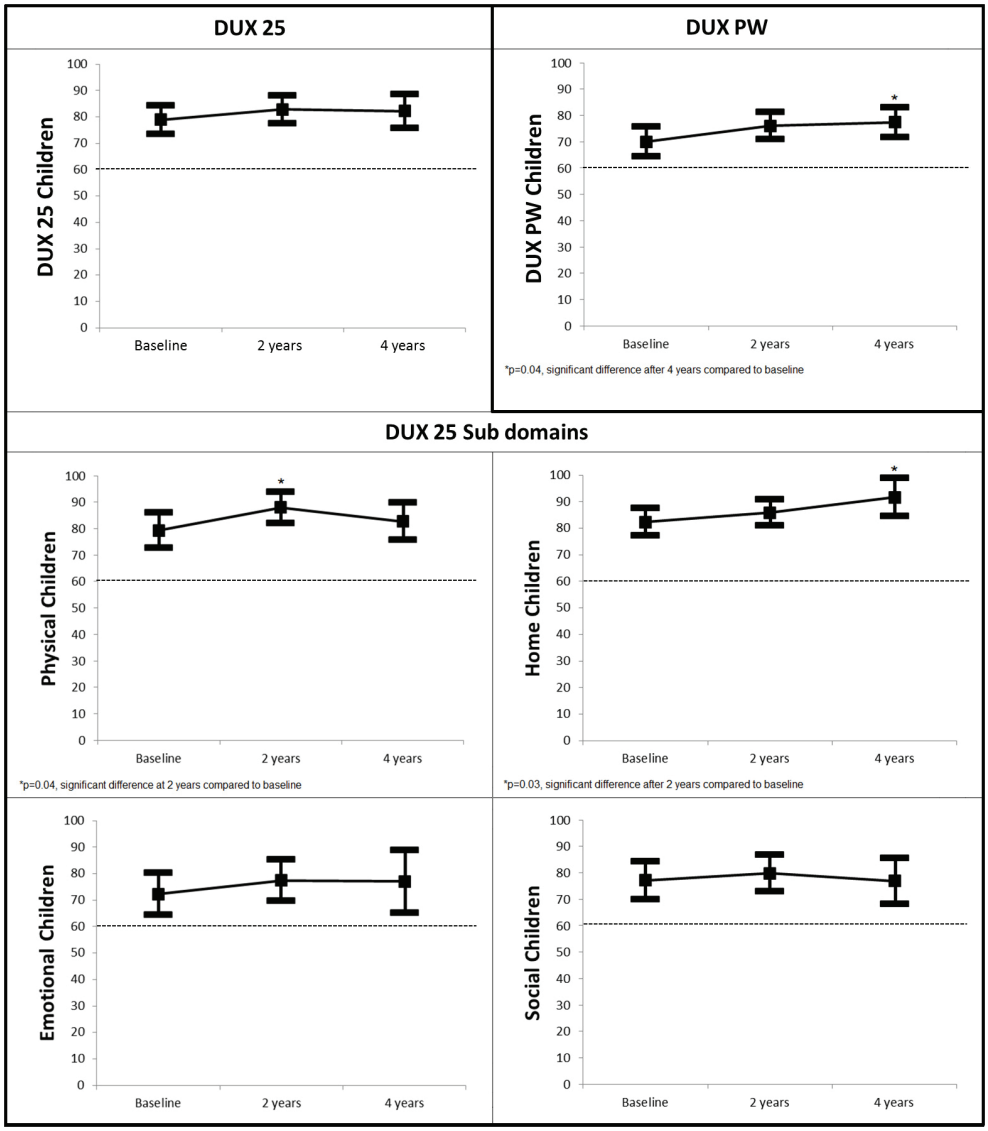

DUX 25
DUX 25 Children
100
90
80
70
60
50
40
30
20
10
0
Baseline
2 years
4 years
DUX PW
DUX PW Children
*
*p=0.04, significant difference after 4 years compared to baseline
DUX 25 Sub domains
Physical Children
*
*p=0.04, significant difference at 2 years compared to baseline
Home Children
*
*p=0.03, significant difference after 2 years compared to baseline
Emotional Children
Social Children

Figure 3A.

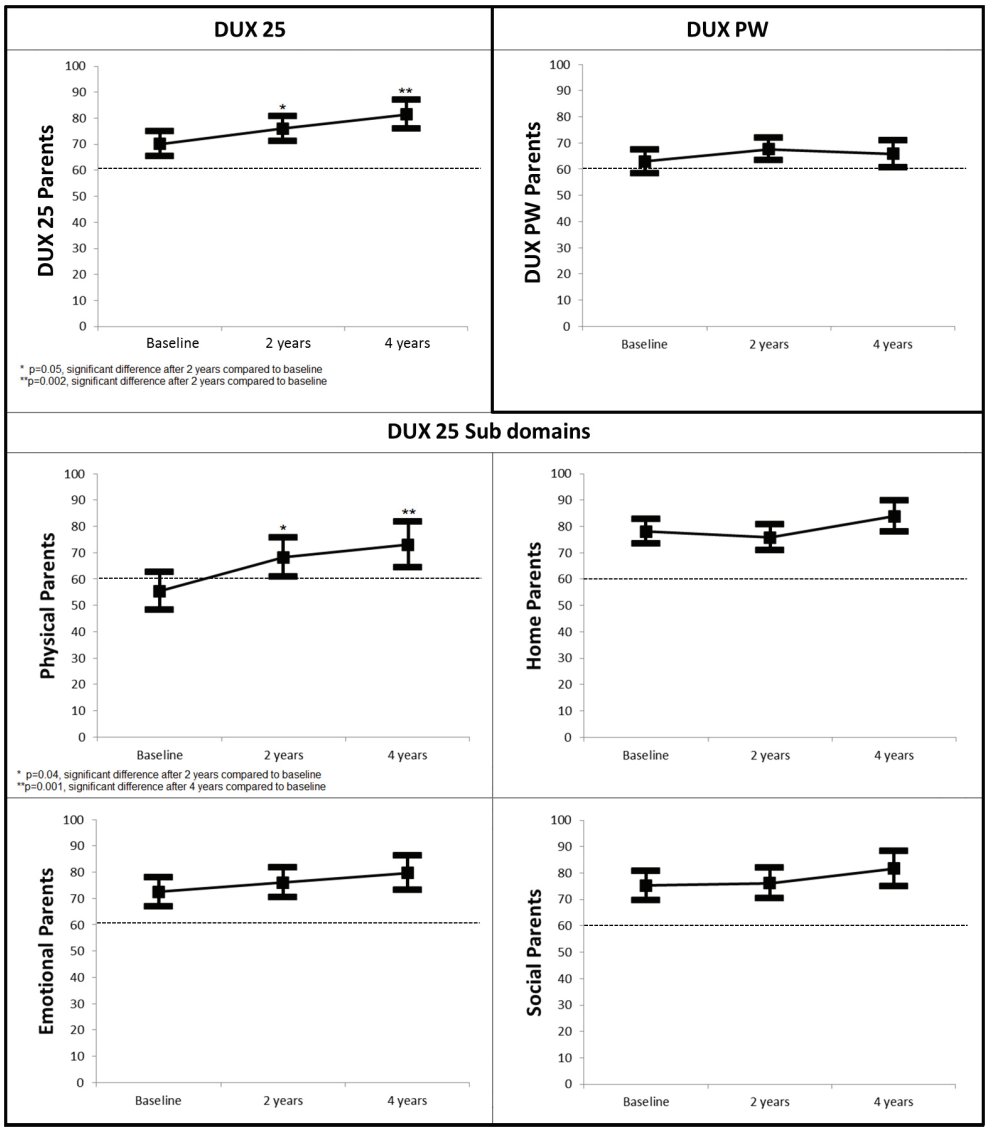


Figure 3B.

Figure 3A and 3B.
This figure presents mean scores and their CI 95% on the DUX25, subdomains of the DUX25, and DUXPWS according to children (Figure 3A) and their parents (Figure 3B) at baseline and during 4 years of continuous GH-treatment. Scores on the DUX25, the DUXPW and all subdomains are corrected for age. Significant differences between baseline and after 2 and 4 years of GH-treatment are indicated in the figure. The lower limit of normal is indicated by a dashed line.

According to parents

Figure 3B shows HRQOL in children with PWS according to parents during 4 years of continuous GH-treatment.

According to parents, HRQOL increased during GH-treatment on the DUX25 total score and all subdomains, but not on the DUXPW, which remained comparable to baseline.

After 2 years of GH-treatment, the DUX25 total score and scores on the Physical subdomain had significantly increased compared to baseline (mean (CI 95%) difference after 2 years compared to baseline 5 (0-12), p=0.05 and 12 (4-22), p=0.005 for the DUX25 and the Physical subdomain, respectively) and these continued to increase significantly during 4 years of GH-treatment (mean (CI 95%) difference after 4 years compared to baseline 11 (4-18), p=0.002 and 18 (7-28), p=0.001 for the DUX25 and the Physical subdomain, respectively). Scores on the Physical subdomain were below the lower limit of normal in the majority of children at start, but were significantly higher than the lower limit after 4 years of GH-treatment ($p<0.05$).

After 4 years of GH-treatment, scores on the Home functioning, Emotional and Social subdomains had all increased compared to baseline, albeit, not significantly (p=0.12, p=0.08 and p=0.11, respectively).

According to parents, scores were significantly inversely related with age on the DUX25, all subdomains and the DUXPW (β=-3.5, $p<0.0001$ for the DUX25, β=-4.7, p=0.002 for the Physical subdomain, β=-2.9, p=0.006 for the Home subdomain, β=-3.7, p=0.001 for the Emotional subdomain, β=-2.8, p=0.02 for the Social subdomain and β=-1.9, p=0.03 for the DUXPW score). Parents of children with a deletion rated their children's HRQOL higher than parents of children with other genotypes on the DUX25, and the Home and Social subdomains (for the DUX25: mean (CI 95%) difference between children with a deletion and an mUPD 11 (0-22), p=0.04), and between children with a deletion and an ICD 28 (7-50), p=0.01; for the Home subdomain: mean (CI 95%) difference between children with a deletion and an ICD 30 (7-54), p=0.013 and for the Social subdomain: mean (CI 95%) difference between children with a deletion and an mUPD 15 (2-28), p=0.003). Children with a higher BMI SDS, had a significantly lower score on the Physical subdomain than children with lower BMI SDS (β=-6.9, p=0.04) and children with higher IGF-I SDS had a significantly higher score on the DUXPW (β=3.8, p=0.03).

There were no significant effects of IQ, head circumference SDS or gender on HRQOL of children with PWS according to parents.

Discussion

Our study is the first to investigate HRQOL in children with PWS according to both children and parents. In addition it assessed the effects of GH-treatment on HRQOL in children with PWS during 2 years in an RCT and during 4 years of continuous GH-treatment. We show that

prior to start of GH-treatment, children with PWS rate their HRQOL higher than their parents and than children with various other diseases. Furthermore, according to children and parents, HRQOL improved significantly in GH-treated children with PWS, while it decreased or remained similar to baseline in untreated controls with PWS. This positive effect of GH-treatment on HRQOL in children with PWS sustained during long-term GH-treatment, according to both children and parents.

Children with PWS rated their HRQOL higher than children with other diseases and even healthy children, already at baseline. In a study investigating HRQOL in 9 untreated children with PWS (age range 5-14 years)[26], HRQOL was found to be lower in children with PWS compared to a reference population. However, in that study HRQOL was rated by parents only. A possible explanation for the high HRQOL reported by children in our study is the intelligence level of children with PWS. Mild to moderate intellectual impairment is common in patients with PWS[27], while the HRQOL reference values are based on children without intellectual impairment. Comparison of HRQOL in children with PWS to a normal reference population may be less reliable due to the inequality in intelligence. On the other hand, our results also show that the child's IQ has a significant positive relation with the child-reported HRQOL, which means that children with PWS with an IQ level closer to normal reported a higher HRQOL than those with a lower IQ. This indicates that children with PWS consider themselves quite happy children, despite the difficulties that go with the syndrome.

The improvement of HRQOL during GH-treatment is in line with findings from another study, in which HRQOL was assessed during 2 years of GH-treatment in 13 adults with PWS[28]. Two years of GH-treatment improved HRQOL, both according to patients and parents. A Finnish GH study in 20 children with PWS (age at baseline 2.0 to 10.3 years) investigated HRQOL after 10 years[29] and found a lower HRQOL in patients with PWS than in a reference population after 10 years of GH-treatment. However, in that study, there was no untreated control group and HRQOL was not measured at baseline and only reported by children and not by their parents. Thus, the effects of GH-treatment could not be analyzed in that study. Improvement of HRQOL during GH-treatment is in line with findings in short children born small for gestational age when measured by short stature-specific questionnaires[30,31].

At baseline, children with PWS reported significantly higher scores on the Physical subdomain and the DUXPW scale than their parents. In addition, during long-term GH-treatment, parents reported a significant negative effect of age on HRQOL in children with PWS, while this was not found in children. Disagreement between parent and child reports on HRQOL in children was also found in other studies in children with other disorders than PWS[32,33]. Parents may be negatively influenced by the burden of care-giving, their own well-being and concerns[34] and children might be positively influenced by adaptation to their illness[35].

There were no effects of GH-treatment on HRQOL in the Social subdomain according to children and parents. Social impairment is typically found in patients with PWS and many of their social behaviors appear to be on the same continuum of social deficits found in autism

spectrum disorder (e.g., social withdrawal, poor peer relationships, lack of empathy)[36]. Both clinical and research reports suggest that children with PWS exhibit poor peer relationships, a lack of friends, immaturity, weakness in coping skills, and a preference for solitary activities[37-40]. This typical behavior is therefore not likely to change. However, despite the fact that social impairment is a common finding in PWS, HRQOL on the Social subdomain was not extremely low according to patients and parents, suggesting that both children and parents seem to cope with this social impairment.

Children with a deletion had a higher HRQOL than children with an mUPD, according to parents. This might be related to the fact that psychiatric problems are also more common in children with an mUPD than in children with a deletion genotype[41].

Not surprisingly, a higher BMI SDS was associated with lower HRQOL on the Physical subdomain, as rated by parents. However, this association was not found in self-reports of the children. They reported a higher HRQOL on the Physical subdomain than their parents, indicating that children with PWS have little sense of the consequences of their weight on their Physical health.

Our study shows that children with PWS report a normal HRQOL of life. Both children and parents report an increase in HRQOL during GH-treatment, while this increase was not found in the randomized untreated children with PWS. During long-term GH-treatment, HRQOL continued to increase even further. Based on our results, we conclude that GH-treatment in children with PWS is not merely an effective treatment for normalizing height and improving body composition, but has also beneficial effects on their HRQOL.

Acknowledgements

We express our gratitude to all children and parents for their enthusiastic participation in our Dutch PWS Cohort study and acknowledge the work of P. M. C. C. van Eekelen, research nurse, Zyrhea C.E. Troeman, psychologist. We thank all pediatricians for their participation, A. A. E. M. (Janielle) van Alfen-van der Velden, Barto J. Otten, Joost Rotteveel, Roelof J. H. Odink, G. C. B. (Karen) Bindels-de Heus, Mariette van Leeuwen, Danny A. J. P. Haring, Wilma Oostdijk, Gianni Bocca, E. C. A. Mieke Houdijk, A. S. Paul van Trotsenburg J. J., Gera Hoorweg-Nijman, Hester van Wieringen, René C. F. M. Vreuls, Petr E. Jira, Eelco J. Schroor, Evelyn van Pinxteren-Nagler, Jan Willem Pilon, L. (Bert) Lunshof. We further thank Pfizer Inc. for the independent research grant for the investigator initiated trial investigating the effects of GH-treatment in children with PWS.

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Behavior in children with Prader-Willi syndrome before and during growth hormone treatment: a randomized controlled trial and longitudinal study

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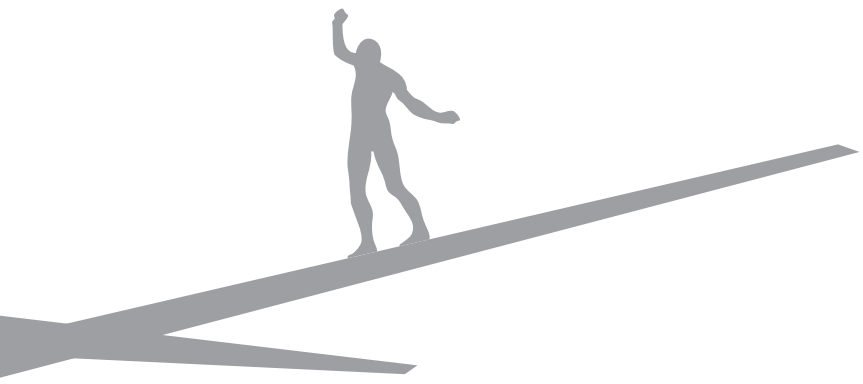
Submitted



Abstract

Background: Information on behavior of children with Prader-Willi syndrome (PWS) and the effect of GH-treatment is scarce. Parents report less problem behavior during GH-treatment.

Objectives: To investigate behavior in children with PWS and the effects of GH-treatment.

