Psychiatric disorders and behavioral problems in children with Prader-Willi syndrome and the effects of growth hormone treatment

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Psychiatric disorders and behavioral problems in children with Prader-Willi syndrome and the effects of growth hormone treatment

Psychiatrische stoornissen en gedragsproblemen bij kinderen met het Prader-Willi syndroom en de effecten van groeihormoonbehandeling

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Voor alle kinderen met Prader-Willi syndroom en hun ouders

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CHAPTER 1

GENERAL INTRODUCTION AND AIMS OF THE THESIS

INTRODUCTION

This thesis includes studies about developmental, behavioral and psychiatric characteristics in children with Prader-Willi syndrome (PWS). Endocrinologists Prader, Labhart, and Willi were the first describing the combination of neonatal hypotonia, short stature, hypogonadism, hyperphagia and obesity, developmental delay and cognitive impairment in 1956 (1). It was not until the 1980s that behavioral and psychiatric problems in adults with PWS were investigated (2). However, studies about psychiatric disorders in children are very limited and the few publishes are case reports.
This chapter provides an overview of the genetic cause, developmental course, behavioral phenotype, and psychiatric disorders in children with PWS. Subsequently, the objectives of the studies described in the following chapters are presented. Finally, an overview is presented of the Dutch PWS Cohort Study.

1.1 Prader-Willi syndrome

PWS is a neurogenetic developmental disorder caused by the absence of expression of genes on the paternally inherited chromosome 15 at the locus q11-q13, due to a paternal deletion (DEL), maternal uniparental disomy (mUPD), imprinting defects or paternal chromosomal translocation. Occurrence is not associated with gender, race or social-economic status. The estimated birth incidence is 1:15,000 (3, 4). Main characteristics are hypotonia, hypogonadism, short stature if not treated with growth hormone, obesity, intellectual disability with an IQ between 50-80 (5) and behavioral problems. 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 (6, 7). Children with PWS have sweet personalities, but the syndrome is also characterized by behavioral problems, social difficulties and psychiatric symptoms (8, 9). At birth, the infant has typically a low birth weight for gestational age and severe hypotonia causing failure to thrive. During childhood, however, mostly between the age of two and five years, there is a change towards increasing weight and 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 solely based on the appearance of a combination of symptoms listed in the consensus diagnostic criteria (10). In 1981, the chromosome affected in patients with PWS was first identified (11). It was shown that the majority of patients with PWS have a deletion in chromosome 15. In 1982, it was discovered that

this deletion only affected the paternally inherited chromosome (12). To date, PWS is defined as the lack of expression of paternally inherited genes sited on chromosome 15 locus q11-13. Another term for the locus 15q11-13 on chromosome 15 is the 'Prader-Willi region'. Expression of the genes in the Prader-Willi region van be due to a deletion, an mUPD, an imprinting center defect (ICD) or a translocation (13-17).

1.2.2 Genomic Imprinting

Genes are segments of deoxyribonucleic acid (DNA), carrying the genetic information that is 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 imprinted or 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 (18).

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

A paternal deletion is the most common chromosomal defect in PWS and present in 70% of patients (13, 15). The defect occurs either as a large type I deletion or as a smaller type II deletion. Maternal uniparental disomy (mUPD) is the second most frequent cause of PWS and has been reported in 25% of patients (19). In children with mUPD, the paternally inherited Prader-Willi region on chromosome 15 is absent, while two copies of the maternally inherited regions on chromosome 15 (which are both imprinted) are present. In a small number of cases (less than 5%), PWS results from an ICD (17, 20). 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. Recent studies showed changing rates of the incidence in genetic subtypes: an increasing percentage up to 50% due to mUPD is seen, probably due to increased maternal age (21, 22). In less than 1% of patients, part of the paternally inherited chromosome is situated on another chromosome, this is called a translocation (13, 23, 24). For a schematic overview of the chromosomal defects, see Figure 2.

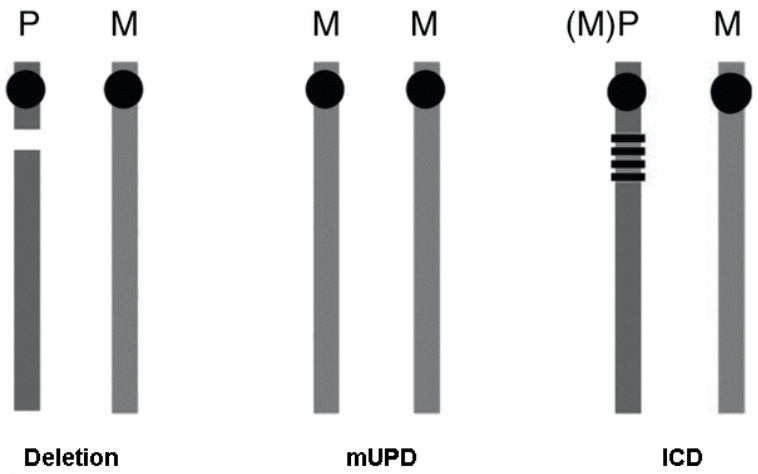

Figure 1. Schematic overview of the chromosomal defects described in Prader-Willi syndrome
P=paternally derived chromosome, M=maternally derived chromosome, mUPD=maternal uniparental disomy, ICD=imprinting center defect

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 mUPD genotypes. Table 1 shows the main differences that were indicated between the two genotypes.

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

Traits	Deletion	mUPD
Cognitive functioning (25-27)		
Performal IQ (PIQ)*	65 (47-86)	62 (42-82)
Verbal IQ (VIQ)*	61 (43-79)	70 (58-82)
Visuospational skills	+	-
Coding ability	+	-
Psychiatric illness (28, 29)	+/-	+
Behavioral problems (30-33)		
Self-injury	+	+/-
Food stealing	+	+/-
Compulsive behavior	+	+/-
Pervasive developmental disorders (PDD)	+/-	+
Post term deliveries (34)	+/-	+
Hypopigmentation (35)	+	-

*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 fetal activity, which is in most cases also notified by obstetricians. Furthermore, the rate of polyhydramnios is elevated. The frequency of induced labor is high in PWS and often results in caesarian section (36, 37). Both premature and post term deliveries are frequently observed, and there is also significantly greater risk of premature birth for babies with PWS due to mUPD compared with babies with PWS due to a deletion (38). Birth weight of 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 (38).

In infancy, the most consistent clinical feature is marked central hypotonia, which causes decreased movements, a head lag, lethargy with decreased arousal, weak or absent cry, and poor reflexes, including a poor suck that leads to feeding difficulties and failure to thrive (39). Problems with thermoregulation and hypogonadism with genital hypoplasia are evident at birth and throughout life (40). Facial dysmorphic features in PWS are a narrow bifrontal diameter, almond shaped yes, a thin downturned upper lip and a narrow nose (10).

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 (7).

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 (41), dual energy-x-ray absorptiometry, and double labeled water (42). Conversely, lean body mass measurements are decreased in PWS infants, correlating with a 30% lower energy expenditure as compared to healthy individuals (42).

Gross motor and language milestones are delayed in infants with PWS. Early milestones are reached on average at double the normal age (43, 44).

1.3.3 The child

In general, children with PWS are described as friendly, easy going and affectionate (45). However, in childhood simultaneously with the change in eating pattern, children with PWS start to show significant maladaptive behavioral and emotional characteristics including temper tantrums, automutilation (skin picking), stubbornness, mood lability, impulsivity, argumentativeness, and inappropriate social behavior (46). Autism spectrum disorder, attention deficit/hyperactivity symptoms, and insistence on sameness are common and of early onset (31, 47). 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 (48-50). Obesity can worsen the sleep disorder (51, 52).

A significant weight gain begins typically during early childhood, between 2 and 4 years of age (53). During later childhood, a seemingly insatiable appetite develops (53-55). 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 (56).

Cognitive disability becomes more evident by school age. The average intelligence quotient (IQ) is 70, but even children with low to normal IQs almost all have learning difficulties (57-59).

1.3.4 The adolescent

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 (60, 61). But, early pubarche and precocious puberty, although more rarely, were also found in these patients (40). 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 (62). Spontaneous growth velocity is impaired and the pubertal growth spurt may be lacking, which both contribute to a decreased adult height. Typical adolescent rebelliousness and behavioral problems are common in PWS, and are particularly food-related. Psychiatric symptoms might occur in adolescence, mostly psychosis and affective disorders. Adults with mUPD are most at risk for a psychosis (28).

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 (63). Morbidity in adults with PWS includes marked obesity, metabolic diseases, sleep apnea and lipolymphoedema. Adults with PWS are generally incapable of living independently (64).

Median adult height is 145 cm for women and 155 cm for men (65, 66). In adults with compromised pubertal development and absent pubertal onset, secondary sex characteristics are often absent or incomplete (65, 67). There are, however, a few case reports of pregnancy in females with PWS (68, 69), but paternity in PWS has never been reported.

The adult population of today was usually diagnosed relatively late, when obesity was already present. So 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, growth hormone 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 (67, 70). However, if severe obesity can be avoided, patients with PWS may have a reasonable life expectancy (71). 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.

1.4 Growth hormone treatment in children with PWS

In 2002, the Dutch national growth hormone (GH) trial for children with Prader-Willi syndrome was started to find the effects of GH on growth, body composition, activity level and psychological development. At first, children were treated in a randomized GH 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 adult height and beyond.

Several studies, including the Dutch national GH trial showed that GH treatment is an effective and safe treatment in children with PWS, both on the short and on the long term (72-88). GH treatment improves height gain, which results in a normal adult height, particularly when GH treatment is started before onset of puberty. GH treatment is also 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 (74, 78, 79, 81-84, 89). Furthermore, GH treatment improves psychomotor development in the very young children with PWS and cognition after long-term GH treatment, and it has psychological and behavioral benefits (27, 44, 87). There were no adverse effects of GH treatment on glucose homeostasis, blood pressure and serum lipids (75, 90, 91).

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.

1.5 Psychiatric disorders

PWS is associated with a higher risk of psychiatric problems (28, 92). Individuals with an mUPD subtype are more prone to a psychosis than those with a DEL (28, 92-94). The prevalence of psychosis varies in earlier reports: one study found psychosis in 100% of adults with an mUPD (28), but more recent studies reported a prevalence between 13 and 62% in adults with mUPD, while this was 13% in adults with a DEL (92, 95, 96). The age at onset of psychotic disorders varied between 13 and 19 years (92), but a Dutch report

described two 8 year old boys with an mUPD who suffered from a psychotic episode (97). The onset of psychotic symptoms are characterized by a sudden onset, agitation anxiety, abnormal thoughts or beliefs, and sometimes by auditory hallucinations (93). Affect disorders is the second major psychiatric disorder in PWS, sometimes combined with a psychosis. Although reports about psychiatric disorders in adults with PWS are increasing, knowledge about the presence and progression of psychiatric problems during childhood is still very limited.

1.6 Behavioral phenotype

Children with PWS are described as friendly, easy going and affectionate during the first years of life (45). However, simultaneously with the change in eating pattern, the behavioral phenotype may also change in childhood showing significant maladaptive behavioral and emotional characteristics including temper tantrums, auto mutilation (skin picking), stubbornness, mood lability, impulsivity, argumentativeness, and inappropriate social behavior (98-105).

During childhood, ritualistic behavior (such as the need to tell, know or ask something, insistence on routines, hoarding and ordering objects and repetitive actions and speech), compulsive symptoms, autism spectrum disorder, and manipulative and controlling behavior are reported (9, 104, 106-110). The prevalence of some behaviors differs between patients with a DEL and mUPD. Maladaptive behavior and skin-picking are more common in those with a DEL than mUPD (111, 112), but the severity of compulsions is not different between the genetic subtypes (113, 114). In contrast, those with an mUPD seems to be more prone for autism spectrum disorder (114-116), but little is known about the earlier development of social cognition, i.e. the Theory of Mind in children with PWS. The critical periods for a deterioration of the behavioral and emotional disturbances in children with intellectual disability are adolescence and young adulthood (117), but there were no specific data for children with PWS. Furthermore, although GH treatment is a routine treatment in most developed countries, no studies had investigated the effect of long-term growth hormone treatment on behavior and adaptive functioning in children with PWS.

1.7 This thesis

Recent studies described the increased prevalence of behavioral and psychiatric disorders in individuals with PWS, mainly in adults. Present study was conducted to investigate behavioral and psychiatric disorders in children and adolescents with PWS to improve our understanding of the mental development and its pathology during childhood and to investigate if early signs and symptoms of psychiatric disorders could prevent a further deterioration of the disorder.

The primary aims of the studies described in this thesis were to investigate:

- if, and if so, what symptoms within the psychiatric domain occur in children with PWS
- age at onset of psychiatric symptoms
- differences in psychiatric symptoms between children with a deletion and mUPD
- development of psychiatric symptoms over time
- predictors and stressors of psychiatric disorders
- cognitive functioning such as visual-motor integration over time
- effect of growth hormone treatment on behavior and adaptive functioning
- if precursors and metabolites of dopamine and serotonin system are predictors of psychiatric symptoms in children with PWS

For this study, children with genetically confirmed PWS, aged 7 to 17 years, who participate in the Dutch PWS Cohort Study were asked for participation. We choose this age range as little is known about psychiatric disorders in childhood and adolescence, but also to warrant that particular questionnaires and tasks could be performed by the children themselves. Structured interviews were performed with children with PWS and their parents once every 2 years between 2011-2013 to assess psychiatric symptoms, behavioral disturbances, and visual-motor integration. An overview of the PD-study and its testing materials is depicted in more detail in Appendix 1. Two chapters in this thesis describe the long-term effects of growth hormone treatment on behavior and adaptive functioning. Participants of these 2 studies originated from the Dutch randomized controlled growth hormone trial. Appendix 2 describes the design for both the Dutch PWS Cohort Study and Dutch randomized controlled growth hormone trial.

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 PWS 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, such as social functioning, mental health and the presence of psychotic symptoms, behavior, adaptive functioning, and visual-motor integration. The

study population consisted of children with PWS, who participated in the Dutch PWS Cohort Study. Study designs are described in Appendix 1 and 2.

Chapter 1 gives an introduction about PWS and the topics described in this thesis.

Chapter 2 describes the prevalence of behavioral problems and psychiatric disorders in children with PWS, at baseline and after 2 years of follow-up.

Chapter 3 reports characteristics of Autism Spectrum Disorder in children with PWS, by outlining the level of social cognitive functioning, i.e. the Theory of Mind, and symptoms of Autism Spectrum Disorder.

Chapter 4 presents behavioral characteristics and the effect of long-term GH treatment in children with PWS studied in a 2-year randomized controlled trial and during 8 years of follow-up.

Chapter 5 describes the adaptive functioning and the effect of long-term GH treatment in children with PWS in a randomized controlled trial and during 7 years of follow-up.

Chapter 6 reports the visual-motor integration in children with PWS, at baseline and after 2 years of follow-up.

Chapter 7 presents depressive symptoms and its relation with dopamine and serotonin system in children with PWS.

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 abbreviations and publications. It further contains the PhD portfolio, CV and acknowledgements.

APPENDIX 1: PSYCHIATRIC DISORDERS IN CHILDREN WITH PWS (PD-STUDY)

In November 2010, the study 'Psychiatric disorders in children with PWS ' (PD-study)' was started parallel to the Dutch PWS Cohort Study (Appendix 2). Children who participated in the Dutch PWS Cohort Study and their parents were invited for assessment of behavior and mental health at baseline and after 2 years. Children were examined at the Sophia Children's Hospital Rotterdam, and in home setting by the author of this thesis. The PD-study was conducted in collaboration with Dr. P.J.L. Collin, child-psychiatrist (Koraalgroep, Gastenhof, Sittard/ de Hondsberg, Oisterwijk), Dr. D. Fekkes, biochemist (Erasmus Medical Center Rotterdam), Prof. dr. W.M.A. Verhoeven, psychiatrist (Vincent van Gogh Institute, Venray), and Prof. dr. J.I.M. Egger, neuropsychologist (Vincent van Gogh Institute, Venray).
The following assessments were performed in the Sophia Children's Hospital Rotterdam and in home setting:

- Diagnostic Interview Schedule for Children (DISC-IV) (118)
- Theory of Mind Test (ToM test) (119)
- Diagnostic Interview Social and Communication disorders (DISCO) (119)
- Compulsive Behaviour Checklist (CBC) (122)
- Visual-motor Integration Test (VMI) (121)
- Children's Depression Inventory (CDI) (123)

Additionally, blood collected during the yearly control was examined to investigate possible predictors in the dopamine and serotonin system correlated, to psychiatric disorders in children with PWS.
Until 01-09-2014, 75 Dutch children were included in the PD-study. Children had to meet the following criteria:

Inclusion criteria:
- Genetically confirmed diagnosis of PWS;
- Age between 7 and 17 years;

Exclusion criteria:
- Non-cooperative behavior

APPENDIX 2: 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 three MD-researchers, a neuroscientist, a psychologist, and a research nurse. Three-monthly, 18 hospitals throughout The Netherlands are visited by one of the two MD-researchers and the research nurse, where children are examined, in collaboration with the local pediatrician or pediatric endocrinologist (Figure 2). 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.

Figure 2. Participating centers

DUTCH GH RCT

Infants

The Dutch GH RCT was performed from April 2002 to October 2009. 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

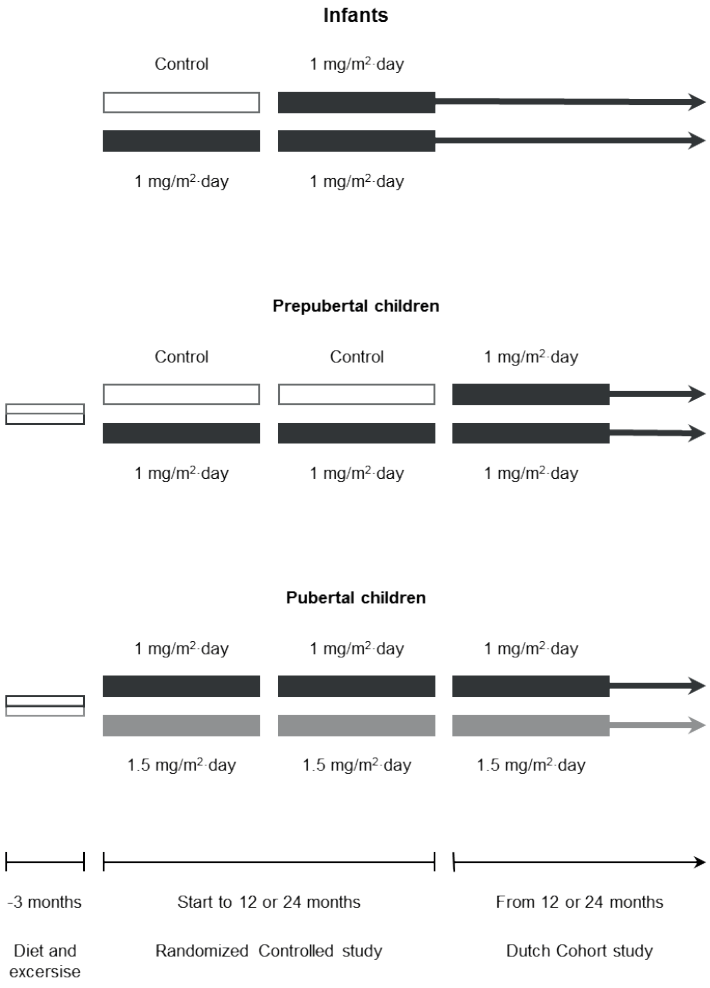

Figure 3. Design of the multicenter randomized controlled GH trial and the Dutch PWS Cohort study

group for the duration of one year (Figure 3). 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 2)

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.

THE ONGOING DUTCH PWS COHORT STUDY

As GH treatment has been shown to improve body composition, bone mineral density, motor functioning, the Dutch GH RCT was stopped in October 2009. Since 2009, 46 infants between 6 months and 3 years were directly included in the Dutch 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. To date, the Dutch PWS Cohort Study has shown the beneficial effect of long-term GH treatment on body composition and cognition, and has resulted in new insight in testicular and ovarian functioning in children with PWS.

Dutch PWS Cohort Study

Until 01-06-2014, 162 Dutch children with PWS were included in the RCT GH trial and the Cohort Study (Figure 3). 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)

REFERENCES

1. Prader A, Labhart A, Willi H 1956 Ein syndrom von adipositas, kleinwuchs, kryptorchidismus und oligophrenie nach myatonieartigem zustand in neugeborenalter. Schweiz. Med. Wochenschr. 86: 1260
2. Greenswag LR 1987 Adults with Prader-Willi syndrome: a survey of 232 cases. Dev. Med. Child Neurol. 29:145-152
3. Akefeldt A, Gillberg C, Larsson C 1991 Prader-Willi syndrome in a Swedish rural county: Epidemiological aspects. Dev. Med. Child Neurol. 33:715-721
4. Vogels A, Van Den Ende J, Keymolen K, Mortier G, Devriendt K, Legius E, Fryns JP 2004 Minimum prevalence, birth incidence and cause of death for Prader-Willi syndrome in Flanders. Eur. J. Hum. Genet. 12:238-240
5. Whittington J, Holland A, Webb T 2009 Relationship between the IQ of people with Prader-Willi syndrome and that of their siblings: evidence for imprinted gene effects. J. Intellect. Disabil. Res. 53:411-418
6. Aughton DJ, Cassidy SB 1990 Physical features of Prader-Willi syndrome in neonates. Am. J. Dis. Child. 144:1251-1254
7. Gunay-Aygun M, Schwartz S, Heeger S, O'Riordan MA, Cassidy SB 2001 The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. Pediatrics 108:E92
8. Dykens EM, Hodapp RM, Walsh K, Nash LJ 1992 Adaptive and maladaptive behavior in Prader-Willi syndrome. J. Am. Acad. Child Adolesc. Psychiatry 31:1131-1136
9. Akefeldt A, Gillberg C 1999 Behavior and personality characteristics of children and young adults with Prader-Willi syndrome: a controlled study. J. Am. Acad. Child Adolesc. Psychiatry 38:761-769
10. Holm VA, Cassidy SB, Butler MG, Hanchett JM, Greenswag LR, Whitman BY, Greenberg F 1993 Prader-Willi syndrome: consensus diagnostic criteria. Pediatrics 91:398-402
11. Ledbetter DH, Riccardi VM, Airhart SD, Strobel RJ, Keenan BS, Crawford JD 1981 Deletions of chromosome 15 as a cause of the Prader-Willi syndrome. N. Engl. J. Med. 304:325-329
12. Butler MG, Palmer CG 1983 Parental origin of chromosome 15 deletion in Prader-Willi syndrome. Lancet 1:1285-1286
13. Cassidy SB 1997 Prader-Willi syndrome. J. Med. Genet. 34:917-923
14. Conroy JM, Grebe TA, Becker LA, Tsuchiya K, Nicholls RD, Buiting K, Horsthemke B, Cassidy SB, Schwartz S 1997 Balanced translocation 46,XY,t(2;15)(q37.2;q11.2) associated with atypical Prader-Willi syndrome. Am J Hum Genet 61:388-394
15. State MW, Dykens EM 2000 Genetics of childhood disorders: XV. Prader-Willi syndrome: genes, brain, and behavior. J. Am. Acad. Child Adolesc. Psychiatry 39:797-800
16. Schulze A, Hansen C, Baekgaard P, Blichfeldt S, Petersen MB, Tommerup N, Brondum-Nielsen K 1997 Clinical features and molecular genetic analysis of a boy with Prader-Willi syndrome caused by an imprinting defect. Acta Paediatr. 86:906-910
17. Buiting K, Barbel Dittrich, Stephanie GroB, Christina Lich, Claudia Farber, Tina Buchholz, Ellie Smith, Bernhard Horsthemke 1998 Sporadic Imprinting Defects in Prader-Willi Syndrome and Angelman Syndrome: Implications for Imprint-Switch Models, Genetic Counseling, and Prenatal Diagnosis. Am. J. Hum. Genet. 63:170-180
18. Driscoll DJ, Miller JL, Schwartz S, Cassidy SB 1993 Prader-Willi Syndrome. Seattle (WA): GeneReviews

19. Mascari MJ, Gottlieb W, Rogan PK, Butler MG, Waller DA, Armour JA, Jeffreys AJ, Ladda RL, Nicholls RD 1992 The frequency of uniparental disomy in Prader-Willi syndrome. Implications for molecular diagnosis. N. Engl. J. Med. 326:1599-1607
20. Buiting K, Saitoh S, Gross S, Dittrich B, Schwartz S, Nicholls RD, Horsthemke B 1995 Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. Nat. Genet. 9:395-400
21. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM 2011 Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32: 1729-1735
22. Whittington JE, Butler JV, Holland AJ 2007 Changing rates of genetic subtypes of Prader-Willi syndrome in the UK. Eur. J. Hum. Genet. 15:127-130
23. Glenn CC, Nicholls RD, Robinson WP, Saitoh S, Niikawa N, Schinzel A, Horsthemke B, Driscoll DJ 1993 Modification of 15q11-q13 DNA methylation imprints in unique Angelman and Prader-Willi patients. Hum. Mol. Genet. 2:1377-1382
24. Bittel DC, Butler MG 2005 Prader-Willi syndrome: clinical genetics, cytogenetics and molecular biology. Expert Rev Mol Med 7:1-20
25. Roof E, Stone W, MacLean W, Feurer ID, Thompson T, Butler MG 2000 Intellectual characteristics of Prader-Willi syndrome: comparison of genetic subtypes. Journal of intellectual disability research : JIDR 44 (Pt 1):25-30
26. Fox R, Yang GS, Feurer ID, Butler MG, Thompson T 2001 Kinetic form discrimination in Prader-Willi syndrome. Journal of intellectual disability research : JIDR 45:317-325
27. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314
28. Boer H, Holland A, Whittington J, Butler J, Webb T, Clarke D 2002 Psychotic illness in people with Prader Willi syndrome due to chromosome 15 maternal uniparental disomy. Lancet 359:135-136
29. Vogels A, De Hert M, Descheemaeker MJ, Govers V, Devriendt K, Legius E, Prinzie P, Fryns JP 2004 Psychotic disorders in Prader-Willi syndrome. Am J Med Genet A 127A:238-243
30. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of child psychology and psychiatry, and allied disciplines 46: 1089-1096
31. Veltman MW, Craig EE, Bolton PF 2005 Autism spectrum disorders in Prader-Willi and Angelman syndromes: a systematic review. Psychiatr. Genet. 15:243-254
32. Hartley SL, Maclean WE, Jr., Butler MG, Zarcone J, Thompson T 2005 Maladaptive behaviors and risk factors among the genetic subtypes of Prader-Willi syndrome. Am J Med Genet A 136:140-145
33. Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP 2006 Pervasive developmental disorders in Prader-Willi syndrome: the Leuven experience in 59 subjects and controls. American journal of medical genetics. Part A 140:1136-1142

34. Butler MG, Sturich J, Myers SE, Gold JA, Kimonis V, Driscoll DJ 2009 Is gestation in Prader-Willi syndrome affected by the genetic subtype? Journal of assisted reproduction and genetics 26: 461-466
35. Spritz RA, Bailin T, Nicholls RD, Lee ST, Park SK, Mascari MJ, Butler MG 1997 Hypopigmentation in the Prader-Willi syndrome correlates with P gene deletion but not with haplotype of the hemizygous P allele. American journal of medical genetics 71:57-62
36. Whittington JE, Butler JV, Holland AJ 2008 Pre-, peri- and postnatal complications in Prader-Willi syndrome in a UK sample. Early human development 84:331-336
37. Bigi N, Faure JM, Coubes C, Puechberty J, Lefort G, Sarda P, Blanchet P 2008 Prader-Willi syndrome: is there a recognizable fetal phenotype? Prenatal diagnosis 28:796-799
38. Dudley O, Muscatelli F 2007 Clinical evidence of intrauterine disturbance in Prader-Willi syndrome, a genetically imprinted neurodevelopmental disorder. Early human development 83:471-478
39. Aughton DJ, Cassidy SB 1990 Physical features of Prader-Willi syndrome in neonates. Am J Dis Child 144:1251-1254
40. Crino A, Schiaffini R, Ciampalini P, Spera S, Beccaria L, Benzi F, Bosio L, Corrias A, Gargantini L, Salvatoni A, Tonini G, Trifiro G, Livieri C 2003 Hypogonadism and pubertal development in Prader-Willi syndrome. Eur J Pediatr 162:327-333
41. Eiholzer U, Blum WF, Molinari L 1999 Body fat determined by skinfold measurements is elevated despite underweight in infants with Prader-Labhart-Willi syndrome. The Journal of pediatrics 134:222-225
42. Bekx MT, Carrel AL, Shriver TC, Li Z, Allen DB 2003 Decreased energy expenditure is caused by abnormal body composition in infants with Prader-Willi Syndrome. J Pediatr 143:372-376
43. Festen DA, Wevers M, de Weerd AW, van den Bossche RA, Duivenvoorden HJ, Otten BJ, Wit JM, Hokken-Koelega AC 2007 Psychomotor development in infants with Prader-Willi syndrome and associations with sleep-related breathing disorders. Pediatr Res 62:221-224
44. Festen DA, Wevers M, Lindgren AC, Bohm B, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 68:919-925
45. Descheemaeker MJ, Vogels A, Govers V, Borghgraef M, Willekens D, Swillen A, Verhoeven W, Fryns JP 2002 Prader-Willi syndrome: new insights in the behavioural and psychiatric spectrum. J. Intellect. Disabil. Res. 46:41-50
46. Dykens EM, Hodapp RM, Walsh K, Nash LJ 1992 Adaptive and maladaptive behavior in Prader-Willi syndrome. Journal of the American Academy of Child and Adolescent Psychiatry 31:1131-1136
47. Wigren M, Hansen S 2005 ADHD symptoms and insistence on sameness in Prader-Willi syndrome. J. Intellect. Disabil. Res. 49:449-456
48. Nixon GM, Brouillette RT 2002 Sleep and breathing in Prader-Willi syndrome. Pediatr. Pulmonol. 34:209-217
49. Hertz G, Cataletto M, Feinsilver SH, Angulo M 1993 Sleep and breathing patterns in patients with Prader Willi syndrome (PWS): effects of age and gender. Sleep 16:366-371
50. Festen DA, de Weerd AW, van den Bossche RA, Joosten K, Hoeve H, Hokken-Koelega AC 2006 Sleep-related breathing disorders in prepubertal children with Prader-Willi syndrome and effects of growth hormone treatment. J Clin Endocrinol Metab 91:4911-4915
51. Hertz G, Cataletto M, Feinsilver SH, Angulo M 1995 Developmental trends of sleep-disordered breathing in Prader-Willi syndrome: the role of obesity. American journal of medical genetics 56: 188-190