Patients and Methods: Fifty pre-pubertal children, aged 3.5 to 14 years were studied in a randomized controlled GH trial during 2 years (RCT), followed by a longitudinal study during 4 years of GH-treatment. Behavior was measured annually by the Developmental Behavior Checklist for children with mental disability (DBC) and a Dutch questionnaire to evaluate social behavioral problems in children, the Children's Social Behavior Questionnaire (CSBQ).

Results: Problem behavior measured by the DBC in children with PWS was similar compared to references with a comparable mental retardation, but scores on the 'Communication disorders' and 'Social disabilities' subscales were relatively high compared to the DBC total score ($p \leq 0.001$ for both). Problem behavior measured by the CSBQ was higher compared to healthy references. Children with PWS scored significantly higher than 0 SDS on the 'Not tuned', 'Contact', 'Orientation', 'Stereotyped' and 'Changes' subscales ($p<0.01$ for all subscales) and significantly higher than +1 SDS on the 'Social understanding' subscale and the CSBQ total score ($p<0.0001$ and $p=0.01$, respectively). Behavioral problems measured by the DBC and the CSBQ increased significantly with age. After correction for age, there were no significant effects of GH-treatment during the RCT and 4 years of GH-treatment.

Conclusions: Children with PWS showed similar problem behavior than a reference population with a comparable mental retardation and more pervasive developmental disorders compared to a healthy population. Problem behavior increases with age in both GH-treated and untreated children with PWS. In contrast to our expectations, our study showed no effect of GH-treatment on behavioral problems in children with PWS.

Introduction

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from the absence of expression of paternally expressed genes, located on chromosome 15 at the locus q11-q13 caused by paternal deletion, maternal uniparental disomy (mUPD), imprinting errors, or by chromosomal translocation[1]. PWS is characterized by a number of signs and symptoms, including muscular hypotonia, hypogonadism, short stature, obesity, psychomotor delay, neurobehavioral abnormalities, and cognitive impairment[2].

During the first years of life, children with PWS are described as friendly, easy going and affectionate[3]. However, between the ages of 2 and 4 years, simultaneously with the change in eating pattern, children with PWS show significant maladaptive behavioral and emotional characteristics including temper tantrums, inappropriate social behavior, automutilation (skin picking), stubbornness, mood lability, impulsivity, argumentativeness, depression and anxiety[4-11]. Obsessive-compulsive symptoms and autism spectrum disorder are also known in children with PWS[10,12,13].

Long-term continuous growth hormone (GH) treatment is an effective and safe treatment for children with PWS[14,15]. GH-treatment has beneficial effects on antropometrics, body composition, cognition, activity level and motor development, but little is known about the effect of GH-treatment on behavioral problems. Two studies described the effect of GH-treatment on behavior in PWS. One study included 12 children (age range 4.5 to 14.6 years) in a cross-over design who were randomized to either GH or placebo intervention for 6 months[16]. The second study included 54 children (age range 4 to 16 years) who were randomized to GH-treatment or control group for 1 year[17]. Both studies could not find behavioral differences between GH-treatment and control groups when they used structured questionnaires. This lack of effect could be due to either a lack of GH effect, or because the study periods were too short to find a significant effect.

In the present study, we investigated the effect of GH-treatment on behavior in 50 children with PWS during a 2-year randomized controlled GH trial (RCT) and during 4 years of continuous GH-treatment with the Developmental Behavior Checklist for children with mental disability (DBC) and a Dutch questionnaire to evaluate social behavioral problems in children, the Children's Social Behavior Questionnaire (CSBQ).

Based on clinical experience and parental reports during GH-treatment, we hypothesized a decrease in problem behavior in GH-treated compared to untreated children with PWS and sustainment of this effect during 4 years of continuous GH-treatment.

Patients and Methods

In April 2002, a multi-center, Randomized Controlled Trial (RCT) was started, investigating the effects of GH-treatment versus no GH-treatment on growth, body composition, activity level, and psychosocial development. After stratification for age and body mass index (BMI) children were randomly assigned to either the GH-treatment group or control group for 2 years. Fifty children were included in this study. All participants fulfilled the following inclusion criteria: (i) genetically confirmed diagnosis of PWS; (ii) age between 3 and 12 years (girls) or 14 years (boys) at start of study; (iii) bone age < 14 years (girls) or 16 years (boys); (iv) prepubertal at start of study, defined as Tanner breast stage < 2 for girls and testicular volume < 4 ml for boys[18]. After the RCT, all children were treated with GH and investigated in the Dutch PWS Cohort study.

Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY), dose 1.0 mg/m2/day, was administered sc once daily at bedtime in children of the treatment group during the RCT and in all children during the Cohort study. All children were naïve to GH-treatment at start of the RCT. During the entire study period, children were seen three-monthly for anthropometric measurements by the PWS team of the Dutch Growth Research Foundation, in collaboration with Dutch pediatric endocrinologists and pediatricians.

Behavior was measured annually in the 50 pre-pubertal children with PWS during the RCT and in the same group of children during 4 years in the Cohort study. Two children dropped out of the Cohort study. One during the first year of GH-treatment because of family problems, and the other during the third year of GH-treatment, because of very high IGF-I levels, even with a low GH dose. The data of these children were included in our analysis until they dropped out.

The study protocols were approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands. Written informed consent was obtained from the parents and from children older than 12 years and assent in children younger than 12 years of age.

Anthropometry and cognition

Height was assessed by a Harpenden stadiometer and weight was assessed on an accurate scale. BMI (kg/m2) was calculated. Height and BMI were converted into standard deviation scores (SDS), according to Dutch references for age[19,20] Growth Analyser Version 3.0 software was used to calculate BMI, height, weight for height and BMI SDS (www.growthanalyser.org). Cognition was measured by WPPSI-R or WISC-R, depending on age, and an intelligence quotient (IQ) was calculated as described earlier[21].

Behavior

Behavior was measured by two parent questionnaires, the DBC[22] and CSBQ[23]. The questionnaires were completed by the main caregiver. The questionnaires could not be completed for

all children because of language or logistic problems.

DBC. This is a 96-items checklist for all levels of mental disabilities, completed by parents or caregivers to assess emotional or behavioral problems over the last 6 months in children aged 4–18 years. It contains five subscales: Disruptive Antisocial, Self Absorbed, Communication disorder, Anxiety, and Social disabilities. A total problem behavior score was calculated by combining all subscale scores. Subscale scores were converted to SDS according to age and gender. We used reference data for Dutch children with mental disability (IQ between 59 and 70), as we considered the IQ of this reference population to resemble children with PWS. The questionnaire employs a 3-point rating scale (0=not true, 1=somewhat or sometimes true, 2=very true or often true) for each item. A higher score on the test, implies more problem behavior.

Items that belonged to the Anxiety subscale were included when we measured the total problem behavior score, but these scores were left out when we analyzed the subscales individually. We considered the items in this subscale not representative for anxiety behavior in children with PWS because some items in this subscale were food related, for example 'lack of appetite' and 'being a picky eater'.

CSBQ. The CSBQ is a 49 item parent questionnaire for healthy Dutch children aged 4-18 years, which aims to assess problem behavior in children with milder forms of pervasive developmental disorders. It covers a wide range of problems in different domains of development, mainly social problems. The CSBQ specifies six problem dimensions: 'not optimally tuned to the social situation (Not tuned)', 'reduced contact and social interest (Contact)', 'difficulties in understanding social information (Social Understanding)', 'orientation problems in time, place or activity (Orientation)', 'stereotyped behavior (Stereotyped)' and 'fear of and resistance to changes (Changes)'. A total social problem behavior score was calculated by combining all subscale scores. The questionnaire employs a 3-point rating scale (0=not true, 1=somewhat or sometimes true, 2=very true or often true) on each item. A higher score implies more problem behavior. Subscale scores and the total score were converted into SDS, according to healthy Dutch references for age and gender.

To investigate the effect of long-term GH-treatment on behavior, we assessed behavior during 4 years of GH-treatment. Children who had been in the control group of the RCT were on average 2 years older at start of GH-treatment, than those who had been in the treatment group of the RCT.

Assay

Serum IGF-I levels were measured in one central laboratory using a immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California). The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age and sex dependency, IGF-I levels were transformed into SDS[24].

Data analysis

Statistical analyses were performed with PASW statistics 18.0 (SPSS Inc. Chicago, IL). Independent samples t-tests were used to compare the baseline characteristics between the GH-treated and the untreated controls. Correlations between scores on the behavioral subscales and the total scores at baseline and age, IQ, BMI SDS, head circumference SDS and IGF-I SDS were calculated by Pearson's correlation coefficient or linear regression analysis. Gender and genotypic differences on the behavioral subscales and the total scores at baseline were calculated by Mann-Whitney U tests and within the item-analysis by chi-square tests.
To analyze the effect of GH-treatment during the RCT and the longitudinal study, Linear Mixed Models for repeated measurements[25] was used with GH-treatment and time as factors (GH-treatment coded as: 1=GH-treatment group; 0=control group; time coded as 0=baseline; 1= after 1 year and 2=after 2 years of study) in the RCT and time (time coded as: 0 = baseline, and 1=after 1 year, 2= after 2 years, 3=after 3 years and 4= after 4 years of GH-treatment) as factor in the longitudinal study.
The effects of age, gender, genotype, serum IGF-I level, anthropometric measurements, IQ on behavior during GH-treatment were determined by using these variables as factors (in case of nominal or ordinal variables) or covariates (in case of scale variables) in the model.

Results

Baseline characteristics

Table 1 shows the baseline characteristics of 50 children. At start of the RCT, the median (interquartile range, iqr) age was 6.2 (4.3 to 7.8) years in the treatment group and 5.8 (4.1 to 7.4) years in the control group (p=0.47). Children had a baseline BMI SDS between 0 and +2 SDS, a head circumference between 0 and -2 SDS and IGF-I levels significantly below 0 SDS (p<0.0001). Seventeen children (34 %) had a deletion of chromosome 15q11-q13, 25 (50 %) an mUPD, and 5 (10 %) an imprinting center defect (ICD). Positive methylation test was demonstrated in the remaining 3 (6 %) patients, but the underlying genetic defect was not identified. There were no significant differences in age, BMI, head circumference, IGF-I and IQ between the treatment and control group.

Behavior at baseline

DBC

Figure 1A shows the SDS of the DBC subscales and the DBC total score at baseline in children with PWS compared to a reference population with a comparable mental retardation.
None of the subscale scores were significantly different from 0 SDS, indicating that problem behavior in children with PWS was similar as in children with a comparable mental retardation. However, the Self-absorbed and the Communication disorders SDS were significantly

Table 1. Baseline characteristics

	Treatment group	Control group	p-value
N	29	21	
Gender (M/F)	15/14	8/13	
Age (years)	6.2 [4.3 to 7.8]	5.8 [4.1 to 7.4]	0.47
BMI SDS	0.7 [0.1 to 1.6]	1.2 [0.6 to 1.8]	0.13
Head circumference SDS	-1.0 [-1.6 to -0.4]	-0.7 [-1.2 to 0.2]	0.26
IGF-I SDS	-1.7 [-2.4 to -1.2]	-1.6 [-2.1 to -1.2]	0.75
IQ	65 [57 to 80]	73 [62 to 81]	0.27
Genetic defect			
-Deletion	12	5	
-UPD	10	15	
-ICD	4	1	
-Unknown	3	0	

higher than the DBC total score SDS, indicating relatively more problems on these subscales compared to the other subscales of the DBC in children with PWS. There were no gender- or genotypic differences on any of the subscales of the DBC.

After correction for age, we found significant effects of IQ on the Self-absorbed subscale score (β=-0.40, p=0.05) and on the Communication disorder subscale score (β=-0.44, p=0.03), indicating that children with a lower IQ showed more self-absorbed behavior and had more communication problems. We did not find any correlations between the DBC total score or its subscales and age, BMI SDS, head circumference SDS or IGF-I SDS.

The highest scores were found on the following items: 'influenceable', 'has temper tantrums', 'impatient', 'enjoys company of younger children or adults instead of peers', 'likes doing things on his/her own', 'skin-picking', 'gets upset by changes in the daily routine', 'keeps a strict order in certain items or activities', 'easily distracted', 'no sense of danger' and 'inactive'. Children with a deletion scored significantly lower on the jealousy item than children with an mUPD. All children with a deletion scored '0', while half of the children with an mUPD scored '1' (45%) or '2' (5%) on this item (p=0.013). There were no differences between children with a deletion or an mUPD on the other items of the DBC and no gender differences.

CSBQ

Figure 1B shows the SDS of the CSBQ subscales and the CSBQ total score SDS at baseline in children with PWS compared to healthy references. Children with PWS scored significantly higher than 0 SDS on all subscales (p<0.01) and scores on the Social Understanding subscale and the CSBQ total score were significantly higher than +1 SDS in children with PWS (p<0.0001 and p=0.01, respectively). This indicates that compared to a healthy Dutch reference population, children with PWS have more pervasive developmental disorders. Boys with PWS were less resistant to changes than girls with PWS, as boys had a mean (SD) SDS of 0.3 (1.3) and girls had a mean (SD) SDS of 1.3 (1.7) on the Changes subscale (p=0.05). We also

found a significant difference between children with a deletion and an mUPD on the Stereotyped subscale, children with a deletion (mean (SD) SDS 1.3 (1.3)) showed more stereotyped behavior than children with an mUPD (mean (SD) SDS 0.5 (1.1)) (p=0.05).
Older children were less optimally tuned to the social situation, (Not tuned: (β=0.33, p=0.03)), were more resistant to changes (Changes: β=0.34, p=0.024 and after correction for gender, β=0.32, p=0.03)), and scored higher on total problem behavior (Total score: β=0.33, p=0.03). Children with a smaller head circumference and lower IGF-I SDS had more problems with orientation in time, place or activity (Orientation subscale: β=-0.31, p=0.05 and β= -0.40, p=0.01 for head circumference SDS and IGF-I SDS, respectively). When we added both variables in the multiple regression model, only the effect of IGF-I SDS remained significant (β=-0.37, p=0.02). Children with lower IGF-I SDS had reduced contact and social interest than children with higher IGF-I SDS (Contact subscale: β=-0.35, p=0.03). A higher BMI-SDS was associated with less difficulties in social understanding (Social Understanding subscale: β= -0.32, p=0.04).
The highest scores were found on the following items of the CSBQ: 'can/will only talk about one's own interests', 'does not understand jokes', 'takes things literally', 'naïve' (these items belong to the Social Understanding subscale), 'requires too much attention', 'has trouble doing two things at the same time', 'mood swings', 'gets angry easily', 'sees no danger', 'does not distinguish between known and unknown people', 'blows things up', 'keeps nagging/ does not know when to stop', and 'stubborn'.

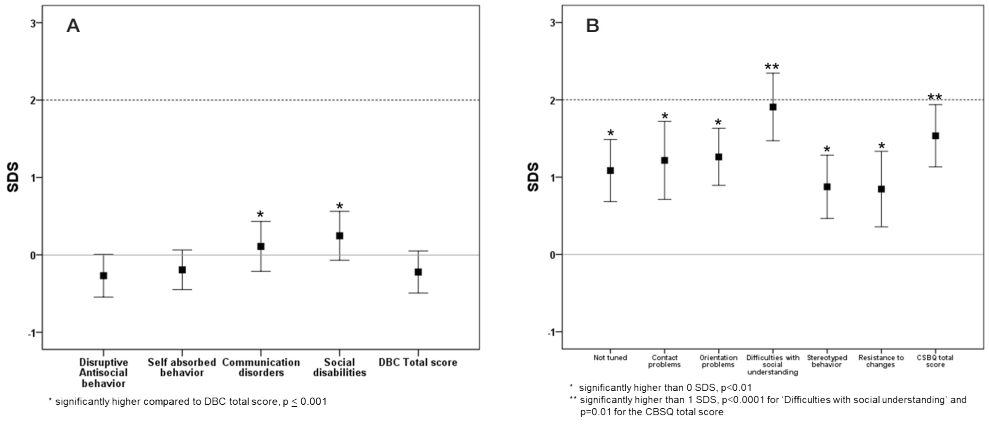


Figure 1A and 1B.
Figure 1A shows the mean SDS of the DBC subscales and the DBC total score SDS and their CI 95% at baseline in children with PWS compared to a reference group with a comparable mental retardation. Figure 1B shows the mean SDS of the CSBQ subscales and the CSBQ total score SDS and their CI 95% at baseline in children with PWS compared to a healthy reference group. 0 SDS is indicated by a straight line and +2 SDS is indicated by a dotted line.

Randomized Controlled Trial

Effect of GH-treatment versus no treatment on behavior

DBC

Figure 2A shows the mean estimated SDS of the DBC subscales, the DBC total score SDS and their 95% confidence intervals after correction for age, in GH-treated versus untreated controls with PWS, during the 2 year-RCT. At baseline there were no significant differences in subscale scores and the DBC total score between the two groups.

As age had a significant effect on behavior measured by the DBC during the RCT (Disruptive antisocial, β=0.15, p=0.001; Self-absorbed, β=0.08, p=0.03; Communication disorder, β=0.15, p=0.02; Social disabilities, β=0.09, p=0.03 and DBC total score, β=0.12, p=0.01), all scores were corrected for age.

After 2 years, scores between the GH-treated and untreated children were not significantly different and there were no significant differences compared to baseline in both groups. This indicates that, after correction for age, problem behavior measured by the DBC remained similar to children with a comparable mental retardation, regardless of treatment or no treatment with GH.

We found no significant effects of gender or genetic subtype on the subscales or the DBC total score over time.

CSBQ

Figure 2B shows the mean estimated SDS of the CSBQ subscales, CSBQ total score SDS and their 95% confidence intervals at baseline and in GH-treated versus controls with PWS, during the 2 year-RCT. At baseline there were no significant differences in subscale scores and the CSBQ total score between the two groups.

As age had a significant effect on behavior measured by the CSBQ during the RCT (Not tuned, β=0.17, p=0.03; Contact, β=0.17, p<0.1; Understanding, β=0.23, p=0.01; Stereotyped, β=0.35, p=0.001 and CSBQ total score, β=0.22, p=0.02), all scores were corrected for age.

After 2 years, scores between the GH-treated and untreated children were not significantly different and there were no significant differences compared to baseline in both groups. This indicates that, after correction for age, problem behavior measured by the CSBQ remained higher compared to healthy children, regardless of treatment or no treatment with GH.

Boys had less problems on the Contact subscale than girls (β=-0.87, p=0.04), in both GH-treated and untreated children with PWS. We found no significant effects of genetic subtype on the subscales or the CSBQ total score over time.

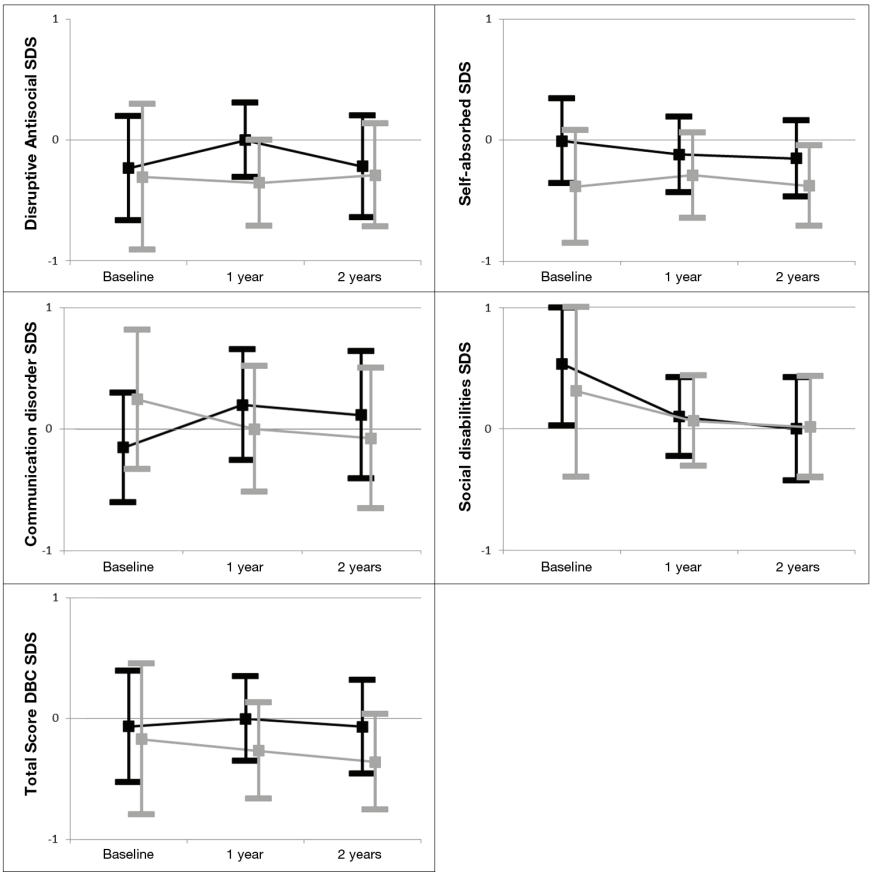

Disruptive Antisocial SDS
Self-absorbed SDS
Communication disorder SDS
Social disabilities SDS
Total Score DBC SDS
1
0
-1
Baseline
1 year
2 years

Figure 2A.

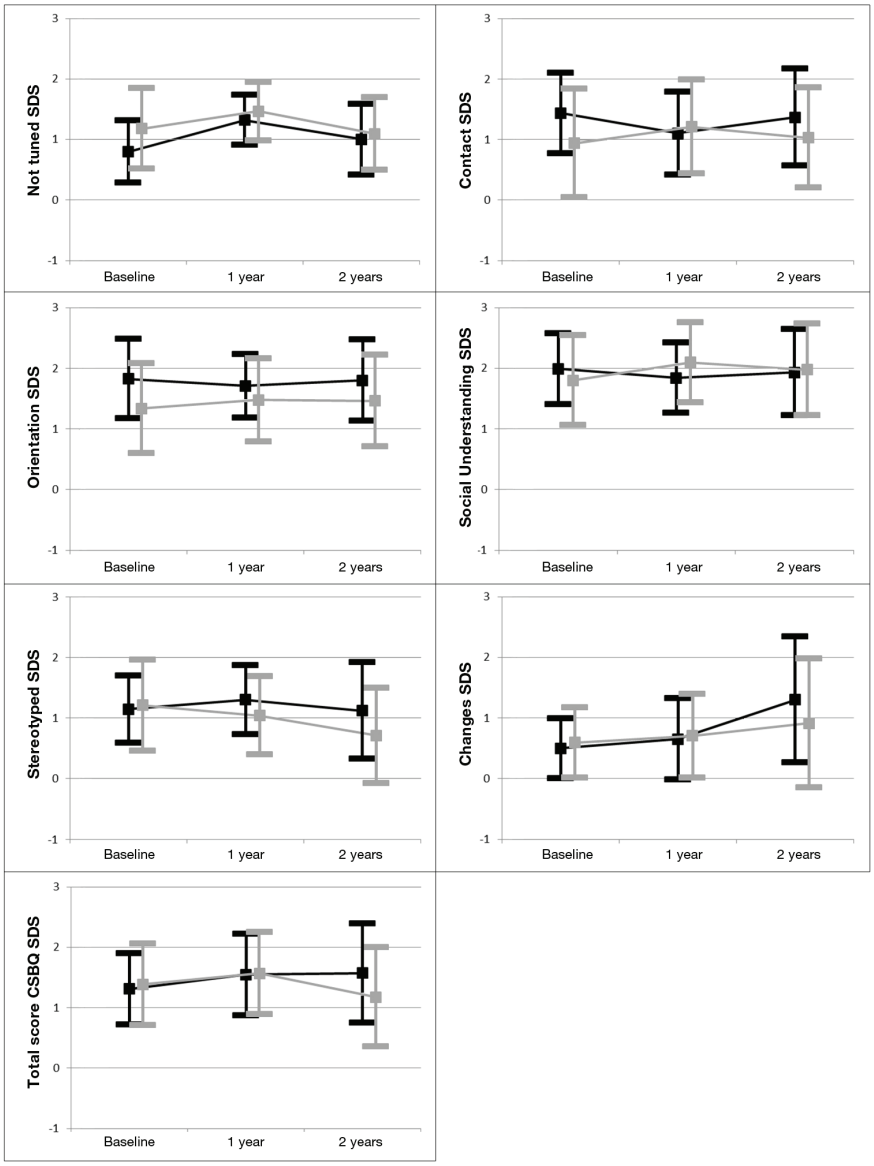


Figure 2B.

Figure 2A and 2B.
This figure shows the mean estimated SDS of the DBC subscales and the DBC total score (figure 2A), the mean estimated SDS of the CSBQ and the CSBQ total score (figure 2B) and their 95% confidence intervals after correction for age during the 2 year-RCT in GH-treated children (in black) versus randomized controls (in gray) with PWS. 0, 1, and 2 SDS are indicated by straight lines.

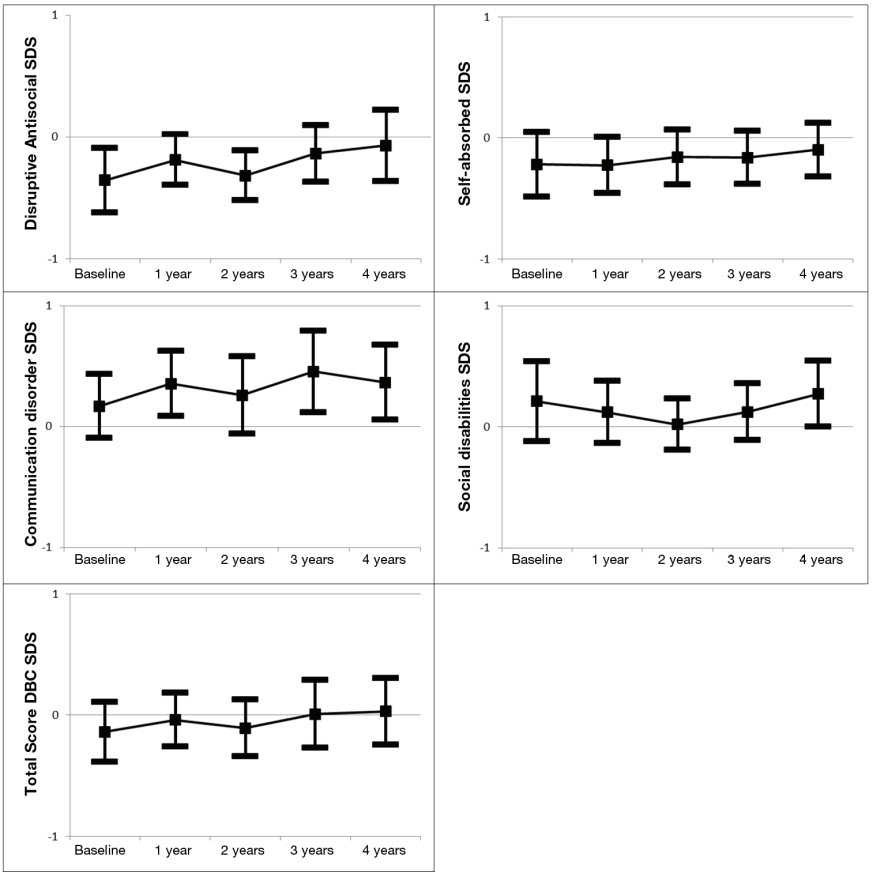

Disruptive Antisocial SDS
Self-absorbed SDS
Communication disorder SDS
Social disabilities SDS
Total Score DBC SDS
1
0
-1
Baseline
1 year
2 years
3 years
4 years

Figure 3A.

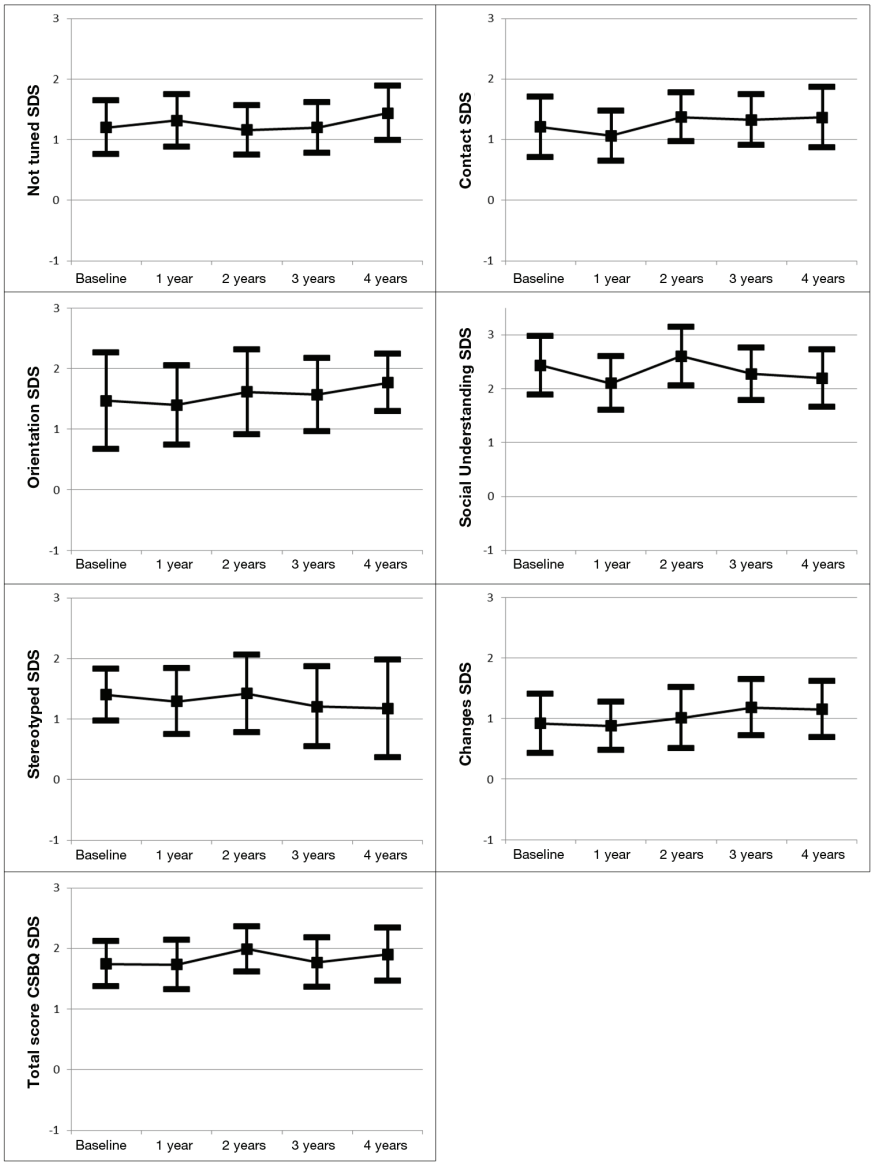


Figure 3B.

Figure 3A and 3B.
This figure shows the mean estimated SDS of the DBC subscales and the DBC total score (figure 3A), the mean estimated SDS of the CSBQ subcales and the CSBQ total score (figure 3B) and their 95% confidence intervals after correction for age during 4 years of GH-treatment in children with PWS. 0, 1, and 2 SDS are indicated by straight lines.

Long term GH-treatment

DBC

Figure 3A shows the mean estimated SDS of the DBC subscales, the DBC total score SDS and their 95% confidence intervals after correction for age during 4 years of GH-treatment. During 4 years of GH-treatment, scores on all subscales and the DBC total score did not significantly change, after correction for age.

CSBQ

Figure 3B shows the mean estimated SDS of the CSBQ subscales, the CSBQ total score SDS and their 95% confidence intervals after correction for age during 4 years of GH-treatment. During 4 years of GH-treatment, scores on all subscales and the CSBQ total score did not significantly change, after correction for age.

Discussion

Our study is the first to investigate the effect of GH-treatment on behavior in children with PWS during a 2-year randomized controlled trial and during 4 years of continuous GH-treatment. In contrast to our expectations based on parental reports and our clinical experience, we did not find a reduction in problem behavior measured by the DBC and CSBQ in GH-treated children compared to untreated controls with PWS. Problem behavior measured by the DBC and CSBQ increased with increasing age, regardless of GH-treatment or not. After correction for age, behavioral problems were stable and remained similar to baseline during 4 years of GH-treatment. Children with PWS showed similar problem behavior as a reference population with a comparable mental retardation, but more pervasive developmental disorders compared to a healthy population, also during GH-treatment.

Next to parental reports and our clinical experience with respect to improvements of behavior during GH-treatment, our expectation to find a positive effect of GH-treatment on behavior in children with PWS was also based on our recent finding that GH-treatment improves cognitive functioning in these children, especially in the performance area[21]. However, it seems that behavior in children with PWS is not clearly influenced by GH-treatment, while cognition is. Findings in short children born small for gestational age (SGA) confirm this assumption. In short children born SGA, a significant improvement on cognition was found during GH-treatment, while attention deficits, especially accurateness and impulsiveness did not change during GH-treatment[26]. The authors concluded that attention deficits were related to being born SGA, and we might draw a similar conclusion for behavioral problems being related to PWS.

Another explanation why we could not find an effect of GH-treatment on behavior might be the type of questionnaires we used in our study. Parents had to score behavior of their chil-

dren with PWS on a 3-point scale ('0=not true', '1=sometimes or somewhat true' or '2= often true or very true') in both questionnaires. On this sort of scale it is only possible for parents to indicate if certain behavior occurs often, sometimes or never in their child. As PWS is associated with certain typical behavioral problems, for example temper tantrums, obsessive-compulsive and preservative behavior[2], all children display this behavior to a greater or lesser extent. These typical behavioral problems might get milder during GH-treatment, but will probably never completely disappear. Thus, parents will never score '0' on these particular behavioral problems, thereby saying that this behavior never occurs in their child. It is therefore very likely, that also if the behavioral problems in their child decreased during GH-treatment, parents scored in the same way as before start of treatment. However, as parents did notice subtle changes, they reported these during their visits to our hospital. Our results are in line with those of a 1-year GH-controlled study[17], in which parents reported improved behavior during GH-treatment in open interviews, while this was not the case in the untreated controls. Structured behavioral questionnaires after 6 and 12 months, however, did also not show differences in behavior between GH-treated and untreated patients.