52. Festen DA, Wevers M, de Weerd AW, van den Bossche RA, Duivenvoorden HJ, Otten BJ, Wit JM, Hokken-Koelega AC 2007 Psychomotor development in infants with Prader-Willi syndrome and associations with sleep-related breathing disorders. Pediatr. Res. 62:221-224
53. Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold JA, Kimonis V, Dykens E, Butler MG, Shuster JJ, Driscoll DJ 2011 Nutritional phases in Prader-Willi syndrome. Am J Med Genet A 155A:1040-1049
54. Zipf WB, Berntson GG 1987 Characteristics of abnormal food-intake patterns in children with Prader-Willi syndrome and study of effects of naloxone. The American journal of clinical nutrition 46:277-281
55. Holland AJ, Treasure J, Coskeran P, Dallow J 1995 Characteristics of the eating disorder in Prader-Willi syndrome: implications for treatment. Journal of intellectual disability research : JIDR 39 (Pt 5):373-381
56. Stephenson JB 1980 Prader-Willi syndrome: neonatal presentation and later development. Developmental medicine and child neurology 22:792-795
57. Dykens EM, Hodapp RM, Walsh K, Nash LJ 1992 Profiles, correlates, and trajectories of intelligence in Prader-Willi syndrome. Journal of the American Academy of Child and Adolescent Psychiatry 31:1125-1130
58. Gross-Tsur V, Landau YE, Benarroch F, Wertman-Elad R, Shalev RS 2001 Cognition, attention, and behavior in Prader-Willi syndrome. J. Child Neurol. 16:288-290
59. Whittington J, Holland A, Webb T, Butler J, Clarke D, Boer H 2004 Academic underachievement by people with Prader-Willi syndrome. J. Intellect. Disabil. Res. 48:188-200
60. Butler MG 1990 Prader-Willi syndrome: current understanding of cause and diagnosis. American journal of medical genetics 35:319-332
61. Siemensma EP, van Alfen-van der Velden AA, Otten BJ, Laven JS, Hokken-Koelega AC 2012 Ovarian function and reproductive hormone levels in girls with prader-willi syndrome: a longitudinal study. J. Clin. Endocrinol. Metab. 97:E1766-1773
62. Eiholzer U, l'Allemand D, Rousson V, Schlumpf M, Gasser T, Girard J, Gruters A, Simoni M 2006 Hypothalamic and gonadal components of hypogonadism in boys with Prader-Labhart- Willi syndrome. J Clin Endocrinol Metab 91:892-898
63. Clarke DJ, Boer H, Chung MC, Sturmey P, Webb T 1996 Maladaptive behaviour in Prader-Willi syndrome in adult life. Journal of intellectual disability research : JIDR 40 (Pt 2):159-165
64. Partsch CJ, Lammer C, Gillessen-Kaesbach G, Pankau R 2000 Adult patients with Prader-Willi syndrome: clinical characteristics, life circumstances and growth hormone secretion. Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society 10 Suppl B:S81-85
65. Greenswag LR 1987 Adults with Prader-Willi syndrome: a survey of 232 cases. Developmental medicine and child neurology 29:145-152
66. Wollmann HA, Schultz U, Grauer ML, Ranke MB 1998 Reference values for height and weight in Prader-Willi syndrome based on 315 patients. European journal of pediatrics 157:634-642
67. Laurance BM, Brito A, Wilkinson J 1981 Prader-Willi Syndrome after age 15 years. Archives of disease in childhood 56:181-186
68. Akefeldt A, Tornhage CJ, Gillberg C 1999 'A woman with Prader-Willi syndrome gives birth to a healthy baby girl'. Developmental medicine and child neurology 41:789-790
69. Schulze A, Mogensen H, Hamborg-Petersen B, Graem N, Ostergaard JR, Brondum-Nielsen K 2001 Fertility in Prader-Willi syndrome: a case report with Angelman syndrome in the offspring. Acta Paediatr 90:455-459

70. Sinnema M, Maaskant MA, van Schrojenstein Lantman-de Valk HM, van Nieuwpoort IC, Drent ML, Curfs LM, Schrander-Stumpel CT 2011 Physical health problems in adults with Prader-Willi syndrome. Am J Med Genet A 155A:2112-2124
71. Carpenter PK 1994 Prader-Willi syndrome in old age. Journal of intellectual disability research : JIDR 38 (Pt 5):529-531
72. Mascari MJ, Gottlieb W, Rogan PK, Butler MG, Waller DA, Armour JA, Jeffreys AJ, Ladda RL, Nicholls RD 1992 The frequency of uniparental disomy in Prader-Willi syndrome. Implications for molecular diagnosis. The New England journal of medicine 326:1599-1607
73. Lindgren AC, Hellstrom LG, Ritzen EM, Milerad J 1999 Growth hormone treatment increases CO(2) response, ventilation and central inspiratory drive in children with Prader-Willi syndrome. European journal of pediatrics 158:936-940
74. Haqq AM, Stadler DD, Jackson RH, Rosenfeld RG, Purnell JQ, LaFranchi SH 2003 Effects of growth hormone on pulmonary function, sleep quality, behavior, cognition, growth velocity, body composition, and resting energy expenditure in Prader-Willi syndrome. The Journal of clinical endocrinology and metabolism 88:2206-2212
75. Lindgren AC, Hagenas L, Ritzen EM 1999 Growth hormone treatment of children with Prader-Willi syndrome: effects on glucose and insulin homeostasis. Swedish National Growth Hormone Advisory Group. Horm Res 51:157-161
76. Tauber M, Barbeau C, Jouret B, Pienkowski C, Malzac P, Moncla A, Rochiccioli P 2000 Auxological and endocrine evolution of 28 children with Prader-Willi syndrome: effect of GH therapy in 14 children. Horm Res 53:279-287
77. Angulo M, Castro-Magana M, Mazur B, Canas JA, Vitollo PM, Sarrantonio M 1996 Growth hormone secretion and effects of growth hormone therapy on growth velocity and weight gain in children with Prader-Willi syndrome. J Pediatr Endocrinol Metab 9:393-400
78. Lindgren AC, Hagenas L, Muller J, Blichfeldt S, Rosenborg M, Brismar T, Ritzen EM 1998 Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favourably. Acta Paediatr 87:28-31
79. Lindgren AC, Hagenas L, Muller J, Blichfeldt S, Rosenborg M, Brismar T, Ritzen EM 1997 Effects of growth hormone treatment on growth and body composition in Prader-Willi syndrome: a preliminary report. The Swedish National Growth Hormone Advisory Group. Acta Paediatr Suppl 423:60-62
80. Hauffa BP 1997 One-year results of growth hormone treatment of short stature in Prader-Willi syndrome. Acta Paediatr Suppl 423:63-65
81. Davies PS, Evans S, Broomhead S, Clough H, Day JM, Laidlaw A, Barnes ND 1998 Effect of growth hormone on height, weight, and body composition in Prader-Willi syndrome. Arch Dis Child 78: 474-476
82. Eiholzer U, Gisin R, Weinmann C, Kriemler S, Steinert H, Torresani T, Zachmann M, Prader A 1998 Treatment with human growth hormone in patients with Prader-Labhart-Willi syndrome reduces body fat and increases muscle mass and physical performance. Eur J Pediatr 157:368-377
83. Myers SE, Carrel AL, Whitman BY, Allen DB 2000 Sustained benefit after 2 years of growth hormone on body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome. J Pediatr 137:42-49
84. Carrel AL, Moerchen V, Myers SE, Bekx MT, Whitman BY, Allen DB 2004 Growth hormone improves mobility and body composition in infants and toddlers with Prader-Willi syndrome. J Pediatr 145: 744-749

85. Ritzen EM, Lindgren AC, Hagenas L, Marcus C, Muller J, Blichfeldt S 1999 Growth hormone treatment of patients with Prader-Willi syndrome. Swedish Growth Hormone Advisory Group. J Pediatr Endocrinol Metab 12 Suppl 1:345-349
86. Eiholzer U, L'Allemand D, Schlumpf M, Rousson V, Gasser T, Fusch C 2004 Growth hormone and body composition in children younger than 2 years with Prader-Willi syndrome. J Pediatr 144: 753-758
87. Whitman B, Carrel A, Bekx T, Weber C, Allen D, Myers S 2004 Growth hormone improves body composition and motor development in infants with Prader-Willi syndrome after six months. J Pediatr Endocrinol Metab 17:591-600
88. Eiholzer U, l'Allemand D 2000 Growth hormone normalises height, prediction of final height and hand length in children with Prader-Willi syndrome after 4 years of therapy. Horm Res 53:185-192
89. de Lind van Wijngaarden RF, Siemensma EP, Festen DA, 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 AC 2009 Efficacy and safety of long-term continuous growth hormone treatment in children with Prader-Willi syndrome. J. Clin. Endocrinol. Metab. 94:4205-4215
90. Festen DA, van Toorenenbergen A, Duivenvoorden HJ, Hokken-Koelega AC 2007 Adiponectin levels in prepubertal children with Prader-Willi syndrome before and during growth hormone therapy. J Clin Endocrinol Metab 92:1549-1554
91. de Lind van Wijngaarden RF, Cianflone K, Gao Y, Leunissen RW, Hokken-Koelega AC 2010 Cardiovascular and metabolic risk profile and acylation-stimulating protein levels in children with Prader-Willi syndrome and effects of growth hormone treatment. J Clin Endocrinol Metab 95: 1758-1766
92. Vogels A, Matthijs G, Legius E, Devriendt K, Fryns JP 2003 Chromosome 15 maternal uniparental disomy and psychosis in Prader-Willi syndrome. J. Med. Genet. 40:72-73
93. Clarke DJ 1993 Prader-Willi syndrome and psychoses. Br. J. Psychiatry 163:680-684
94. Soni S, Whittington J, Holland AJ, Webb T, Maina EN, Boer H, Clarke D 2008 The phenomenology and diagnosis of psychiatric illness in people with Prader-Willi syndrome. Psychol. Med. 38:1505-1514
95. Soni S, Whittington J, Holland AJ, Webb T, Maina E, Boer H, Clarke D 2007 The course and outcome of psychiatric illness in people with Prader-Willi syndrome: implications for management and treatment. J. Intellect. Disabil. Res. 51:32-42
96. Sinnema M, Einfeld SL, Schrander-Stumpel C, Maaskant MA, Boer H, Curfs LMG 2011 Behavioral phenotype in adults with Prader-Willi syndrome. Res. Dev. Disabil. 32:604-612
97. Collin PJL, Boer H, Vogels A, Curfs LMG 2005 Psychose bij kinderen met het Prader-Willi syndroom. Tijdschr Psychiatr 47:325-328
98. Curfs LM, Hoondert V, van Lieshout CF, Fryns JP 1995 Personality profiles of youngsters with Prader-Willi syndrome and youngsters attending regular schools. J. Intellect. Disabil. Res. 39 (Pt 3):241-248
99. Curfs LM, Verhulst FC, Fryns JP 1991 Behavioral and emotional problems in youngsters with Prader-Willi syndrome. Genet. Couns. 2:33-41
100. Dykens EM, Cassidy SB 1995 Correlates of maladaptive behavior in children and adults with Prader-Willi syndrome. Am. J. Med. Genet. 60:546-549
101. Dykens EM, Hodapp RM, Walsh K, Nash LJ 1992 Adaptive and maladaptive behavior in Prader-Willi syndrome. J. Am. Acad. Child Adolesc. Psychiatry 31:1131-1136

102. Dykens EM, Kasari C 1997 Maladaptive behavior in children with Prader-Willi syndrome, Down syndrome, and nonspecific mental retardation. Am. J. Ment. Retard. 102:228-237
103. Dykens EM, Leckman JF, Cassidy SB 1996 Obsessions and compulsions in Prader-Willi syndrome. Journal of Child Psychology and Psychiatry 37:995-1002
104. Einfeld SL, Smith A, Durvasula S, Florio T, Tonge BJ 1999 Behavior and emotional disturbance in Prader-Willi syndrome. Am. J. Med. Genet. 82:123-127
105. Stein DJ, Keating J, Zar HJ, Hollander E 1994 A survey of the phenomenology and pharmacotherapy of compulsive and impulsive-aggressive symptoms in Prader-Willi syndrome. J. Neuropsychiatry Clin. Neurosci. 6:23-29
106. Dykens EM, Rosner BA 1999 Refining behavioral phenotypes: personality-motivation in Williams and Prader-Willi syndromes. Am. J. Ment. Retard. 104:158-169
107. Morgan JR, Storch EA, Woods DW, Bodzin D, Lewin AB, Murphy TK 2010 A preliminary analysis of the phenomenology of skin-picking in Prader-Willi syndrome. Child Psychiatry Hum. Dev. 41: 448-463
108. Clarke DJ, Boer H, Whittington J, Holland A, Butler J, Webb T 2002 Prader-Willi syndrome, compulsive and ritualistic behaviours: the first population-based survey. Br. J. Psychiatry 180:358-362
109. Reddy LA, Pfeiffer SI 2007 Behavioral and emotional symptoms of children and adolescents with Prader-Willi Syndrome. J. Autism Dev. Disord. 37:830-839
110. Dimitropoulos A, Blackford J, Walden T, Thompson T 2006 Compulsive behavior in Prader-Willi syndrome: examining severity in early childhood. Res. Dev. Disabil. 27:190-202
111. Dykens EM, Cassidy SB, King BH 1999 Maladaptive behavior differences in Prader-Willi syndrome due to paternal deletion versus maternal uniparental disomy. Am. J. Ment. Retard. 104:67-77
112. Cassidy SB, Forsythe M, Heeger S, Nicholls RD, Schork N, Benn P, Schwartz S 1997 Comparison of phenotype between patients with Prader-Willi syndrome due to deletion 15q and uniparental disomy 15. Am. J. Med. Genet. 68:433-440
113. Dimitropoulos A 2003 An investigation of childhood rituals and compulsive behavior in children with Prader-Willi syndrome. Dissertation Abstracts International: Section B: The Sciences and Engineering 63:3493
114. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
115. Veltman MW, Thompson RJ, Roberts SE, Thomas NS, Whittington J, Bolton PF 2004 Prader-Willi syndrome--a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. Eur. Child Adolesc. Psychiatry 13:42-50
116. Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP 2006 Pervasive developmental disorders in Prader-Willi syndrome: the Leuven experience in 59 subjects and controls. American Journal of Medical Genetics Part A 140:1136-1142
117. Steinhausen HC, Eiholzer U, Hauffa BP, Malin Z 2004 Behavioural and emotional disturbances in people with Prader-Willi Syndrome. J. Intellect. Disabil. Res. 48:47-52
118. Van Berckelaer-Onnes IA, Noens, I, Dijkxhoorn, Y. 2008 Diagnostic Interview for Social and Communication Disorders: Nederlandse Vertaling.
119. Steerneman PM, C. 2009 ToM test-R Handleiding. Antwerpen-Apeldoorn: Garant
120. Ferdinand RF, van der Ende, J. 1998 Nederlandse vertaling van de DISC-IV; Diagnostic Interview Schedule for Children. Rotterdam: Afdeling kinder-en jeugdpsychiatrie van het Sophia Kinderziekenhuis.

121. Beery KE, Beery NA 2004 The Beery-Buktenica developmental test of visual-motor integration: Beery VMI with supplemental deveopmental tests of visual perception and motor coordination: Administration, scoring and teaching manual. . 6th ed. Minneapolis: NCS Pearson Inc
122. Gedye A 1992 Recognizing obsessive-compulsive disorder in clients with developmental disabilities. . Habilitative Mental Healthcare Newsletter II:73-77
123. Timbremont B, Braet C, Roelofs J 2008 Children's Depression Inventory Manual. Amsterdam: Pearson Assessment and Information B.V.

CHAPTER 2

PSYCHIATRIC DISORDERS IN CHILDREN WITH PRADER-WILLI SYNDROME

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ABSTRACT

Psychiatric disorders such as psychosis are highly prevalent in adults with Prader-Willi syndrome (PWS). However, knowledge about the presence and progression of psychiatric disorders in children with PWS is very limited.

Sixty-one children with PWS aged 7 to 17 years were tested using the Diagnostic Interview Schedule for Children (DISC) and Compulsive Behaviour Checklist (CBC), and 38/61 were retested after 2 years. Prevalence of psychiatric disorders and the association with age, gender, genetic subtype, and total IQ were assessed. In addition, occurrence and characteristics of compulsions were determined.

Prior to the study, two boys were known with psychotic symptoms and treated with antipsychotics. At baseline, none scored positive for psychotic disorder. During the follow-up, only one boy with known psychotic symptoms required a dose adjustment of his antipsychotic medication. After 2 years, none of the children had a psychotic disorder according to the DISC. Oppositional defiant disorder (ODD) was the most common diagnosis and present in 20% of children with PWS, and this was not associated with age (β= -0.081,p=0.546), gender (β= 0.013,p=0.923), genetic subtype (β=-0.073,p=0.584), or total IQ (β=-0.150,p=0.267). The most common compulsions were hoarding and fixed hygiene sequences.

In our large group of 61 children with PWS, the majority had no psychotic disorder and no progression was found during 2-year follow-up. ODD was present in 20% of children. No changes in the prevalence of psychiatric disorders were found during the 2-year follow-up study and genetic subtype was not related to psychosis, depression, or ODD.

INTRODUCTION

Prader-Willi syndrome (PWS) was first described in 1956 (1). The estimated birth incidence rate is 1:15.000 (2, 3). The genetic cause lies in the absence of expression of genes on the paternal chromosome 15q11-q13 (4). Deletion (DEL) occurs in approximately 70% of people with PWS, 25% is affected by maternal uniparental disomy (mUPD) and the rest is due to imprinting defects or translocation in the PWS region. PWS is characterized by hypotonia, short stature, hypogonadism, intellectual disability, behavioral problems and higher risk for psychosis.

The prevalence of a psychiatric disorder in individuals with intellectual disability is higher than in healthy references. Almost 30% of adolescents and young adults with an intellectual disability meet the criteria for at least one DSM-IV diagnosis (5). Anxiety, mood disorder and disruptive disorder occur in 18%, 3% and 16%, resp. (5). PWS is also associated with a higher risk of psychiatric problems (6-8). Individuals with the mUPD subtype are more prone to a psychosis than those with a DEL (6, 7, 9). Different prevalences of psychosis has been reported: although one study found psychosis in 100% of adults with an mUPD (6), more recent studies reported prevalences between 13 and 62% in adults with mUPD, while this was 13% in adults with a DEL (7, 10, 11). The age at onset of psychotic disorders varied between 13 and 19 years (7), but a Dutch report described two 8 year old boys with an mUPD who suffered from a psychotic episode (12). Knowledge about the presence and progression of psychiatric problems during childhood in PWS remains scarce, as most studies outlining psychiatric problems were performed in adults with a wide age range.

Behavioral problems in PWS include hyperphagia, skin picking, temper tantrums, stubbornness, manipulative and controlling behavior, obsessive-compulsive features, and difficulties in changing routines (13-16). The prevalence of some behaviors differs between those with a DEL and mUPD. Maladaptive behavior and skin picking are more common in those with a DEL than mUPD (17, 18), but the severity of compulsions is not different between the genetic subtypes (19, 20). The critical periods for a deterioration of the behavioral and emotional disturbances in children with intellectual disability are adolescence and young adulthood (21), but there is no data specific for children with PWS.

We hypothesized that psychiatric disorders such as psychosis and depression, oppositional defiant disorder (ODD) and compulsions are present in children with PWS, and that their prevalence of psychiatric disorders increases with age. We expected that subjects with mUPD would be more prone to psychosis and those with DEL to depression and oppositional defiant disorder.

METHODS

For the present study, we invited parents of children aged 7 to 17 years who were enrolled in the Dutch PWS Cohort Study, to participate in this study. The Dutch PWS Cohort Study is a study investigating long-term effects of growth hormone treatment in children with PWS (22). Screening for psychiatric disorders was performed twice, i.e. at baseline and after 2 years.

A total of 69 children with PWS were eligible for participation, 61 of whom agreed to participate in the first assessment, resulting in a response rate of 88%. In the second assessment, 44 children who also completed the first assessment were eligible for participation and 38 children completed the second assessment, resulting in a response rate of 86% (17 children excluded due to a follow-up period less than 2 years). Parents of 6 children did not respond. None of the participants dropped out because of lack of internet to complete the tests, severe illness, or psychiatric disorders such as psychosis. The genetic diagnosis of PWS was confirmed in all participants by methylation test, the genetic subtype was known in all but 2 participants. All participants were treated with Genotropin 1 mg/m^2/day. This study was approved by Medical Ethics Committee of the Erasmus Medical Center Rotterdam. Written informed consent was obtained from all parents or carers, informed consent was obtained from children of 12 years or older, and assent below the age of 12 years.

Screening for psychiatric disorders

The Dutch translation of the Diagnostic Interview Schedule for Children (DISC) (23) is a structured diagnostic interview to investigate more than 30 psychiatric diagnoses using the DSM-IV (24) and ICD-10 (25) in children and adolescents. The DISC has been used as a screening instrument for psychiatric disorders to prevent extensive psychiatric evaluations (26). Major diagnostic modules of the DISC are anxiety, mood disorders, disruptive behavior disorders, substance use, schizophrenia, and miscellaneous disorders. Disruptive behavior disorders include ADHD, oppositional defiant disorder and conduct disorders.

Most DISC questions are short and not very complicated. The DISC assesses the presence of diagnoses occurring within the past month, the past year, and between the age of 5 years and the current year ('whole-life' module). Responses to DISC questions are mostly limited to 'yes' and 'no', although some have an additional 'sometimes'/ 'somewhat' response option or a close-ended frequency choice.

The paper-version of DISC is known to be an extensive interview by virtue of its length, branching and skipping instructions, and multiple time frames (current, past year, and lifetime questions). The internet-based DISC has the facility to skip modules or sections

automatically, depending on the answer given or the modules selected for the study. The use of the internet-based DISC minimizes therefore interviewer and editor error.
In this study, we used the parental self-administered internet-based version of the DISC, which covers an age range of 6 to 17 years. The child version was too complicated for children with an intellectual disability. Parents completed the DISC in home setting at start and after approximately 2 years. An advantage of the internet version is the convenience to complete the questionnaire at the preferred moment. To limit the load for the parents, we restricted the questionnaire to the most relevant diagnoses and excluded the modules 'substance use disorders' and 'miscellaneous disorders' (diagnosing anorexia nervosa/bulimia nervosa, elimination disorders, pica, trichotillomania). Five major disorders were assessed: anxiety, disruptive behavior, mood, schizophrenia and tic. As the module schizophrenia included the major criteria for other psychotic disorders, namely delusions, hallucinations, and grossly disorganized or catatonic behavior, we studied the presence of these items to screen for other psychotic disorders. We did not calculate the scores of the 'whole-life' module because of the unreliable answers of parents to recall behavior of their child from the age of 5 years onwards, which could be more than 10 years ago for our oldest adolescents. During the study, children with suspected psychiatric problems were referred to a child-psychiatrist (PC).

Compulsions

People with intellectual disability have a different compulsive profile than healthy references. For that reason, we used a test specifically designed for people with intellectual disability, the Compulsive Behavior Checklist (CBC) (27). The CBC was administered via a structured interview with parents. The CBC describes the 25 items of compulsions that are grouped according to 5 categories: ordering (arranging objects), completeness (closing doors), cleaning (washing hands), checking/touching (tapping floor), and grooming. We assessed the item 'insists on activities/chores at a certain time' twice, namely including food-related and non-food related compulsions. In other words, if a child insisted on having dinner at 6 pm, this was scored as a positive answer in the food-related, but negative in the non-food related way.
The CBC also aligns the extent of interference with daily life and the response to parents' interruption of a compulsion. Each item and extent of the interference with daily life were scored as nominals (absent=0, present=1). The child's response after interruption of a compulsion by parents was scored as ordinals (0=never, 1= rare, 2=occasional, 3=often).

Cognitive functioning

Cognitive testing was annually performed in prepubertal children and biennially in pubertal children using the Wechsler Intelligence Scale for Children-Revised (WISC-R)

by an experienced psychologist (28). The method of cognitive testing in our children with PWS has been described in detail (29).

Data-analyses

Age and intelligence at baseline are presented as median and interquartile range (IQR). The prevalence of the most common diagnoses in children with PWS is presented. Wilcoxon rank sum test was used to test the characteristics of the study group at start and after 2 years. McNemar's test was used to test the difference between prevalence of diagnoses within the DISC at start and after 2 years. Multiple linear regression was performed to assess the association between age, gender (1=boys, 2=girls), genetic subtype (1=deletion, 2=mUPD), intelligence, and presence of a psychiatric disorder (0=absent, 1=present). Data of the Compulsive Behavior Checklist were listed and occurrence of compulsions were presented as percentages. McNemar's test was used to test the difference in occurrence of compulsions, extent of interference in daily life, and child's response after interruption of a compulsion by parents at start and after 2 years. Multiple linear regression tested the association between occurrence of ODD and extent of interference of compulsions in daily life and genetic subtype, after adjustment for age and gender. Mann-Whitney U test was used to test the difference in skin-picking between genetic subtypes. Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL). A p-value of <0.05 (two-tailed) was considered statistically significant.

RESULTS

Baseline

Median (interquartile range (IQR)) age of the 61 children with PWS was 11.8 (8.8-14.3) years and 36 (59%) were male (Table 1). Twenty-five had DEL (41%), 33 mUPD (54%), one an imprinting defect, and in two children the genetic subtype was unknown. Median (IQR) total IQ was 62 (55-73). Family psychiatric history was absent in 40 children (66%), present in first-degree relatives in 7 (11%) children (i.e. 1 parent with psychosis, 2 parents with depression, 2 sibs with ADHD and 2 sibs with autism spectrum disorder) and in second-degree relatives in 11 children (18%) (i.e. 4 with depression, 3 with autism spectrum disorder, 3 with ADHD, and one with borderline personality disorder).

Psychosis

Prior to the study, two boys with mUPD had psychotic symptoms at the age of 8 and 9 years and had started with antipsychotics and antidepressants (PC). Psychotic or mood disorders were absent in the family of both children. Fifty-nine of 61 children (97%) were

Table 1. Characteristics of the study group

	Baseline
N (male)	61 (36)
Age (years)	11.8 (8.8-14.3)
Genetic subtype (n (%))	
Deletion	25 (41)
UPD	33 (54)
ICD/Translocation	1
Unknown	2
Total IQ	62 (55-73)
Symptoms of children with psychotropic medication	
Psychotic symptoms	2
Irritability, anxiety, temper tantrums	2
Severe rigidity	1

Age and total IQ are expressed in median (IQR); genetic subtype and symptoms of children with psychotropic medication are expressed in N.

never diagnosed with a psychotic disorder. Based on the DISC, none had schizophrenia or other psychotic symptoms, including adolescents older than 13 years.

Depression

One 10-year old boy with a DEL (2%) who did not use psychotropic medication scored positive for depression. Family psychiatric history of this boy was absent for mood disorders.

Other psychiatric disorders

Prior to the study, two other boys had started with antipsychotics (one also combined with antidepressants) because of increased irritability, anxiety and temper tantrums with aggressiveness without psychotic symptoms at the age of 9 and 11 years (PC). Due to severe rigidity in routines and repeated questioning, one 13-year old girl had started with a selective serotonin reuptake inhibitor.

Oppositional Defiant disorder (ODD)

According to the DISC, 14 (22%) children scored positive for disruptive behavior disorders and 2 (3%) for anxiety and disruptive behavior disorder. Ten of 16 children (16% of total group) with a disruptive behavior scored positive for ODD, 1 (2% of total group) for ODD and ADHD, and 1 (2% of total group) for ODD and conduct disorders. ODD was the most common disorder and found in 20% of children with PWS. The presence of symptoms within ODD are presented in Figure 1 for children who fulfilled the criteria of

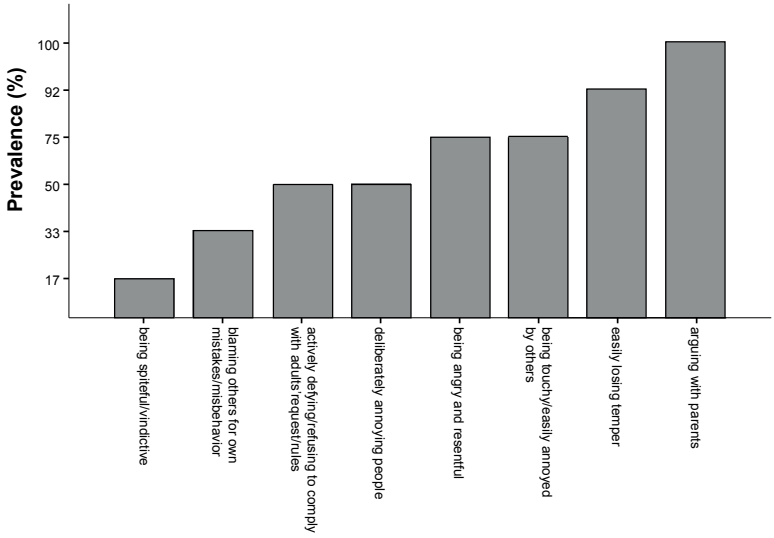

Figure 1. Symptoms within oppositional defiant disorder (ODD) for children with PWS who fulfilled the criteria of ODD.

ODD. The presence of ODD was not associated with age (β= -0.081, p=0.546), gender (β= 0.013, p=0.923), genetic subtype (β= -0.073, p=0.584) or total IQ (β= -0.150, p=0.267).

Other diagnoses

Three (5%) children scored positive for anxiety disorder, 1 (2%) for anxiety and mood disorder, but 41 (67%) had no psychiatric diagnosis. Anxiety disorders consisted of specific phobias for needles and thunder, and separation anxiety. Two of 16 children (3% of total group) scored positive for attention deficit hyperactivity disorder (ADHD), and 2 (3% of total group) for conduct disorders. The most common diagnoses are depicted in Table II. None of the children scored positive for panic disorders, agoraphobia, generalized anxiety disorder, social phobia, obsessive-compulsive disorder within the module anxiety, tic disorders, or mania/hypomania within the module mood disorders.

Compulsions according to the Compulsive Behavior Checklist (CBC)

Compulsions are depicted in Table III. The most prominent compulsions were hiding, collecting or hoarding objects (37%), and insisting on doing hygienic steps in a fixed sequence and starting at beginning of sequence if interrupted (27%). Thirty-six percent of children met the item 'insists activities/chores on a certain time' including food-related activities, but 15% met this item without food-related activities.

Skin picking occurred in 68% of the children and no significant difference in prevalence was found between children with DEL and mUPD. In 27% of children with PWS, compul-

Table 2. Most common psychiatric disorders in children with PWS

	Disruptive behaviour disorders						Anxiety disorders			
	ODD		ADHD		Conduct disorder		Specific phobia		Separation anxiety	
	Start	After 2 yrs	Start	After 2 yrs	Start	After 2 yrs	Start	After 2 yrs	Start	After 2 yrs
Percentage (n)	20 (12)	21 (8)	5 (3)	11 (4)	5 (3)	5 (2)	5 (3)	3 (1)	3 (2)	3 (1)
DEL/mUPD/ICD	5/7/0	6/1/1	0/2/1	0/3/1	1/2/0	1/1/0	1/2/0	1/0/0	1/1/0	0/1/0
Total IQ	58 (53-73)	64 (54-79)	70	67 (56-85)	51	53	51	54	60	61

Total IQ is expressed in median (IQR). ICD= Imprinting Center Defect. Numbers at start: n=61, numbers after 2 years: n=38.

Table 3. Compulsions in children with PWS (n=61)

Items	Occurrence in %			
Ordering compulsions				
Objects in a certain pattern	18			
Arranges certain items in one spot	20			
Wants chair in fixed arrangement	7			
Wants/arranges peers to sit in certain chairs	5			
Uses same chair or location when in particular room	15			
Insists on activities/chores at a certain time				
food related	36			
non-food related	15			
Completeness/Incompleteness compulsions				
Insists on closing doors, cupboards	12			
Removes all items from closet, purse etc.	2			
Repeatedly removes then replaces items	3			
Empties/wants containers emptied	7			
Puts garments on and off; hangs up and removes clothes	0			
Insists on certain chore, resists letting others	15			
Cleaning/Tidiness compulsions				
Fixed hygiene sequence; restarts if interrupted	27			
Cleans body part(s) excessively	3			
Insists on picking up bits; 'lint picking'	2			
Often picks/rips objects if not prevented	12			
Insists certain ('cleaning') activity be done	2			
Hides away, collects/hoards particular objects	37			
Checking/Touching compulsions				
Repeatedly opens and closes doors/drawers	0			
Touches or taps items repeatedly	2			
Touching/stepping pattern	2			
Unusual sniffing	2			
Deviant grooming compulsions				
Picks at face/body to point of gouging skin	68			
Checks hair, face/excessive mirror checking	2			
Inappropriately cuts/pulls/calmly pulls our hair	7			
Extent of interference with daily living				
Compulsions take more than an hour a day (if not prevented)	27			
Compulsions significantly interfere with the person's normal routine	20			
Compulsions significantly interfere with usual social activities	15			
Compulsions significantly interfere with relationship with others	12			
The child's response after interruption of a compulsion by parents	*Never*	*Rare*	*Occasional*	*Often*
Halts momentarily, then resumes compulsive activity	25	5	8	62
Waits until caregiver left the immediate area, then resumes	30	5	8	57
Becomes angry, may hit or kick caregiver who intervene	50	20	20	10
Becomes upset, may bite self, hit self, or headbang	83	3	12	2

sions took more than an hour a day if not prevented, in 20% it interfered with the child's normal routine, in 13% with usual social activities, and in 12% in relationships with others. Most common responses after parents' interruption of a compulsion was halting momentarily, before resuming compulsive activity (63%), or waiting with resuming until the caregiver left the immediate area (57%). Seventy percent of children with PWS never or rarely became angry, and would not hit or kick the parent who intervened, and 83% neither became upset, nor bite themselves or head banged (Table III). No association was found between occurrence of ODD in the past year and extent of interference by compulsions in daily life, i.e. compulsions taking more than one hour per day (β= -0.083, p=0.615), compulsions interfering with normal routine (β= 0.120, p=0.507), compulsions interfering with social activities (β= -0.019, p=0.923), and compulsions interfering with relationships with others (β= 0.205, p=0.254), resp. The occurrence of compulsions were not different between the genetic subtypes. These results were adjusted for gender and age, as it is known that rituals and routines are part of the normal development in childhood.

Follow-up after 2 years

In the second assessment after 2 years, 38 children participated and this group did not significantly differ from the initial group in gender, genetic subtype and total IQ (p=1.00, p=0.317, p=0.614, resp.).

Psychosis

The two boys with known psychotic symptoms prior to the present study were still on antipsychotic medication. One of them had reappearing psychotic symptoms in the pre-psychotic phase and required a dose adjustment due to a higher bodyweight. None of the other children had signs of schizophrenia or other psychotic symptoms during 2 years of follow-up.

Depression

One 14-year old boy with a DEL who scored negative for depression at start, scored positive for depression after 2 years but did not use psychotropic medication.

Other psychiatric disorders

ODD

The prevalence of ODD did not significantly differ during the study (Table II). After 2 years, 6 of 38 children (16%) scored positive for disruptive behavior disorders, and 4 (11%) for anxiety disorders and disruptive behavior disorders. The ODD diagnosis of some children had changed after 2 years: 4 children remained positive, 4 children who

scored positive at start had a negative score, 4 children who scored negative at start had a positive score, and 26 children remained negative.

Other diagnoses

The prevalence of ADHD, conduct disorders, and anxiety did not significantly differ from start of the study (Table II). Three of 38 children (8%) scored positive for anxiety disorders, 1 (3%) for mood disorders, but 22 (58%) did not have signs of psychiatric disorders. After 2 years, none of the children scored positive for panic disorders, agoraphobia, generalized anxiety disorder, social phobia, obsessive-compulsive disorder within the module anxiety, tic disorders, or mania/hypomania within the module mood disorders.

Compulsions according to the CBC

Four boys (11%) who scored negative at start for compulsive disorders had a positive score after 2 years. Three were known with a DEL and one had an mUPD. After 2 years, no significant difference was found in the frequency of the compulsions, extent of the interference in daily life, or response to parents' interruption of a compulsion.

DISCUSSION

In this study, we investigated the presence and progression of psychiatric disorders in a large group of 61 children with PWS aged 7 to 17 years in a 2-year longitudinal study. Schizophrenia or other psychotic disorders were rare in children with PWS: two of 61 children (3%) had psychotic symptoms prior to the study and 59 of 61 children (97%) did not have psychotic symptoms. Concerning the other psychiatric disorder disorders, oppositional defiant disorder (ODD) was most common (20%), followed by ADHD (5%), conduct disorders (5%), specific phobia (5%) and separation anxiety (5%). The two most frequent compulsions were hiding objects or collecting/hoarding particular objects (37%), fixed hygiene sequence, and restarting if interrupted (27%). Although most children resumed the compulsions after interruption or after the parents left the immediate area, most children were not aggressive towards the parents or themselves. The prevalence of psychiatric disorders did not increase by age. Psychosis was not associated with the mUPD subtype, and depression and ODD were not associated with DEL.

Psychosis

In contrast to our expectations, we did not find schizophrenia or other psychotic symptoms in the majority of the 61 children with PWS. This is in contrast to earlier studies reporting a high prevalence of 13% to 62% for psychosis in adults with PWS caused by an mUPD (7, 10, 11). These findings could be explained by several factors.

First, the aim of our study was to investigate the presence of psychiatric disorders in children with PWS. Our study group is therefore young and it is likely that our participants were less vulnerable for psychosis and mood disorders. However, the age at onset of psychosis has been reported in the age range of 13 to 19 years (7), but none of our participants above the age of 13 years had a psychosis except one boy with psychotic symptoms. Second, all children with PWS in our study were followed closely and referred to a child-psychiatrist (PC) when psychopathology was presumed. Early start of psychotropic medication and adjusted guidance by a multidisciplinary team may have prevented a psychosis. In our study, two children who were showing mood instability, increased irritability, and anxiety were treated with antipsychotics and remained stable during the 2-year follow-up. This is in line with a previous study, which reported no psychotic episodes in adults with PWS after a psychosis or those who were at risk for psychosis due to the mUPD subtype (30). Antipsychotics such as Risperidone and Aripiprazole has been described to be effective in reducing irritability in children and adolescents with autism (31, 32). Because it turned out that the boy with relapsing psychotic symptoms in the pre-psychotic phase required a dose adjustment, we advise regular check calculations of psychotropic dosage in order to prevent relapse of psychotic symptoms, especially in case of weight gain. Third, the absence of schizophrenia or other psychotic symptoms in the majority of our children might be due to improved diagnostic tools and treatment in PWS, including the beneficial effect of long-term growth hormone treatment on the mental state. Our study group had been treated with growth hormone for many years prior to this study. It can not be excluded that long-term growth hormone played a role in the neurodevelopment, leading to a lower risk for psychotic symptoms. We previously demonstrated that growth hormone treatment improved cognition in children with PWS, particularly in those with lower cognitive functioning (33). Another research group showed that growth hormone enhances cell-to-cell communication in the central nervous system by increasing connexin-43 expression, which is a biomarker for gap-junction formation in the brain (34). Also, the current generation of children with PWS seems to differ from the earlier generation, i.e. in body composition and development (22) due to the early detection and multidisciplinary care of physiotherapy, speech therapy, diets and family support.

It is remarkable that the 2 boys with psychosis prior to this study were known with an mUPD. The association between mUPD and psychosis has been described previously (6, 7). In our analysis this association was not significant, most likely due to the small numbers of children with psychosis.

ODD

No studies have screened for ODD in PWS. We found an ODD prevalence of 20% in children with PWS, which was not associated with genetic subtype. It is well known

that people with mild to moderate intellectual disability have less compensational skills to cope with daily challenges of life compared to healthy peers, and that this leads to behavioral problems. One explanation might be that some symptoms of ODD, such as arguing with adults or easily losing temper, result from overestimation by the environment. The prevalence of ODD in children with PWS was in the same range as it has been reported that ODD occurs in 16% of individuals with intellectual disability and 3-4% of children without an intellectual disability (5). A child with ODD causes stress in a family (35, 36). It is therefore important to be aware of the high prevalence of ODD in children with PWS in order to prevent overestimation of the child's capacity with regard to language skills, emotional skills and social functioning. It also means that families require practical advices how to approach their child. Overestimation could be decreased by using adjusted learning programs at school, breaking down large tasks into clearly defined small steps, or allowing more time to rest. Communication with the child with PWS should be clear and concise by describing tasks simply, using short sentences, and avoiding abstract language (29).

Compulsions

We found a high rate of hoarding/collecting objects and insisting on (hygienic) routines in children with PWS. This is in line with earlier studies showing the presence of compulsions in individuals with PWS (14, 37). In children with PWS aged 7 to 17 years, we did not find a difference in the extent of compulsions between the genetic subtypes, which is in line with a study in young children with PWS (37).

We assessed the item 'insists on activities/chores at the same time' twice, namely for food-related and non-food related 'compulsions'. It is doubted if food-related compulsions should be seen as compulsions, as it seems that hyperphagia is due to pathology in appetite regulating hormones (e.g. ghrelin or oxytocin disturbances). However, children with PWS show a compulsive aspect in hyperphagia such as being rigid about the time of eating and what is being eaten. Also, the smallest changes in the eating ritual could lead to mood fluctuations, temper tantrums, and aggressiveness. Thus, the item 'insists on activities/chores at the same time' for food-related is presented in this study to show the impact of food on this behavior.

We interviewed parents with the Compulsive Behavior Checklist (CBC) to assess the compulsive profile in their children. In the CBC, we also asked the parents to indicate how frequent the child continues with the compulsion after an interruption. Many parents answered with 'always'. However, 'often' was the highest score in the questionnaire. It is thus very likely that the results underestimated the child's response to interruption of a compulsion.

Although skin picking is considered not to be a compulsive disorder but rather behavior specific to PWS (38), this item was assessed to determine its prevalence. In our study,

skin picking was present in two-third of children with PWS and no significant difference in prevalence was found between children with DEL and mUPD. These findings are in contrast to reports describing more skin picking in children with a DEL than mUPD (17, 39, 40).

Our study results have implications for the treatment and guidance of children with PWS. However, our study is limited by the lack of a control group. In the Netherlands, as in most Western European countries, growth hormone treatment is nowadays standard treatment for children with PWS. This challenges the possibility to conduct a new RCT in children without GH treatment. However, countries where GH treatment has not been standardized yet, do have the possibility to investigate the prevalence of psychiatric disorders in children with PWS without growth hormone treatment.
In our study, screening for psychiatric disorders have been performed twice during 2 years. Although the tests were applied twice, all children were regularly examined in the Dutch PWS Cohort Study by the Dutch PWS team and pediatricians. During the consultations, behavior and mental health were always evaluated. It is therefore unlikely that major psychiatric disorders were missed.

In conclusion, in our large group of 61 children with PWS, the majority of children had no schizophrenia or other psychotic symptoms and no progression of psychotic symptoms was found during 2-year follow-up. Oppositional defiant disorder (ODD) was the most common diagnosis and found in 20% of children with PWS. The most common compulsions were hoarding and fixed hygiene sequence. No changes in the prevalence of psychiatric disorders were found during the 2-year follow-up study and genetic subtype was not related to psychosis, depression, or ODD.

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REFERENCES

1. Prader A, Labhart A, Willi H 1956 Ein syndrom von adipositas, kleinwuchs, kryptorchidismus und oligophrenie nach myatonieartigem zustand in neugeborenalter. Schweiz. Med. Wochenschr. 86: 1260
2. Vogels A, Van Den Ende J, Keymolen K, Mortier G, Devriendt K, Legius E, Fryns JP 2004 Minimum prevalence, birth incidence and cause of death for Prader-Willi syndrome in Flanders. Eur. J. Hum. Genet. 12:238-240
3. Akefeldt A, Gillberg C, Larsson C 1991 Prader-Willi syndrome in a Swedish rural county: Epidemiological aspects. Dev. Med. Child Neurol. 33:715-721
4. Webb T, Whittington J, Clarke D, Boer H, Butler J, Holland A 2002 A study of the influence of different genotypes on the physical and behavioral phenotypes of children and adults ascertained clinically as having PWS. Clin Genet 62:273-281
5. de Ruiter K 2013 Five-Year Development of Psychopathology in Young People with Intellectual Disabilities. In: Faculteit der Psychologie en Pedagogiek. Amsterdam: Vrije Universiteit Amsterdam; 77-98
6. Boer H, Holland A, Whittington J, Butler J, Webb T, Clarke D 2002 Psychotic illness in people with Prader Willi syndrome due to chromosome 15 maternal uniparental disomy. Lancet 359:135-136
7. Vogels A, Matthijs G, Legius E, Devriendt K, Fryns JP 2003 Chromosome 15 maternal uniparental disomy and psychosis in Prader-Willi syndrome. J. Med. Genet. 40:72-73
8. Cooper SA, Smiley E, Morrison J, Williamson A, Allan L 2007 Mental ill-health in adults with intellectual disabilities: prevalence and associated factors. Br. J. Psychiatry 190:27-35
9. Clarke DJ 1993 Prader-Willi syndrome and psychoses. Br. J. Psychiatry 163:680-684
10. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32:1729-1735
11. Soni S, Whittington J, Holland AJ, Webb T, Maina E, Boer H, Clarke D 2007 The course and outcome of psychiatric illness in people with Prader-Willi syndrome: implications for management and treatment. J. Intellect. Disabil. Res. 51:32-42
12. Collin PJL, Boer H, Vogels A, Curfs LMG 2005 Psychose bij kinderen met het Prader-Willi syndroom. Tijdschr Psychiatr 47:325-328
13. Morgan JR, Storch EA, Woods DW, Bodzin D, Lewin AB, Murphy TK 2010 A preliminary analysis of the phenomenology of skin-picking in Prader-Willi syndrome. Child Psychiatry Hum. Dev. 41: 448-463
14. Clarke DJ, Boer H, Whittington J, Holland A, Butler J, Webb T 2002 Prader-Willi syndrome, compulsive and ritualistic behaviours: the first population-based survey. Br. J. Psychiatry 180:358-362
15. Akefeldt A, Gillberg C 1999 Behavior and personality characteristics of children and young adults with Prader-Willi syndrome: a controlled study. J. Am. Acad. Child Adolesc. Psychiatry 38:761-769
16. Reddy LA, Pfeiffer SI 2007 Behavioral and emotional symptoms of children and adolescents with Prader-Willi Syndrome. J. Autism Dev. Disord. 37:830-839
17. Dykens EM, Cassidy SB, King BH 1999 Maladaptive behavior differences in Prader-Willi syndrome due to paternal deletion versus maternal uniparental disomy. Am. J. Ment. Retard. 104:67-77
18. Cassidy SB, Forsythe M, Heeger S, Nicholls RD, Schork N, Benn P, Schwartz S 1997 Comparison of phenotype between patients with Prader-Willi syndrome due to deletion 15q and uniparental disomy 15. Am. J. Med. Genet. 68:433-440

19. Dimitropoulos A 2003 An investigation of childhood rituals and compulsive behavior in children with Prader-Willi syndrome. Dissertation Abstracts International: Section B: The Sciences and Engineering 63:3493
20. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
21. Steinhausen HC, Eiholzer U, Hauffa BP, Malin Z 2004 Behavioural and emotional disturbances in people with Prader-Willi Syndrome. J. Intellect. Disabil. Res. 48:47-52
22. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Schroor EJ, Van Alfen AA, Van Leeuwen M, Van Pinxteren-Nagler E, Van Wieringen H, Vreuls RC, Zwaveling-Soonawala N, de Ridder MA, Hokken-Koelega AC 2013 Eight Years of Growth Hormone Treatment in Children With Prader-Willi Syndrome: Maintaining the Positive Effects. J. Clin. Endocrinol. Metab. 98:4013-4022
23. Ferdinand RF, van der Ende, J. 1998 Nederlandse vertaling van de DISC-IV; Diagnostic Interview Schedule for Children. Rotterdam: Afdeling kinder-en jeugdpsychiatrie van het Sophia Kinderziekenhuis.
24. American Psychiatric Association 2000 DSM-IV-TR. Washington, DC: American Psychiatric Publishing
25. World Health Organization 1993 The ICD-10 Classification of Mental and Behavioural Disorder: Diagnostic Criteria for Research. In. Geneva: World Heatlh Organization
26. Shaffer D, Fisher P, Lucas CP, Dulcan MK, Schwab-Stone ME 2000 NIMH Diagnostic Interview Schedule for Children Version IV (NIMH DISC-IV): description, differences from previous versions, and reliability of some common diagnoses. J. Am. Acad. Child Adolesc. Psychiatry 39:28-38
27. American Psychiatric Association 1994 Diagnostic and Statistical Manual of Mental Disorders. 4 th ed. Washington, DC.
28. Wechsler D 2002 Wechsler Intelligence Scale For Children (Dutch Version), manual. Third edition ed. London, United Kingdom: Harcourt Assessment
29. Lo ST, Siemensma E, Collin P, Hokken-Koelega A 2013 Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. Res. Dev. Disabil. 34:2764-2773
30. Larson FV, Whittington J, Webb T, Holland AJ 2013 A longitudinal follow-up study of people with Prader-Willi syndrome with psychosis and those at increased risk of developing psychosis due to genetic subtype. Psychol. Med.:1-5
31. McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, Arnold LE, Lindsay R, Nash P, Hollway J, McDougle CJ, Posey D, Swiezy N, Kohn A, Scahill L, Martin A, Koenig K, Volkmar F, Carroll D, Lancor A, Tierney E, Ghuman J, Gonzalez NM, Grados M, Vitiello B, Ritz L, Davies M, Robinson J, McMahon D 2002 Risperidone in children with autism and serious behavioral problems. N. Engl. J. Med. 347:314-321
32. Owen R, Sikich L, Marcus RN, Corey-Lisle P, Manos G, McQuade RD, Carson WH, Findling RL 2009 Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. Pediatrics 124:1533-1540
33. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi

Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314

34. Aberg ND, Carlsson B, Rosengren L, Oscarsson J, Isaksson OG, Ronnback L, Eriksson PS 2000 Growth hormone increases connexin-43 expression in the cerebral cortex and hypothalamus. Endocrinology 141:3879-3886
35. Wulffaert J, Scholte EM, Van Berckelaer-Onnes IA 2010 Maternal parenting stress in families with a child with Angelman syndrome or Prader-Willi syndrome. J Intellect Dev Disabil 35:165-174
36. Mazaheri MM, Rae-Seebach RD, Preston HE, Schmidt M, Kountz-Edwards S, Field N, Cassidy S, Packman W 2013 The impact of Prader-Willi syndrome on the family's quality of life and caregiving, and the unaffected siblings' psychosocial adjustment. J. Intellect. Disabil. Res. 57:861-873
37. Dimitropoulos A, Blackford J, Walden T, Thompson T 2006 Compulsive behavior in Prader-Willi syndrome: examining severity in early childhood. Res. Dev. Disabil. 27:190-202
38. Feurer ID, Dimitropoulos A, Stone WL, Roof E, Butler MG, Thompson T 1998 The latent variable structure of the Compulsive Behaviour Checklist in people with Prader-Willi syndrome. J. Intellect. Disabil. Res. 42 (Pt 6):472-480
39. Dykens EM, Roof E 2008 Behavior in Prader-Willi syndrome: relationship to genetic subtypes and age. J. Child Psychol. Psychiatry 49:1001-1008
40. Flores CG, Valcante G, Guter S, Zaytoun A, Wray E, Bell L, Jacob S, Lewis MH, Driscoll DJ, Cook EH, Jr., Kim SJ 2011 Repetitive behavior profiles: Consistency across autism spectrum disorder cohorts and divergence from Prader-Willi syndrome. J Neurodev Disord 3:316-324

CHAPTER 3

THEORY OF MIND AND SYMPTOMS OF AUTISM SPECTRUM DISORDER IN CHILDREN WITH PRADER-WILLI SYNDROME

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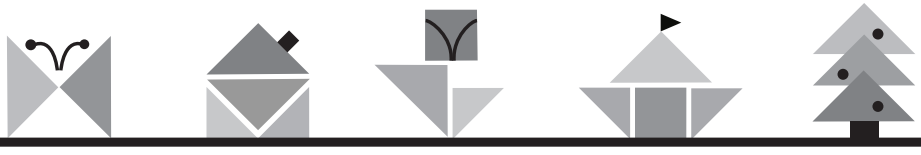


ABSTRACT

In order to evaluate the social cognitive functioning in children with Prader-Willi syndrome (PWS), Theory of Mind (ToM) and symptoms of Autism Spectrum Disorder were evaluated. Sixty-six children with PWS aged 7 to 17 years were tested using the Theory of Mind test-R and the Diagnostic Interview for Social Communication disorders. We tested the correlation between Total ToM Standard Deviation Score (Total ToM SDS) and genetic subtype of paternal deletion or maternal uniparental disomy, and total IQ, verbal IQ and performal IQ. Prevalence and symptoms of Autism Spectrum Disorder were assessed. Median (interquartile range) of total ToM SDS of those aged 7 to 17 years was -3.84 (-5.73, -1.57). Their Total ToM SDS correlated with total IQ (β= 0.662, p< 0.001,adj. R^2=0.407), in particular with verbal IQ (β= 0.502, p= 0.001, adj.R^2=0.409), but not with performal IQ (β= 0.241, p>0.05, adj.R^2=0.259). No difference in Total ToM SDS was found between children with deletion and maternal uniparental disomy (β=-0.143, p>0.05, adj. R^2= -0.016). Compared to the reference group of healthy children aged 7 to 12 years, children with PWS in the same age group had a median ToM developmental delay of 4 (3-5) years. One third of children with PWS scored positive for Autism Spectrum Disorder. Most prominent aberrations in Autism Spectrum Disorder were focused on maladaptive behavior. Our findings demonstrate a markedly reduced level of social cognitive functioning which has consequences for the approach of children with PWS, i.e. adjustment to the child's level of social cognitive functioning.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurogenetic developmental disorder caused by the absence of paternal expression of genes in chromosome 15 at the locus q11-q13, either by paternal deletion (DEL), maternal uniparental disomy (mUPD), imprinting defects or paternal chromosomal translocation (1). Its main characteristics are hypotonia, hypogonadism, short stature, obesity, intellectual disability and a specific behavioral phenotype.

Earlier studies described various behavioral characteristics, such as hyperphagia and food preoccupation, skin picking, compulsive behavior, and psychotic disorders (2-5).
Differences in behavioral phenotype have been linked to the genetic subtype of individuals with PWS. Those with mUPD seem more prone to Autistic Spectrum Disorder (ASD) than individuals with DEL (6-8). A recent study showed that impaired social functioning in subjects with mUPD was similar to that in subjects with Autism Spectrum Disorder (9).

Children with ASD have an impaired Theory of Mind (ToM) development (10). The ToM describes the cognitive capacity to infer in the mental states of oneself (11) and others, and is an essential ability in social cognitive functioning and a core cognitive feature of ASD. The Theory of Mind has not been previously investigated in detail in children with PWS. One study tested three aspects of the ToM in a small group of young children with PWS, who were participating as one of the comparison groups in their major study of ToM in children with Williams syndrome (12). They found that children with PWS performed better in false belief, an element of the ToM, than children with Williams syndrome. Another study found that the level of social adjustment, using the Social Attribution Task, in adults with PWS were more poorly than in those with comparable intellectual disability but similar to those with a pervasive developmental disorder (13).
In order to evaluate the social cognitive functioning in children with Prader-Willi syndrome, the Theory of Mind and Autism Spectrum Disorder and their correlation were evaluated.
We postulated that ToM development was delayed in most children with PWS, and expected that ToM scores would be less abnormal in children with DEL. We also hypothesized that children with DEL are less prone to ASD than children with mUPD. For that reason, we assessed the prevalence and symptoms of Autism Spectrum Disorder in these children.

METHODS

For the present study, we invited participants aged 7 to 17 years who participate in the Dutch PWS Cohort Study, a study regarding the long-term effects of growth hormone treatment in children with PWS (14). In the Dutch PWS Cohort Study, all participants were treated with Genotropin 1 mg/m^2/day, cognitive functioning was measured biennially, and anthropometry was performed annually.
For the present study, a total of 76 children with PWS were eligible for participation, 66 of whom agreed to participate, resulting in a response rate of 87%.
The genetic diagnosis of PWS was confirmed in all participants by methylation testing. The genetic subtype was known in all but 2 participants.

ToM

The Dutch ToM test-R (15) is a validated diagnostic instrument for healthy children between the age of 4 to 12 years old (10). It consists of 14 illustrated short stories which are divided into 3 developmental stages. Stage 1 covers recognition of emotions and the difference of reality and surreality (e.g. "actually" cycling and "dreaming" about cycling). Stage 2 covers the first manifestations of "belief": "first-order belief" and "false belief". "First-order belief" is the ability to understand one's own mental state as "I think". "False belief" is the individual's comprehension of another person's mistaken belief; for example if a character has not been informed that an object has been moved to another place. An example for testing the "false belief" is the following situation: person 1 places an object in box 1 and leaves the scene. Person 2 transfers the object to box 2. When person 1 returns, the investigator asks the child where person 1 would look for the object. "False belief" is acquired if the child's answer is box 1. If the child's answer is box 2, it suggests that the child is not able to make a judgment about another person's false expectation. Stage 3 consists of questions about the "second-order belief", i.e. inference of someone's belief about another's belief (e.g., "John thinks that Mary thinks it is going to rain and therefore she is taking the umbrella when she is going outdoors…").
The ToM- test-R includes 14 stories with a total of 33 questions and 3-pretence exercises. Scores are either 0 (failed) or 1 (passed), leading to a maximum of 36 points. A maximum of 12 points can be scored per stage. The ToM test-R has been validated for healthy children without intellectual disability in the age range of 4 to 12 years.
In order to search for a screening instrument for ASD in children with PWS, the ToM test-R was chosen to evaluate social cognitive functioning.
The ToM test-R was performed at the children's home or residence and the scores were verified by another researcher, which resulted in similar scores.

DISCO

The Dutch translation of the Diagnostic Interview Social and Communication disorders (DISCO), 11th revision, was used to diagnose ASD. DISCO (16) is a standardized semi-structured, interviewer-based questionnaire for diagnosing ASD, and is based on the original validated DISCO of 2003 (17). It can accurately identify children with ASD, including children with intellectual disability (18).

Only parents of children with PWS were interviewed for this study. DISCO was chosen because it diagnoses disorders within the broader "autism spectrum" (17). The diagnoses of the DISCO are based on DSM-IV-TR, the Diagnostic and Statistical Manual of Mental Disorders, fourth edition Text Revision (19).

To reduce the duration of the test, questions used for the computer algorithms were asked during the interview. On average, the interview took 1.5 hours. It included the DISCO-sections on motor skills, communication skills and social-developmental skills, imagination, repetitive and stereotyped activities, maladaptive behavior, and interviewer's judgement of quality.