In our study, behavioral problems in children with PWS measured by the DBC were comparable to those of children with mental retardation. However, in children with PWS scores on the socially related subscales of the DBC, 'communication disorders' and 'social disabilities', were higher than those on the DBC total score. This indicates that there was relatively more socially-related problem behavior in children with PWS. This was confirmed by our results on the CSBQ, which is a checklist that is developed to assess social problem behavior in children with normal intelligence, in particular pervasive developmental disorders (PDD). We found that children with PWS experienced significantly more PDD than healthy Dutch references and in particular problems with orientation in time, place or activity and understanding social information. Social impairment is typically found in patients with PWS and many of their social behaviors appear to be on the same continuum of social deficits found in autism spectrum disorder (e.g., social withdrawal, poor peer relationships, lack of empathy)[27]. Poor peer relationships, a lack of friends, immaturity, weakness in coping skills, and a preference for solitary activities are all reported in children with PWS[6,28-30]. One other study assessing PDD in children and adults with PWS, compared patients with PWS to controls with intellectual disabilities[31]. It showed elevated scores on a PDD questionnaire in patients with PWS compared to the controls. Like in our study, there was no significant difference in PDD score between PWS individuals with a deletion and mUPD genotype. The finding that children with PWS had particularly difficulties with understanding social information and were more insensitive to the social situation than references was also found in a previous study in children with PWS evaluating associations between sleep related breathing disorders and behavior[32].

Older age was associated with more problem behavior, measured by both the DBC and CSBQ. It has indeed been reported that problem behavior increases in children with PWS from infancy to childhood and further into adolescence[33].

A higher BMI SDS was associated with less problem behavior in children with PWS, both at baseline and during GH-treatment, indicating a better social understanding, being less resistant to changes and being more socially interested. Less maladaptive behavior was also found in other studies in adolescents and adults with PWS with a higher BMI[6].
Our study shows that problem behavior in children with PWS is in many aspects similar as in children with a comparable mental retardation. However, they display more pervasive developmental disorders compared to a healthy population. Problem behavior increases with age in both GH-treated and untreated children with PWS alike. Thus, in contrast to our expectations, our study shows no effect of GH-treatment on behavioral problems in children with PWS.

Acknowledgements

We express our gratitude to all children and parents for their participation in our Dutch PWS RCT and Cohort studies and acknowledge the work of P. M. C. C. van Eekelen, research nurse. We thank Pfizer Inc. for the independent research grant for the investigator initiated trial investigating the effects of GH-treatment in children with PWS.

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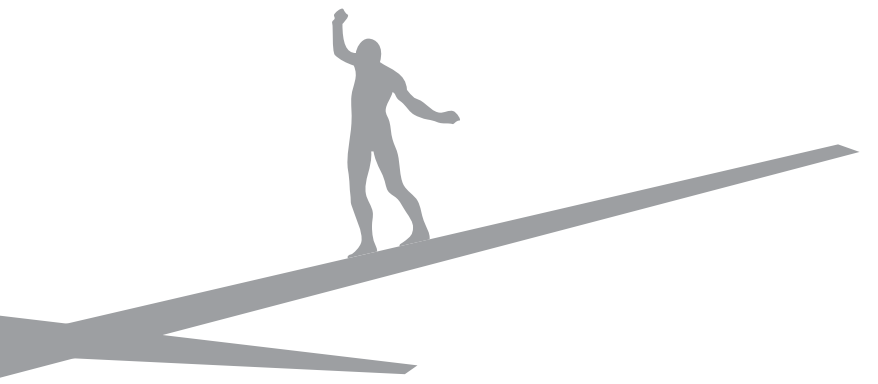
8

General discussion, conclusions and directions for future research



General discussion

In 2002, the Dutch national randomized controlled growth hormone trial in children with Prader-Willi syndrome (PWS) was started, as knowledge about different aspects of the PWS phenotype and the effects of growth hormone (GH) on these aspects were scarce. After participating in the randomized controlled GH trial, children are followed during GH-treatment in the Dutch PWS Cohort study until they reach final height, to study long-term efficacy and safety of GH-treatment. These Dutch PWS studies have markedly improved our knowledge about PWS and have led to better care for these children. Up to date, the inclusion of infants with PWS (below 3 years of age) in the PWS Cohort study continues.
This thesis describes several aspects of children with PWS, such as adrenarche, pubarche, gonadal function, cognition, health related quality of life and behavior, and the effects of GH-treatment on these aspects, in the large Dutch cohort of children with PWS. In this chapter, results are compared and discussed in view of current literature. Subsequently, clinical implications and directions for future research are given.

8.1 Adrenarche and Pubarche

Adrenarche refers to the increase in adrenocortical androgen production in mid-childhood, most specifically dehydroepiandrosterone-sulfate (DHEAS)[1], which leads to the appearance of the clinical signs of adrenarche: adult type body odor, oily hair, acne and comedones, axillary and pubic hair. Pubarche is the appearance of pubic hair and premature pubarche (PP) is the appearance of pubic hair before 8 years in girls or 9 years in boys[2]. High serum levels of DHEAS and PP were found in small groups of children with PWS[3-6]. However, until we performed our study, it was unknown at what age DHEAS levels were increased, and the development of pubarche over time had not yet been described in these children. We therefore assessed serum DHEAS levels and determined the age at onset of pubarche, development of pubic hair and the prevalence of premature pubarche, in comparison with healthy controls. Our study showed that median serum DHEAS levels in children with PWS were higher than in healthy age-matched controls at ages 3 to 10 years, increased with increasing age and were higher in boys. The median age at onset of pubarche was 9.0 years, which is younger than in healthy peers, and the prevalence of PP was 30% in girls and 16.1% in boys. After its early onset, pubarche developed in a normal pace until pubic hair stage 4 (P4) (according to Tanner[7,8]), but slowed down or arrested thereafter. Some children did not reach P5 and none reached P6.
Two recent studies, one in male and the other in female patients with PWS, also investigated DHEAS levels and reported high levels in children between 5 and 15 years, in line with our findings[9,10]. In adolescents and adults with PWS, however, they reported DHEAS levels in the

normal range. Our observation that children with PWS reached P2 and P3, but not P4 and P5 at a significantly earlier age, is in line with these findings.
As most children with PWS have hypothalamic dysfunction, our data indicate that the increase of DHEAS levels can occur with less, or even without stimulation via the pituitary-adrenal axis. This indication is supported by the varying DHEAS levels we found in this study, in children who were previously diagnosed with central adrenal deficiency by overnight single-dose metyrapone tests[11]. The observation that almost no progression of pubarche occurred after stage P4, might indicate that a normal pituitary-gonadal axis is required to achieve an adult stage of pubic hair.
In general, high serum DHEAS levels are associated with weight gain and obesity and are often seen in combination with hyperinsulinism and increased IGF-I levels[12,13]. We found no association between body composition, such as BMI and fat mass percentage, or glucose homeostasis parameters and serum DHEAS levels in children with PWS. These results are in line with the only other study evaluating the effect of obesity parameters on serum adrenal steroid levels in children with PWS[3]. In our study, there was also no significant influence of IGF-I levels or GH-treatment on DHEAS levels and no difference in duration of GH-treatment between children with PP and normal timing of pubarche. These findings are comparable with findings in another study in children with PWS, where they found that before and during GH-treatment, there were no significant correlations between IGF-I levels and DHEAS, and that the increase in DHEAS levels during GH-treatment, was due to the increase in age[3]. Also in a large group of short children born small for gestational age, there was no effect of GH-treatment on DHEAS levels[14].

In conclusion, our study shows that compared to healthy children, children with PWS have higher serum DHEAS levels from 3 to 10 years of age, are significantly younger than healthy peers at onset of pubarche and have a higher prevalence of premature pubarche, but an impairment of the development of pubarche after P4.

8.2 Male gonadal function

As most symptoms of PWS are considered to result from hypothalamic dysfunction, hypogonadism in PWS was hypothesized to be hypogonadotropic[15,16]. A few studies described hypogonadotropic hypogonadism in adults with PWS[17-19], but later on, it was shown that primary gonadal dysfunction could also contribute to the abnormal pubertal development in male patients with PWS[9,20,21]. These findings suggested a decreased gonadal function in adolescence and adulthood[20,22]. Until recently, data about gonadal function in boys with PWS were mainly cross-sectional and it was unclear at what age the gonadal dysfunction became prominent. We therefore performed a longitudinal study on hormonal levels of the pituitary-

testicular axis in boys with PWS during childhood, puberty and adolescence and compared these to healthy references.

Our study showed that boys with PWS have normal inhibin B levels between 6 months and 10 years of age, but after onset of puberty, inhibin B levels decline to below the 5th percentile, while FSH levels increase to above the 95th percentile. Testosterone levels increased, but remained below the 5th percentile and LH levels increased, but not above the 95th percentile. In childhood, inhibin B is produced by immature Sertoli cells. However, during puberty, when Sertoli cells mature, inhibin B production is dependent on normal spermatogenesis[23]. Our findings suggest that patients with PWS have a normal Sertoli cell function during childhood, with normal inhibin B levels during that period, but a failure of the Sertoli cells, spermatogenic cells and/or the interaction between these cells after onset of puberty. The low testosterone levels in combination with normal LH levels in most, and high levels in some boys, indicate moderate Leydig cell dysfunction that also became apparent after onset of puberty. The decline in inhibin B, the increase of FSH and LH levels, the fact that inhibin B production is stimulated by gonadotrophins[24,25] and the report of a normal mini-puberty in male infants with PWS[22], indicate that not hypogonadotropic hypogonadism, but primary testicular failure, mainly of the tubular compartment, is the main cause of hypogonadism in PWS. This was confirmed in recent cross-sectional studies in adolescents and adults with PWS[9,26].

Normal inhibin B levels before puberty, and undetectable levels thereafter, have also been found in boys with Sertoli-cell-only testes[23]. Thus, Sertoli-cell-only-testes might very well explain our findings and were indeed found in testicular biopsies during orchiopexy in male patients with PWS[27,28].

The results of our study indicate that there is no, or severely impaired spermatogenesis in adolescents with PWS, which might be caused by a genetic defect. It has been suggested that the C15orf2 gene, which is located in the PWS region of chromosome 15 and paternally expressed in human testes and the fetal brain and thus not expressed in PWS, plays a role in spermatogenesis in healthy subjects[29-31].

As our results indicate defective spermatogenesis in male patients with PWS, fertility is highly unlikely. Up to date, fatherhood in PWS has never been described. However, patients with PWS do have sexual and relational interests[32], which might be in line with the low normal testosterone levels. It is therefore recommended that medical doctors, parents and care-takers of patients with PWS remain alert. Defective spermatogenesis in male PWS patients could be further investigated by semen analysis, but as far as we know, this has never been done in patients with PWS, probably because of ethical constraints.

GH-treatment did not affect Sertolli cell function in boys with PWS, as inhibin B levels were similar before and during GH-treatment. These results are in line with findings in prepubertal boys and young men born small for gestational age[33].

Next to a defect in the Sertoli cell compartment, we found low testosterone levels and normal or high LH levels indicating a moderate Leydig cell defect in male patients with PWS. Patients

with hypothalamic hypogonadism and thus low testosterone levels have low bone mineral density and an increased risk to develop osteoporosis[34]. Furthermore, considerable evidence exists that testosterone enhances and maintains muscle mass and reduces fat mass[35,36]. Therefore testosterone replacement therapy may be beneficial in the prevention of osteoporosis, management of obesity and improvement of body composition in male adolescents with PWS. We recommend testosterone replacement therapy in adolescents with PWS according to the schedule presented in Appendix 1. This needs to be done under strict clinical supervision, because replacement of sex hormones in male adolescents with PWS has been associated with aggravation of behavior abnormalities in some patients[5,37]. However, it is reassuring that the one study that investigated the effects of hormonal replacement in boys with PWS, found no detrimental effects on behavior[38].

In conclusion, our study indicates primary testicular failure, mainly in the tubular compartment, which becomes apparent after the onset of puberty. Normal spermatogenesis is therefore highly unlikely in male patients with PWS. The majority showed no classical hypogonadotropic hypogonadism.

8.3 Female gonadal function

Hypogonadism has also been described in female patients with PWS[5,19], but information about gonadal function in female patients was scarce, and only investigated in cross-sectional studies[10,39]. We longitudinally assessed serum levels of anti-Müllerian hormone (AMH), gonadotrophins, estradiol (E_2), inhibin B and A, and pubertal development in girls and female adolescents with PWS.

Our study showed normal AMH and inhibin B levels, indicating that the primordial follicle pool and number of small antral follicles are conserved in girls and female adolescents with PWS. We found no classical hypogonadotropic hypogonadism, but low levels of E_2 and inhibin A indicate that maturation of follicles and progression of pubertal development are impaired, probably due to dysregulation of LH secretion. As these impairments were not absolute, ovulation and thus conception cannot be ruled out in individual female adolescents with PWS. This is in line with previously described pregnancies in female patients with PWS[40,41].

Disturbed reproductive function in females with PWS does not result from hypogonadotropic hypogonadism, because we found high normal FSH and low normal LH levels. There is also no hypergonadotropic hypogonadism like in girls with Turner syndrome, because LH levels were low normal and AMH levels normal. Findings in females with PWS are also different from those in patients with polycystic ovary syndrome, because of the normal AMH levels. Thus, in females with PWS an unique combination of hormone levels was found.

The combination of delayed pubertal development and disturbed folliculogenesis was also

found in female mice lacking the Magel2 gene[42], which is located in the PWS critical region on chromosome 15. This gene is also involved in circadian rhythms[43], and loss of the gene might result in dysregulation of LH secretion and disturbed timing of LH peaks. Further investigation showed that the Magel2 gene also contributes to other characteristics of the PWS phenotype. Magel2-null mice had decreased activity[44], obesity with increased body fat and decreased lean mass[45]. Recently, the effects of loss of Magel2 on other endocrine functions, i.e. the HPA-axis, glucose homeostasis and the GH-axis were also shown, being more severe in Magel2-null female mice than in male mice[46].

Progression of pubertal development after onset of puberty in girls and female adolescents with PWS in our study was delayed compared to healthy peers, or even incomplete. This was also found in other studies[5,10]. Most of the adolescents had primary or secondary amenorrhea and a few developed oligoamenorrhea, which is in line with the low normal E_2 levels. Because of the arrest in pubertal development and the low E_2 levels, we recommend hormonal replacement therapy in adolescents with PWS to prevent osteoporosis and other consequences of prolonged hypoestrogenism, according to the treatment schedule in Appendix 2.

The results of our study indicate that contraceptive therapy might be considered in girls with PWS. However, as most girls with PWS are treated with GH until final height, interference with growth has to be taken into account if contraceptive therapy is considered. As we found that AMH levels in girls and female adolescents with PWS stabilized from 8 years of age, we advise to assess serum AMH levels at or above this age. Previous studies in girls with Turner syndrome and females with secondary oligoamenorrhea showed that specificity and sensitivity for infertility in case of a serum AMH level below 1.1 µg/l were respectively 86% and 96% for girls with Turner syndrome[47] and 85% and 100% for females with secondary oligomenorrhea[48]. If the AMH level is 1.1 µg/l or higher and the girl with PWS is still prepubertal, there is no need for contraceptive measurements, but if the AMH level turns out to be 1.1 µg/l or higher and the girl is pubertal (Tanner M2 or higher), fertility cannot be ruled out. The decision whether to start contraceptive therapy depends on several additional factors such as the girl's behavior; is she outgoing or seeking attention, her mental state; is she severely mentally retarded or not and environmental circumstances; does she live in an institute or at home. Figure 1 shows a flow chart which may support the decision to start contraceptive therapy.

In conclusion, our study shows that the primordial follicle pool and number of small antral follicles are conserved in girls and female adolescents with PWS. We found no classical hypogonadotropic hypogonadism. However, maturation of follicles and progression of pubertal development are impaired, which might be due to dysregulation of LH secretion. As these impairments are not absolute, ovulation and thus conception cannot be ruled out in individual female adolescents with PWS.

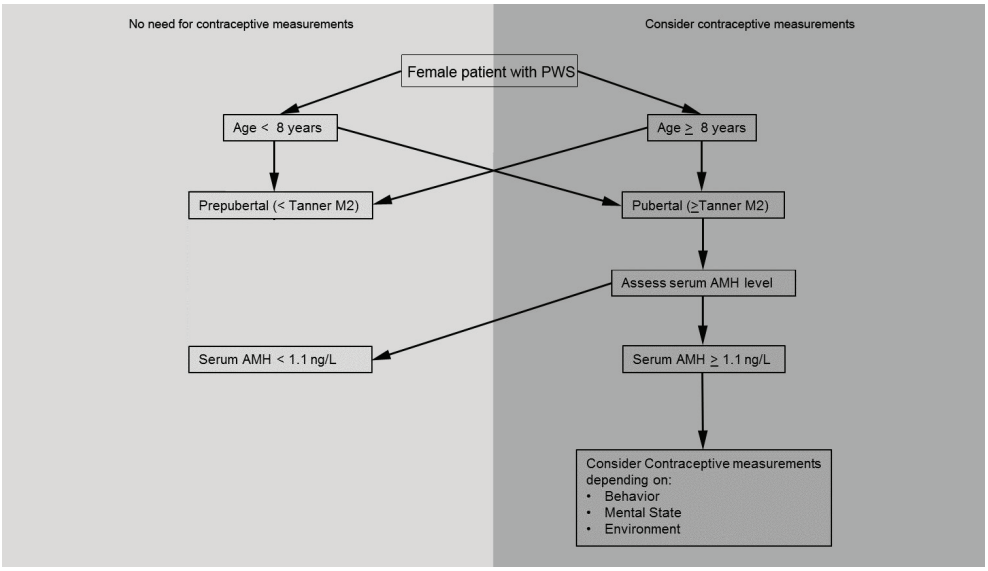


Figure 1.
Flow chart to support consideration of contraceptive therapy

8.4 Gonadal dysfunction in male and female patients with PWS, are there similarities?

Onset of puberty was timed normally in both male and female patients and both showed a delay in pubertal development after puberty onset, in combination with a defect in gametogenesis. These defects in gametogenesis could be explained by lack of one of the paternally expressed genes located in the PWS critical region on chromosome 15 in both sexes. In both male and female patients with PWS, we found no classical hypogonadotropic hypogonadism, as both had high FSH levels compared to references. Furthermore, LH levels remained relatively low considering the testosterone levels in males and E_2 levels in females.
However, male and female patients do differ in the outcome of reproductive function, as menarche, menses and even pregnancy have been reported in females with PWS, and, as far as we know, no men with PWS have fathered a child. This could be explained by the fact that in women the size of the primordial follicle pool is already determined before birth, while in men the germ cell production has to be maintained later in life.