The DISCO interview was performed in the hospital. Most of the items required an answer for past behavior (questions on "ever") and present behavior (questions on "current"). As the diagnoses of ASD did not differ between the "current" and "ever" sections, we used only the "current" classification in the DISCO analysis. The assessment was scored manually by a trained observer (L.S.) using the algorithms of revised DISCO 11th revision.

Cognitive functioning

To assess intelligence, we used a short form of the Wechsler Intelligence Scale for Children-Revised, Dutch version (WISC-R). These subtests were vocabulary and similarities (verbal IQ subtests), block design and picture arrangement (performance IQ subtests). Children over 7 years of age were tested by an experienced psychologist with expertise in testing children with PWS (20). Good correlations have been found between the short-form IQ and the full-scale IQ for the WISC-R (21-23).

The subtests' scores were expressed as standard deviation scores (SDS), based on normalized standard scores with a mean of 10, ranging from 1 (-3 SDS) to 19 (+3 SDS), on the basis of Dutch population data for the same age (20). Total IQ score was calculated according to an equation based on the Dutch outpatient population reference (total IQ = 45.3 + 2.91 x vocabulary standard score + 2.50 x block design standard score), as has been used in other studies (24-26). Similarly, the vocabulary subtest was taken as the verbal IQ, the Block design as performance IQ.

This study was approved by Medical Ethics Committee of the Erasmus Medical Center Rotterdam. Written informed consent was obtained from all parents or carers, informed

consent was obtained from children of 12 years of age onwards, and assent below the age of 12 years.

Data Analysis

In the total study group aged 7 to 17 years, Total ToM scores in SDS (Total ToM SDS) are presented as median and interquartile range (IQR). The correlation of Total ToM SDS with genetic subtype of DEL or mUPD (coded as 1 = DEL, 2 = mUPD), and total IQ, verbal IQ and performance IQ was tested using multiple linear regression. Adjustments were made for age and gender. Spearman's correlation was used to test the correlation between Total ToM SDS and IQ in children with PWS between 7-17 years. The difference in delay in ToM-age and Verbal IQ in children with PWS aged 7 to 12 years was tested with the Mann-Whitney U test. This age range was used because validated age-appropriate peer norms do only exist until the age of 12 years. The ToM developmental delay was calculated by subtracting ToM developmental age from the calendar age (ToM developmental delay = Age – ToM developmental age).
Children with PWS with a ToM score less than the mean for a four-year-old healthy child, were scored as a four-year-old child. The validated reference scores end at the age of 12. In the reference scores from the age of 10 onwards, an overall plateau for ToM score is shown indicating that ToM development in healthy children is completed at the age of 10 years. Therefore, we presumed that ToM development is completed from 10 years of age onwards in healthy children. We calculated the Total ToM SDS for those aged 13 to 17 using the reference score for healthy 12 years olds. The median (IQR) scores per stage of the ToM were calculated to evaluate which stages were impaired in children with PWS.

With regard to DISCO analyses, we firstly analyzed the individual DISCO results to diagnose the presence or absence of ASD. Fisher's exact test was used to test whether the diagnosis of ASD was different between genetic subtypes, and between the diagnosis of ASD and gender. Secondly, we assessed DISCO symptoms which occurred in more than fifty percent of the PWS group. To determine the relation between the most prominent symptoms and ToM SDS, and most prominent symptoms and genetic subtype, the Fisher's exact test was used. For this analysis, correction for multiple testing was made and a p-value of 0.006 was considered as significant. Thirdly, we assessed symptoms in the ASD which occurred in less than ten percent (least frequent symptoms) of our study group.
Mann-Whitney U test was used to test the association between BMI SDS and Total ToM SDS (group 0= Total ToM SDS >2, group 1= Total ToM SDS<2), and the association between BMI SDS and ASD diagnosis (group 0=negative ASD diagnosis, group 1 = positive ASD diagnosis).
The correlation of Total ToM SDS and ASD was tested using Fisher's exact test.

Statistical analyses were performed with SPSS 20·0 (SPSS Inc., Chicago, IL).
All statistical tests were two-sided, and a p-value ≤ 0.05 was considered statistically significant.

RESULTS

Characteristics

We included a total of 66 children with confirmed PWS and a median (IQR) age of 11 (9-14) years; twenty-five children with DEL (38%), 34 with mUPD (52%) and 5 with an imprinting defect (7%). Characteristics of the study group are presented in Table 1.

Table 1. Characteristics of the study group

	PWS
N	66
Age (yr)	11 (9, 14)
Gender	
Male	36 (55%)
Female	30 (45%)
Height SDS	0.09 (-1.07, 1.05)
Weight for height SDS	1.12 (0.14, 1.12)
BMI SDS	1.44 (0.46, 2.44)
Total IQ	61 (54, 74)
Age (years) at start GH treatment	4.66 (2.47, 6.72)
Duration (years) of GH treatment	7.00 (5.00, 7.25)
Genetic subtype	
Deletion	25 (38%)
UPD	34 (52%)
ICD	5 (7%)
Unknown	2 (3%)

Data are expressed as median (IQR); genetic subtype is expressed in N (%); BMI = Body Mass Index

ToM

Sixty-six children were tested with the ToM test-R. Six children were excluded from testing due to severe attention deficiency and/or insufficient language development. These six children did not significantly differ from the rest of the group in terms of age, gender, genetic subtype, total IQ and body mass index SDS.

Median Total ToM SDS in the group aged 7 to 17 years was -3.84 (-5.73, -1.57). Their Total ToM SDS was correlated with total IQ (β= 0.662, p< 0.001, adj. R^2= 0.407), in particular with verbal IQ (β=0.502, p= 0.001, adj. R^2=0.409) but not with performance IQ (β= 0.241, p>0.05, adj. R^2=0.259). Total IQ and verbal IQ remained significant after additional adjustment for gender and age. Total ToM SDS was not correlated with genetic subtype

(β=-0.143, p>0.05, adj.R^2 = -0.016). Total ToM SDS was significantly correlated with IQ in the total group aged 7-17, r= 0.592, p <0.001.

Figure 1a shows the distribution of Total ToM SDS per age. As an IQ score of 70 is the cut-off level for intellectual disability, we labeled total IQ score as total IQ > 70 or total IQ <70. In contrast to children with an IQ <70, children with an IQ > 70 tend to score a ToM score > -2 SDS. Figure 1b shows the distribution of Total ToM SDS per age, divided in those with an ASD diagnosis and those without.

In the subgroup of 39 children aged 7 to 12 years, the median (IQR) age was 9 (7-11) years. Their median ToM developmental age was 5 (4-6) years, resulting in a median delay of ToM development of 4 (3-5) years. The ToM developmental delay increased with age (p<0.001) and the verbal IQ decreased with age (p=0.05). The delay of ToM per age in children is depicted in Figure 2a, the Verbal IQ per age in Figure 2b.

The ToM test indicates of 3 stages of development which can reach a maximum score of 12 per stage. The median (IQR) score for stage 1 of the ToM test, the recognition of emotions and difference of reality and surreality, was 10 (9-11), for stage 2, the 'first-order belief' and 'false belief' the score was 8 (6-10) and for stage 3, the 'second-order belief', it was 5 (2-8).

DISCO

Parents of 66 children were interviewed using the DISCO. Twenty-four out of 66 children (36%) scored positively for ASD, 7/25 (28%) having DEL and 14/34 (58%) having mUPD. The prevalence of ASD diagnosis did not differ between children with DEL and mUPD (Fisher's exact, 2-tailed, p>0.05).

ASD diagnosis was neither associated with total IQ (Fisher's exact, 2-tailed, p>0.05), and not different between boys and girls (Fisher's exact, 2-tailed, p>0.05).

The most prominent aberrations within ASD which occurred in at least fifty percent of children with PWS were maladaptive behavior and routines. Children tended to interrupt conversations, talk to strangers and skin pick. In addition, children tended to pretence play alone and were taking literal interpretation of expressions. Parents found their children with PWS clumsy and poor in motor coordination (Figure 3a).

Less than ten percent of the parents reported that their child had problems in making contact with others shown such as nodding their head, reacting to visitors, and cuddling with family. Repetitive activities as motor restlessness and fascination for light, sparkling object, sounds, spinning objects, parts of objects, abstract characteristics of objects as color or shape, and restriction of food were also only present in less than ten percent in our group (Figure 3b).

None of the most prominent symptoms were related to Total ToM SDS and the prevalence of these symptoms did not significantly differ between the DEL and mUPD group.

Figure 1a. Total ToM SDS in children with PWS, labeled by Total IQ

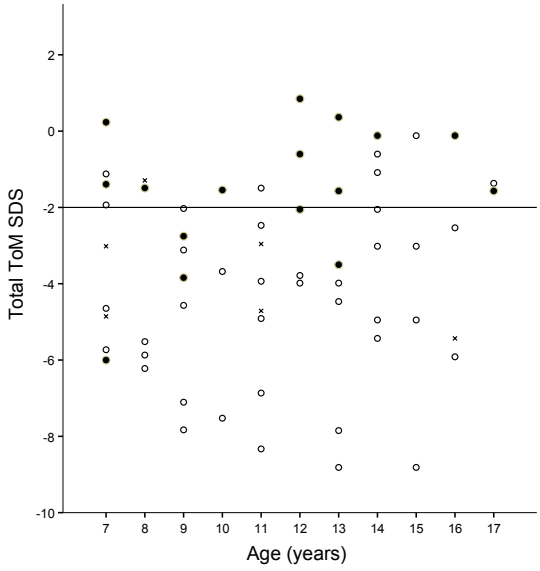
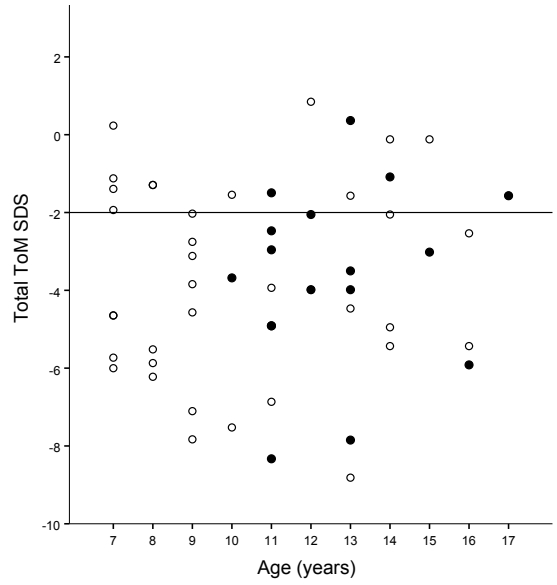


Figure 1b. Total ToM SDS in children with PWS, labeled by ASD diagnosis



Total ToM SDS = Total Theory of Mind Standard Deviation Score, PWS= Prader-Willi Syndrome, TIQ = Total IQ, ASD= Autism Spectrum Disorder. Total IQ: x = Unknown, ○ = TIQ <70, ● = TIQ >70. ASD: ○ = negative, ● = positive.

Figure 2a. Delay in ToM development

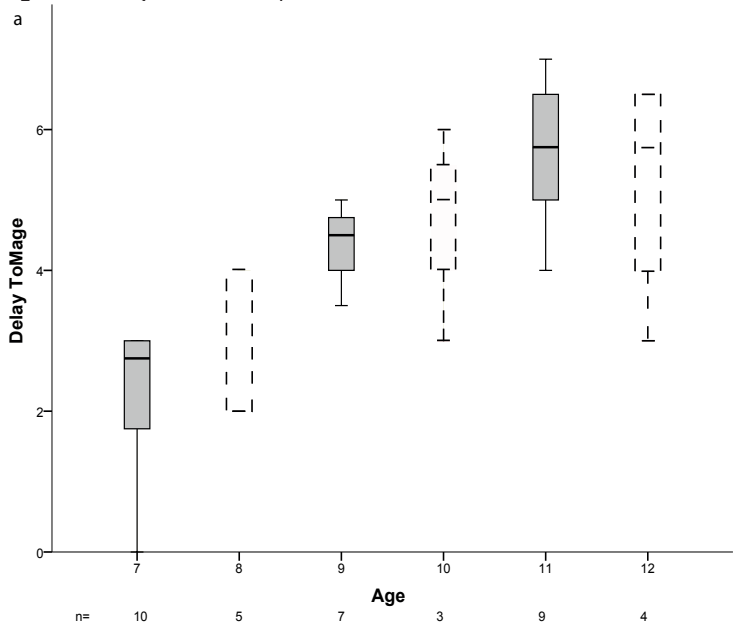


Figure 2b. Verbal IQ in Standard Score (SS)

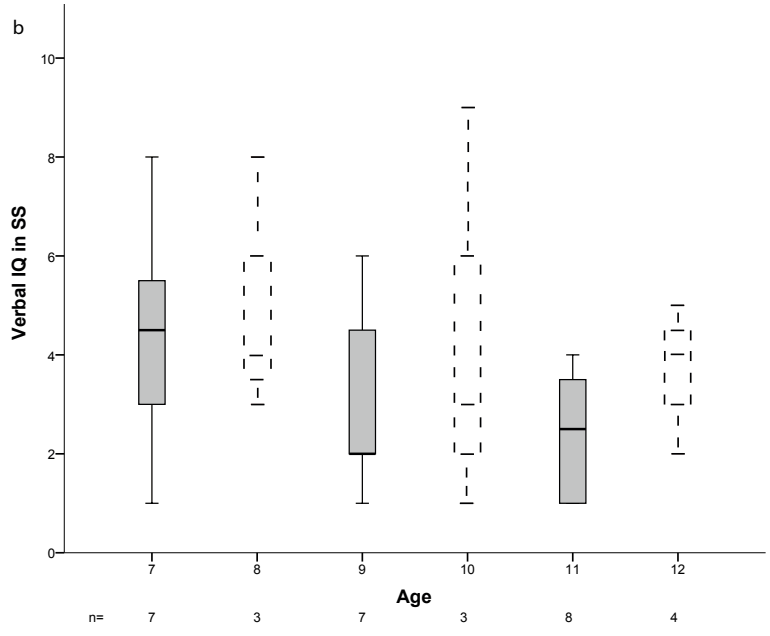
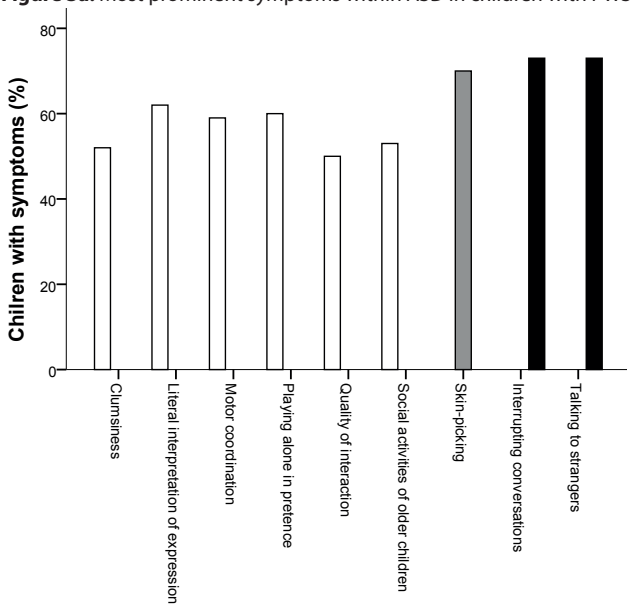


Figure 2 depicts the boxplots at age 7-12; data of those aged 8, 10 and 12 are depicted in the background as these groups were small (n = 3, 3, and 4, resp).

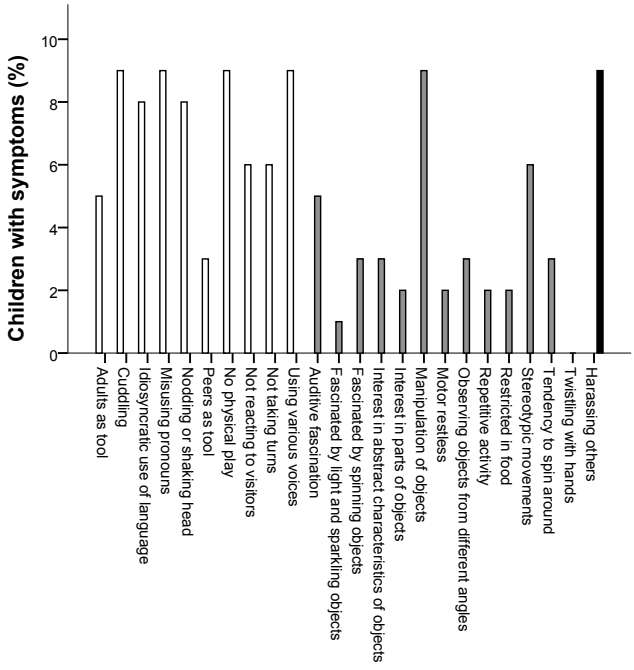
Figure 3a. Most prominent symptoms within ASD in children with PWS using the DISCO



DISCO

□ Developmental skills, ▩ Repetitive and stereotypical activities, ■ Maladaptive behavior

Figure 3b. Least frequent symptoms within ASD in children with PWS using the DISCO



DISCO

□ Developmental skills, ▩ Repetitive and stereotypical activities, ■ Maladaptive behavior

No association was found between BMI SDS and Total ToM SDS, and BMI SDS and ASD diagnosis, both p-values > 0.05.The presence of ASD in children with PWS did not differ significantly between those with a ToM SDS <-2 (impaired) and those with ToM SDS >-2 (normal), p>0.05.

DISCUSSION

In order to evaluate social functioning in children with PWS, we tested the Theory of Mind (ToM), i.e. the ability to infer in the mental state of oneself and others. In addition, we tested for Autism Spectrum Disorder. Our results show that children with PWS have severely impaired ToM. ToM score was positively related with Total IQ, especially with verbal IQ. We found no difference in ToM score between children with DEL and those with mUPD.
Additionally, 36% of children with PWS scored positively for Autism Spectrum Disorder (ASD) and there was no significant difference in prevalence of ASD between genetic subtypes of DEL and mUPD in our group. Within ASD, symptoms of maladaptive behavior were most prominent in children with PWS.

ToM

No study has yet reported the ToM in children with PWS. ToM testing is useful to detect the child's ability to empathize and therefore their ability in social cognitive functioning. To determine the impairment in ToM, we calculated the ToM developmental delay. In aged 7 to 12 years, median ToM developmental delay was 4 years and the delay was increasing as the child aged. Because 6 of the 66 children did not accomplish the ToM test-R due to attentive deficiency and language impairment, the median ToM developmental delay in children with PWS might be even larger. Based on these results, we advise family and carers to be aware of the younger ToM developmental age of the child with PWS.

To evaluate the degree of impairment in ToM, we studied the 3 stages of the ToM test. The fairly high score of 10 out of 12 in stage 1 of the ToM test, recognition of emotions and difference in reality and surreality, suggests that these cognitive abilities are not major difficulties in these children.
The median score of 8 out of 12 for stage 2, a test of "first-order belief" and "false belief", suggests that miscommunication could occur during interaction with children with PWS when "first-order belief" and "false belief" are used. It is therefore essential to name what one is thinking and not to use words figuratively.
The poor score for stage 3, "second-order belief" shows that thoughts as "person 1 did that because person 1 thought that person 2 thought…" are impaired and poorly de-

veloped in children with PWS. The ToM test is a validated test; however, the individual stages of the ToM have not been specifically validated. Although the individual stages of the ToM provides an insight in the development of each stage of the Theory of Mind, results of the individual stages should be interpreted with caution.

We found that ToM score was positively related to total IQ, in particular to verbal IQ, which is in accordance with earlier reports on ToM testing in subjects with an intellectual disability (10, 27, 28). This raises the question if the impaired ToM score in this study group is affected by PWS specifically, or by the intellectual disability due to PWS. Future studies in children with various types of intellectual disability are needed to delineate whether the ToM developmental delay in children with PWS is related to the syndrome or to the intellectual disability.

Our findings on ToM are essential in the approach of these children. Both children with DEL and mUPD are delayed in ToM development, which might result in overestimation of their empathetic abilities. This is in line with earlier findings (7), except that these investigators found the problem mainly in children with mUPD. Due to the relatively stronger verbal development of children with mUPD compared to those with DEL, children with mUPD are often overestimated in their ability to feel empathy for others. As major impairments were in the manifestations of the ToM, starting from 'first belief' and 'false belief', carers should adjust their communication and mention why and what they are thinking. For example, instead of saying "Clean up your toys" to the child with PWS, the caretaker should say: "Put your toys in the basket after five minute, because grandmother will visit us and I want the house to be tidy". Communicating as in the last sentence will be much clearer for children with PWS because the caretaker's thinking is said aloud which is much easier for the child to understand why it should clean up the toys. This insight into the social cognitive development of the child with PWS is important to understand the child and to adjust ones attitude to the child's needs to prevent frustration and bursts of emotional disturbance such as temper tantrums.

Autism Spectrum Disorder

In our study, thirty-six percent of children with PWS fulfilled the criteria of ASD, more precisely 29% of children with DEL and 41% of those with mUPD. Although these percentages between the genetic subtypes seem to differ, this was not significant. These percentages correspond to the reported overall ASD rate of 36.5%; in which 29% of the individuals had DEL and 43.8% in individuals an mUPD (6, 29). These percentages were significantly different. One explanation could be the effect of growth hormone therapy in children with PWS. Most participants in earlier studies were adults, who had not been treated with growth hormone during childhood (6, 7). Nowadays, growth hormone

treatment is part of the routine care for Dutch children with PWS. It has been shown that growth hormone improves the lean body mass and physical strength in children with PWS (14, 30). Another study showed that growth hormone treatment prevents deterioration of certain cognitive skills on the short term and improves cognitive functioning including abstract reasoning and visuospatial skills, especially in those with mUPD who had a greater deficit (26). Therefore, as growth hormone treatment improves cognitive functioning especially in children with mUPD, a smaller difference in prevalence of ASD between the genetic subtypes might be result.

Remarkably, none of the children between the age of 7 to 9 scored positively for ASD. Various factors might explain this finding. Firstly, the behavioral phenotype and the developmental delay of PWS becomes more clear as the child ages and the difference in cognitive ability increases between the child with PWS and their siblings or peers. Thus, symptoms in ASD might be easier to detect at an older age.
Secondly, before the age of 10, the behavioral phenotype and developmental delay might be more accepted by family and carers. During infancy and childhood, the child with PWS improves with major steps in their development: e.g. they manage to eat independently after a period of tube feeding; they become physically stronger and learn to walk or even to do sports (e.g. cycling on tricycle, doing judo) after severe neonatal hypotonia. It is therefore likely, that the medical disorder of PWS is "overshadowing" the autistic symtomatology (31). Family and carers are therefore accepting much more of the behavioral phenotype during childhood, because the child improves significantly in developmental skills within the first ten years.

Our study group consists of children with PWS with a fairly normal BMI SDS. We show that BMI SDS is not related with the level of Theory of Mind and ASD rates. As children with PWS who are treated with growth hormone are less obese and have a normal stature, their improved appearance will contribute to the risk of overestimation of their empathetic abilities by the environment.
No correlation was found between Total ToM SDS and most prominent symptoms, we conclude that the ToM test-R is not a valuable screening instrument for ASD in children with PWS, presumably due to the emphasis on maladaptive behavior in this group.

Overall

Because the Theory of Mind considers the level of empathy, it evaluates the social cognitive functioning, which is a dysfunction in ASD. Our results, however, show that children with PWS have a different behavioral phenotype compared to children with typical autism. The most prominent symptoms within ASD were symptoms in maladaptive behavior and routines. On the other hand, some of the major autistic characteristics such

as obsessions about light and sound were barely seen in children with PWS. Neither did they struggle to make contact with others, especially when the person was accompanied with a pet. However, they seem to have difficulty in sensing a stranger's personal space and tend to invade it without noticing. We may therefore conclude that impairments in social cognitive functioning in children with PWS are related to impairments in social reciprocity (6, 7) and not in making contact.
Ritualistic and compulsive behavior, another main feature of ASD, were described in earlier studies in individuals with PWS (32, 33), but we did not find these. It might be that the early signs of ASD in PWS rely on these symptoms of ritualistic and compulsive behavior, and that the sensitivity of the DISCO, which was used to test for ASD in this study, was insufficient to detect them. Future studies concerning screening for Autism Spectrum Disorder in PWS should therefore focus on rigidity in routine and structure, and compulsive behavior in children with PWS.

In our study group, 39% of children had a deletion and 52% had a mUPD. Earlier studies reported a DEL: mUPD ratio of 70:30 (34, 35). This higher frequency of mUPD type PWS is most likely related to a higher maternal age, in line with previous studies (36, 37).

As PWS is a rare disorder, our sample size is relatively small, especially in the subgroup analyses. This might be considered as a limitation of the study. However, because our study group consisted of children and adolescents only, it is a relatively large group in terms of a pediatric PWS population.

In conclusion, the ToM development is severely delayed in children with PWS, and those aged 7 to 12 years, an average ToM developmental delay of 4 years is found. Maladaptive behavior seems to be the most prominent behavior within Autism Spectrum Disorder in children with PWS. These findings highlight the need to adjust to their level of social cognitive functioning to prevent overestimation in children with PWS.

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REFERENCES

1. Cassidy SB 1997 Prader-Willi syndrome. J. Med. Genet. 34:917-923
2. Morgan JR, Storch EA, Woods DW, Bodzin D, Lewin AB, Murphy TK 2010 A preliminary analysis of the phenomenology of skin-picking in Prader-Willi syndrome. Child Psychiatry Hum. Dev. 41: 448-463
3. Boer H, Holland A, Whittington J, Butler J, Webb T, Clarke D 2002 Psychotic illness in people with Prader Willi syndrome due to chromosome 15 maternal uniparental disomy. Lancet 359:135-136
4. Vogels A, De Hert M, Descheemaeker MJ, Govers V, Devriendt K, Legius E, Prinzie P, Fryns JP 2004 Psychotic disorders in Prader-Willi syndrome. Am J Med Genet A 127A:238-243
5. Dimitropoulos A, Feurer ID, Butler MG, Thompson T 2001 Emergence of compulsive behavior and tantrums in children with Prader-Willi syndrome. Am. J. Ment. Retard. 106:39-51
6. Veltman MW, Thompson RJ, Roberts SE, Thomas NS, Whittington J, Bolton PF 2004 Prader-Willi syndrome--a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. Eur. Child Adolesc. Psychiatry 13:42-50
7. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
8. Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP 2006 Pervasive developmental disorders in Prader-Willi syndrome: the Leuven experience in 59 subjects and controls. American Journal of Medical Genetics Part A 140:1136-1142
9. Dimitropoulos A, Ho A, Feldman B 2012 Social Responsiveness and Competence in Prader-Willi Syndrome: Direct Comparison to Autism Spectrum Disorder. J. Autism Dev. Disord.
10. Muris P, Steerneman P, Meesters C, Merckelbach H, Horselenberg R, van den Hogen T, van Dongen L 1999 The TOM test: a new instrument for assessing theory of mind in normal children and children with pervasive developmental disorders. J. Autism Dev. Disord. 29:67-80
11. Baron-Cohen S, Leslie AM, Frith U 1985 Does the autistic child have a "theory of mind"? Cognition 21:37-46
12. Tager-Flusberg H, Sullivan K 2000 A componential view of theory of mind: evidence from Williams syndrome. Cognition 76:59-90
13. Koenig K, Klin A, Schultz R 2004 Deficits in social attribution ability in Prader-Willi syndrome. J. Autism Dev. Disord. 34:573-582
14. de Lind van Wijngaarden RF, Siemensma EP, Festen DA, 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 AC 2009 Efficacy and safety of long-term continuous growth hormone treatment in children with Prader-Willi syndrome. J. Clin. Endocrinol. Metab. 94:4205-4215
15. Steerneman PM, C. 2009 ToM test-R Handleiding. Antwerpen-Apeldoorn: Garant
16. Van Berckelaer-Onnes IA, Noens, I, Dijkxhoorn, Y. 2008 Diagnostic Interview for Social and Communication Disorders: Nederlandse Vertaling.
17. Wing L, Leekam SR, Libby SJ, Gould J, Larcombe M 2002 The Diagnostic Interview for Social and Communication Disorders: background, inter-rater reliability and clinical use. J. Child Psychol. Psychiatry 43:307-325
18. Maljaars J, Noens I, Scholte E, Van Berckelaer-Onnes I 2011 Evaluation of the criterion and convergent validity of the Diagnostic Interview for Social and Communication Disorders in young and low-functioning children. Autism

19. American Psychiatric Association 2000 DSM-IV-TR. Washington, DC: American Psychiatric Publishing
20. van Haassen P, de Bruyn E, Pijl Y, Poortinga Y, Lutje Spelberg H, van der Steene G, Coetsier P, Spoelders-Claes R, Stinissen J 1986 Wechsler Intelligence Scale for Children-Revised (Dutch Version), Manual. Lisse, The Netherlands: Swets, Zeitlinger BV
21. Herrera-Graf M, Dipert ZJ, Hinton RJ 1996 Exploring the effective use of the Vocabulary/Block design short form with a special school population. . Educational and Psychological Measurement 56:522-528
22. Talkington LW, Rieker GA 1969 A short form of the WISC for use with the mentally retarded. Psychol. Rep. 25:461-462
23. Tsushima WT 1994 Short form of the WPPSI and WPPSI-R. J. Clin. Psychol. 50:877-880
24. Hokken-Koelega A, Hackeng WH, Stijnen T, Wit JM, De Muinck Keizer-Schrama SM, Drop SL 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J. Clin. Endocrinol. Metab. 71:688-695
25. van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC 2004 Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. Journal of Clinical Endocrinoly and Metabolism 89:5295-5302
26. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314
27. Abbeduto L, Short-Meyerson K, Benson G, Dolish J 2004 Relationship between theory of mind and language ability in children and adolescents with intellectual disability. J. Intellect. Disabil. Res. 48:150-159
28. Lorusso ML, Galli R, Libera L, Gagliardi C, Borgatti R, Hollebrandse B 2007 Indicators of theory of mind in narrative production: a comparison between individuals with genetic syndromes and typically developing children. Clin Linguist Phon 21:37-53
29. Veltman MW, Craig EE, Bolton PF 2005 Autism spectrum disorders in Prader-Willi and Angelman syndromes: a systematic review. Psychiatr. Genet. 15:243-254
30. Festen DA, de Lind van Wijngaarden R, van Eekelen M, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Randomized controlled GH trial: effects on anthropometry, body composition and body proportions in a large group of children with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 69:443-451
31. Christopher Fillberg RH, Hans-Christoph Steinhausen 2006 A Clinician's Handbook of Child and Adolescent Psychiatry. Cambridge: Cambridge University Press
32. Clarke DJ, Boer H, Whittington J, Holland A, Butler J, Webb T 2002 Prader-Willi syndrome, compulsive and ritualistic behaviours: the first population-based survey. Br. J. Psychiatry 180:358-362
33. Greaves N, Prince E, Evans DW, Charman T 2006 Repetitive and ritualistic behaviour in children with Prader-Willi syndrome and children with autism. J. Intellect. Disabil. Res. 50:92-100
34. Cassidy SB 1984 Prader-Willi syndrome. Curr. Probl. Pediatr. 14:1-55
35. Dykens EM, Roof E 2008 Behavior in Prader-Willi syndrome: relationship to genetic subtypes and age. J. Child Psychol. Psychiatry 49:1001-1008

36. Whittington JE, Butler JV, Holland AJ 2007 Changing rates of genetic subtypes of Prader-Willi syndrome in the UK. Eur. J. Hum. Genet. 15:127-130
37. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32:1729-1735

CHAPTER 4

BEHAVIOR IN CHILDREN WITH PRADER-WILLI SYNDROME BEFORE AND DURING GROWTH HORMONE TREATMENT: A RANDOMIZED CONTROLLED TRIAL AND 8-YEAR LONGITUDINAL STUDY

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ABSTRACT

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

Forty-two pre-pubertal children, aged 3.5 to 14 years were studied in a randomized controlled GH trial (RCT) during 2 years, followed by a longitudinal study during 8 years of GH treatment. Behavior was measured annually by the Developmental Behavior Checklist (DBC) and a Dutch questionnaire to evaluate social behavioral problems, the Children's Social Behavior Questionnaire (CSBQ).

Problem behavior measured by the DBC in children with PWS was similar compared to peers with comparable intellectual disability. Scores on 'Social disabilities' subscale were however significantly higher compared to the DBC total score ($p<0.01$). A lower IQ was associated with more self-absorbed behavior, more communication problems and more problem behavior in general. Problem behavior measured by the CSBQ was similar compared to peers with comparable intellectual disability, but children with PWS scored significantly higher on the 'Not tuned', 'Understanding', and 'Stereotyped' subscales than the CSBQ total score ($p<0.05$ for all subscales and $p=0.001$ for 'Not tuned' subscale). There were no significant effects of GH treatment during the RCT and 8 years of GH treatment.

Social problems were most pronounced within problem behavior in PWS. In contrast to our expectations and parents reports, our study shows no improvement but also no deterioration of behavioral problems in children with PWS during long-term GH treatment.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from the absence of expression of paternally expressed genes 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, in childhood simultaneously with the change in eating pattern, children with PWS start to show significant maladaptive behavioral and emotional characteristics including temper tantrums, automutilation (skin picking), stubbornness, mood lability, impulsivity, argumentativeness, and inappropriate social behavior (4-11). Obsessive-compulsive symptoms and autism spectrum disorder are also known in children with PWS (10, 12-14).

Long-term continuous growth hormone (GH) treatment is an effective and safe treatment for children with PWS (15). GH treatment has beneficial effects on anthropometrics, 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 untreated control group with a cross-over in the second year for the control group (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 42 children with PWS during a 2-year randomized controlled GH trial (RCT) and during 8 years of continuous GH treatment. First, we used the Developmental Behavior Checklist for children with intellectual disability (DBC) to assess problem behavior in children with PWS compared to children with comparable intellectual disability. As we suspected that most problems would be within social skills, we also used a Dutch questionnaire to evaluate social behavioral problems compared to children with comparable intellectual disability, 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 a sustained effect during 8 years of continuous GH treatment.

METHODS

In April 2002, a multicenter, 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 in children with PWS (Multicenter, randomized, controlled growth hormone study in children with Prader Willi Syndrome: effects on growth, body composition, activity level and psychosocial development. IS-RCTN49726762/NTR628). After stratification for age and body mass index (BMI), children were randomly assigned to either the GH treatment group or no treatment-group for 2 years. Details of the allocated group were given on cards contained in sequentially numbered, opaque, sealed envelopes, which were generated by an independent statistician. Twenty-four children were randomly assigned to the GH treatment group, 18 children to the untreated group.

Forty-two 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 (10). After the RCT, all children were treated with GH and investigated in the Dutch PWS Cohort study.

The primary objective of our study was to investigate the effects of GH treatment on behavior. The secondary objective was to study factors associated with this behavior. Behavior was measured annually in the 42 pre-pubertal children with PWS during the RCT and during 8 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. Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY), dose 1.0 mg/m2/day (~0.033 mg/kg/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. 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. This study was conformed with the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning Human and Animal Rights.

Behavior

Behavior was measured by two parent questionnaires, the Developmental Behavior Checklist for children with intellectual disability (DBC) (18) and a Dutch questionnaire to evaluate social behavioral problems in children with intellectual disability, the Children's

Social Behavior Questionnaire (CSBQ) (18, 19). We choose these two questionnaires to compare in more detail behavior in children with PWS and children with intellectual disability (DBC), and to investigate problem behavior related to pervasive developmental disorders in children with PWS compared to children with intellectual disability (CSBQ). It has been shown that part of the behavioral characteristics are based on social behavioral problems (14, 20-23). The questionnaires were completed by the main caregiver and were completed in the Sophia Children's hospital in Rotterdam, the Netherlands, or in home setting. 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 intellectual disability, 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 intellectual 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 of the Anxiety subscale were included in 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 and aims to assess problem behavior in children with milder forms of pervasive developmental disorders. It has standardized norms for Dutch children aged 4-18 years without intellectual disability and with intellectual disability (IQ between 51 and 70). The CSBQ covers a wide range of problems in different domains of development, mainly social problems. It 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 Dutch references with intellectual disability, and for the CSBQ also into SDS according to healthy Dutch references.

To investigate the effect of long-term GH treatment on behavior, we assessed behavior during 8 years of GH treatment. Children who had been in the untreated 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. All children had at least 8 years of GH treatment: children who started in the control group had 8 years of GH treatment at the end of our study, but children who started in the treated group had 10 years of continuous GH treatment (2 years during the RCT followed by 8 years in the longitudinal study).

Anthropometry and cognition

Secondary endpoint of this study was to assess which factors were associated with the behavioral characteristics. Height was assessed by a Harpenden stadiometer and weight by an accurate scale. BMI (kg/m^2) was calculated. Height and BMI were converted into standard deviation scores (SDS), according to Dutch references for age (24, 25). Growth Analyser Version 3.0 software was used to calculate BMI, height, weight for height and BMI SDS (www.growthanalyser.org). IGF-1 levels were not included in this study due to changing assays and reference values over the last years. In the Netherlands, GH treatment is standard treatment for all children with PWS until they reach final height. No GH stimulation test was therefore performed before the start of GH therapy.

Cognition was measured by WPPSI-R or WISC-R, depending on age, and an intelligence quotient (IQ) was calculated as described earlier (26).

Data Analysis

Statistical analyses were performed with SPSS 20.0 (SPSS Inc. Chicago, IL). Age, BMI SDS, head circumferences, and IQ are presented as median and interquartile range (IQR). Friedman's ANOVA was used to test for differences within the subscales of the DBC. Correlations between scores on the behavioral subscales and the total scores at baseline and age, IQ, BMI SDS and head circumference SDS were calculated by Spearman'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.

To analyze the effect of GH treatment during the RCT and the longitudinal study, Linear Mixed Models for repeated measurements (27) 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, …, 8= after 8 years of GH treatment) as factors in the longitudinal study. As the CSBQ has standardized norms for both children with and without intellectual disability, scores on all subscales and the

CSBQ total score were also standardized compared to these two reference groups in the longitudinal study.

The effects of age, gender, genotype, 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

At start of the RCT, the median (interquartile range, IQR) age of 42 children with PWS was 6.4 (4.9 to 7.6) years (Table 1). Children had a baseline BMI SDS between 0 and +2 SDS and a head circumference between 0 and -2 SDS. Sixteen children (38 %) had a deletion of chromosome 15q11-q13, 20 (48 %) an mUPD, and 3 an imprinting center defect. Three children had a positive methylation test, but the underlying genetic defect was unknown. There were no significant differences in age, BMI, head circumference, and IQ between the treatment (n=24) and untreated group (n=18). The baseline characteristics for the treatment and untreated group are presented in Table 1.

Table 1. Baseline characteristics

	Total group	GH-treated	Untreated controls	p-value
N (male)	42 (19)	24 (12)	18 (7)	
Age (years)	6.4 (4.9, 7.6)	6.0 (4.6, 7.5)	6.7 (4.9, 7.7)	0.438
Height SDS	-2.6 (-3.3, -2.0)	-2.4 (-3.1, -1.8)	-2.5 (-3.1, -1.9)	0.899
Weight for height SDS	1.3 (0.4, 2.0)	0.8 (0.1, 1.8)	1.6 (0.9, 2.3)	0.071
BMI SDS	1.1 (0.4, 1.7)	0.8 (0.1, 1.6)	1.3 (0.9, 1.8)	0.084
Head circumference SDS	-0.8 (-1.4, -0.4)	-0.9 (-1.7, -0.3)	-0.7 (-1.2, -0.2)	0.461
IQ	65 (59, 83)	62 (59, 75)	74 (62, 84)	0.323
Genetic subtype				
Deletion	16 (38%)	11 (46%)	5 (28%)	
mUPD	20 (48%)	8 (33%)	12 (67%)	
Imprinting	3	2	1	
Unknown	3	3	0	
Age at start of GH treatment (years)	7.0 (5.5, 8.9)	6.0 (4.6, 7.5)	8.7 (6.9, 10.2)	

Age, BMI SDS, Head circumference SDS and IQ are expressed in median (IQR); genetic subtype is expressed in n(%), GH=growth hormone. The p-value is shown for analyses between the GH-treated group and untreated controls.

Behavior at baseline

DBC standardized for children with intellectual disability

None of the subscale scores were significantly different from 0 SDS, indicating that problem behavior in children with PWS was not different from children with comparable intellectual disability.

We investigated if children with PWS had particular characteristics within their problem behavior which were more prominent than their overall problem behavior. Social Disabilities SDS was significantly higher than the other subscales (Disruptive Antisocial behavior and Self absorbed behavior: p= 0.004, Communication disorders: p=0.046), indicating relatively more social problems compared to the other subscales of the DBC in children with PWS (Figure 1A).

Children with a smaller head circumference had more problems in social abilities (β=-0.356, p=0.003) and scored higher on total problem behavior (β=-0.244, p=0.048). We found significant associations between the IQ and the Self-absorbed subscale score (β=-0.022, p=0.023) and the Communication disorder subscale score (β=-0.024, p=0.029), indicating that children with a lower IQ showed more self-absorbed behavior and had more communication problems. A lower IQ was associated with a higher DBC total score indicating more problem behavior (β=-0.024, p=0.020), also after correction for head circumference (β=-0.022, p=0.026). We did not find any association between DBC total score or its subscales and gender, genetic subtype, and BMI SDS. Behavioural problems were not correlated with age at start of GH treatment. The highest scores were found on the following items of the DBC: 'keeps a strict order in certain items or activities', 'easily crying', 'easily distracted', 'inactive', 'no sense of danger', 'likes doing things on his/her own'.

CSBQ standardized for children with intellectual disability

Children with PWS scored similar to children with intellectual disability in all subscales. We also investigated if children with PWS had particular characteristics within problem behavior related to pervasive developmental disorder which were more prominent than their overall problem behavior. Scores on the Not Tuned, Social Understanding and Stereotyped behavior were significantly higher than the CSBQ total score ($p<0.05$ for Understanding and Stereotyped subscales and p=0.001 for Not tuned subscale) (Figure 1B).

Children with a smaller head circumference had more difficulties in orientation (Orientation: β=-0.352, p=0.037). Children with a lower IQ had more difficulties in social understanding (Understanding: β=-0.028, p=0.045), had more problems in stereotyped behavior (Stereotyped: β=-0.034, p=0.045) and scored higher on total problem behavior

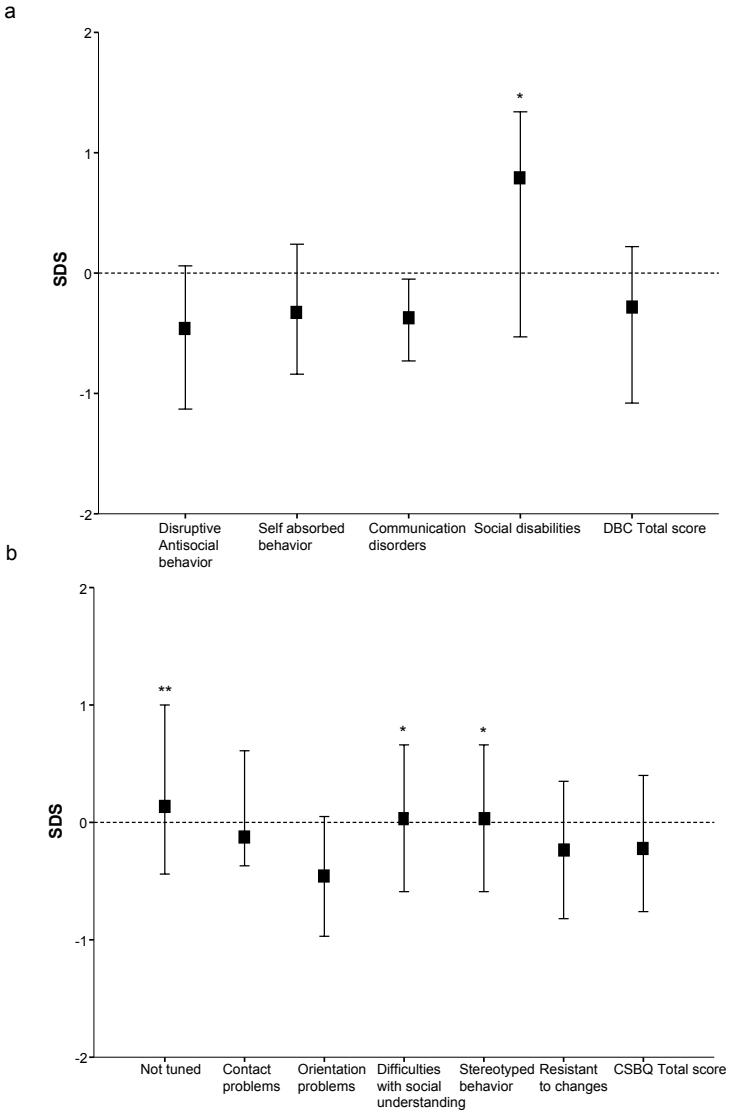

Figure 1. Behavior at baseline

Fig. 1A Behavior at baseline measured by the DBC, standardized for children with intellectual disability
This figure shows the median SDS of the DBC subscales and the DBC total score SDS and their interquartile range (IQR) at baseline in children with PWS compared to a reference group with a comparable intellectual disability. 0 SDS is indicated by a dotted line.
Fig. 1B Behavior at baseline measured by the CSBQ, standardized for children with intellectual disability
This figure shows the median SDS of the CSBQ subscales and the CSBQ total score SDS and their IQR at baseline in children with PWS compared to a reference group with a comparable intellectual disability. 0 SDS is indicated by a dotted line.

(β=-0.031, p=0.033). Older children had more problems in social understanding (Understanding: β=-0.203, p=0.004) and in stereotyped behavior (Stereotyped: β=0.247, p=0.004). Children with a deletion had more difficulties in social understanding (Understanding: β=-0.769, p=0.015 and after correction for IQ and age, β=-0.683, p=0.025), had more stereotyped behavior (Stereotyped: β=-0.937, p=0.015 and after correction for IQ and age, β=-0.832, p=0.025) and were more resistant to changes (Changes: β=-0.856, p=0.025) than children with an mUPD. We did not find any association between the other subscales or CSBQ total score and gender or BMI SDS. Behavioural problems were not correlated with age at start of GH treatment. 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', 'has trouble doing two things at the same time', 'needs to be reminded what needs to be done', 'easily changing mood', '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'.

Randomized Controlled Trial

Effects of GH treatment versus no treatment on behavior

DBC standardized for children with intellectual disability

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

After 2 years, scores between the GH-treated and untreated children were not significantly different. This indicates that GH treatment had no effect and that problem behavior measured by the DBC remained similar to children with a comparable intellectual disability in both groups. We found no significant effects of gender or genetic subtype on the DBC total score over time.

CSBQ standardized for children with intellectual disability

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

After 2 years, scores between the GH-treated and untreated children were not significantly different. This indicates that GH treatment had no effect and that social problem behavior measured by the CSBQ remained similar to children with intellectual disability

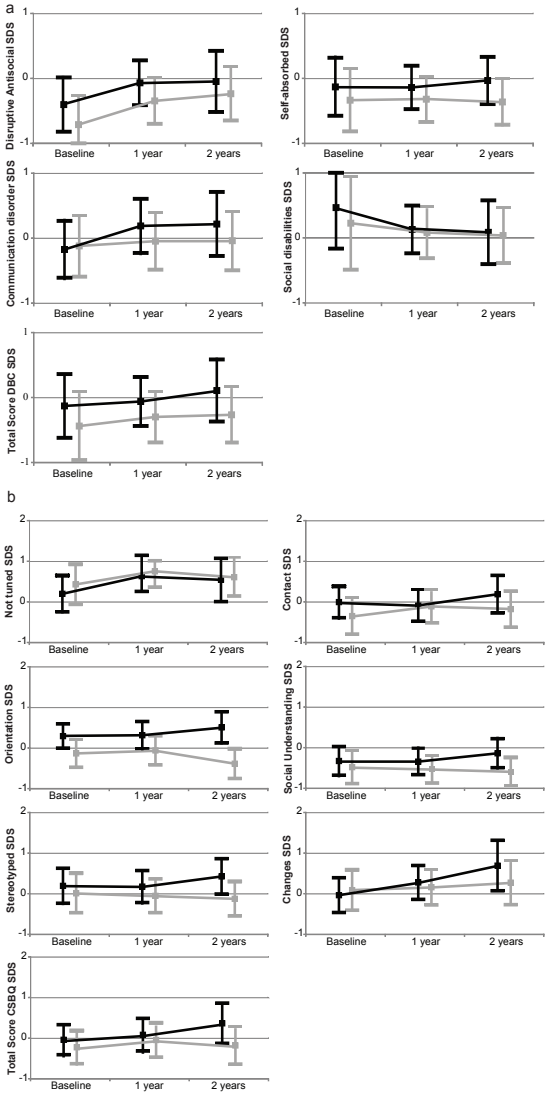

Figure 2. Behavior during the RCT

Fig. 2A DBC during RCT, standardized for children with intellectual disability
This figure shows the mean estimated SDS of the DBC subscales and the DBC total score and their 95% confidence intervals during the 2 year-RCT in 24 GH-treated children (in black) versus 18 randomized untreated controls (in gray) with PWS. The DBC is standardized for children with intellectual disability. No significant difference was found in all subscales between baseline and after 2 years.
Fig. 2B CSBQ during RCT, standardized for children with intellectual disability
This figure shows the mean estimated SDS of the CSBQ and the CSBQ total score and their 95% confidence intervals during the 2 year-RCT in 24 GH-treated children (in black) versus 18 randomized untreated controls (in gray) with PWS. The CSBQ is standardized for children with intellectual disability. No significant difference was found in all subscales between baseline and after 2 years.

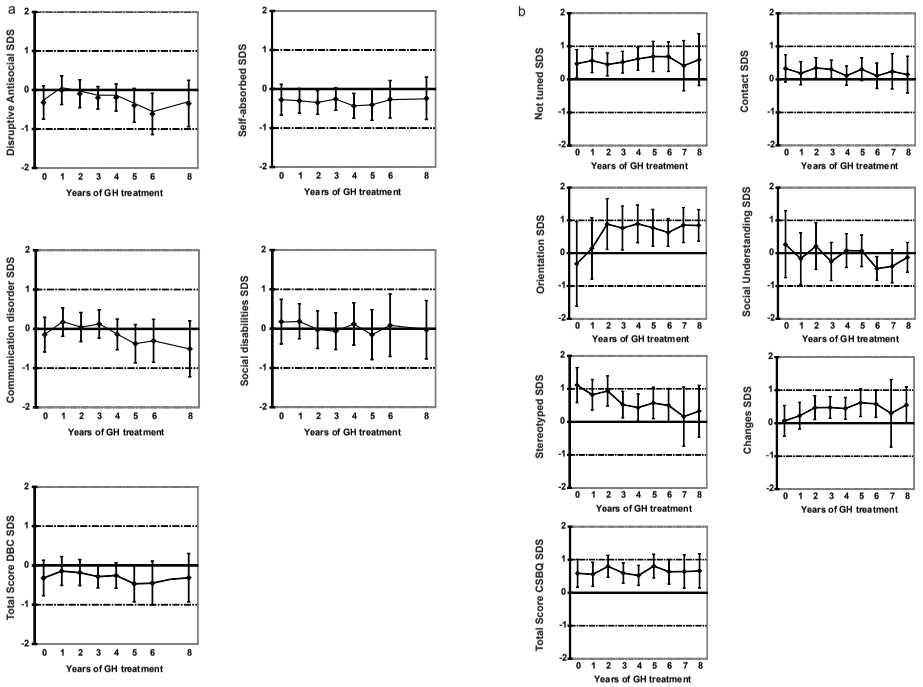

Figure 3. Behavior during 8 years of GH treatment

Fig. 3A DBC during 8 years of GH treatment, standardized for children with intellectual disability

This figure shows the mean estimated SDS of the DBC subscales and the DBC total score and their 95% confidence intervals after correction for age during 8 years of GH treatment in 42 children with PWS. 0 SDS is indicated. A score above 0 SDS implies more problem behavior. Data of those at 7 years GH treatment is not depicted as this group was small (n=11). The DBC is standardized for children with intellectual disability. No significant difference was found in all subscales between start and after 8 years of GH treatment.

Fig. 3B CSBQ during 8 years of GH treatment, standardized for children with intellectual disability

This figure shows the mean estimated SDS of the CSBQ subscales and the CSBQ total score and their 95% confidence intervals after correction for age during 8 years of GH treatment in children with PWS. 0 SDS is indicated. A score above 0 SDS implies more problem behavior. The CSBQ is standardized for children with intellectual disability. No significant difference was found in all subscales between start and after 8 years of GH treatment.

in both groups. We found no significant effects of gender and genetic subtype on the subscales or the CSBQ total score over time.

Long-term GH treatment

DBC standardized for children with intellectual disability

During 8 years of GH treatment, scores on all subscales and the DBC total score did not significantly change (Figure 3A).

CSBQ standardized for children with intellectual disability

During 8 years of GH treatment, scores on all subscales and the CSBQ total score did not significantly change (Figure 3B).

CSBQ standardized for healthy children

After 8 years of follow-up, the CSBQ total score was significantly higher than at baseline (estimated mean SDS (95% confidence interval) after 8 years versus at baseline: 1.686 (0.856, 2.517) versus 0.708 (0.032, 1.385), p= 0.042). However, there was no difference between children who started with GH treatment or no treatment at baseline (p=0.139). After 8 years of follow-up, scores on the other subscales of the CSBQ were not significant different from baseline. The estimated mean SDS (95% confidence interval) per subscale after 8 years of follow-up and at baseline were as follow: Not tuned (1.280 (0.286, 2.273) versus 1.181 (0.650, 1.711), p=0.986), Contact (1.331 (0.734, 2.126) versus 1.430 (0.426, 2.235), p=0.617), Orientation (2.131 (1.264, 2.998) versus 1.661 (1.050, 2.273), p=0.984), Social Understanding (2.587 (1.784, 3.390) versus 2.012 (1.352, 2.672), p=0.297), Stereotyped (1.208 (-0.081, 2.497) versus 2.130 (1.416, 2.844), p=0.280), Changes (1.646 (0.812, 2.480) versus 0.737 (0.060, 1.414), p=0.051).

We previously reported the effects of 8 years GH treatment on somatic outcomes such as body composition (15).

DISCUSSION

Our study investigated the effect of GH treatment on behavior in children with PWS during a 2-year randomized controlled trial and during 8 years of continuous GH treatment. In contrast to 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. Behavioral problems were stable and remained similar to baseline during 8 years of GH treatment. Children with PWS showed similar problem

behavior as a reference population with a comparable intellectual disability, also during GH treatment.

Against our expectations, we could not find an effect of GH treatment on behavior. 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 long-term GH treatment improves cognitive functioning in these children. In our earlier study, we showed that GH treatment prevents deterioration of certain cognitive skills in children with PWS on the short-term and significantly improves abstract reasoning and visuo-spational skills during four years of GH treatment. Furthermore, children with a greater deficit had more benefit from GH treatment (26). Thus, it seems that behavior in children with PWS is not clearly influenced by GH treatment, in contrast to the cognitive functioning. The earlier findings of a beneficial effect of GH treatment on cognition are in line with those in short children born small for gestational age (SGA). In short children born SGA, a significant improvement of cognition was found during long-term GH treatment, while attention deficits, especially accurateness and impulsiveness did not change during GH treatment (28, 29). 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 children 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. On the other hand, most parents did notice subtle changes, which they reported during their visits to our hospital. These are in line with those of a 2-year GH-controlled study in children with PWS (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 with a 3-point scale after 6 and 12 months, however, did also not show differences in behavior between GH treated and untreated patients. As reported by several studies, GH treatment has a beneficial

effect on muscle strength and tone (30, 31). Concerns have been raised if these improvements could worsen characteristics of PWS, such as deterioration of temper tantrums or aggressiveness. One study showed that abrupt-ceasing of GH treatment for six months led to a successive deterioration in behavioral problems in children with PWS (32). In another 12-month, randomized double-blind, placebo-controlled, cross-over study in 12 children with PWS, no improvement or deterioration in behavior was found either using the Behavior Assessment System for Children (BASC) and Child Behavior Checklist (CBCL), except of a significant increase on the hyperactivity scale on the BASC (16). Our study did not find an improvement in behavior, but it might be argued that we did also not find a deterioration during 8 years of GH treatment as normally seen over time, which strengthens the arguments for the use of GH treatment in children with PWS to improve body composition, motor abilities and cognition (15, 26, 30). To our knowledge, our study is the first study investigating the long-term effect of GH treatment in a large group of 42 children with PWS. As we did find a beneficial effect of GH treatment on cognition in our earlier study, in which intelligence was scored in a broad scale (26), it cannot be excluded that long-term GH treatment has an effect in preventing deterioration of problem behavior as the questionnaires used might not be sufficiently sensitive. Future research investigating the effect of long-term GH treatment on behavior should include more sensitive questionnaires and/or a control group.

In our study, behavioral problems in children with PWS measured by the DBC were comparable to those of children with intellectual disability. However, scores on the socially related subscale of the DBC, 'Social disabilities', were higher than the other subscales. This indicates that there is 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 in more detail, in particular pervasive developmental disorders (PDD). Children with PWS experienced particularly more problems in being tuned to the social situation, understanding social information and stereotyped behavior. 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) (33). Poor peer relationships, lack of friends, immaturity, weakness in coping skills, and a preference for solitary activities are all reported in individuals with PWS (1, 6, 14, 34, 35). One other study assessing PDD in children and adults with PWS, compared patients with PWS to controls with intellectual disabilities (20). Patients with PWS showed elevated scores on a PDD-questionnaire in patients with PWS compared to the controls.

Our finding of more problems in social behaviour has implications in the approach and guidance of children with PWS. Although the current (GH) treatment and multidisciplinary support has improved the bodycomposition and therefore the appearance of

this group children, we should be aware not to overestimate them in their social abilities. Children with PWS are less capable in dealing with daily challenges and overestimation could easily lead to stress and agitation causing misbehavior as temper tantrums or increased compulsive behavior. It is therefore important to determine the social skills of children with PWS, especially when overestimation by the environment is suspected. Remarkably, we found more problems in social understanding, stereotyped behavior and resistance to changes in children with a deletion compared to children with an mUPD. Earlier studies reported an increased prevalence of autistic characteristics in children with mUPD (20, 23, 36). It might be that parents of children with an mUPD are more aware of the autistic features within PWS, which has led to a more structured approach in daily life. Parents of children with a deletion might not have expected these autistic features and therefore reported more problems within PDD.
In our study group, 38% of children had a deletion and 48% an mUPD. Earlier studies reported a DEL: mUPD ratio of 70:30 (1, 37). The higher frequency of the mUPD type in our study group is most likely related to an increasing maternal age in the Netherlands, which in line with other countries in Western Europe (38, 39).
Although problem behavior in children with PWS was not significantly different compared to children with similar intellectual disability over time, it was significantly increased compared to healthy references. This suggests that problem behavior deteriorates over time in children with intellectual disability and that this is not PWS-specific.

Our study shows that problem behavior in children with PWS is in many aspects similar to children with a comparable intellectual disability. However, they display more problems in social understanding. Problem behavior remains stable in both GH-treated and untreated children with PWS. Thus, in contrast to our experience and parents reports, our study shows no improvement but also no deterioration of behavioral problems in children with PWS during long-term GH treatment.