8.5 Cognition

There was little information about cognitive development in infants and children with PWS, and no information about the effect of GH-treatment on cognition in children with PWS above 3 years of age, for a period beyond 6 months. In our randomized controlled GH trial, we investigated baseline cognition and the effect of GH-treatment versus no treatment on cognitive functioning in children with PWS. Furthermore, we studied cognitive functioning during 4 years of continuous GH-treatment in the PWS Cohort study. Our results demonstrated that GH-treatment prevented deterioration of certain cognitive skills on the short-term and significantly improved abstract verbal reasoning and visuospatial skills during 4 years of GH-treatment. Children with an mUPD had lower visuospatial skills at baseline, but showed a larger improvement of these skills after 4 years of GH-treatment than children with a deletion genotype. Furthermore, GH-treatment had a greater effect on abstract verbal reasoning and visuospatial skills in children with lower cognitive functioning at baseline.

In line with our findings, effects of GH-treatment on cognition were found in infants and adults with PWS, children with GH deficiency, short children born SGA and children with Down syndrome[49-55]. One study in children with PWS found no effect of GH-treatment[56], but this might be due to a small patient number and short period of treatment.

GH receptors are located throughout the brain and GH and IGF-I affect the genesis of neurons, astrocytes, endothelial cells and oligodendrocytes, induce cell genesis in the adult brain and enhance cell to cell communication in the central nervous system[57-59]. These findings, in combination with the effects of GH-treatment in our RCT and long-term study, indicate plasticity of the human brain and local activity of GH and IGF-I. Our positive findings in children with PWS might also have implications for children with cerebral deficits and warrant further detailed studies.

A recent MRI study compared brain development in children with GH deficiency (GHD) and children with idiopathic short stature (ISS), to determine the effect of GH deficiency on the brain[60]. They found abnormalities in white matter fiber density and neural volumes in children with GH deficiency compared to ISS, which were associated with impaired cognitive function and motor skills. Furthermore, lower IGF-I and IGFBP-3 levels were both associated with abnormalities of certain brain areas and lower cognitive skills. Unfortunately, the effect of GH-treatment on brain development was not investigated in that study, but these findings support that the growth hormone-IGF-I axis plays a role in the normal brain and cognitive development. This might explain the positive effects of GH-treatment on cognitive functioning in the prepubertal children with PWS as shown in our present study and on mental and motor development in infants with PWS in our previous study[49].

Before the start of GH-treatment, age was negatively associated with cognition, and untreated children with PWS showed a deterioration of cognitive functioning compared to healthy peers. During 4 years of GH-treatment, the mean total IQ score (TIQ) increased from 66 to

70. This means that after 4 years of GH-treatment only half of the children with PWS in our study were mentally retarded according to the DSM-IV[61]. Another study investigating the effects of GH-treatment on cognition in small children born small for gestational age found a comparable increase in TIQ[54]. These results implicate that early intervention with GH could improve brain development and thereby impaired cognitive functioning, which might have major implications for daily life of patients with PWS.

Our study demonstrates that GH-treatment prevents deterioration of certain cognitive skills in children with PWS in the short term and significantly improves abstract reasoning and visuospatial skills during four years of GH-treatment. Furthermore, children with a greater deficit had more benefit from GH-treatment.

8.6 Health related quality of life

Information about health related quality of life (HRQOL) in children with PWS was scarce and was never investigated when interpreted by both children and their parents. Furthermore, nothing was known about the effect of GH-treatment on HRQOL in children with PWS, or HRQOL during a longer period of GH-treatment. Our study investigated HRQOL according to both children and parents in children with PWS. In addition it assessed the effects of GH-treatment on HRQOL in children with PWS during 2 years in an RCT and during 4 years of continuous GH-treatment.

We showed that prior to start of GH-treatment, children with PWS rate their HRQOL higher than their parents and than children with various disorders. Furthermore, according to children and parents, HRQOL improved significantly in GH-treated children with PWS, while it decreased or remained similar to baseline in untreated children with PWS. This positive effect of GH-treatment on HRQOL in children with PWS was sustained during long-term GH-treatment, according to both children and parents.

HRQOL reported by prepubertal children with PWS, was never described before. We found that children with PWS rated their HRQOL higher than children with other diseases and even healthy children. In a study investigating HRQOL in children with PWS reported by parents, HRQOL was found to be lower in children with PWS compared to a reference population[62]. An explanation for the high HRQOL reported by the children in our study might be the lower intelligence level of children with PWS. Mild to moderate intellectual impairment is common in these patients[63], thus comparison of HRQOL in children with PWS to a reference population with normal intelligence may be less appropriate. On the other hand, children with PWS with an IQ level closer to normal reported a higher HRQOL than those with a lower IQ. This indicates that children with PWS consider themselves quite happy children, despite the difficulties that go with the syndrome.

The improvement of HRQOL during GH-treatment is in line with findings in another study in adults with PWS[64] and with findings in short children born small for gestational age[65,66].
There were no effects of GH-treatment on HRQOL in the Social subdomain according to children and parents. Social impairment is typically found in patients with PWS and many of their social behaviors appear to be on the same continuum of social deficits as found in autism spectrum disorder (e.g., social withdrawal, poor peer relationships, lack of empathy)[67]. Both clinical and research reports suggest that children with PWS exhibit poor peer relationships, a lack of friends, immaturity, weakness in coping skills, and a preference for solitary activities[68-71]. This typical behavior is therefore not likely to change with GH. However, despite the fact that social impairment is a common finding in PWS, HRQOL on the Social subdomain was not extremely low according to patients and parents, suggesting that both children and parents seem to cope with this social impairment.
According to parents, children with a deletion had a higher HRQOL than children with an mUPD, which might be related to the fact that most parents know that psychiatric problems are more common in children with an mUPD[72] and that this knowledge colors their interpretation of their children's HRQOL. Furthermore, parents of children with a higher BMI SDS gave their child a lower score on the Physical subdomain of the DUX25. These associations between HRQOL and genotype or obesity were not found in the self-reports of children. Disagreement between parent and child reports on HRQOL in children was also found in children with other disorders[73,74]. Parents may be negatively influenced by the burden of caregiving, their own well-being and concerns[75] and children might be positively influenced by adaptation to their illness[76]. However, children with PWS even reported a higher HRQOL on the Physical subdomain than their parents, which indicates that children with PWS have little sense of the consequences of their increased weight on their physical health. Thus, our findings imply that strict supervision is needed in children with PWS when it comes to eating habits and the prevention of obesity, even when children are treated with GH.

Our study shows that children with PWS report a normal HRQOL, even higher than their HRQOL reported by parents. According to children and parents, HRQOL increases during GH-treatment, in contrast to untreated children with PWS. The effects sustain during long-term GH-treatment.

8.7 Behavior

Information about behavior or the effect of GH-treatment on behavior in children with PWS was very scarce. Our study investigated the effect of GH-treatment on behavior in children with PWS during our 2-year randomized controlled GH trial and during 4 years of continuous GH-treatment.

The results of this study showed that children with PWS display similar problem behavior as a reference population with a comparable mental retardation, but more pervasive developmental disorders compared to a healthy population. In contrast to our expectations, which were based on parental reports and our clinical experience, we did not find a reduction in problem behavior in GH-treated children compared to untreated controls with PWS. Problem behavior increased with increasing age, regardless of GH-treatment or not. After correction for age, behavioral problems were stable and remained similar to baseline during 4 years of GH-treatment.

Behavior in children with PWS was not clearly influenced by GH-treatment, while we did find a significant effect of GH on cognitive functioning in our patients. A recent fMRI study in patients with PWS, suggested that altered patterns of communication between the frontal and parietal cortex and deactivation of brain activity in the anterior region of the ventromedial prefrontal cortex during a cued task switching procedure, might contribute to the typical behavioral problems in patients with PWS, including temper outbursts, repetitive questions and attention deficits[77]. Our findings indicate that GH-treatment has no or little influence on the activity in these areas of the brain.

A recent MRI study that compared brain development between children with isolated GH deficiency (IGHD) and children with ISS, found abnormal brain development in children with IGHD, but not in children with ISS and concluded that these developmental abnormalities were associated with GH deficiency in children with IGHD[60]. It also described that there were cognitive deficits in children with IGHD, but not in children with ISS, which led to the conclusion that the abnormal brain development which was associated with GH deficiency, resulted in cognitive deficits. However, that study did not find any behavioral disorders, both in children with IGHD and ISS, implicating that GH deficiency does not necessarily cause behavioral problems. This might explain why we did not find an effect of GH-treatment on behavior in children with PWS. Findings in short children born SGA confirm this, as in short children born SGA, a significant improvement in cognition was found during GH-treatment, while attention deficits, especially accurateness and impulsiveness did not change during GH-treatment[54,78]. The authors concluded that attention deficits were related to being born SGA, and we might draw a similar conclusion for behavioral problems in relation to PWS.

Another explanation why we could not find an effect of GH-treatment on behavior measured by standard parental questionnaires, although parents did report changes during the yearly visits, might be that answers to the standard questionnaires had to be given on a 3-point scale. Retrospectively, we realized that this type of scale it is not suitable for the registration of subtle changes in behavior, which might have occurred in children with PWS during GH-treatment. It is very likely, that parents scored the questionnaires in the same way before and during GH-treatment because typical problem behavior will not completely disappear in children with PWS, so they will never score '0' (not true). Our results were in line with those of another study[79]. Older age, and surprisingly a lower BMI, were associated with more problem behavior. Both

associations were also reported in other studies[70,80]. This has implications for the treatment of children with PWS growing into adolescence and adulthood. As children grow older, more attention needs to be paid to their behavioral problems, even during GH-treatment, and, although a strict diet is implied because of health related issues, there also needs to be a balance with respect to the development of behavioral disorders.

In conclusion, our study shows that problem behavior in children with PWS is similar to that in a reference population with a comparable mental retardation but that children with PWS display more pervasive developmental disorders compared to a healthy population. Problem behavior increases with age in both GH-treated and untreated children with PWS. Thus, in contrast to our expectations, our study did not show an effect of GH-treatment on behavioral problems in children with PWS.

8.8 General conclusions

In the present thesis, we described several aspects of the PWS phenotype, such as adrenarche, pubarche, gonadal function, cognition, health related quality of life and behavior, and the effects of GH-treatment on these aspects, in a large cohort of children with PWS participating in the Dutch PWS studies (Figure 2).
Our results demonstrate that children with PWS have younger or normal ages at onset of adrenarche, pubarche and puberty, but delayed development, compared to healthy references. Most male and female adolescents do not reach the adult stage of pubic hair development and show an impaired gametogenesis. Male adolescents have a testicular dysfunction, mainly in the tubular compartment, which results in impaired or absent spermatogenesis and in female adolescents there is an impaired maturation of follicles. As the impairment in male adolescents seems more severe than in female adolescents, fertility in males is highly unlikely, while this cannot be ruled out in females with PWS. There is no classical hypogonadotropic hypogonadism in most children and adolescents with PWS.
Furthermore, our results show cognitive impairment in children with PWS, with a mean total IQ of 66 and higher scores on performance subtests in children with the deletion genotype. HRQOL is normal in children with PWS and higher when reported by children than by their parents. Behavioral problems are similar as in children with a comparable mental retardation, but children with PWS display more pervasive developmental disorders than healthy children. GH-treatment prevents further deterioration of cognitive functions in the short term and significantly improves cognition in the long term, especially the performance functions. Children with a greater deficit in IQ show greater benefits of GH-treatment. Next to the beneficial effects of GH-treatment on cognition, GH improves HRQOL, mainly on physical aspects, according to both children and their parents. Behavior, however, did not change during GH-treatment.

Adrenache and Pubarche

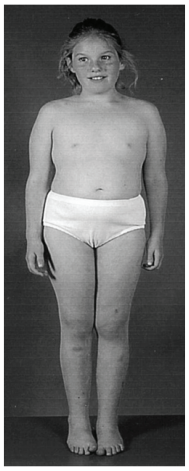
- Higher DHEAS levels in prepubertal children
- Younger age at onset of pubarche
- Impaired development of pubarche after P4
- GH-treatment: no effect

Female gonadal function

- Primordial follicle pool is conserved
- Normal number of small antral follicles
- Impaired maturation of follicles
- No classical hypogonadism
- Impaired progression of puberty
- GH-treatment: no effect

Male gonadal function

- Impairment in the Sertoli cell compartment: normal spermatogenesis is highly unlikely
- Moderate impairment in the Leydig cell compartment
- No hypogonadotropic hypogonadism in most patients
- GH-treatment: no effect



Health related quality of life

- Baseline:
  - Normal
  - Higher by self-report than by parental report
- GH-treatment:
  - Increase in GH-treated, not in untreated children
  - Sustainment of positive effect on the long-term

Cognition

- Baseline:
  - Mean (CI95%) IQ 66 (60-72)
  - Higher scores on performal subtests in deletion genotype
- GH-treatment:
  - Prevention of deterioration on the short-term
  - Significant improvement on the long-term
  - Greater deficit = greater benefit of GH-treatment

Cognition

- Baseline:
  - Similar problem behaviour as in children with comparable mental retardation
  - More PDD than in healthy references
- GH-treatment:
  - No effect during short- or longterm

Figure 2.
General conclusions (picture published with permission of the girl and her parents)

8.9 Directions for future research

We are the first to present serum DHEAS levels and the development of pubarche in a large group of children and adolescents with PWS. We measured serum DHEAS levels cross-sectionally, but it may be worthwhile to study serum DHEAS levels longitudinally, in combination with the development of pubarche, to learn more about the etiology of early adrenarche and find the consequences of early adrenarche in the long term. Early adrenarche is associated with the metabolic syndrome (MBS). The MBS is a cluster of metabolic risk factors that predispose to cardiovascular diseases and are thought to share a common underlying pathophysiological process. Features generally included in the MBS constellation are insulin resistance or glucose intolerance, dyslipidemia, hypertension and abdominal obesity. MBS has been associated with an increased risk of type 2 diabetes and CVD in large population based studies[81,82]. In previous studies we found no insulin resistance in children with PWS, but we did find dyslipidemia and a high body fat percentage in most, and at least one component of the MBS in about 40% of infants and children with PWS[83,84]. It is therefore important to keep track of metabolic risk factors in combination with androgen levels and body composition in children with PWS, especially when they have clinical signs of premature adrenarche such as PP.

We investigated gonadal function in boys and girls with PWS and found that fertility in male patients is highly unlikely, but it cannot be ruled out in female patients. Further follow-up of gonadal function in adolescents with PWS into adulthood is needed to predict fertility in patients with PWS and thus individual need for anti-contraceptive treatment or measures.

Our studies showed beneficial effects of GH-treatment on cognitive functioning and health related quality of life. Against our expectations, we did not find an effect of GH-treatment on behavior. Research in children with disorders other than PWS indicated that this might be due to the different brain structures involved in cognition or behavior. It is therefore interesting to perform brain imaging studies in patients with PWS and look for relations between brain abnormalities and cognitive dysfunctions or behavioral problems.

Appendix 1: Suggestion for hormonal replacement therapy in male adolescents with PWS

The start of hormonal substitution is recommended in the following situations:

- From 14 years of age and serum testosterone levels < 2 nmol/l.
- From 16 years of age and serum testosterone levels < 5 nmol/l
- From 18 years of age and serum testosterone levels < 10 nmol/l

Level[1]	Oral (Andriol®) (daily dose in mg)	Intramuscularly (Sustanon®)	Transdermal (Tostran®)[2] (daily dose in mg)
1	40	50 mg every 4 weeks	10
2	80	100 mg every 4 weeks	20
3	120	100 mg every 3 weeks	30
4	[3] →→→→→→→	150 mg every 3 weeks	40
5	[3] →→→→→→→	200 mg every 3 weeks	50
6	[3] →→→→→→→	250 mg every 3 weeks	60

[1]The duration of each level is 6 months. [2]The use of transdermal testosterone to induce puberty is empirical, because there is little clinical experience. Close follow-up of effects in case of treatment is therefore recommended. Skin contact of transdermal testosterone by females (caretakers, family members, class mates or roommates) must be avoided. [3] Not recommended, choose another treatment

Appendix 2: Suggestion for hormonal replacement therapy in female adolescents with PWS

The start of hormonal substitution is recommended in the following situations:

- From 13 years of age: if Tanner breast stage is M1, combined with an estradiol level below 50 pmol/l. (In case of questionable breast development, one might perform a breast ultrasound) Start treatment at level 1.
- From 13 years of age: in case of an arrest of pubertal development (the same Tanner stage during more than 1 year). Starting level of treatment depends on pubertal Tanner stage and serum estradiol level.
- From 16 years of age: in case of primary amenorrhea in combination with normal breast development, start treatment at level 4 or 5 (prior to start of treatment at level 3 or 4: consider medroxyprogesterone[2] for 7 days to generate withdrawal bleeding).
- From 16 years of age: in case of secundary amenorrhea or oligomenorrhea, start treatment at level 5.

Level[1]	Ethinylestradiol	17-β estradiol[4]
1	0.05 ug/kg/dag	5 ug/kg/dag
2	0.10 ug/kg/dag	10 ug/kg/dag
3[2, 3]	0.15 ug/kg/dag	15 ug/kg/dag
4[2, 3]	0.20 ug/kg/dag	20 ug/kg/dag
5[3] (adult)	Microgynon 30 ® continuous (oral contraceptive function)	Femoston 1/5 mg continuous (no contraceptive function)
	Consider transdermal therapy ethinylestradiol 600 ug, norelgestromine 6 mg (contraceptive function)	

[1]The duration of level 1, 2, 3 and 4 is 6 months. [2]Consider medroxyprogesterone for 7 days prior to start of treatment to generate withdrawal bleeding. [3]In level 3 and higher, estradiol therapy needs to be combined with Medroxyprgesterone 5 mg per day, during 15 days per month. [4]Preferred treatment

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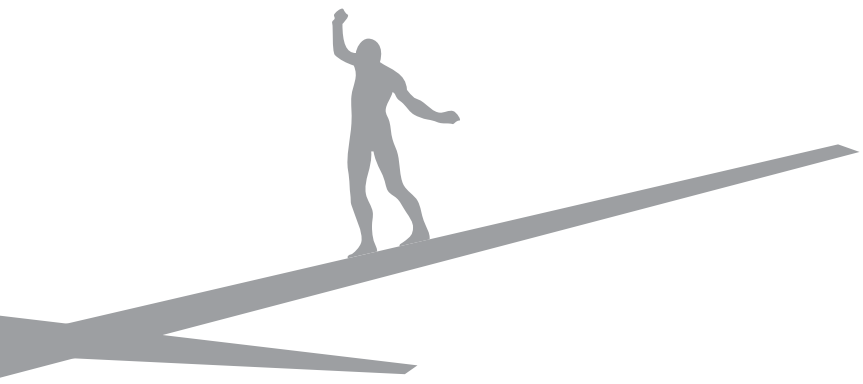
9

Summary / Samenvatting



Summary

This doctoral dissertation provides a detailed account of the various studies performed to improve the knowledge of Prader-Willi syndrome (PWS) and the care for patients with PWS. Studies were embedded in the Dutch National Randomized Controlled trial and Dutch PWS Cohort study. This chapter summarizes these studies and their most important outcomes.

Chapter 1

This chapter provides an introduction in PWS by discussing the genetic cause, symptoms in different phases of life, growth hormone treatment and unresolved issues, such as adrenarche and pubarche, gonadal function of boys and girls, cognition, health related quality of life and behavior. This chapter further presents the aims and outline of this thesis.

Chapter 2

Early adrenarche, the early increase in circulating androgen concentrations, resulting in early appearance of adult type body odor, acne and comodones, oily hair and pubic and/or axillary hair, has been described in children with PWS, but was often studied retrospectively, in small groups, or in mixed groups with adults and children. Information about serum androgen levels in children with PWS was very limited and knowledge about the age at onset and the prevalence of premature adrenarche in PWS was also scarce. We prospectively followed 120 children (6 months-17 years) included in the Dutch PWS Cohort study and assessed levels of serum dehydroepiandrosterone (DHEAS) in 97 children with PWS, which were compared to a control group comprising of 335 healthy age-matched prepubertal children. Furthermore, in all children, the age at onset of pubarche and various pubic hair stages and prevalence of premature pubarche (PP) were determined. We found that median serum DHEAS levels were significantly higher in children with PWS than in healthy age-matched controls at ages 3 to 10 years. The median [P10-P90] age at onset of pubarche in children with PWS was 9.04[6.75-11.84] years in PWS girls and 10.31[8.65-12.29] years in boys, which wa significantly younger than in healthy peers. The prevalence of PP in children with PWS was 30.0% in girls and 16.1% in boys. In conclusion, our study shows that compared to healthy children, children with PWS have significantly higher DHEAS levels from 3 to 10 years of age. They are younger at onset of pubarche and have a higher prevalence of PP. DHEAS levels in PWS are higher in boys than in girls and increase with age, while GH-treatment, BMI and fat mass have no effect on DHEAS levels. Our findings suggest an earlier maturation of the zona reticularis of the adrenal glands in children with PWS.

Chapter 3

The pathophysiology of hypogonadism in boys with PWS was uncertain. In addition, it remained unclear at what age gonadal dysfunction arised, because no longitudinal data in a large group of boys and adolescents with PWS were available. We evaluated gonadal func-

tion over time and the effect of GH-treatment in 66 boys with PWS participating in the Dutch PWS Cohort study, by longitudinal assessment of inhibin B, FSH, testosterone and LH levels in prepubertal boys and male adolescents with PWS. Serum inhibin B, FSH, LH and testosterone levels were compared with reference values. Our results showed that boys with PWS had normal inhibin B levels between 6 months and 10 years of age, but after onset of puberty, inhibin B levels declined to less than the 5th percentile and FSH levels increased to above the 95th percentile. Two years after the onset of puberty and in young adults, inhibin B levels were significantly lower and FSH levels significantly higher than at onset of puberty. Testosterone levels increased, but remained below the 5th percentile and LH levels increased, but not above the 95th percentile. Age showed a significant correlation with inhibin B levels after 9 years of age and GH-treatment had no significant effect on inhibin B levels. In conclusion, our study shows that hypogonadotropic hypogonadism is not the main reason of hypogonadism in most boys with PWS. Only some boys have a combination of gonadal dysfunction and decreased functioning of the hypothalamic- pituitary-gonadal axis. In the majority of male adolescents with PWS, primary testicular dysfunction, especially in the tubular compartment, and moderate Leydig cell dysfunction underlie hypogonadism.

Chapter 4

Clinical signs of hypogonadism, such as hypoplasia of labia minora and/or clitoris, were reported in girls with PWS. The etiology of hypogonadism in girls with PWS remained uncertain because longitudinal information about gonadal function was lacking. We evaluated gonadal function longitudinally in girls and female adolescents with PWS by longitudinal assessment of anti-Müllerian hormone (AMH), gonadotrophins, estradiol (E_2), inhibin B and A and pubertal development in 61 girls and female adolescents with PWS participating in the Dutch PWS Cohort study. Serum AMH, gonadotrophins, E_2 and inhibin B and A levels were compared with reference values. Our results showed that AMH levels in girls and female adolescents with PWS were comparable to reference levels between 6 months and 22 years of age. From 10 years of age, FSH and LH levels increased to above the 5th percentile of reference levels. E_2 and inhibin B levels were in the low normal range in the majority of girls, whereas inhibin A levels were low, but detectable in almost half the female adolescents with PWS. The median age at puberty onset was similar, but the median ages at attaining Tanner M3 and M4 were significantly higher in girls with PWS than in healthy references. In conclusion, findings in our study indicate that the primordial follicle pool is conserved in girls with PWS, throughout puberty and adolescence. We did not find classical hypogonadotropic hypogonadism. Our findings further indicate defects in the maturation of follicles and in the progression of pubertal development in female adolescents with PWS, which might be related to dysregulation of LH secretion. However, as these defects are not absolute, ovulation and thus conception cannot be ruled out in an individual female adolescent with PWS. Therefore, contraceptive therapy might be considered if clinical and laboratory findings and circumstances request this.

Chapter 5

There was no information about the effect of GH-treatment on cognition in children with PWS above 3 years of age, beyond a period of 6 months. We therefore studied 50 pre-pubertal children, aged 3.5 to 14 years in the randomized controlled GH trial during 2 years, followed by a longitudinal study during 4 years of GH-treatment. Cognitive functioning was measured biennially by a short form of the WPPSI-R or WISC-R test, depending on age. Total IQ (TIQ) score was estimated based on 2 subtest scores. We found that during the RCT, mean SD-scores of all subtests and mean TIQ score remained similar compared to baseline in GH-treated children with PWS, while in untreated controls, mean subtest SD-scores and mean TIQ score decreased and became lower compared to baseline. After 4 years of GH-treatment, mean SD-scores on the Similarities and Block design subtests were significantly higher than at baseline and scores on Vocabulary and TIQ scores remained similar compared to baseline. At baseline, children with a maternal uniparental disomy had a significantly lower score on the Block design subtest, but a larger increment on this subtest during 4 years of GH-treatment than children with a deletion. Lower baseline scores correlated significantly with higher increase in Similarities and Block design SD-scores. In conclusion, our study shows that GH-treatment prevents deterioration of certain cognitive skills in children with PWS on the short term and significantly improves abstract verbal reasoning and visuospatial skills during 4 years of GH-treatment compared to a reference population. The more children lag behind, the more benefit they had from GH-treatment. Based on our results, we conclude that GH-treatment in children with PWS is not merely an effective treatment for normalizing height and improving body composition, but has also a beneficial effect on their cognitive functioning.

Chapter 6

Information about health-related-quality-of-life (HRQOL) or the effect of GH-treatment on HRQOL in children with PWS was scarce. We therefore evaluated HRQOL in children with PWS and investigated the effect of GH-treatment in 25 pre-pubertal children, aged 6 to 14 years in a randomized controlled GH trial (RCT) during 2 years, followed by a longitudinal study during 4 years of GH-treatment. HRQOL was measured biennially by self and parental reports on a generic questionnaire (DUX25), that contained four subdomains (Physical, Home, Social and Emotional) and a PWS-specific questionnaire (DUXPW). We found that children with PWS scored significantly higher than their parents on the Physical subdomain and on the DUXPW and they rated their HRQOL higher than healthy, chronically ill, obese or growth impaired children, especially on the Physical subdomain. During the RCT, HRQOL increased significantly compared to baseline according to GH-treated children on the Physical and Home subdomains and on the DUXPW total score and according to parents on the DUX25 total score and the Physical subdomain. Scores in untreated children with PWS decreased or remained similar to baseline. During 4 years of GH-treatment, HRQOL increased significantly compared to baseline according to children on the DUXPW and on the Home subdomain and according to parents

on the DUX25 and Physical subdomain. In conclusion, our study shows that children with PWS report a normal HRQOL. Both children and parents report an increase in HRQOL during GH-treatment, while this increase was not found in the randomized untreated children with PWS. During long-term GH-treatment, HRQOL continued to increase even further. Based on our results, we conclude that GH-treatment in children with PWS is not merely an effective treatment for normalizing height and improving body composition, but has also beneficial effects on their HRQOL.

Chapter 7

Long-term continuous GH-treatment is an effective and safe treatment for children with PWS. GH-treatment has beneficial effects on antropometrics, body composition, cognition, activity level and motor development, but little was known about the effect of GH-treatment on behavioral problems, although parents of children with PWS reported less problem behavior during GH-treatment. In this study, we investigated behavior in children with PWS and the effects of GH-treatment in 50 pre-pubertal children, aged 3.5 to 14 years in a randomized controlled GH trial during 2 years (RCT), followed by a longitudinal study during 4 years of GH-treatment. Behavior was measured annually by the Developmental Behavior Checklist for children with mental disability (DBC) and a Dutch questionnaire to evaluate social behavioral problems in children, the Children's Social Behavior Questionnaire (CSBQ). We found that problem behavior measured by the DBC in children with PWS was similar compared to references with a comparable mental retardation, but scores on the 'Communication disorders' and 'Social disabilities' subscales were relatively high compared to the DBC total score. Problem behavior measured by the CSBQ was higher compared to healthy references. Children with PWS scored significantly higher than 0 SDS on the subscales that measured social inappropriate and stereotyped behavior and on the subscales that measured problems with making contact and orientation and being resistant to changes. They scored significantly higher than +1 SDS on the subscales that measured problems with social understanding and the CSBQ total score. Behavioral problems measured by the DBC and the CSBQ increased significantly with age. After correction for age, there were no significant effects of GH-treatment during the RCT and 4 years of GH-treatment. In conclusion, our study shows that problem behavior in children with PWS is in many aspects similar as in children with a comparable mental retardation. However, they display more pervasive developmental disorders compared to a healthy population. Problem behavior increases with age in both GH-treated and untreated children with PWS alike. Thus, in contrast to our expectations, our study showed no significant effect of GH-treatment on behavioral problems in children with PWS.

Chapter 8

In the general discussion, we discuss our findings in a broader context. We present our general conclusions and the chapter ends with suggestions for further research.

Samenvatting

Dit proefschrift beschrijft verschillende studies die verricht zijn om de kennis van het Prader-Willi syndroom (PWS) te vergroten en de zorg voor patiënten met PWS te verbeteren. De studies zijn uitgevoerd binnen het kader van de landelijke groeihormoon studies voor kinderen met PWS. In dit hoofdstuk wordt een samenvatting gegeven van deze studies en van de belangrijkste bevindingen in deze studies.

Hoofdstuk 1

Dit hoofdstuk is een korte introductie van het PWS en beschrijft de genetische oorzaken van het syndroom, de verschillende symptomen per levensfase, groeihormoonbehandeling en onderwerpen waarover binnen PWS nog weinig bekend is. Verder wordt in dit hoofdstuk besproken wat de doelstellingen en indeling van dit proefschrift zijn.

Hoofdstuk 2

Vroege adrenarche, een vroegtijdige stijging van circulerende androgeen concentraties in het bloed, welke resulteren in vroegtijdige ontwikkeling van een volwassen lichaamsgeur, acne en comedonen, vettig haar, axillaire beharing en pubarche (pubis beharing), werd al eerder beschreven bij kinderen met PWS. Echter de studies waarin dit werd beschreven waren vaak retrospectief en gebaseerd op kleine of gemengde groepen bestaande uit zowel kinderen als volwassenen. Er was bij kinderen met PWS weinig bekend over androgeen spiegels in het bloed, de leeftijd waarop de adrenarche aanvangt en de prevalentie van premature pubarche (PP). In onze PWS Cohort studie werden 120 kinderen in de leeftijd van 6 maanden tot 17 jaar prospectief gevolgd. Bij 97 van deze kinderen hebben we bloedspiegels van het androgeen dehydroepiandrosteron sulfaat (DHEAS) bepaald en deze vergeleken met spiegels van 335 gezonde leeftijdsgenoten. Verder hebben we bij alle kinderen de leeftijd bepaald waarop de adrenarche aanvangt en de ontwikkeling van de pubarche en de prevalentie van PP onderzocht. Uit ons onderzoek kwam naar voren dat vergeleken met leeftijdsgenoten, DHEAS waarden significant hoger waren bij kinderen met PWS in de leeftijd van 3 tot 10 jaar. De mediane leeftijd waarop bij meisjes met PWS de adrenarche begon was 9.04 jaar en bij de jongens was dit 10.31 jaar. Zowel bij jongens als meisjes was dit significant jonger dan bij leeftijdsgenoten zonder PWS. De prevalentie van PP bij kinderen met PWS was 30.0% bij meisjes en 16.1% bij jongens. Concluderend hebben kinderen met PWS in de leeftijd van 3 tot 10 jaar hogere bloedspiegels van DHEAS dan leeftijdsgenoten, zijn kinderen met PWS jonger bij de start van de pubarche en is er bij kinderen met PWS een hogere prevalentie van PP. Verder worden serum DHEAS waarden beïnvloed door zowel leeftijd en geslacht, oudere kinderen hebben hogere DHEAS waarden dan jongere en jongens hebben hogere waarden dan meisjes, en hebben groeihormoonbehandeling, BMI en vetmassa geen effect op serum DHEAS spiegels. Deze bevindingen suggereren een vroegere rijping van de zona reticularis van de bijnieren van kinderen met PWS.

Hoofdstuk 3

De pathofysiologie van hypogonadisme bij jongens met PWS was tot op heden nog onduidelijk. Het was eveneens onduidelijk op welke leeftijd deze gonadale disfunctie ontstond. Dit werd mede veroorzaakt doordat er geen longitudinale data over de gonadale functie bij een grote groep jongens en jongvolwassen mannen met PWS beschikbaar waren. In onze studie hebben we bij 66 jongens met PWS die participeerden in de PWS Cohort studie bloedspiegels van inhibine B, FSH, testosteron en LH gedurende een langere periode vervolgd en vergeleken met de bloedspiegels van leeftijdsgenoten. Onze resultaten lieten zien dat jongens met PWS in de leeftijd van 6 maanden tot 10 jaar normale inhibine B spiegels in hun bloed hadden, maar dat de spiegels tijdens de puberteit daalden tot onder het 5de percentiel vergeleken met leeftijdsgenoten. Tegelijkertijd stegen FSH spiegels tot boven het 95ste percentiel. Twee jaar na de start van de puberteit en in jongvolwassen mannen waren de inhibine B spiegels significant lager en de FSH spiegels significant hoger dan bij de start van de puberteit. Testosteron spiegels stegen, maar bleven onder het 5de percentiel en ook de LH spiegels stegen, maar niet boven het 95ste percentiel. Er was een significante correlatie tussen de leeftijd en de inhibine B spiegel na de leeftijd van 9 jaar en groeihormoonbehandeling had geen effect op de inhibine B spiegels. Concluderend kunnen we stellen dat onze studie laat zien dat bij de meeste jongens met PWS, hypogonadoptroop hypogonadisme niet de voornaamste oorzaak van hypogonadisme is. Bij sommige jongens zou een combinatie van gonadale disfunctie en een verminderde functie van de hypothalame-hypofysaire-gonadale as een rol kunnen spelen, maar bij de meeste mannelijke adolescenten met PWS is de onderliggende oorzaak van hun hypogonadisme een primaire testiculaire disfunctie, en dan voornamelijk in het tubulaire compartiment, en is er daarnaast sprake van een matige Leydig cel disfunctie.