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REFERENCES

1. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ 2012 Prader-Willi syndrome. Genet Med 14:10-26
2. Holm VA, Cassidy SB, Butler MG, Hanchett JM, Greenswag LR, Whitman BY, Greenberg F 1993 Prader-Willi syndrome: consensus diagnostic criteria. Pediatrics 91:398-402
3. Descheemaeker MJ, Vogels A, Govers V, Borghgraef M, Willekens D, Swillen A, Verhoeven W, Fryns JP 2002 Prader-Willi syndrome: new insights in the behavioural and psychiatric spectrum. J. Intellect. Disabil. Res. 46:41-50
4. Curfs LM, Hoondert V, van Lieshout CF, Fryns JP 1995 Personality profiles of youngsters with Prader-Willi syndrome and youngsters attending regular schools. J. Intellect. Disabil. Res. 39 (Pt 3):241-248
5. Curfs LM, Verhulst FC, Fryns JP 1991 Behavioral and emotional problems in youngsters with Prader-Willi syndrome. Genet. Couns. 2:33-41
6. Dykens EM, Cassidy SB 1995 Correlates of maladaptive behavior in children and adults with Prader-Willi syndrome. Am. J. Med. Genet. 60:546-549
7. Dykens EM, Hodapp RM, Walsh K, Nash LJ 1992 Adaptive and maladaptive behavior in Prader-Willi syndrome. J. Am. Acad. Child Adolesc. Psychiatry 31:1131-1136
8. Dykens EM, Kasari C 1997 Maladaptive behavior in children with Prader-Willi syndrome, Down syndrome, and nonspecific mental retardation. Am. J. Ment. Retard. 102:228-237
9. Dykens EM, Leckman JF, Cassidy SB 1996 Obsessions and compulsions in Prader-Willi syndrome. Journal of Child Psychology and Psychiatry 37:995-1002
10. Einfeld SL, Smith A, Durvasula S, Florio T, Tonge BJ 1999 Behavior and emotional disturbance in Prader-Willi syndrome. Am. J. Med. Genet. 82:123-127
11. Stein DJ, Keating J, Zar HJ, Hollander E 1994 A survey of the phenomenology and pharmacotherapy of compulsive and impulsive-aggressive symptoms in Prader-Willi syndrome. J. Neuropsychiatry Clin. Neurosci. 6:23-29
12. Akefeldt A, Gillberg C 1999 Behavior and personality characteristics of children and young adults with Prader-Willi syndrome: a controlled study. J. Am. Acad. Child Adolesc. Psychiatry 38:761-769
13. Dykens EM, Rosner BA 1999 Refining behavioral phenotypes: personality-motivation in Williams and Prader-Willi syndromes. Am. J. Ment. Retard. 104:158-169
14. Lo ST, Siemensma E, Collin P, Hokken-Koelega A 2013 Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. Res. Dev. Disabil. 34:2764-2773
15. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Schroor EJ, Van Alfen AA, Van Leeuwen M, Van Pinxteren-Nagler E, Van Wieringen H, Vreuls RC, Zwaveling-Soonawala N, de Ridder MA, Hokken-Koelega AC 2013 Eight Years of Growth Hormone Treatment in Children With Prader-Willi Syndrome: Maintaining the Positive Effects. J. Clin. Endocrinol. Metab. 98:4013-4022
16. Haqq AM, Stadler DD, Jackson RH, Rosenfeld RG, Purnell JQ, LaFranchi SH 2003 Effects of growth hormone on pulmonary function, sleep quality, behavior, cognition, growth velocity, body composition, and resting energy expenditure in Prader-Willi syndrome. Journal of Clinical Endocrinoly and Metabolism 88:2206-2212
17. Whitman BY, Myers S, Carrel A, Allen D 2002 The behavioral impact of growth hormone treatment for children and adolescents with Prader-Willi syndrome: a 2-year, controlled study. Pediatrics 109:E35

18. Dekker MC, Nunn RJ, Einfeld SE, Tonge BJ, Koot HM 2002 Assessing emotional and behavioral problems in children with intellectual disability: revisiting the factor structure of the developmental behavior checklist. J. Autism Dev. Disord. 32:601-610
19. Hartman CA, Luteijn E, Serra M, Minderaa R 2006 Refinement of the Children's Social Behavior Questionnaire (CSBQ): an instrument that describes the diverse problems seen in milder forms of PDD. J. Autism Dev. Disord. 36:325-342
20. Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP 2006 Pervasive developmental disorders in Prader-Willi syndrome: the Leuven experience in 59 subjects and controls. American Journal of Medical Genetics Part A 140:1136-1142
21. Dimitropoulos A, Ho A, Feldman B 2012 Social Responsiveness and Competence in Prader-Willi Syndrome: Direct Comparison to Autism Spectrum Disorder. J. Autism Dev. Disord.
22. Dykens EM 2004 Maladaptive and compulsive behavior in Prader-Willi syndrome: new insights from older adults. Am. J. Ment. Retard. 109:142-153
23. Veltman MW, Thompson RJ, Roberts SE, Thomas NS, Whittington J, Bolton PF 2004 Prader-Willi syndrome--a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. Eur. Child Adolesc. Psychiatry 13:42-50
24. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr. Res. 47:316-323
25. Fredriks AM, van Buuren S, Wit JM, Verloove-Vanhorick SP 2000 Body index measurements in 1996-7 compared with 1980. Arch. Dis. Child. 82:107-112
26. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314
27. West BT 2009 Analyzing longitudinal data with the linear mixed models procedure in SPSS. Evaluation & the health professions 32:207-228
28. van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC 2004 Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. Journal of Clinical Endocrinoly and Metabolism 89:5295-5302
29. van der Reijden-Lakeman IE, de Sonneville LM, Swaab-Barneveld HJ, Slijper FM, Verhulst FC 1997 Evaluation of attention before and after 2 years of growth hormone treatment in intrauterine growth retarded children. J. Clin. Exp. Neuropsychol. 19:101-118
30. Festen DA, Wevers M, Lindgren AC, Bohm B, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 68:919-925
31. Reus L, Pelzer BJ, Otten BJ, Siemensma EP, van Alfen-van der Velden JA, Festen DA, Hokken-Koelega AC, Nijhuis-van der Sanden MW 2013 Growth hormone combined with child-specific motor training improves motor development in infants with Prader-Willi syndrome: a randomized controlled trial. Res. Dev. Disabil. 34:3092-3103
32. Bohm B, Ritzen EM, Lindgren AC 2014 Growth hormone treatment improves vitality and behavioural issues in children with Prader-Willi syndrome. Acta Paediatr.

33. Koenig K, Klin A, Schultz R 2004 Deficits in social attribution ability in Prader-Willi syndrome. J. Autism Dev. Disord. 34:573-582
34. Clarke DJ, Boer H, Chung MC, Sturmey P, Webb T 1996 Maladaptive behaviour in Prader-Willi syndrome in adult life. J. Intellect. Disabil. Res. 40 (Pt 2):159-165
35. van Lieshout CF, de Meyer RE, Curfs LM, Koot HM, Fryns JP 1998 Problem behaviors and personality of children and adolescents with Prader-Willi syndrome. J. Pediatr. Psychol. 23:111-120
36. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
37. Dykens EM, Roof E 2008 Behavior in Prader-Willi syndrome: relationship to genetic subtypes and age. J. Child Psychol. Psychiatry 49:1001-1008
38. Whittington JE, Butler JV, Holland AJ 2007 Changing rates of genetic subtypes of Prader-Willi syndrome in the UK. Eur. J. Hum. Genet. 15:127-130
39. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32:1729-1735

CHAPTER 5

BENEFICIAL EFFECTS OF LONG-TERM GROWTH HORMONE TREATMENT ON ADAPTIVE FUNCTIONING IN INFANTS WITH PRADER-WILLI SYNDROME

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ABSTRACT

The aim of this study was to investigate the effect of growth hormone treatment on adaptive functioning in children with Prader-Willi syndrome. Vineland Adaptive Behavior Scale (VABS) was assessed during a randomized controlled trial (RCT) and after 7 years of growth hormone treatment. In the RCT, 75 children (42 infants and 33 prepubertal children) with Prader-Willi syndrome were included. Subsequently, 53 children were treated with long-term growth hormone. Our study demonstrates a marked delay in adaptive functioning in infants and children with Prader-Willi syndrome, which was associated with older age and lower intelligence. Results of the multiple linear regression show that the earlier growth hormone treatment was started during infancy, the better the adaptive skills were on the long-term.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurogenetic developmental disorder caused by the absence of expression of genes on the paternally inherited chromosome 15 at the locus q11-q13, due to a paternal deletion (DEL), maternal uniparental disomy (mUPD), imprinting defects or paternal chromosomal translocation. Main characteristics are hypotonia, hypogonadism, short stature, obesity, intellectual disability and behavioral problems.

The average IQ in PWS varies between 50 and 80 (1, 2). Consequently, individuals with PWS have impairments in language, social and motor abilities (2-5). The ability to deal with daily activities required in the personal and sociocultural environment is defined as adaptive functioning and is an essential part of the behavioral phenotype (6). In addition, the adaptive functioning is related to age. It varies among genetic syndromes and is dependent on the level of intellectual disability (7).
Only few studies investigated the adaptive functioning in persons with PWS. A previous study in PWS concluded that motor abilities are more impaired than communication, daily living skills and socialization when tested with the Vineland Adaptive Behavior Scale (VABS) in a mixed age-group (7). Another study showed that those with mUPD scored lower in daily living skills than those with DEL (8). However, this study group was not age-specific, because participants were aged 3 to 50 years, and it was not reported whether growth hormone (GH) treatment was used. Impairments in adaptive functioning have a great impact on the independence of an individual with PWS and the burden for the family. Insight in the adaptive functioning of children with PWS will therefore improve the understanding and guidance of this group, by families and professionals in medical care.

In the last decade, children with PWS have been treated with growth hormone in many developed countries. In our previous studies, we found a beneficial effect of GH treatment on motor skills (9, 10). Long-term GH treatment improves also cognitive functioning in children with PWS (11). One study found that a higher intelligence correlated with a higher level of adaptive functioning as measured with the Vineland Adaptive Behavioral Scale (VABS) in people with intellectual disability (12). However, no study investigated if the improved motor and cognitive skills during GH treatment also result in improvement in adaptive functioning in children with PWS.

In this study, we hypothesized that GH treatment might have beneficial effects on the development of adaptive functioning in children with PWS. In addition, we expected that a younger age at start of GH treatment would result in better adaptive functioning. We therefore investigated the level of adaptive functioning without GH treatment,

the effect of GH treatment and variables associated with the development of adaptive functioning during long-term GH treatment.

METHODS

Participants

We included 75 children with PWS, 42 infants and 33 prepubertal children. All participants had a genetically confirmed diagnosis of PWS and were naïve to GH treatment at start of the RCT. All infants were below the age of 3.5 years. Prepubertal children fulfilled the following inclusion criteria: 1) age between 3.5 and 12 years or 14 years in girls and boys, resp.; 2) Tanner breast stage of 2 or less for girls and testicular volume less than 4 ml for boys (13). Characteristics about the participants are shown in the Results section.

Measures

Vineland Adaptive Behavior Scale (VABS)

The Dutch version of the VABS was used to determine the level of adaptive behavior in children with PWS (6). The VABS is a standardized structured interview with the primary caretaker to assess the adaptive behavior in four domains, which are communication (receptive, expressive, and written), daily living skills (personal, domestic, and community), socialization (interpersonal relationships, play and leisure time, and coping skills), and motor skills (gross and fine). VABS-items were rated on a 3-point rating scale (0=never, 1=sometimes or partially, 2=usually or habitually). Items that were not permitted to children with PWS, such as food preparation or bathing independently, were scored as 0 as these resulted in a similar practical consequence, namely that parents need to supervise and help in performing the task. The VABS has a high reliability and validity (14). The VABS expanded form provides a detailed analysis of the level of adaptive functioning and is suitable for use in case of intellectual disability (15). Tests were performed by a qualified medical investigator (STL).

Cognitive functioning

Cognitive testing was annually performed in prepubertal children and biennially in pubertal children. All cognitive measurements described in this study were performed by an experienced psychologist. The psychologist was blinded for the randomization.
Cognitive functioning in children between the age of 0-3.5 years was assessed with the Bayley Scales of Infants II-NL (BSID II) (16) and results have been described (5). The scores were expressed in mental and motor developmental age (in months). The mental scale consists of items in relation to visual and auditory information processing, language

development, memory, eye-hand coordination, imitation and problem solving and the motor scale in gross and fine motor skills.
Cognitive functioning in children between 3.5-7 years was tested with the Wechsler Preschool and Primary Scale of Intelligence – Revised Dutch version (WPPSI-R) short form (17), including the four subtests of Vocabulary and Similarities (verbal IQ tests), and Block design and Picture completion (performal IQ tests).
In children over 7 years of age, a Wechsler Intelligence Scale for Children-Revised (WISC-R) was performed (18). The method of cognitive testing in prepubertal children with PWS has been described in detail (11). In short, total IQ score was calculated according to an equation based on the Dutch outpatient population reference (total IQ = 45.3 + 2.91 x Vocabulary standard score + 2.50 x Block design standard score), as has been used in other studies (11, 19, 20). The vocabulary subtest was taken as the verbal IQ, the Block design as performal IQ.

Procedures

Randomized Controlled Trial

In April 2002, a multicenter randomized controlled trial (RCT) was started in infants and prepubertal children with PWS (21). The trial investigated 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, infants and children were randomly assigned to either GH treatment (1 mg/m^2/day) or no treatment. The RCT lasted one year for infants and two years for prepubertal children. Adaptive and cognitive functioning were measured annually during the RCT.

Follow-up during 7 years of continuous GH-treatment

After the RCT, all infants and children were treated with GH and followed in the Dutch PWS Cohort Study (10). Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY; 1 mg/m^2/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.

To investigate the effect of long-term GH treatment on adaptive functioning, parents of children who had been treated with GH for 7 years and who were still on GH treatment were asked to participate in a VABS test in 2013. In 2013, 42 children of the former infant group were eligible for the reassessment of the VABS test but six children were excluded: one child had died, three children had dropped-out during the follow-up study, and two participants did not respond. In the former prepubertal group, 33 children were eligible for the reassessment of the VABS test but sixteen children were excluded: ten had

stopped GH treatment due to attainment of adult height, and six children had stopped during the follow-up study for various reasons.

The study was approved by Medical Ethics Committee of the Erasmus Medical Center Rotterdam. Written informed consent was obtained from all parents or caretakers and children of 12 years and older, and assent of children younger than 12 years.

Data analysis

Developmental age equivalent to the raw score of the VABS test was used as the age of adaptive functioning. The delay in developmental age in years was calculated by the formula: age - developmental age. Non-parametric testing was used, and median and interquartile range (IQR) are presented for age and developmental age.

Mann-Whitney U test was used to test the difference in developmental age in all four domains of the VABS between the GH-group and untreated group during the RCT. To analyze the long-term effects of GH treatment, Linear Mixed Modeling was used with GH treatment and time as factors (GH treatment coded as: 1=GH treatment group, 0= untreated group; time coded as: 0= start of RCT, 1= one year after start of the RCT, 2= two years after start of the RCT, 3 = after 7 years of GH treatment).
Multiple linear regression was performed at start of the RCT and after 7 years of GH treatment. At baseline, we studied the association between delay in adaptive functioning with the variables age, mental developmental age and motor developmental age in infants with PWS, after adjusting for gender (coded as: 1=boys, 2=girls) and genetic subtype (coded as: 1=deletion, 2=mUPD). Total IQ, verbal IQ and performal IQ were used as variables of intelligence in prepubertal children with PWS. To investigate the association between age at start of GH treatment and delay on adaptive functioning independently of the change in IQ over time, we adjusted for baseline IQ and IQ in 2013.

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL). All statistical tests were two-sided. A p-value of <0.05 was considered statistically significant.

RESULTS

Randomized Controlled Trial

Baseline characteristics

In the RCT, 75 children with PWS (42 infants and 33 prepubertal children) were included (Table 1). At start of the RCT, the median (IQR) age was 1.58 (1.15-2.81) years in infants

Table 1. Baseline characteristics and adaptive functioning

	Infants	Prepubertal children
N (male)	42 (23)	33 (15)
Age (years)	1.58 (1.15-2.81)	6.66 (5.13-7.49)
Genetic subtype		
Deletion	20	15
UPD	21	14
ICD/Translocation	0	2
Unknown	1	2
Cognitive functioning		
Developmental mental age (years)	1.2 (0.8-1.7)	
Developmental motor age (years)	0.9 (0.7-1.3)	
Total IQ		68 (61-85)
Adaptive functioning (years)		
Communication	1.25 (0.85-1.56)	3.42 (2.67-4.34)
delay	*0.53 (0.22-0.81)*	*2.61 (1.92-4.00)*
Daily living skills	1.21 (0.77-1.79)	3.75 (2.96-4.75)
delay	*0.50 (0.25-0.78)*	*2.57 (1.76-3.74)*
Socialization	1.00 (0.75-1.42)	3.08 (2.21-4.04)
delay	*0.71 (0.48-1.26)*	*3.09 (2.52-4.58)*
Motor skills	0.87 (0.60-1.56)	3.00 (2.54-4.50)
delay	*0.72 (0.53-1.08)*	*3.17 (2.16-4.57)*

Data are expressed as median (IQR); genetic subtype is expressed in N.

and 6.66 (5.13-7.49) years in prepubertal children. In the infant group, 20 (48%) had a deletion, 21 (50%) an mUPD, and in one (2%) the underlying genetic defect was not identified. In the prepubertal children, 15 (45%) had a deletion, 14 (42%) an mUPD, 2 (6%) an imprinting center defect (ICD), and in 2 (6%) the underlying genetic defect was not identified. Median (IQR) mental developmental age of the infants was 1.2 (0.8-1.7) years and median motor developmental age 0.9 (0.7-1.3) year. In prepubertal children, the median (IQR) total IQ was 68 (61-85).

Table 1 shows the developmental age and delay per domain for infants and prepubertal children. Figure 1 shows the correlation between age and developmental age for all participants at baseline. For communication, daily living skills and socialization the developmental age is normally similar to the age until the maximum of 19 years. In the PWS group, the maximal age was 14 years, so normally the developmental age should be 14 years. However, none of the children with PWS scored higher than a developmental age of 8 years. This maximal developmental age is depicted in Figure 1 at 8 years, with the arrow representing the expected developmental age in healthy children. In contrast, the developmental age in motor skills normally increases until the age of 6 years and should remain similar after the age of 6 years (represented by the arrow). As shown in Figure 1, most children with PWS did not reach the age-appropriate developmental age, espe-

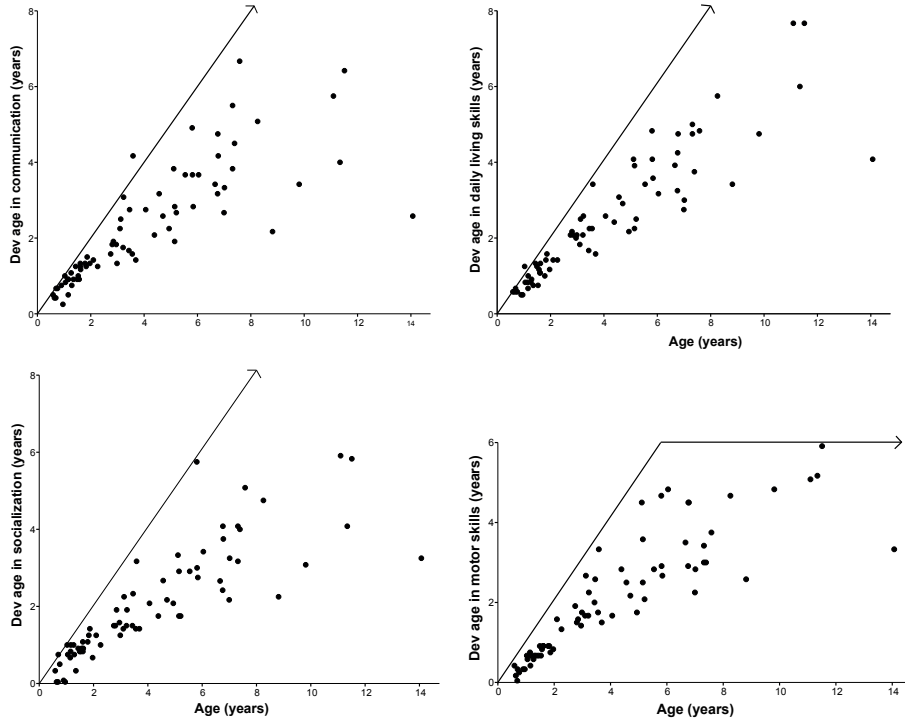

Figure 1. The relation between age and developmental age per domain of the VABS test at baseline. Dev age= developmental age. The line of identity (45°) represents the maximal developmental age in normal development and is similar to age, except in motor skills in which the maximal developmental age is 6 years.

cially after infancy. With increasing age, the gap between the developmental age and age-appropriate developmental age increased and a wide variation of developmental age was found, especially in the domain communication and socialization.

In infants, older age was associated with more delay in all domains of adaptive functioning (communication: p= <0.001, daily living skills: p= <0.001, socialization: p= <0.001, motor skills: p= <0.001, resp.) (Table 2a). A lower mental developmental age (years) was associated with more delay in adaptive functioning (communication: p= <0.001, daily living skills: p= <0.005, socialization: p= <0.05, resp.), except in motor skills (p= 0.392). A lower motor developmental age (years) was also associated with more delay in communication (p= <0.05) and motor skills (p= <0.001).

In prepubertal children, older age was associated with more delay in adaptive functioning (communication: p= <0.001, daily living skills: p= 0.001, socialization: p= <0.001, motor skills: p= <0.001, resp.) (Table 2b). A lower total IQ was associated with more delay in adaptive functioning (communication: p= 0.001, daily living skills: p= 0.001, socialization: p= 0.001, motor skills: p= <0.001, resp.), especially a lower verbal IQ (com-

Table 2. Delay in adaptive functioning at baseline.

A. Infants (n=42)

	Delay in communication						Delay in daily living skills					
	Model A		Model B		Model C		Model A		Model B		Model C	
	ß	P	ß	P	ß	P	ß	P	ß	P	ß	P
Age	0.771	**<0.001**	1.292	**<0.001**	1.739	**<0.001**	0.831	**<0.001**	1.092	**<0.001**	1.452	**<0.001**
Mental dev age			-0.653	**<0.001**	-0.674	**<0.001**			-0.328	0.033	-0.463	0.004
Motor dev age					-0.458	**0.011**					-0.255	0.166
Overall P-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Adj. R^2	0.579		0.726		0.854		0.687		0.718		0.811	

	Delay in socialization						Delay in motor skills					
	Model A		Model B		Model C		Model A		Model B		Model C	
	ß	P	ß	P	ß	P	ß	P	ß	P	ß	P
Age	0.883	**<0.001**	1.178	**<0.001**	1.495	**<0.001**	0.803	**<0.001**	1.090	**<0.001**	1.802	**<0.001**
Mental dev age			-0.433	**0.004**	-0.387	**0.023**			-0.360	0.032	-0.100	0.392
Motor dev age					-0.388	0.052					-1.048	**<0.001**
Overall P-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Adj. R^2	0.684		0.744		0.785		0.626		0.664		0.891	

Dev age = developmental age. Age, dev ages, and delay in years. Adjusted for gender and genetic subtype.

B. Prepubertal children (n=33)

	Delay in communication						Delay in daily living skills					
	Model A		Model B		Model C		Model A		Model B		Model C	
	ß	P	ß	P	ß	P	ß	P	ß	P	ß	P
Age	0.917	**<0.001**	0.644	**<0.001**	0.645	**<0.001**	0.903	**<0.001**	0.564	**0.001**	0.562	**0.001**
Total IQ			-0.455	**0.001**					-0.494	**0.001**		
Verbal IQ					-0.295	**0.027**					-0.374	**0.010**
Performal IQ					-0.237	0.076					-0.207	0.190
Overall P-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Adj. R^2	0.738		0.760		0.747		0.703		0.690		0.676	

	Delay in socialization						Delay in motor skills					
	Model A		Model B		Model C		Model A		Model B		Model C	
	ß	P	ß	P	ß	P	ß	P	ß	P	ß	P
Age	0.922	**<0.001**	0.656	**<0.001**	0.654	**<0.001**	0.930	**<0.001**	0.623	**<0.001**	0.617	**<0.001**
Total IQ			-0.437	**0.001**					-0.464	**<0.001**		
Verbal IQ					-0.344	**0.010**					-0.443	**<0.001**
Performal IQ					-0.168	0.190					-0.085	0.407
Overall P-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Adj. R^2	0.758		0.766		0.756		0.790		0.828		0.841	

Dev age = developmental age. Age, dev ages, and delay in years. Adjusted for gender and genetic subtype.

munication: p= <0.05, daily living skills: p= <0.05, socialization: p= 0.01, motor skills: p= <0.001, resp.).
Neither gender, nor genetic subtype were associated with delay in adaptive functioning in infants and prepubertal children.

Short-term GH treatment

GH treatment for 1 or 2 years, in infants and prepubertal children respectively, had no effect on delay in adaptive functioning versus no treatment (in communication (p= 0.645, p= 0.262, resp.), daily living skills (p= 0.295, p= 0.366, resp.), socialization (p= 0.605, p= 0.219, resp.) and motor skills (p= 0.727, p= 0.311, resp.).

Long-term continuous GH treatment

Former infant group

Thirty-six children of the former infant group were investigated by the VABS test after at least 7 years of GH treatment. Median (IQR) age of the former infant group was 10.18 (8.59-12.96) years. The developmental age in communication was 4.88 (3.99-6.35) years, in daily living skills 5.09 (3.88-5.79) years, in socialization 3.58 (2.58-4.17) years, and in motor skills 3.58 (3.08-4.02) years. Median (IQR) duration of GH treatment had been 7.79 (6.24-9.86) years. After 7 years of GH treatment, the delay in the former GH-treated infant group of the RCT was significantly less than in the former untreated group for all four domains, i.e. for communication (p<0.001), daily living skills (p<0.001), socialization (p<0.001) and motor skills (p<0.001), indicating that long-term GH treatment improves adaptive functioning if GH treatment is started in infancy. The multiple linear regression showed that an earlier age at start of GH during infancy was associated with less developmental delay in communication (β= 0.533, p= 0.018), daily living skills (β= 0.440, p= 0.041), socialization (β= 0.503, p= 0.048) and motor skills (β= 0.706, p= 0.003) (Table 3), after adjustment for change in IQ in time. In other words, an earlier age at start of GH treatment in infancy was associated with improved adaptive functioning.

Former prepubertal group

In seventeen children of the former prepubertal children group, VABS test was performed after long-term GH treatment. Median (IQR) age of participants of the former prepubertal group was 15.87 (15.14-16.34) years. They had a developmental age in communication of 4.92 (3.66-7.66) years, daily living skills 6.66 (5.58-8.13) years, socialization 4.75 (3.38-5.83) years, and motor skills 3.92 (3.34-4.50) years. Median (IQR) duration of GH treatment had been 8.75 (8.56-10.73) years.

Table 3. Delay in adaptive functioning after 7 years of GH treatment in children with PWS.

A. Children who started GH treatment in infancy (n=36)

	Delay in communication		Delay in daily living skills		Delay in socialization		Delay in motor skills	
	Model		Model		Model		Model	
	ß	P	ß	P	ß	P	ß	P
Mental dev age at start	-0.209	0.324	-0.186	0.368	-0.267	0.276	-0.065	0.765
Motor dev age at start	0.156	0.470	0.436	**0.045**	0.438	0.085	-0.212	0.343
Total IQ in 2013	-0.591	**<0.001**	-0.471	**<0.001**	-0.333	**0.022**	-0.520	**<0.001**
Age at start of GH	0.533	**0.018**	0.440	**0.041**	0.503	**0.048**	0.706	**0.003**
Overall P-value	<0.001		<0.001		<0.001		<0.001	
Adj. R^2	0.677		0.675		0.547		0.635	

Dev age = developmental age. Age, dev ages and delay in years.

B. Children who started GH treatment in childhood (n=17)

	Delay in communication		Delay in daily living skills		Delay in socialization		Delay in motor skills	
	Model		Model		Model		Model	
	ß	P	ß	P	ß	P	ß	P
Total IQ at start	-0.622	**0.019**	-0.353	0.341	-0.521	0.146	-0.063	0.849
Total IQ in 2013	-0.421	0.131	0.190	0.658	0.353	0.384	0.651	0.123
Age at start of GH	0.114	0.642	-0.257	0.528	-0.249	0.511	-0.456	0.238
Overall P-value	0.020		0.706		0.447		0.409	
Adj. R^2	0.571		-0.166		-0.004		0.022	

No effect of long-term GH treatment was found in delay of adaptive functioning between the former GH-treated and the untreated group of the RCT, except for daily living skills (p= 0.012).

No association was found between delay in adaptive functioning and age at start of GH treatment, after adjustment for difference in IQ in time (total IQ at start and total IQ in 2013). The results between delay in adaptive functioning and age at onset of GH treatment were as follow: in communication: β=0.114, p=0.642, daily living skills: β= -0.257, p=0.528, socialization: β= -0.249, p=0.511, and motor skills: β= -0.456, p=0.238.

DISCUSSION

In order to improve the daily care for children and adolescents with PWS, it is important to know the level of adaptive functioning of these children, and which variables can influence this functioning. Our results demonstrate a marked delay in adaptive functioning in children with PWS, which increased with age and was inversely associated with IQ. Gender and genetic subtype had no significant effect on the delay in adaptive functioning. No effect of short-term GH treatment was found on adaptive functioning. However, children with long-term GH treatment who started GH treatment at a younger age during infancy had a significantly better adaptive functioning.

Results in relation to previous research

The marked delay in adaptive functioning shows the need to adapt the approach of children with PWS. Children aged 14 years had a developmental age of around 6 years in communication and socialization, indicating that the adaptive functioning of prepubertal children with PWS is less than half of healthy children. Social functioning in children with PWS has been described in earlier studies, and was also found to be significantly impaired compared to healthy peers (2, 22, 23). Additionally, social disabilities in PWS are more impaired than in other children with intellectual disability, indicating that the impaired social functioning is specific for PWS (24, 25).