Hoofdstuk 4

Bij meisjes met PWS werden tekenen van hypogonadisme gerapporteerd, zoals hypoplasie van de labia minora en/of clitoris. De etiologie van hypogonadisme bleef echter onduidelijk bij deze meisjes, omdat longitudinale studies naar de gonadale functie bij meisjes met PWS ontbraken. Wij evalueerden daarom de gonadale functie bij 61 meisjes en adolescenten met PWS die participeerden in de Nederlandse PWS Cohort studie, door bij deze patiënten gedurende een langere periode spiegels in het bloed te meten van anti-Müllerian hormoon (AMH), gonadotrophines, oestradiol, inhibine B en A. Daarnaast onderzochten we de puberteitsontwikkeling bij deze meisjes en adolescenten. De AMH, gonadotrophine, oestradiol en inhibine B en A spiegels werden vergeleken met leeftijdsgenoten zonder PWS. Onze resultaten lieten zien dat AMH spiegels van meisjes met PWS in de leeftijd van 6 maanden tot 22 jaar vergelijkbaar waren met die van leeftijdsgenoten zonder PWS. Vanaf de leeftijd van 10 jaar namen de waarden van FSH en LH in het bloed toe tot boven het 5de percentiel in vergelijking met leeftijdsgenoten. Zowel de oestradiol als inhibine B spiegels waren laag nor-

maal vergeleken met leeftijdsgenoten. Inhibine A spiegels waren laag, maar niet onmeetbaar laag, in bijna de helft van de vrouwelijke adolescenten met PWS. De mediane leeftijd bij de start van de puberteit was vergelijkbaar met die van leeftijdsgenoten zonder PWS, maar de mediane leeftijd bij het bereiken van de M3 en M4 stadia volgens Tanner, was significant hoger dan bij leeftijdsgenoten. Op basis van deze resultaten kunnen we concluderen dat de primordiale follikel voorraad aanwezig is bij meisjes en vrouwelijke adolescenten met PWS. Er is geen sprake van een klassieke vorm van hypogonadotroop hypogonadisme. Onze bevindingen wijzen er op dat er defecten zijn in de maturatie van follikels en in de ontwikkeling van de puberteit bij vrouwelijke adolescenten met PWS. Dit zou veroorzaakt kunnen worden door een disregulatie in de LH secretie. Echter, deze defecten zijn niet absoluut en daarom is ovulatie en dus de mogelijkheid van conceptie niet uitgesloten bij een individuele patiënte met PWS. Daarom zou contraceptieve therapie overwogen moeten worden wanneer klinische factoren, hormonale waarden, in combinatie met bepaalde omstandigheden dit vereisen.

Hoofdstuk 5

Er was nog weinig bekend over het effect van groeihormoon behandeling op het cognitief functioneren van kinderen met PWS. Met name bij kinderen PWS ouder dan 3 jaar die langer dan 6 maanden met GH werden behandeld. We hebben daarom het effect van groeihormoonbehandeling op bepaalde cognitieve functies onderzocht in een gerandomiseerde en gecontroleerde groeihormoon studie gedurende 2 jaar bij 50 kinderen met PWS in de leeftijd van 3.5 tot 14 jaar. Na afloop van de gerandomiseerde studie werden de kinderen verder vervolgd in de PWS Cohort studie waarin we het effect van 4 jaar groeihormoonbehandeling op deze cognitieve functies hebben onderzocht. De mate van cognitief functioneren hebben we jaarlijks bepaald met behulp van verkorte versies van de WPPSI-R of WISC-R afhankelijk van de leeftijd van de kinderen. De totale IQ score hebben we berekend op basis van de uitslag van 2 subtesten. We vonden dat tijdens de 2 jaar durende gerandomiseerde en gecontroleerde studie de gemiddelde SD-scores op alle subtesten stabiel bleven bij kinderen met PWS die behandeld worden met groeihormoon, terwijl de gemiddelde SD-scores van kinderen met PWS die niet behandeld worden verslechterden. Gedurende 4 jaar groeihormoonbehandeling namen de gemiddelde SD-scores op 2 subtesten, Overeenkomsten en Blokpatronen, significant toe en bleven gemiddelde SD-scores op de andere subtest, Woordenschat, en de totale IQ score, stabiel. Aan het begin van de studie scoorden kinderen met een maternale uniparentele disomie significant slechter op de subtest Blokpatronen dan kinderen met een deletie, maar zij lieten eveneens een grotere verbetering zien op deze subtest gedurende 4 jaar groeihormoonbehandeling. We vonden een significante correlatie tussen lagere scores aan het begin van de studie en een grotere verbetering gedurende 4 jaar groeihormoonbehandeling op de subtests Overeenkomsten en Blokpatronen. We concluderen op basis van onze resultaten dat groeihormoon behandeling bij kinderen met PWS verslechtering van bepaalde cognitieve functies gedurende behandeling op de korte termijn voorkòmt, en

dat gedurende 4 jaar behandeling het abstract verbaal redeneren en de visuospatiele vaardigheden verbeteren wanneer dit wordt vergeleken met leeftijdsgenoten zonder PWS. Hoe meer de kinderen met PWS achter lopen ten opzichte van hun leeftijdsgenoten, hoe meer zij profiteren van het positieve effect van groeihormoonbehandeling. Groeihormoonbehandeling bij kinderen met PWS is dus niet alleen een effectieve behandeling wanneer het gaat om het normaliseren van de lengte en het verbeteren van de lichaamssamenstelling, maar heeft ook een positief effect op het cognitief functioneren van deze kinderen.

Hoofdstuk 6

Er was zeer weinig bekend over de aan ziekte en gezondheid gerelateerde kwaliteit van leven bij kinderen met PWS en over het effect van groeihormoon behandeling op de kwaliteit van leven van deze kinderen. Wij hebben daarom de aan ziekte en gezondheid gerelateerde kwaliteit van leven onderzocht bij 25 prepubertaire kinderen met PWS in de leeftijd van 6 tot 14 jaar voordat zij startten met groeihormoonbehandeling en we hebben het effect van groeihormoonbehandeling bij deze kinderen onderzocht gedurende een 2 jaar durende gerandomiseerde en gecontroleerde groeihormoonstudie. Na afloop van de gerandomiseerde studie werden de kinderen verder vervolgd in de PWS Cohort studie, zodat we het effect van 4 jaar groeihormoonbehandeling op de kwaliteit van leven konden onderzoeken. Kwaliteit van leven hebben we tweejaarlijks gemeten met behulp van 2 vragenlijsten, een generieke vragenlijst (DUX25) die 4 subdomeinen bevat (Lichamelijk, Thuis, Sociaal en Emotioneel) en een ziekte specifieke PWS vragenlijst (DUXPW). Deze vragenlijsten werden door zowel de kinderen zelf als hun ouders ingevuld. Uit ons onderzoek kwam naar voren dat kinderen met PWS significant hoger scoorden dan hun ouders op het Lichamelijke subdomein en op de DUXPW en dat kinderen met PWS significant hoger scoorden dan gezonde, chronisch zieke en obese kinderen en kinderen met groeiproblemen. Dit gold vooral voor het Lichamelijke subdomein. Tijdens de gerandomiseerde en gecontroleerde groeihormoonstudie bleek dat kinderen met PWS die behandeld werden met groeihormoon hun kwaliteit van leven een hogere score gaven na 2 jaar, voornamelijk op de subdomeinen Lichamelijk en Thuis en op de DUXPW totaal score. Ook de ouders gaven de kwaliteit van leven van hun kinderen die groeihormoon kregen een hogere score na 2 jaar studie, voornamelijk op de DUX25 totaal score en het Lichamelijke subdomein. Scores van onbehandelde kinderen met PWS namen af of bleven gelijk aan de score aan het begin van de studie. Volgens kinderen met PWS nam gedurende 4 jaar groeihormoonbehandeling hun kwaliteit van leven significant toe. Vergeleken met de start van de studie scoorden zij na 4 jaar significant hoger op de DUXPW en het Huis subdomein. Ook volgens de ouders van kinderen met PWS nam de kwaliteit van leven van hun kinderen gedurende 4 jaar groeihormoonbehandeling toe. Vergeleken met de start van de studie scoorden zij na 4 jaar significant hoger op de DUX25 totaal score en het Lichamelijke subdomein. We concluderen op basis van onze bevindingen dat kinderen met PWS een normale kwaliteit van leven rapporteren. Zowel kinderen als hun ouders rapporteren

een toename in kwaliteit van leven tijdens groeihormoon behandeling, terwijl dit niet het geval is bij onbehandelde kinderen. Tijdens 4 jaar groeihormoon behandeling neemt de kwaliteit van leven zelfs nog verder toe. Groeihormoonbehandeling bij kinderen met PWS is dus niet alleen een effectieve behandeling wanneer het gaat om het normaliseren van de lengte en het verbeteren van de lichaamssamenstelling, maar heeft ook een positief effect op de aan ziekte en gezondheid gerelateerde kwaliteit van leven van deze kinderen.

Hoofdstuk 7

Gedragsproblemen spelen een belangrijke rol bij kinderen met PWS, maar er was nog weinig bekend over het effect van groeihormoonbehandeling op het gedrag van deze kinderen, ondanks dat ouders wel gedragsverbetering rapporteren tijdens groeihormoonbehandeling. We hebben daarom het effect van groeihormoonbehandeling op het gedrag onderzocht in een gerandomiseerde en gecontroleerde groeihormoon studie gedurende 2 jaar bij 50 kinderen met PWS in de leeftijd van 3.5 tot 14 jaar. Na afloop van de gerandomiseerde studie werden de kinderen verder vervolgd in de PWS Cohort studie waarin we het effect van 4 jaar groeihormoon behandeling op het gedrag hebben onderzocht. Het gedrag van de kinderen werd jaarlijks geëvalueerd met behulp van 2 oudervragenlijsten, de gedragsvragenlijst voor verstandelijk beperkte kinderen (VOG) en de vragenlijst voor inventarisatie van sociale problemen bij kinderen (VISK). Uit ons onderzoek kwam naar voren dat probleemgedrag bij kinderen met PWS gemeten met de VOG overeen kwam met kinderen met een vergelijkbare mentale retardatie. Echter, scores op de subschalen communicatieproblemen en sociale problemen waren bij kinderen met PWS relatief hoog ten opzichte van de VOG totaalscore. Probleemgedrag gemeten met de VISK was significant hoger vergeleken met gezonde leeftijdsgenoten. Kinderen met PWS scoorden significant hoger dan 0 SDS op de subschalen onaangepast gedrag, contact mijdend, oriëntatieproblemen, stereotiep gedrag en verzet tegen veranderingen en significant hoger dan +1 SDS op de subschaal die problemen met sociaal begrip meet en de totale VISK score. Gedragsproblemen gemeten met de VOG en de VISK namen significant toe met de leeftijd. Na correctie voor leeftijd vonden we geen significante effecten van groeihormoonbehandeling, zowel tijdens de gerandomiseerde studie als gedurende 4 jaar groeihormoon behandeling. We concluderen op basis van onze bevindingen dat probleemgedrag bij kinderen met PWS vergelijkbaar is als bij kinderen met een mentale retardatie door een andere oorzaak. Daarnaast vertonen kinderen met PWS meer pervasieve ontwikkelingsstoornissen dan gezonde kinderen. Probleemgedrag bij kinderen met PWS neemt toe met de leeftijd in zowel groeihormoon behandelde als onbehandelde kinderen. Dus in tegenstelling tot onze verwachtingen laten onze resultaten geen effect van groeihormoon behandeling op gedragsproblemen zien.

Hoofdstuk 8

In de algemene discussie bespreken we onze bevindingen in een bredere context. We presenteren onze algemene conclusies en het hoofdstuk eindigt met suggesties voor verder onderzoek.

10

List of abbrevations
List of co-authors and affiliations
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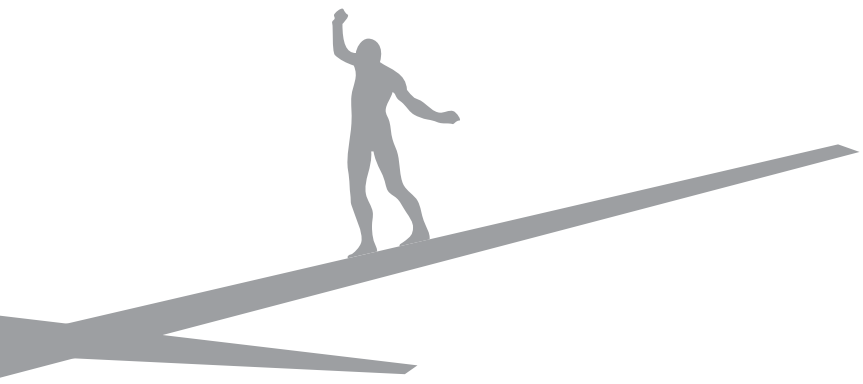

List of abbreviations

ACTH	corticotropin
AHI	apnea hypopnoea index
AMH	anti-Müllerian hormone
ASP	acylation stimulating protein
BA	bone age
BMAD	bone mineral apparent density
BMD	bone mineral density
BMI	body mass index
CA	calendar age
CAI	central adrenal insufficiency
CRH	corticotropin-releasing hormone
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DNA	deoxyribonucleic acid
E_2	estradiol
FSH	follicle stimulating hormone
FSIQ	full scale intelligence quotient
FT4	free thyroxine
GH	growth hormone
GHD	growth hormone deficiency
GHRH	growth hormone-releasing hormone
GnRH	gonadotrophin releasing hormone
HDL	high density lipoproteine
HOMA-IR	homeostatic model assessment of insulin resistance
HRQOL	health related quality of life
IGF-BP3	insuline like growth factor binding factor 3
IGF-I	insulin like growth factor I
IGHD	isolated growth hormone deficiency
IHH	isolated hopygonadotropic hypogonadism
IQ	intelligence quotient
ISS	idiopatic short stature
KS	Klinefelter syndrome
LBM	lean body mass
LDL	low density lipoproteine
LH	luteinizing hormone
MBS	metabolic syndrome

MRI	magnetic resonance imaging
mUPD	maternal uniparental disomy
OSAS	obstructieve slaap apneu syndroom
PDD	pervasive developmental disorders
PIQ	performal IQ
PP	premature pubarche
PRL	prolactine
PWS	Prader-Willi syndrome
RCT	randomized controlled trial
REM	rapid eye movement
SCO	Sertoli cell only
SGA	small for gestational age
SRBD	sleep related breathing disorder(s)
SULT2A1	sulfotransferase
T3	trijoodthyronine
TIQ	total IQ
TRH	thyrotropin-releasing hormone
TSH	thyrotropin
VIQ	verbal IQ
WIPPSI-R	Wechsler Preschool and Primary Scale of Intelligence-Revised
WISC-R	Wechsler Intelligence Scale for Children-Revised
ZR	zona reticularis

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List of publications

1. de Lind van Wijngaarden RFA, **Siemensma EPC**, Festen DAM, Otten BJ, van Mil EG, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Bocca G, Houdijk EC, Hoorweg-Nijman JJ, Vreuls RC, Jira PE, van Trotsenburg AS, Bakker B, Schroor EJ, Pilon JW, Wit JM, Drop SL, Hokken-Koelega ACS. Efficacy and safety of long-term continuous growth hormone treatment in children with Prader-Willi syndrome. *Journal of Clinical Endocrinology and Metabolism. 2009 Nov;94(11):4205-15.*

2. **Siemensma EPC**, de Lind van Wijngaarden RFA, Otten BJ, de Jong FH, Hokken-Koelega ACS. Pubarche and serum dehydroepiandrosterone sulfate levels in children with Prader-Willi syndrome. *Clinical Endocrinology. 2011 Jan;75:83–89.*

3. **Siemensma EPC**, de Lind van Wijngaarden RFA, Otten BJ, de Jong FH, Hokken-Koelega ACS . Testicular failure in boys with Prader-Willi syndrome: longitudinal studies of reproductive hormones. *Journal of Clinical Endocrinology and Metabolism. 2012 Mar;97(3):E452-459.*

4. **Siemensma EPC**, Tummers-de Lind van Wijngaarden RFA, Festen DAM, Troeman ZC, van Alfen-van der Velden AAEM, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Houdijk ECAM, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Willem Pilon J, Lunshof LB, Hokken-Koelega ACS. Beneficial effects of growth hormone treatment on cognition in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study. *Journal of Clinical Endocrinology and Metabolism. 2012 Jul;97(7):2307-14.*