For daily living skills, the highest acquired level was nearly 8 years, which is also very much delayed. For motor skills, the maximal score for developmental age is normally reached at the age of 6 years. Because some children scored a developmental age between 5 and 6 years at an older age, we presume that some children with PWS are able to reach a nearly maximal developmental age in motor skills, but at a later age.

Some children were able to perform some adaptive skills themselves such as personal care or helping in the house. However, this does not necessarily relieve the parental care. The majority of parents still need to supervise the children to make sure that they do their task in the right way and within a certain time limit, e.g. before the school bus arrives. Additionally, due to the PWS-specific characteristics (26) as hypotonia and repetitive behaviour, adaptive tasks might not be completed within the time limits in daily life which also requires special attention of parents. Studies about parental stress are very limited. One study described that parents of children with PWS are mostly stressed in case of behavioural problems (24) and had more somatization, phobic anxiety, obsessive compulsion, and anxiety problems than parents of healthy children who were matched in age, gender and IQ (22). Therefore, support for parents should incorporate knowledge about the delay of adaptive functioning in children with PWS and the consequences in daily life.

In the present study, short-term GH treatment had no effect on adaptive functioning in children with PWS. After long-term GH treatment, however, at a younger age during infancy starting GH treatment improved adaptive functioning.
Such results were not found in prepubertal children, who started GH treatment after infancy. The lack of power in the prepubertal group might explain this result, as mentioned under the section Limitations. Alternatively, it might be that GH has a limited effect on adaptive functioning when it is started after infancy. These children might have gained such a large delay in adaptive functioning, that subsequent GH treatment did not affect adaptive functioning.

A lower intelligence was correlated with more developmental delay in adaptive functioning; regarding communication, daily living skills and socialization. Also, after 7 years of GH treatment a higher IQ was associated with less delay in adaptive functioning. Such an association was not found for baseline IQ, suggesting that adaptive functioning during long-term GH treatment is associated with current IQ and not with baseline IQ. Also, the largest effect of GH treatment on adaptive functioning was reached if GH treatment was started at a younger age during infancy. Thus, our data may indicate that GH treatment does not only improve bodycomposition (10) and intelligence (11), but also adaptive functioning after long-term treatment, particularly if it is started during infancy.

In the present study we only focused on children with PWS who were treated with long-term GH. As GH therapy is currently the standard treatment in most developed countries, children and young adults nowadays show major differences due to a combination of GH treatment (10), early diagnosis, and early start of medical and rehabilitative interventions. For example, communication skills are not only related to verbal intelligence, but also to the muscular facial anatomy and strength (3). GH is known to increase the muscle tone and promote physical activity (5), and therefore promotes articulation and motor control for optimal development of communication skills, which additionally promotes social interaction and physical tasks. These effects might be cumulative over time. Together with the early start of multidisciplinary care such as speech therapy, communication skills improve. Therefore, characteristics of children with PWS nowadays might differ significantly from adults with PWS who were diagnosed later in childhood/adulthood and did not receive suitable treatment and guidance.

Limitation and further research

Our study results have implications for the treatment of children with PWS. Early diagnosis of PWS provides the opportunity to start GH treatment and rehabilitative therapies at an early age to improve adaptive skills as much as possible, leading to more

independency of the person with PWS and less burden for the family in supporting the daily activities. However, our study is limited by the relative low number of prepubertal children in the long-term study as a result of discontinuation of GH due to attainment of adult height. A larger group of prepubertal children without GH treatment at start of the RCT would have resulted in a larger group with long-term GH treatment. In the Netherlands, as in most developed countries, GH treatment is nowadays a standard treatment for children with PWS. This challenges the possibility to conduct a new RCT in children without GH treatment. However, countries in which GH treatment has not been standardized yet, have the possibility to investigate the effect of GH treatment on adaptive functioning in a larger group.

In conclusion, children with PWS have a marked delay in adaptive functioning, which increases with age. A lower IQ is associated with more delay in adaptive functioning. For that reason, infants and children with PWS require adjusted guidance. Our study shows that starting GH treatment at an earlier age during infancy leads to better adaptive skills on the long-term.

REFERENCES

1. Whittington J, Holland A, Webb T 2009 Relationship between the IQ of people with Prader-Willi syndrome and that of their siblings: evidence for imprinted gene effects. J. Intellect. Disabil. Res. 53:411-418
2. Lo ST, Siemensma E, Collin P, Hokken-Koelega A 2013 Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. Res. Dev. Disabil. 34:2764-2773
3. Akefeldt A, Akefeldt B, Gillberg C 1997 Voice, speech and language characteristics of children with Prader-Willi syndrome. J. Intellect. Disabil. Res. 41 (Pt 4):302-311
4. Dimitropoulos A, Ferranti A, Lemler M 2013 Expressive and receptive language in Prader-Willi syndrome: report on genetic subtype differences. J. Commun. Disord. 46:193-201
5. Festen DA, Wevers M, Lindgren AC, Bohm B, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 68:919-925
6. Sparrow SS, Balla DA, Cicchetti D 1984 Vineland Adaptive Behavior Scales-Revised. Circle Pines, Minnesota: American Guidance Service
7. Di Nuovo S, Buono S 2011 Behavioral phenotypes of genetic syndromes with intellectual disability: comparison of adaptive profiles. Psychiatry Res. 189:440-445
8. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
9. Festen DA, de Lind van Wijngaarden R, van Eekelen M, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Randomized controlled GH trial: effects on anthropometry, body composition and body proportions in a large group of children with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 69:443-451
10. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Schroor EJ, Van Alfen AA, Van Leeuwen M, Van Pinxteren-Nagler E, Van Wieringen H, Vreuls RC, Zwaveling-Soonawala N, de Ridder MA, Hokken-Koelega AC 2013 Eight Years of Growth Hormone Treatment in Children With Prader-Willi Syndrome: Maintaining the Positive Effects. J. Clin. Endocrinol. Metab. 98:4013-4022
11. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314
12. Reschly DJ, Myers TG, Hartel CR 2002 Mental Retardation: Determining Eligibility for Social Security Benefits. Washington, DC: National Academies Press
13. Tanner JM, Whitehouse RH 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch. Dis. Child. 51:170-179
14. de Bildt A, Kraijer D, Sytema S, Minderaa R 2005 The psychometric properties of the Vineland Adaptive Behavior Scales in children and adolescents with mental retardation. J. Autism Dev. Disord. 35:53-62

15. Balboni G, Pedrabissi L, Molteni M, Villa S 2001 Discriminant validity of the Vineland Scales: score profiles of individuals with mental retardation and a specific disorder. Am. J. Ment. Retard. 106: 162-172
16. Van der Meulen B, Ruiter S, Lutje Spelberg H, Smrkovsky M 2002 Bayley Scales of Infant Development - II (Dutch Version), manual. Lisse, The Netherlands: Swets & Zeitlinger BV
17. van der Steene G, A. B 1997 Wechsler Preschool and Primary Scale of Intelligence - Revised (Dutch Version), manual. Lisse, the Netherlands: Swets & Zeitlinger BV
18. Wechsler D 2002 Wechsler Intelligence Scale For Children (Dutch Version), manual. Third edition ed. London, United Kingdom: Harcourt Assessment
19. Hokken-Koelega A, Hackeng WH, Stijnen T, Wit JM, De Muinck Keizer-Schrama SM, Drop SL 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J. Clin. Endocrinol. Metab. 71:688-695
20. van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC 2004 Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. Journal of Clinical Endocrinoly and Metabolism 89:5295-5302
21. Festen DA, de Lind van Wijngaarden R, van Eekelen M, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Randomized controlled GH trial: effects on anthropometry, body composition and body proportions in a large group of children with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 69:443-451
22. Skokauskas N, Sweeny E, Meehan J, Gallagher L 2012 Mental health problems in children with prader-willi syndrome. J Can Acad Child Adolesc Psychiatry 21:194-203
23. Dimitropoulos A, Ho A, Feldman B 2012 Social Responsiveness and Competence in Prader-Willi Syndrome: Direct Comparison to Autism Spectrum Disorder. J. Autism Dev. Disord.
24. Wulffaert J, Scholte EM, Van Berckelaer-Onnes IA 2010 Maternal parenting stress in families with a child with Angelman syndrome or Prader-Willi syndrome. J Intellect Dev Disabil 35:165-174
25. Koenig K, Klin A, Schultz R 2004 Deficits in social attribution ability in Prader-Willi syndrome. J. Autism Dev. Disord. 34:573-582
26. Greaves N, Prince E, Evans DW, Charman T 2006 Repetitive and ritualistic behaviour in children with Prader-Willi syndrome and children with autism. J. Intellect. Disabil. Res. 50:92-100

CHAPTER 6

VISUAL-MOTOR INTEGRATION IN CHILDREN WITH PRADER-WILLI SYNDROME

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Submitted

ABSTRACT

Background

Prader-Willi syndrome (PWS) is characterized by hypotonia, hypogonadism, short stature, obesity, behavioral problems, intellectual disability, and delay in language, social and motor development. There is very limited knowledge about visual-motor integration in children with PWS.

Method

Seventy-three children with PWS aged 7 to 17 years were included. Visual-motor integration was assessed using the Beery Visual-motor Integration test at the start of the study and after two years. The association between visual-motor integration and age, gender, genetic subtype, and intelligence was assessed.

Results

Children with PWS scored 'very low' (-3 standard deviations) in visual-motor integration and 'below average' (-1 standard deviation) in visual perception and motor coordination compared to typically developing children. Visual-motor integration was higher in children with a deletion (β= -0.170, p=0.037), in older children (β=0.222, p=0.009), and in those with a higher total IQ (β=0.784, p<0.001). Visual perception was higher with a deletion (β= -0.193, p=0.044) and higher IQ (β= -0.618, p<0.001), but motor coordination was only higher with a higher total IQ (β= 0.429, p=0.001). Visual perception and motor coordination were not associated with age or gender. There was a trend for visual-motor integration decline over the 2 year follow-up period (p=0.099). Visual perception and motor coordination did not change over the follow-up period.

Conclusions

Visual-motor integration is very poor in children with PWS. Children scored higher on the time-limited subtests for visual perception and motor coordination than the combined test for visual-motor integration. Separation of visual-motor integration tasks into pure visual or motor tasks and allowing sufficient time to perform the tasks might improve daily activities, both at home and at school.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurogenetic developmental disorder due to the lack of expression of genes on the paternally inherited chromosome 15 at the locus q11-q13, due to deletion (DEL), maternal uniparental disomy (mUPD), imprinting defects or chromosomal translocation (1). PWS is characterized by hypotonia, hypogonadism, short stature, obesity, behavioral problems, intellectual disability, and developmental disabilities. Motor deficit is an important characteristic of PWS and is previously reported during pregnancy as lack in foetal activity by mothers and gynaecologists. After birth and during the first years of life, central hypotonia is the most marked characteristic. It causes decreased movements, a head lag, lethargy with decreased arousal, weak or absent cry, and poor reflexes, including a poor suck that leads to feeding difficulties and failure to thrive (2). Also during infancy and childhood, impaired gross motor skills and fine motor skills are reported in children with PWS, resulting in delayed developmental milestones (3-6). Some studies investigated motor development and its relation to body composition and growth hormone treatment in children with PWS (3, 6, 7). However, knowledge about visual-motor integration capacity of children with PWS remained very limited.

Visual-motor integration (VMI) shows the extent in which visual perception (interpretation of visual stimuli) and finger-hand movement are coordinated (8). VMI is an important contributor to academic skills which require eye-hand coordination, such as writing, mathematic formulations, and drawing. It requires accurate perception of visual spatial objects and monitoring of one's own movement. VMI skills are thus embedded in daily activities of school-aged children. Insight into VMI in childhood is therefore clinically relevant to optimize the approach and guidance of daily activities at school in children with PWS. To our knowledge, VMI in PWS has only been studied in two studies using the *Beery-Buktenecia Developmental Test of Visual-motor Integration*. The first research group concluded that VMI was more impaired in children and adolescents with PWS than age-matched typically-developing youngsters (9). The second research group found that individuals with the smaller type II DEL performed better on visual processing than those with the larger type I DEL or mUPD (10). Both studies were performed in a relatively small group of individuals with PWS (21 and 12, resp.) and no separate assessment of visual processing and motor coordination were performed. The present study was conducted to investigate the level of VMI in more detail in a large group of children with PWS.

The IQ-range in individuals with PWS varies between 50 to 80 (11, 12). VMI-testing in 5-year old children with 22q11-deletion syndrome, who have a similar IQ as children with PWS, showed that VMI performance was below average compared to typically

developing peers (13). In children with 22q11-deletion syndrome, VMI and visual skills were correlated with total IQ, but not with motor coordination.
Earlier studies have found children with PWS to be stronger in performing visual spatial tasks than their peers with similar IQ (9), with individuals with a DEL scoring higher on the VMI than those with an mUPD (9, 14). However, these studies were performed in a group with a wide age-range, or in instances where it was not known if the participants were treated with growth hormone.
In our previous study, we found that visual processing deteriorated during 2 years in children without growth hormone treatment, but improved significantly after long-term growth hormone treatment, especially in those with a greater deficit (15). As growth hormone treatment is now a standard treatment in most developed countries, this might suggest that long-term growth hormone treatment not only improves visual processing, but might also improve VMI.

The aim of our study was to determine the level of VMI. Secondly, we investigated which factors predicted the level of VMI in children with PWS. We hypothesized that VMI in children with PWS is impaired compared to healthy references, but that children with DEL score higher than those with mUPD. In addition, we expected that VMI performance and visual perception would correlate with total IQ, and motor coordination with performal IQ and that the level of VMI would decline over time in children with PWS compared to healthy references.

METHODS

For the present study, we enrolled children aged 7 to 17 years who participated in the ongoing Dutch PWS Cohort study, a study investigating the long-term effects of growth hormone treatment in children with PWS (16). Testing for visual-motor integration was performed at baseline and after 2 years.
A total of 81 children with PWS were eligible for participation, 75 of whom agreed to participate, resulting in a response rate of 93%. In the second assessment, 54 children who also completed the first assessment were eligible to participate, and 52 children completed the second assessment, resulting in a response rate of 96%. Twenty-one children were excluded from the second assessment due to the follow-up period being less than 2 years, one died, and one child did not participate.
The genetic diagnosis of PWS was confirmed in all participants by methylation testing. The genetic subtype was known in all but four participants. All children were treated with growth hormone (Genotropin 1 mg/m^2/day). This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center in Rotterdam. Written

informed consent was obtained from all parents or caretakers and children of 12 years of age onwards, and assent below the age of 12 years.

Visual-motor integration

Visual-motor integration was tested with the *Beery-Buktenecia Developmental Test of Visual-motor Integration)(full-form) (*Beery-VMI), which is designed to assess to which extent individuals can integrate their visual and motor abilities by copying geometric forms, organized in a developmental sequence. The Beery-VMI is valid for individuals aged 2 to 100 years to assess the written visual-motor functioning (8). The child was asked to copy thirty geometric forms, organized from simple to more complicated forms. No time limit was required for this test. Visual-motor integration has been investigated in 5-year old children with 22q11-deletion syndrome and small groups of children with PWS, confirming its feasibility (9, 10, 13).

Visual Perception

The *VMI Supplemental Test for Visual Perception* is a test to assess visual performance by reducing motor requirements of the task to a minimum (8). A geometric form was presented to the child and we asked the child to point to the exact same geometric form within a group of other forms, organized from simple to more complicated. The testing time was limited to three minutes for thirty items.

Motor Coordination

The *VMI Supplemental Test for Motor Coordination* is a test to assess motor performance by reducing visual perceptual demands to a minimum (8). The child was asked to trace the stimulus forms with a pencil, staying between the double lined paths. Visual perception was minimised in this subtest by providing starting dots and paths as strong visual guides for the required motor performance. The test consists of thirty items ranging from simple to more complicated forms. All of the geometric forms were the same as the geometric figures used in the Beery-VMI. The testing time was limited to five minutes for thirty items.

Only pencil and paper were allowed for all three tests. No corrections were allowed. All tests were only performed if the child could complete the three example forms after a brief description of the particular task. All three tests consisted of the same 30 geometric figures. The Beery-VMI is well validated and has a sound reliability of 0.92 for VMI, 0.91 for visual perception and 0.90 for motor coordination (8).

Cognitive functioning

Cognitive testing was annually performed in prepubertal children and biennially in pubertal children using the short form of the Wechsler Intelligence Scale for Children-Revised (WISC-R) (17). All cognitive measurements described in this study were performed by an experienced psychologist. The methods of cognitive testing in prepubertal children has been described in detail (12). In summary, total IQ score was calculated according to an equation based on a Dutch outpatient reference population (total IQ = 45.3 + 2.91 x vocabulary s-score + 2.50 x block design s-score), as used in other studies (15, 18, 19). The vocabulary subtest was used to assess verbal IQ, and the block design subtest was used for performal IQ.

Data-Analysis

Age and intelligence at baseline are presented as median and interquartile range (IQR). Results for VMI, visual perception and motor coordination are expressed as a standard score provided by the manual of the Beery-VMI according to an individual's age. Standard scores consist of equal units of measurement with a mean of 100 and a standard deviation of 15. The standard score of performance was compared to a healthy reference group included in the manual, i.e. 'average' performance (standard score 90-109, includes 50% of the age group, equals 0 standard deviation), 'below average' (standard score 80-89, 16% of the age group, equals -1 standard deviation), 'low' (standard score 70-79, 7% of the age group, equals -2 standard deviation), 'very low' (standard score <70, 2% of the age group, equals -3 standard deviation) (8). The equivalent developmental age to the raw score was calculated using the Beery VMI Raw Score Age Equivalents table (8). Multiple linear regression was performed to investigate the association between VMI and age, gender (1=boys, 2=girls), genetic subtype (1= DEL, 2=mUPD), total IQ, verbal IQ, and performal IQ. McNemar's test was used to test for differences in gender and genetic subtype at baseline and after 2 years. Wilcoxon signed ranks test was used to test for differences in total IQ and standard scores for VMI, visual perception and motor coordination between the group at start and after 2 years. Statistical analyses were performed with SPSS 20·0 (SPSS Inc., Chicago, IL). A p-value of <0.05 (two-tailed) was considered statistically significant.

RESULTS

Baseline

Median (interquartile range (IQR)) age of the 75 children with PWS was 11.8 (8.7, 14.1) years and 34 (45%) were male (Table 1). Thirty-one (41%) had DEL, 36 (48%) mUPD, four had an imprinting defect, and in four children the genetic subtype remained unknown.

Table 1. Characteristics of the study group at baseline

	Baseline
N (male)	75 (34)
Age (years)	11.8 (8.7, 14.1)
Genetic subtype (n (%))	
Deletion	31 (41%)
UPD	36 (48%)
ICD	4
Unknown	4
Total IQ	62 (56, 78)
Age at start of GH treatment (years)	4.5 (2.2, 6.8)
Duration of GH treatment (years)	7.0 (6.0, 8.0)

GH = growth hormone. Age, total IQ, age at start and duration of GH treatment are expressed in median (IQR).

Median (IQR) total IQ was 62 (56, 78), age at start of growth hormone treatment 4.5 (2.2, 6.8) years and duration of growth hormone treatment 7.0 (6.0, 8.0) years. Characteristics of the study group are presented in Table 1.

Visual-motor integration

Children with PWS scored 'very low' (-3 standard deviations) in VMI with a median (IQR) standard score of 62 (45, 81). They scored 'below average' (-1 standard deviation) in visual perception and motor coordination with median scores of 81 (62, 89) and 80 (63, 93), resp. The VMI standard scores of individual children with PWS according to age are presented in Figure 1 and the age equivalent to the VMI standard score according to age in Figure 2.

Multiple linear regression showed that VMI was higher in children with a DEL (β=-0.170, p=0.037), in older children (β=0.222, p=0.009) and in those with a higher total IQ (β=0.784, p<0.001) (Table 2), but there was no difference in VMI between boys and girls (β=0.065, p=0.418). Higher verbal IQ (β=0.419, p<0.001) and performal IQ (β=0.469, p<0.001) were both associated with higher VMI performance. Visual perception and motor coordination were higher in children with a higher total IQ (β=0.618, p<0.001 and β=0.429, p=0.001, resp.). Visual perception was higher in children with higher verbal (β=0.339, p=0.005) and performal IQ (β=0.398, p=0.003), but there was no association between motor coordination and verbal (β=0.252, p=0.095) or performal IQ (β=0.236, p=0.146). Visual perception was also higher in children with a DEL (β=-0.193, p=0.044).

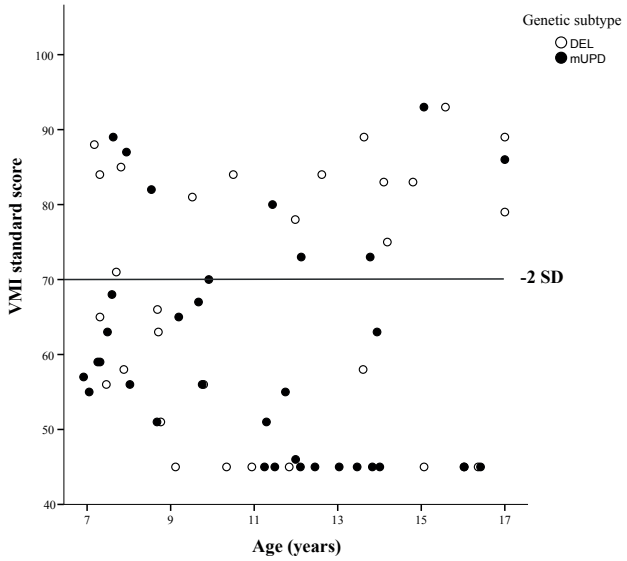

Figure 1. VMI performance in children with PWS

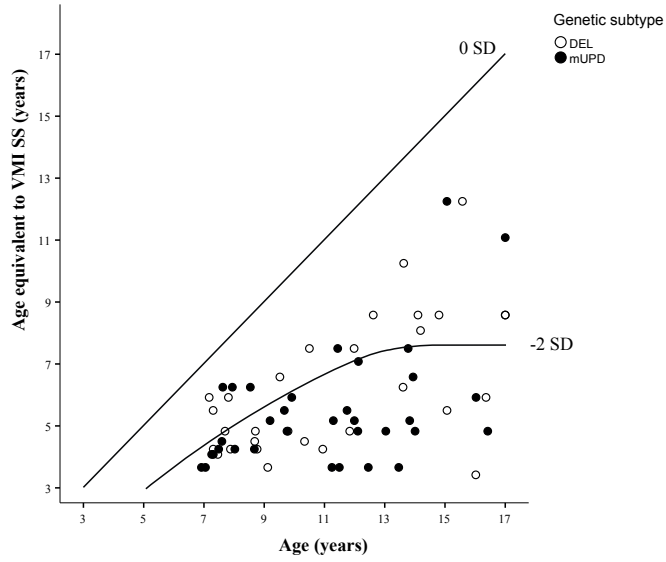

Figure 2. VMI performance expressed as age equivalent in children with PWS
SS = standard score. The line of identity (45°) represents the line of 0 standard deviation (SD) and is similar to age. The line below represents the -2 SD line for typically developing children.

Table 2. VMI in children with PWS

	VMI				Visual Perception				Motor Coordination			
	Model A		Model B		Model A		Model B		Model A		Model B	
	ß	P	ß		ß	P	ß	P	ß	P	ß	P
Age	0.222	**0.009**	0.270	**0.004**	-0.082	0.404	-0.055	0.605	-0.038	0.753	-0.005	0.970
DEL vs mUPD	-0.170	**0.037**	-0.164	0.054	-0.193	**0.044**	-0.157	0.112	-0.059	0.615	-0.038	0.757
Total IQ	0.784	**<0.001**			0.618	**<0.001**			0.429	**0.001**		
Verbal IQ			0.419	**<0.001**			0.339	**0.005**			0.252	0.095
Performal IQ			0.469	**<0.001**			0.398	**0.003**			0.236	0.146
Overall P-value	<0.001		<0.001		<0.001		<0.001		0.004		0.021	
Adj. R^2	0.602		0.598		0.452		0.456		0.173		0.140	

VMI= visual-motor integration. VMI, visual perception, and motor coordination are expressed in standard scores. Genetic subtype is classified as follow: DEL=1, mUPD=2. Adjusted for gender.

After 2 years of follow-up

In the second assessment after 2 years, 52 children participated and this group did not significantly differ from the initial group in gender, genetic subtype and total IQ (p= 0.317, p= 1.000, p= 0.203, resp.).

There was a trend for VMI decline over the 2 year follow-up (z= -1.651, p= 0.099). There was no change in visual perception (z= -0.233, p= 0.815) and motor coordination (z= -0.622, p= 0.534) after 2 years of follow-up.

DISCUSSION

Our study shows that visual-motor integration (VMI) in children with PWS is very poor compared to typically developing children. The majority of our group had a standard deviation score of less than -3. VMI was higher in children with a deletion, in older children and in those with a higher total IQ. However, there was no difference in VMI between boys and girls. Children scored higher when visual perception and motor coordination were tested separately. Visual perception and motor coordination were positively associated with total IQ, but only VMI and visual perception were positively associated with verbal and performal IQ. During the 2 year follow-up, the visual perception and motor coordination did not change, but there was a trend for VMI to decline.

VMI is the ability to integrate visual perception and motor skills. We tested VMI by asking children to copy a figure, for which both visual perception and motor coordination were required. Visual perception and motor coordination were also tested separately to

determine if these skills were impaired on their own. As expected, VMI was considerably impaired in our study group which resulted in a -3 standard deviation score compared to typically developing children. Unexpectedly, the children had lower scores in the no time-limit performance of the Beery-VMI than in the other time-limited subtests. As Beery-VMI tests the integration of visual perception and motor coordination, the results suggest that VMI is more difficult for children with PWS than performing a simplified task, either visual or motor, even in the case of time restriction. This poor VMI performance explains at least part of the difficulties in daily functioning faced by people with PWS, such as the weakness in writing skills. Based on the results of our study, writing could better taught by training the visual and motor skills separately. For example, the visual differences between letters could be trained first followed by motor tasks. The actual writing of letters should be the last step of the learning process as VMI is the most difficult step for these children. It is likely that poor VMI skills also contribute to difficulties in other motor coordination skills, e.g. getting dressed. For daily practice, visual support could be provided by using pictograms.

The majority of children were eager to finish the tests beyond the permitted time. In our analyses, we only used the scores within the time-limit in order to compare the results with typically developing peers. But observationally, children were able to score higher on the visual and motor tests if there was no time-limit. Future studies on VMI in children with PWS could investigate their maximal ability by recording both scores with limited and unlimited time setting for the visual and motor tests. Hypotonia, a typical feature of PWS, might partly explain why individuals with PWS need more time to complete a motor task.

We expected that VMI would decline over time and thus that the difference in VMI skills would increase between children with PWS and their healthy peers. We found, however, that there was a trend in VMI decline, as this was at the border of significance. It is most likely that the time period of 2 years was too short to show any difference in individual VMI performance.

VMI performance was strongly associated with total IQ, which is in line with an earlier report in children with intellectual disability (20). This study suggested that intelligence might be particularly related to the planning stage of the VMI task and not so much to the motor component of the task. Hypothetically, altered sensory perception due to derangement of neurotransmitter balance involving the hypothalamic regions could have delayed the processing speed in individuals with PWS (21). In our study group, 2 boys with an mUPD of 15 and 17 years old scored unexpectedly higher on VMI than their peers with the same genetic subtype. Both boys started growth hormone treatment around the age of 9 years, were treated for 6 to 7 years, and had a total IQ of 86 and 90, resp. Their IQ was in the highest 10% of the total study group.

We did not find a difference in VMI performance between boys and girls, which is in contrast to a previous study in children with intellectual disability reporting that girls performed better than boys (20). However, another study in children with 22q11-deletion also found no difference in VMI performance between boys and girls (13).
In our study group, 41% of children had a deletion and 48% an mUPD. Earlier studies reported a DEL: mUPD ratio of 70:30 (22, 23). The higher frequency of the mUPD type in our Dutch study group is most likely related to an increased maternal age, which is in line with previous studies in Western Europe (24, 25).

As the purpose of this study was to investigate the level of VMI in a large group of children with PWS, we did not include a comparison group with children with intellectual disability. Our study, therefore, does not differentiate which characteristics are 'Prader-Willi specific'. However, children with DEL scored higher on VMI and visual perception than those with mUPD, which is in line with an earlier finding that children with DEL have stronger visual skills (9, 14). Earlier studies showed a beneficial effect of growth hormone treatment on motor skills and IQ (3, 6, 7, 15). However, our study was not designed to investigate the effect of growth hormone treatment on VMI.

In conclusion, visual-motor integration performance was very poor (-3 standard deviations) in a large group of 73 children with PWS compared to healthy references. VMI was higher in children with a deletion, in older children and in those with a higher total IQ. Separation of visual-motor integration tasks into pure visual or motor tasks and allowing sufficient time to perform the tasks might improve daily school activities such as writing. Our findings provide further insight into the neurocognitive deficiencies in children with PWS and how to approach these.