5. **Siemensma EPC**, van Alfen-van der Velden AAEM, Otten BJ, Laven JS, Hokken-Koelega ACS. Ovarian function and reproductive hormone levels in girls with Prader-Willi syndrome: a longitudinal study. *Journal of Clinical Endocrinology and Metabolism. 2012 Sep;97(9):E1766-73.*

6. **Siemensma EPC**, van Rijn M, Festen DAM, Hokken-Koelega ACS. Beneficial effect of growth hormone treatment on health related quality of life in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study. *Submitted.*

7. **Siemensma EPC**, Festen DAM, Hokken-Koelega ACS. Behavior in children with Prader-Willi syndrome before and during GH-treatment: a randomized controlled trial and longitudinal study. *Submitted.*

PhD Portfolio

Summary of PhD training

Department of Pediatrics, subdivision of Endocrinology, Erasmus MC Rotterdam

Research School:	Molecular Medicine Postgraduate School (MolMed)
PhD period:	August 2008-July 2012
Promotor:	Prof. dr. A.C.S. Hokken-Koelega

General courses	**Year**
Biomedical English writing and communication, MolMed, Erasmus MC	2008
Good clinical practice, Erasmus MC	2009
Integrity in medical research, Medical Ethics and Philosophy, Erasmus MC	2009
Missing values in clinical research, NIHES, Erasmus MC	2010

Specific courses	
SNP's and human diseases, MolMed, Erasmus MC	2008
Systemic literature retrieval in Pubmed, Erasmus MC	2008
Thomson Reuters Endnote, Erasmus MC	2008
Adobe InDesign CS5, MolMed, Erasmus MC	2011
Adobe Photoshop and Illustrator CS5, MolMed, Erasmus MC	2011

Seminars and workshops	
Annual Pediatric Research day, Erasmus MC-Sophia	2008-2010
Weekly research meeting, department of Pediatric Endocrinology, Erasmus MC-Sophia	2008-2012
Annual MolMed Day, MolMed, Erasmus MC	2009-2010
Annual PhD Day, Erasmus MC	2009-2011

International conferences	
LWPES/ESPE 8th Joint Meeting, New York USA (oral presentation)	2009
ESPE 49th Annual Meeting, Prague, Czech Republic (poster presentation)	2010
IGF/GRS Society 5th International Congress, New York, USA (oral presentation)	2010

ESPE 50th Annual Meeting, Glasgow, Scotland (poster presentation)	2011
Standardized Assessment of Child Psychopathology: New Developments, Symposium Erasmus MC, Rotterdam	2011
4e Northern-European Neuro-Endocrine Group (NENEG) meeting (oral presentation)	2011
ESPE 51th Annual Meeting, Leipzig, Germany (2 poster presentations)	2012

National conferences

Symposium Stichting Kind & Groei: "Nieuwe inzichten in problemen bij kinderen met het Prader-Willi syndroom, eventuele behandelingen en lange-termijn resultaten van groeihormoonbehandeling", Rotterdam (oral presentation)	2009
Landelijke Adviesgroep vergadering (2 oral presentations)	2010-2011
Symposium Stichting Kind & Groei: Voorlichtingsmiddag voor ouders van kinderen met Prader-Willi syndroom, Utrecht (oral presentation)	2011
11e Nationaal Autisme Congres	2011
Met het oog op Mentale Retardatie, PAOK Erfelijke en Aangeboren Aandoeningen, Máxima Medisch Centrum Veldhoven (oral presentation)	2012

Teaching activities

Supervising Master Thesis of Carla Koopman	2009-2010
Supervising Master Thesis of Marjon van Rijn	2009-2010
Organisation of the Annual IMC Weekendschool day "Growth and Development", Rotterdam	2010-2012
Educational lecture minor students, Pediatric Endocrinology, Erasmus MC Rotterdam	2012

Research proposals

Psychiatrische stoornissen bij kinderen en jongvolwassenen met het Prader-Willi syndroom-Psychiatric Disorders study (PD studie)	2010
Brain structure and -development in children with Prader-Willi syndrome (MRI studie)	2010

Curriculum vitae

Elbrich Siemensma was born in Heinkenszand, the Netherlands, on January 26th in 1979. She passed her secondary school exam (Atheneum) at “St. Bonifatiuscollege” in Utrecht in 1997. In that same year she started medical school at “Limburg Universitair Centrum (LUC)” in Diepenbeek, Belgium. She obtained her bachelor’s degree (in Dutch: Eerste Kandidaatsjaar Geneeskunde) in 1998. In 1999 she went to Nijmegen, the Netherlands to study social sciences, direction pedagogy (in Dutch: Pedagogische Wetenschappen”) at the Radboud University Nijmegen. She obtained her master’s degree in social sciences in 2003. In that same year, she continued medical school at the Radboud University in Nijmegen and graduated on the theoretical part in 2005. She started her internships in that same year and obtained her medical degree in 2007. She started working as a pediatric resident in 2008 at Erasmus-MC Sophia Children’s Hospital in Rotterdam. After 6 months she started working on a clinical research project (The PWS-study) at the Dutch Growth and Research Foundation and the department of pediatric endocrinology of Erasmus-MC Sophia Children’s Hospital in Rotterdam (promotor Prof. dr. A.C.S. Hokken-Koelega). The research project resulted in this thesis. Elbrich started her psychiatric residency in July 2012 at Erasmus-MC in Rotterdam, to become a child and adolescent psychiatrist. Elbrich lives together with her fiancé Sander van Schaik in Rotterdam, the Netherlands.

Dankwoord

Het schrijven van een proefschrift en het doen van onderzoek was voor mij een zeer leerzame, gezellige, spannende, soms moeilijke en drukke, maar alles bij elkaar een hele bijzondere tijd! De afgelopen jaren heb ik met veel plezier gewerkt aan mijn onderzoek. Dat heb ik voor een groot deel te danken aan de vele mensen om mij heen die mij de afgelopen jaren geholpen hebben. Daar wil ik iedereen van harte voor bedanken, en enkele personen in het bijzonder.

Als eerste wil ik alle kinderen met PWS en hun ouders bedanken voor hun deelname aan dit onderzoek. Sommigen van jullie doen zelfs al meer dan 10 jaar mee! Zonder jullie inzet zou geen van deze onderzoeken mogelijk zijn geweest. Lieve kinderen met PWS, ik heb genoten van jullie eerlijkheid, openheid en spontaniteit. Beste ouders van kinderen met PWS, van uw geduld en doorzettingsvermogen heb ik veel kunnen leren, dank u wel. Ik zal de ontmoetingen met jullie allemaal niet snel vergeten.

Mijn promotor, prof. dr. A.C.S. Hokken-Koelega. Beste Anita, je bood mij de kans om onderzoek te doen bij een unieke groep kinderen en binnen een unieke organisatie, de Stichting Kind & Groei. Je bent een bevlogen, hard werkende, integere, zeer precieze kinderarts en wetenschapper met een groot verantwoordelijkheidsgevoel voor de mensen die bij het werk betrokken zijn, of dat nu patiënten, ouders of werknemers zijn. Ik heb ontzettend veel van je kunnen leren de afgelopen jaren en daarvoor dank ik je van harte.

Prof. dr. F.H. de Jong. Graag wil ik u bedanken voor uw bereidheid plaats te nemen als secretaris in de kleine commissie en voor de snelle beoordeling van mijn manuscript. Daarnaast mijn hartelijke dank voor het bepalen van de hormoonwaarden en voor uw constructieve adviezen bij het schrijven van twee artikelen.

Prof. dr. S.L.S. Drop. U wil ik graag bedanken voor de snelle beoordeling van mijn proefschrift en voor uw deelname in de kleine commissie. Daarnaast wil ik hartelijk bedanken voor de samenwerking en de leerzame momenten bij onze ‘endobesprekingen’ op maandagmiddag.

Prof. dr. A.J. van der Lelij. U wil ik graag bedanken voor de snelle beoordeling van mijn proefschrift en voor uw deelname in de kleine commissie.

Overige leden van de promotiecommissie, heel hartelijk dank voor uw bereidheid plaats te nemen in de grote commissie.

Dr. B.J. Otten. Beste Barto, bedankt voor samenwerking binnen de PWS studie. Het plezier waarmee je werkt met de kinderen straalt van je af en werkt aanstekelijk. Van de manier

waarop je omgaat met de kinderen en hun ouders en de zeer positieve reacties daarop heb ik veel kunnen leren. Naast klinische leermomenten op de poli heb ik veel van je geleerd over de onderwerpen waarover we samen artikelen hebben geschreven. Je was altijd erg betrokken bij het schrijfproces en dat heb ik zeer gewaardeerd.

Mw. dr. A. A. E. M. van Alfen-van der Velden. Beste Janielle, ook jij van harte bedankt voor de samenwerking binnen de studie en op de polikliniek. Het nabespreken van de polibezoeken en de overlegmomenten tussendoor zijn zeer leerzaam voor mij geweest.

Graag wil ik alle kinderartsen van de deelnemende centra bedanken voor hun inzet en betrokkenheid tijdens onze bezoeken op de polikliniek. Daarnaast dank ik alle kinderartsen in Nederland die ouders en kinderen met PWS bij ons hebben aangemeld en gestimuleerd om deel te nemen aan de PWS studie.

Alle co-auteurs van de manuscripten in dit proefschrift wil ik van harte bedanken voor de samenwerking.

Pfizer bv Nederland wil ik bedanken voor de financiële ondersteuning, in het bijzonder Joli van der Lans en Marlies Papone, jullie bedankt voor de prettige samenwerking.

De Vereniging Trustfonds Erasmus Universiteit Rotterdam wil ik bedanken voor hun financiële bijdragen aan de jaarlijkse ESPE congresbezoeken.

Dr. A.W. de Weerd en Mw. R.A.S van den Bossche. Bedankt voor het verrichten en plannen van de polysomnografieën.

Drs. J.P. Sluimer. Bedankt voor het verrichten en analyseren van de DXA-scans.

Prof. dr. J. van Doorn en dr. W. Hackeng wil ik hartelijk danken voor de laboratoriumbepalingen.

Inge Maitimu en haar collega's wil ik graag bedanken voor het uitvoeren van alle IGF-bepalingen.

Dr. M.W.G. Nijhuis – van den Sanden en Mw. drs. L. Reus. Beste Ria en Linda, bedankt voor de fijne samenwerking omtrent het onderzoek naar fysiotherapeutische behandeling voor jonge kinderen met PWS.

Drs. S. Rasenberg en mw. J. Veen. Beste Sylvia en José, hartelijk dank voor de orthopedagogische en dieetkundige adviezen.

Dr. M.A.J. de Ridder wil ik graag danken voor haar bijdrage aan de statistische analyses.

Dr. E.L.T. van den Akker, J.C. van der Heijden, Dr. Y.B. de Rijke en Dr. E.F. Gevers, bedankt voor de prettige samenwerking op de afdeling kinderendocrinologie en voor de aanvullingen en tips tijdens de leerzame 'endobesprekingen'.

Mariëlle van Eekelen. Lieve Mariëlle, jij bent van het begin af aan de steun en toeverlaat van alle kinderen en hun ouders die deelnemen aan de studie, maar daarnaast zeker ook van de arts-onderzoeker. Vooral in het begin toen alles voor mij nog nieuw en onduidelijk was, vond ik het geweldig om van je kennis en ervaring gebruik te kunnen maken. Je grenzeloze inzet voor de studie, je openheid, eerlijkheid en bescheidenheid sieren je, ik kijk met een dankbaar gevoel terug op onze samenwerking.

De (arts-)onderzoekers binnen het PWS team. Graag wil ik alle vorige en huidige (arts-)onderzoekers binnen het PWS team bedanken. Dederieke, Roderick, Akvile, Sinddie, Nienke en Renske. Dederieke, meer dan 10 jaar geleden heb jij de studie opgezet. Door de goede basis die jij gelegd hebt, hebben we de studie in de loop der jaren steeds kunnen uitbreiden. Je betrokkenheid bij de studie, de artikelen en natuurlijk de kinderen, is nog steeds heel groot, waarvoor dank. Roderick, jij hebt me toen ik begon binnen de studie de kneepjes van het vak geleerd, je nam uitgebreid te tijd om mij wegwijs te maken, terwijl je op dat moment ook nog een proefschrift te schrijven had, daarvoor ben ik je heel dankbaar. Akvile, Sinddie, Nienke, en Renske, door jullie komst kan er vier keer zo hard aan het onderzoek gewerkt worden, dat is geweldig voor alle kinderen met PWS. Dank voor jullie collegialiteit en gezelligheid daar op de bovenverdieping.

De psychologen binnen het PWS-team. Anne, Marit en Zyrhea. Jullie wil ik bedanken voor jullie geduld, doorzettingsvermogen en flexibiliteit, dat was bij het afnemen en plannen van de psychologische tests soms hard nodig.

Alle endo-collega's, Annemieke, Emile, Daniëlle, Darya, Florentien, Gerthe, Judith, Laura, Nienke, Petra, Ralph, Renske, Roderick, Ruben, Sandra, Sinddie en Yvonne. Jullie bedankt voor jullie collegialiteit en gezelligheid op ESPE congressen, tijdens etentjes en weekendjes.

Alle andere collega onderzoekers van het Sophia, de SOV'ers. Ook jullie bedankt voor jullie collegialiteit en gezelligheid tijdens SOV-diners, -borrels, en –weekendjes.

De medewerkers van de Stichting Kind & Groei, Connie, Eefje, Francine, Gladys, Ineke, Iris, Janneke, Jolanda, Jose, Laura, Lydia, Marianne, Rosadinde, Sander, Sandra en Sunita. Ik dank jullie voor de fijne tijd op deze mooie werkplek. Ineke, jij in het bijzonder bedankt voor

je hulp bij de organisatie van de vele activiteiten bij de SKG. Of het nu een symposium betrof, of het opnieuw inrichten van de bovenverdieping, je energie en enthousiasme zorgden altijd voor een fantastisch eindresultaat. Sander, jou wil ik bedanken voor je interesse, steun, hulp bij computer problemen en gezelligheid tijdens de vele, vaak late uurtjes op zolder.

Lieve jaarclubgenootjes en 'meiden van de studie', in Nijmegen is het allemaal begonnen en ik hoop dat mijn vriendschap met jullie allemaal nog heel lang zal meegaan. Dank voor jullie belangstelling en vriendschap.

Lieve Annemieke, paranimf. Ongeveer gelijk begonnen we aan ons onderzoek bij 'de Stichting'. Ik zal je enthousiaste glimlach missen, die er elke morgen weer voor me was als ik weer eens net iets later binnenkwam. Jouw vriendschap tijdens onze afgelopen onderzoeksjaren heeft mijn promotietraject glans gegeven. Door de vele uren die we samen doorbrachten op zolder, maar ook zeker daarbuiten. Ik vind het geweldig (en ontzettend terecht) dat het je gelukt is een opleidingsplek binnen de kindergeneeskunde te bemachtigen. Heel erg bedankt dat je naast me staat als paranimf.

Lieve Minke, zus en paranimf. Ik hoefde er niet lang over na te denken wie ik als paranimf wilde vragen. Je bent al mijn hele leven mijn grote zus, en altijd bereid mij te helpen. Ik bewonder je doorzettingsvermogen, daardoor ben je nu niet alleen milieukundige, maar ook diëtiste, en je kracht, dat maakt dat je na het plotselinge verdriet van afgelopen jaar de draad weer op kunt pakken. Heel erg bedankt dat je naast me staat als paranimf.

Lieve schoonfamilie, Frank, Harriët, Miels, Saskia, Jon en natuurlijk Anne en Emma. Heel erg bedankt dat jullie er altijd voor ons zijn, voor jullie interesse en medeleven in vrolijke en minder vrolijke tijden.

Lieve Joost, Marjet, Theo, Taeke-Jan en Fedde. Wat heb ik een geluk met zo'n lieve familie. Dank jullie wel dat jullie er voor mij zijn. Joost, grote broer, je had een andere studierichting voor mij in petto en had daar zelfs een studiebeurs van 500 gulden per maand voor over. Misschien is het feit dat mijn promotie op Woudestein plaatsvindt toch een troost.

Lieve papa en mama. Jullie onuitputtelijke steun, enthousiasme en interesse, samen met de onvoorwaardelijke liefde die jullie mij geven, maken dat ik zo ver gekomen ben. Of ik nu in België geneeskunde wilde studeren, pedagogiek of toch verder wilde met geneeskunde in Nijmegen, of wilde promoveren in Rotterdam, jullie stonden altijd achter mij. Dank jullie wel.

Lieve Sander, toen ik je in België voor het eerst zag wist ik het eigenlijk al, wij horen bij elkaar. Alle jaren erna is dat gevoel alleen maar sterker geworden. Ik vind het geweldig om mijn leven

met je te delen en dat je er altijd voor me bent. Je bevlogenheid in je vak en je durf om je eigen bureau te beginnen inspireren mij. Je hebt me de afgelopen periode ontzettend geholpen met het maken van mijn proefschrift, waaronder de prachtige lay-out van de voorkant (nu ben ik 't niet vergeten), waarvoor ik je zeer dankbaar ben. Je hebt weleens gezegd dat het een cliché is om je liefde als laatste in je proefschrift te bedanken, maar ik zou niet weten waar je meer thuis zou horen.