REFERENCES

1. Cassidy SB 1997 Prader-Willi syndrome. J. Med. Genet. 34:917-923
2. Aughton DJ, Cassidy SB 1990 Physical features of Prader-Willi syndrome in neonates. Am J Dis Child 144:1251-1254
3. Reus L, Pelzer BJ, Otten BJ, Siemensma EP, van Alfen-van der Velden JA, Festen DA, Hokken-Koelega AC, Nijhuis-van der Sanden MW 2013 Growth hormone combined with child-specific motor training improves motor development in infants with Prader-Willi syndrome: a randomized controlled trial. Res. Dev. Disabil. 34:3092-3103
4. Ehara H, Ohno K, Takeshita K 1993 Growth and developmental patterns in Prader-Willi syndrome. J. Intellect. Disabil. Res. 37 (Pt 5):479-485
5. Greenswag LR 1987 Adults with Prader-Willi syndrome: a survey of 232 cases. Dev. Med. Child Neurol. 29:145-152
6. Festen DA, Wevers M, Lindgren AC, Bohm B, Otten BJ, Wit JM, Duivenvoorden HJ, Hokken-Koelega AC 2008 Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. Clin. Endocrinol. (Oxf). 68:919-925
7. Carrel AL, Myers SE, Whitman BY, Eickhoff J, Allen DB 2010 Long-term growth hormone therapy changes the natural history of body composition and motor function in children with prader-willi syndrome. J. Clin. Endocrinol. Metab. 95:1131-1136
8. Beery KE, Beery NA 2004 The Beery-Buktenica developmental test of visual-motor integration: Beery VMI with supplemental deveopmental tests of visual perception and motor coordination: Administration, scoring and teaching manual. . 6th ed. Minneapolis: NCS Pearson Inc
9. Dykens EM 2002 Are jigsaw puzzle skills 'spared' in persons with Prader-Willi syndrome? J. Child Psychol. Psychiatry 43:343-352
10. Butler MG, Bittel DC, Kibiryeva N, Talebizadeh Z, Thompson T 2004 Behavioral differences among subjects with Prader-Willi syndrome and type I or type II deletion and maternal disomy. Pediatrics 113:565-573
11. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
12. Lo ST, Siemensma E, Collin P, Hokken-Koelega A 2013 Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. Res. Dev. Disabil. 34:2764-2773
13. Duijff S, Klaassen P, Beemer F, Swanenburg de Veye H, Vorstman J, Sinnema G 2012 Intelligence and visual motor integration in 5-year-old children with 22q11-deletion syndrome. Res. Dev. Disabil. 33:334-340
14. Veltman MW, Thompson RJ, Roberts SE, Thomas NS, Whittington J, Bolton PF 2004 Prader-Willi syndrome--a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. Eur. Child Adolesc. Psychiatry 13:42-50
15. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314

16. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Schroor EJ, Van Alfen AA, Van Leeuwen M, Van Pinxteren-Nagler E, Van Wieringen H, Vreuls RC, Zwaveling-Soonawala N, de Ridder MA, Hokken-Koelega AC 2013 Eight Years of Growth Hormone Treatment in Children With Prader-Willi Syndrome: Maintaining the Positive Effects. J. Clin. Endocrinol. Metab. 98:4013-4022
17. Wechsler D 2002 Wechsler Intelligence Scale For Children (Dutch Version), manual. Third edition ed. London, United Kingdom: Harcourt Assessment
18. van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC 2004 Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. Journal of Clinical Endocrinoly and Metabolism 89:5295-5302
19. Hokken-Koelega AC, van Pareren YK, Arends N 2005 Effects of growth hormone treatment on cognitive function and head circumference in children born small for gestational age. Horm. Res. 64 Suppl 3:95-99
20. Memisevic H, Sinanovic O 2012 Predictors of visual-motor integration in children with intellectual disability. Int. J. Rehabil. Res. 35:372-374
21. Akefeldt A, Ekman R, Gillberg C, Mansson JE 1998 Cerebrospinal fluid monoamines in Prader-Willi syndrome. Biol. Psychiatry 44:1321-1328
22. Cassidy SB 1984 Prader-Willi syndrome. Curr. Probl. Pediatr. 14:1-55
23. Dykens EM, Roof E 2008 Behavior in Prader-Willi syndrome: relationship to genetic subtypes and age. J. Child Psychol. Psychiatry 49:1001-1008
24. Whittington JE, Butler JV, Holland AJ 2007 Changing rates of genetic subtypes of Prader-Willi syndrome in the UK. Eur. J. Hum. Genet. 15:127-130
25. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32:1729-1735

CHAPTER 7

DEPRESSIVE SYMPTOMS AND THE DOPAMINE AND SEROTONIN SYSTEM IN CHILDREN WITH PRADER-WILLI SYNDROME

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Submitted

ABSTRACT

Introduction

Depressive illness is more common in adults with Prader-Willi syndrome (PWS) than in the general population. Data on the presence of depression and its symptoms in children with PWS are unknown. Also, information on depressive symptoms in relation with precursors and metabolites of the dopamine and serotonin system in children with PWS is lacking.

Methods

In 68 children with genetically confirmed PWS, aged 7 to 17 years, the precursors and metabolites of the dopamine (i.e. tyrosine and HVA, resp.) and serotonin system (i.e. tryptophan) in plasma were determined during 2 years and compared with healthy controls. Depressive symptoms were assessed by the Dutch version of the Children's Depression Inventory.

Results

None of the children with PWS fulfilled the criteria for depression.

Dopamine system: Tyrosine-ratio (tyrosine versus competing amino acids) and HVA-levels were normal, but both declined over 2 years ($p<0.001$ and $p=0.105$, resp.). More depressive symptoms were correlated with a lower tyrosine-ratio ($\rho=-0.457$, $p=0.011$) and tended to be correlated with a lower HVA-level ($\rho=-0.346$, $p=0.061$).

Serotonin system: Tryptophan-ratio was higher ($p<0.001$) in children with PWS than in controls and tryptophan-ratio increased over 2 years ($p<0.002$), but did not correlate with depressive symptoms.

Children with PWS had significantly higher plasma levels of aromatic amino acids (tyrosine, tryptophan and phenylalanine), in contrast to normal branched-chain amino acid-levels (isoleucine, leucine and valine).

Conclusion

In our study group, none of the children with PWS fulfilled the criteria for depression. Children with more depressive symptoms had a lower tyrosine-ratio. Tryptophan-ratio was higher than in healthy controls and increased over time, but was not related to depressive symptoms. Our findings warrant future studies.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurogenetic developmental disorder resulting from the lack of expression of genes on the paternal chromosome 15 at locus q11-q13, caused by deletion (DEL), maternal uniparental disomy (mUPD), imprinting defect, or chromosomal translocation (1). The estimated birth incidence rate is 1:15.000 (2, 3). PWS is characterized by hypotonia, hypogonadism, hyperphagia, obesity, intellectual disability, and behavioral problems. The phenotype of PWS has been explained by hypothalamic dysfunction and dysregulation of neurotransmitters. PWS is also associated with a higher risk of psychiatric problems such as depression (4, 5).

Only few studies have investigated depression in people with PWS. Depression occurs approximately in 12% of adults with PWS, mostly combined with psychotic symptoms (4, 5). It has been reported that subjects with DEL are more prone to a depression than those with an mUPD (5). No studies have yet investigated depressive symptoms in children with PWS.

In case of a depressive illness in PWS, a selective serotonin reuptake inhibitor (SSRI) is the most frequently prescribed antidepressant (4). Most antidepressants increase the synaptic level of the neurotransmitters serotonin and dopamine in the brain. As aberrations in the levels of neurotransmitters in these systems might be involved in depression in PWS (6, 7), it is important to investigate plasma precursors and/or metabolite levels of these neurotransmitters to acquire more insight in the dopamine and serotonin system in children with PWS.

Dopamine system: Tyrosine is essential in the formation of catecholamines such as dopamine, and norepinephrine. Plasma tyrosine has to enter the brain via the blood-brain barrier for the formation of dopamine in the brain (Figure 1). At the blood-brain barrier, tyrosine competes with tryptophan, phenylalanine, isoleucine, leucine and valine (competing amino acids) to pass this barrier (8). After entering the brain, tyrosine is firstly converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (Figure 1). Subsequently, L-DOPA is converted to dopamine by decarboxylation, and then mainly to homovanillic acid (HVA) and in a smaller percentage to norepinephrine. In humans, HVA is the major dopamine metabolite. After passing the blood-brain barrier, HVA is measurable in plasma. As a result, approximately 40% of plasma HVA (pHVA) originates from the brain and serves therefore as a proxy for central dopamine neuronal metabolism and hence central dopamine activity (9). Thus, a lower level of pHVA indicates a lower concentration of central dopamine. To estimate the level of dopamine in the brain, the ratio of tyrosine to competing amino acids passing the blood-brain barrier, expressed as Tyr-ratio, and the plasma level of the major dopamine metabolite HVA can be used

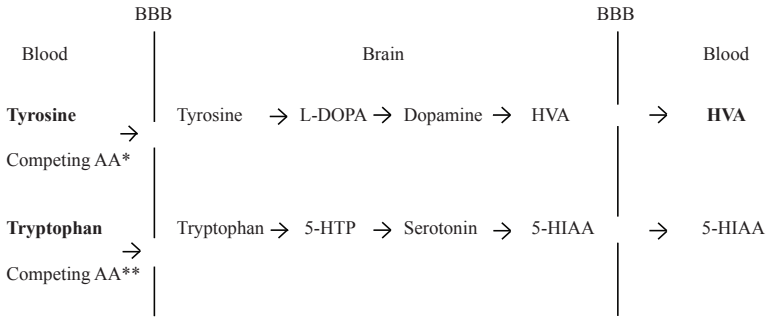

Figure 1. Dopamine and serotonin system
BBB= blood-brain barrier; Competing AA* are tryptophan, phenylalanine, isoleucine, leucine and valine, competing AA** are tyrosine, phenylalanine, isoleucine, leucine and valine. L-DOPA = L-3,4-dihydroxyphenylalanine; HVA = homovanillic acid; 5-HTP = 5-hydroxytryptophan; 5-HIAA= 5-hydroxyindoleacetic acid. Neurotransmitter precursors and metabolite analyzed in this article are depicted in bold.

(9). A lower Tyr-ratio suggests a decreased availability of tyrosine for the formation of dopamine in the brain.

Serotonin system: Serotonin is biochemically derived from tryptophan in the brain, which is one of the eight essential amino acids that cannot be synthesized in the human body and must be supplied by diet. Plasma tryptophan has to enter the brain via the same blood-brain barrier as tyrosine for the subsequent formation of serotonin (Figure 1). Tryptophan is firstly converted to 5-hydroxytryptophan by tryptophan hydroxylase and subsequently to serotonin by aromatic amino acid decarboxylase (Figure 1). The action is terminated by reuptake in the presynaptic element or by metabolization by monoamine oxidase to the metabolite 5-hydroxyindoleacetic acid (5-HIAA). The level of the serotonin precursor tryptophan entering the blood-brain barrier could therefore be an indicator of central serotonin concentration. This is depicted as Trp-ratio (ratio of tryptophan to competing amino acids passing the blood-brain barrier). An earlier study showed that the Trp-ratio is lower in adults with depression than in healthy adults (10). A lower Trp-ratio may cause a lower availability of tryptophan to the central nervous system, resulting in lower serotonin levels in the brain.

To the best of our knowledge, no study has investigated the correlation between depressive symptoms and plasma tyrosine-ratio, HVA and tryptophan-ratio in children with PWS. We, therefore, investigated the depressive symptoms in children with PWS in relation to these biomarkers in this exploratory study. We hypothesized that children with PWS have a higher prevalence of depression with a lower plasma tyrosine-ratio

and HVA-level, and a lower tryptophan-ratio than healthy controls and that these levels deteriorate over time. We expected that children with more depressive symptoms had a lower plasma tyrosine-ratio, HVA-level and tryptophan-ratio.

METHODS

The inclusion criteria for the present study were children with genetically confirmed PWS and aged 7-17 years. Sixty-eight children of the Dutch PWS Cohort Study (11) participated in the present study. All participants were treated with growth hormone 1 mg/m^2/day (Genotropin). Five children were treated with psychotropic medication; two boys were treated with antipsychotics after their first episode with a psychosis and three were treated for increased compulsive behavior, increased irritability and aggression.
The study was approved by Medical Ethics Committee of the Erasmus Medical Center Rotterdam. Written informed consent was obtained from all parents or carers, informed consent was obtained from children of 12 years or older, and assent below the age of 12 years.

Children's Depression Inventory

The Dutch translation of the Children's Depression Inventory (CDI) was used to rate severity and symptoms of depression and/or a dysthymic disorder in children with PWS (12). The CDI has been developed by Maria Kovacs (13) and is based on the Beck Depression Inventory, which is a self-rated instrument for adults (14). It is one of the most common used instruments to screen for depression in children. The CDI is a 27-item, self-rated and symptom-oriented scale suitable for children and adolescents aged 7 to 18 years. The items of the CDI are grouped into five factor areas, including Negative Mood, Interpersonal Problems, Ineffectiveness, Anhedonia, and Negative Self Esteem. Responses to the CDI are rated as: 0 =never or absent, 1=sometimes or somewhat, and 2=always or present. The total score ranges from 0 to 54 with a higher score indicating more symptoms of depression. As the CDI requires insight in one's own thoughts and feelings, only children with sufficient language development and self-awareness participated in this test. For that reason, also the five children with psychotropic medication could not participate in the CDI. The CDI has a high internal consistency and test-retest reliability (13). To minimize the effect of reading mistakes, all items of the CDI were read to the child by a trained medical researcher (SL) who also recorded the answers.

Biochemical analyses

PWS group

Fasting blood samples were taken once a year for 2 years. Within 3 hours, blood was centrifuged for 20 min at 2650 g and 20°C, and the obtained plasma was stored at -80°C until analysis.

Plasma amino acids were analyzed by high performance liquid chromatography (HPLC) using pre-column derivatization with o-phthaldialdehyde (15). The coefficient of intra-variation for all amino acids determined in the present study were below 4% and were highly correlated to levels from other laboratories with a correlation coefficient of r=0.999 (16). Tyr-ratio was calculated by dividing the total tyrosine level by the sum of the competing amino acids, i.e. tryptophan, phenylalanine, isoleucine, leucine, and valine, which compete for the transport of tyrosine through the blood-brain barrier. The Trp-ratio was calculated in the same manner by substituting total tyrosine level for total tryptophan level and by replacing tryptophan for tyrosine as competing amino acid.

Plasma homovanillic acid (pHVA)-levels were measured in 0.4 ml plasma by reversed-phase HPLC after removal of the proteins with 4.2% sulphosalicylic acid. The internal standards were isoprenaline and α-methyl 5-HT. Three µl samples were injected onto a reversed phase column (Zorbax Eclipse XDB-C8, 5 µm particle size, 250 x 3 mm; Agilent Technologies, Waldbronn, Germany) which was protected by a Hypersil ODS guard column (20 x 2.1 mm, 5 µm; Agilent). The HPLC apparatus consisted of an HP 1200 Series quaternary pump, on-line degasser and autosampler (Agilent Technologies, Waldbronn, Germany). The mobile phase consisted of 80 mM sodium acetate, 0.27 mM disodium EDTA, 0.74 mM heptane sulphonic acid and 13% methanol, pH 3.95. The flow rate was set at 0.4 ml/min and the column temperature was 45°C. The effluent was monitored by an electrochemical detector consisting of a VT-03 flow cell (0.33 µl) equipped with a glassy carbon electrode and the Intro controller (Antec Leyden, Leiden, The Netherlands). The oxidation potential was maintained between 0.68 and 0.72 V (versus Ag/AgCl reference electrode), the sensitivity range was 0.2 nA/V and the time constant was set at 2 sec. Noise was reduced with a Link low-pass filter using a cut-off frequency of 0.055 Hz . Data were collected on-line and processed by the Kroma PC Integration Pack data acquisition system (Kontron Instruments, Milan, Italy). Quantification was done by measuring peak heights. The mean recovery (± SD) of HVA added to the plasma samples was 95 ± 7%. Variation coefficients were less than 5% (17).

Healthy controls

Raw data on plasma amino acid levels of healthy controls were provided by Hammarqvist et al (18). In summary, the control group consisted of 10 age-matched healthy children

aged 7-15 years undergoing elective surgery, such as hernia repair, orchidopexy, operation due to hydronephrosis and antireflux operation. Amino acid levels were determined by ion-exchange chromatography, which is described in detail in the original article (18). HPLC and ion-exchange chromatography resulted in similar plasma amino acid levels (r=0.997, p<0.001) (16). No pHVA-levels were assessed in the healthy controls.

Data-analyses

CDI raw scores were calculated as z-scores, with a higher z-score depicting more depressive symptoms. The z-scores were categorized as following: 0= <-2, 1= -2 till -1, 2= -1 till 0, 3= 0 till 1, 4= 1 till 2, 5= >2. Age and Tyr-ratio, pHVA, and Trp-ratio were expressed as median (interquartile range (IQR)). Associations between baseline levels of Tyr-ratio, pHVA, Trp-ratio, and age were calculated with linear regression analysis. Fisher's exact test was used to calculate differences between genders and genotypes. Mann-Whitney U-test was used to test for differences in Tyr-ratio, pHVA, and Trp-ratio between the PWS group and healthy controls. Kruskal-Wallis test was used to analyze the difference between CDI z-scores and Tyr-ratio and Trp-ratio. To analyze the change in Tyr-ratio, pHVA, and Trp-ratio over time, Linear Mixed Models were used, which was adjusted for gender and genetic subtype (gender coded as: 1=boys, 2=girls; genetic subtype coded as: 1=DEL, 2=mUPD) and age.
Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL). All statistical tests were two-sided. A p-value of <0.05 was considered statistically significant.

RESULTS

Baseline

Median (interquartile range (IQR)) age of the total PWS group (n=68) was 11.3 (8.8, 14.2) years and 37 (54%) were male (Table 1). 28 had DEL (41%), 32 mUPD (47%), 4 an imprinting defect, and in 4 children the genetic subtype was unknown.

Assessment of depressive symptoms in children with PWS

Thirty of 68 children (44%) with PWS had sufficient language and self-awareness to complete the Children's Depression Inventory (CDI). Their median (IQR) age was 13.2 (11.2, 15.9) years and 13 (43%) were male. Ten had DEL (33%) and 17 mUPD (57%). Median (IQR) IQ was 68 (59, 83). Depressive symptoms were expressed as z-scores, with a higher z-score representing more depressive symptoms. Four children had a CDI z-score <-2, eleven between -2 and -1, and fifteen between -1 and 0. None of the participants had a z-score higher than 0, and therefore none of the children fulfilled the diagnosis

Table 1. Characteristics of the study groups

	PWS group				Healthy controls*	p-value[2]
	Total group	CDI assessment	No CDI assessment	p-value[1]		
N (male)	68 (37)	30 (13)	38 (25)	0.133	10 (7)	0.495
Age (years)	11.3 (8.8, 14.2)	13.0 (11.2, 15.9)	10.6 (8.6, 13.8)	**0.005**	11.0 (8.5, 12.0)	0.331
IQ	62 (56, 78)	69 (59, 83)	59 (57, 76)	**0.041**	n/a	
Genetic subtype (n (%))						
Deletion	28 (41%)	10	18	0.186		
mUPD	32 (47%)	17	15			
ICD	4	2	2			
Unknown	4	1	3			

Data expressed as median (IQR) or number (%) * Data of the healthy controls (18). P-value[1] represents the p-value between the PWS group with and without CDI assessment, p-value[2] represents the p-value between the total PWS group and healthy controls. N/a= not available.

of depression. More depressive symptoms was associated with an older age (ρ= 0.381, p=0.034), but not with IQ (ρ= 0.212, p=0.270).

Older age was not correlated with a higher IQ (ρ= -0.088, p=0.645). The four most rated symptoms were lack of friends in 17 of 30 children (57%), self-devaluation compared to peers in 8 of 30 (27%), difficulty in making decisions in 6 of 30 (20%), and being tired in 7 of 30 (23%) children. The PWS group without assessment of depressive symptoms (n=33), including 5 children with psychotropic medication, was significantly younger (median (IQR) age of 10.4 (8.5, 13.8) versus 13.2 (11.2, 15.9) years, resp., p=0.004) and had a lower IQ than the group with CDI assessment (median (IQR) IQ of 59 (57, 76) versus 68 (59, 83), resp., p=0.041).

Dopaminergic factors in children with PWS and healthy controls

Total group

In children with PWS, median (IQR) plasma tyrosine was 55 (51, 61) µmol/l, Tyr-ratio 12.3 (10.9, 13.6) and pHVA 42.8 (36.3, 53.1) nmol/l (Table 2). Plasma tyrosine was significantly higher than in healthy controls (p<0.005), but Tyr-ratio was similar (p=0.094). Between the group with and without CDI assessment, no difference was found in Tyr-ratio (p=0.348) and pHVA (p=0.345).

Age was neither associated with Tyr-ratio (β=-0.089, p=0.415) nor with pHVA-level (β=-1.377, p=0.076). No difference in these two parameters was found between children with DEL and mUPD, boys and girls, or children with and without psychotropic medication.

Table 2. Plasma amino acids and HVA in children with PWS at baseline

Amino acids	PWS group			p-value[1]	Healthy controls	p-value[2]
	Total group (n=68)	CDI assessment (n=30)	no CDI assessment (n=38)			
Tyr-ratio	**12.3 (10.9, 13.6)**	12.7 (11.3, 13.9)	11.8 (10.7, 13.5)	0.348	**11.7 (10.2, 12.4)**	0.094
pHVA	42.8 (36.3, 53.1)	43.5 (36.8, 53.9)	41.5 (35.2, 50.9)	0.345	n/a	
Trp-ratio	**8.9 (7.9, 10.3)**	8.9 (7.7, 10.1)	8.9 (8.0, 10.5)	0.450	**6.8 (6.6, 7.7)**	**P<0.001**
Competing amino acids						
Tyrosine	**55 (51, 61)**	58 (52, 61)	53 (49, 60)	0.228	**46 (43, 54)**	**0.004**
Tryptophan	**41 (36, 47)**	42 (36, 46)	40 (37, 51)	0.577	**31 (30, 34)**	**P<0.0001**
Phenylalanine	**47 (44, 51)**	48 (44, 50)	47 (44, 52)	0.861	**42 (39, 47)**	**0.040**
Isoleucine	53 (48, 59)	51 (49, 58)	54 (47, 59)	0.740	51 (41, 57)	0.552
Leucine	106 (94, 113)	106 (95, 115)	106 (94, 114)	0.851	107 (88, 121)	0.660
Valine	203 (189, 224)	205 (181, 224)	200 (195, 226)	0.654	194 (164, 221)	0.213

Plasma amino acids are expressed in µmol/l, pHVA in nmol/l. Data are expressed in median (IQR (=interquartile range)). CDI = Children's Depression Inventory. P-value[1] represents the p-value between the PWS group with and without CDI assessment, p-value[2] represents the p-value between the total PWS group and healthy controls.

PWS children with assessment of depression

Thirty children with PWS completed the CDI assessment of depression in addition to the assessment of Tyr-ratio and pHVA-level. Median (IQR) Tyr-ratio was 12.7 (11.3, 13.9) µmol/l and pHVA 43.5 (36.8, 53.9) nmol/l (Table 2). A higher CDI z-score, indicating more depressive symptoms, was significantly correlated with a lower Tyr-ratio (ρ= -0.457, p=0.011), and tended to be correlated with a lower pHVA-level (ρ= -0.346, p=0.061). Figure 2 shows the Tyr-ratio per subgroup of CDI z-score.

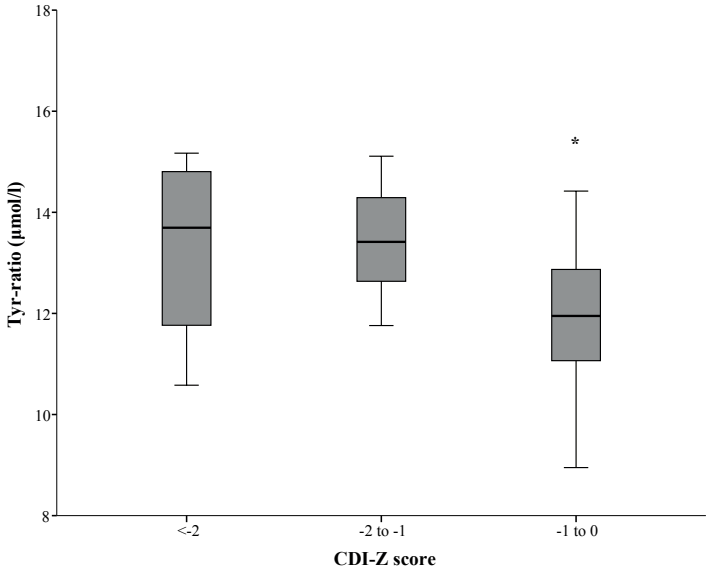

Figure 2. Correlation between tyrosine-ratio and depressive symptoms in children with PWS
Higher CDI z-score represents more depressive symptoms. *A higher CDI Z-score correlated with a lower Tyr-ratio (p=0.010). CDI z-score >0 is not depicted in this figure as none of our participants had a z-score >0.

Follow-up during 2 years

During the follow-up of 2 years in the total group of children with PWS, Tyr-ratio decreased significantly from 12.6 (12.0-13.1) at baseline to 11.8 (11.3-12.4) µmol/l after 2 years (p<0.001). The pHVA-levels tended to decrease from 48.4 (43.7-53.2) at baseline to 43.7 (36.9-50.5) after 2 years (p=0.105).

Serotonergic factor in children with PWS and healthy controls

Total group

In children with PWS, median (IQR) plasma tryptophan was 41 (36, 47) µmol/l and Trp-ratio 8.9 (7.9, 10.3) (Table 2), which were both significantly higher than in healthy controls (p<0.0001 and p<0.001, resp.). No difference was found between the group with and without CDI assessment in Trp-ratio (p=0.450).

Trp-ratio was not associated with age (β=-0.131, p=0.094). No difference in Trp-ratio was found between children with DEL and mUPD, boys and girls, or children with and without psychotropic medication.

PWS children with assessment for depression

Thirty children with PWS completed the CDI assessment for depression in addition to the assessment of Trp-ratio. Median (IQR) Trp-ratio was 8.9 (7.7, 10.1) µmol/l (Table 2). A higher CDI z-score, indicating more depressive symptoms, was not correlated with a lower Trp-ratio (ρ= -0.053, p=0.779).

Follow-up during 2 years

In children with PWS, the Trp-ratio increased significantly from 8.7 (8.4-9.0) at baseline to 9.8 (9.4 -10.3) µmol/l after 2 years (p<0.002).

Competing amino acids

In children with PWS, levels of branched-chain amino acids were similar compared to healthy controls (median (IQR) plasma isoleucine 53 (48, 59), leucine 106 (94, 113), and valine 203 (189, 224) µmol/l), but in contrast, the aromatic amino acids were higher, i.e. median (IQR) plasma tyrosine was 55 (51, 61), tryptophan 41 (36, 47), and phenylalanine 47 (44, 51) µmol/l (p<0.005, p<0.0001, p<0.05, resp.).

DISCUSSION

In our study group, none of the children with PWS fulfilled the criteria for the diagnosis of depression.

Both markers of the dopaminergic system, the plasma tyrosine-ratio, an indicator of tyrosine availability for central dopamine synthesis, and plasma HVA, the major metabolite of dopamine, were normal in children with PWS, but declined over 2 years. More depressive symptoms were correlated with a lower tyrosine-ratio indicating lower tyrosine availability for central dopamine synthesis.

The marker of the serotonin system, the plasma tryptophan-ratio, an indicator of tryptophan availability for central serotonin synthesis, was higher in children with PWS than in controls and increased over 2 years (p<0.002). However, this marker did not correlate with depressive symptoms. Plasma aromatic amino acid-levels were higher in children with PWS than in healthy controls, in contrast to normal levels of the branched-chain amino acids.

Assessment of depressive symptoms

We did not find the diagnosis of depression in our children with PWS aged 7 to 17 years. Lack of friends was the most prominent symptom and reported by approximately half of children with PWS. Weaknesses in social skills is part of the behavioural phenotype of PWS (19-22). Social disabilities have also been reported by parents (19-24). Remarkably,

children with PWS were aware of their limited number of friends. Our study also shows that children with PWS like to be similar as peers and sibs, but their social deficits challenge them to make friends. A quarter of them confirmed feelings of self-devaluation, difficulty in making decisions and being tired. It has been suggested that the tiredness is related to the abnormal body composition and general hypotonia in children with PWS, resulting in moving slowly, being underactive, doing little, e.g. only sitting and watching others (25). Additionally, daytime sleepiness and sleep abnormalities as sleep-related breathing disorders are common features in PWS (26-29), which contributes to being tired.

Three of the 27 items in the Children's Depression Inventory (sleeping problems, lack in appetite, and being worried about pain) rarely occurred in the children with PWS. Children with PWS would therefore potentially score three points less than the maximum score of 30 points. A possible explanation that we did not find depression in our group of children could be the long-term effect of GH treatment. We previously demonstrated that growth hormone treatment improved cognition in children with PWS, particularly in those with lower cognitive functioning (30). It cannot be excluded that long-term growth hormone treatment played a role in the neurodevelopment, and future studies are warranted to show if the current generation of children with PWS who are treated with GH from a young age onwards, have a lower risk of developing depression later in life.

Dopaminergic factors

We found that the plasma Tyr-ratio, an indicator of tyrosine availability for central dopamine synthesis, was relatively low and decreased over time while the plasma level of the dopamine metabolite HVA showed a trend of decreasing levels. In contrast, Akefeldt et al. found higher levels of HVA in cerebrospinal fluid compared to healthy controls (31). Remarkably, all three aromatic amino acids (tyrosine, tryptophan, phenylalanine) were significantly higher in children with PWS than in healthy controls, which was not the case for the branched-chain amino acids (isoleucine, leucine, valine). It is therefore unlikely that these differences in the amino acids are explained by dietary reasons, which would have resulted in aberrations in both aromatic and branched-chain amino acids.

Serotonergic factors

In contrast to our expectation, we found that the Trp-ratio, an indicator of tryptophan availability for central serotonin synthesis, was higher in children with PWS compared to healthy controls. One explanation could be that the serotonin system in children with PWS is compensating for a serotonin deficiency or accelerated breakdown of serotonin in the brain, resulting in increased levels of the serotonin precursor. However,

a serotonin deficiency seems unlikely as Akefeldt et al. found as higher levels of the serotonin metabolite 5-HIAA in cerebrospinal fluid in children and adolescents with PWS compared to a healthy control group, independent of age, body mass index and intelligence (31). Unfortunately, no cerebrospinal fluid was available and thus we were unable to determine 5-HIAA.

It has been shown that serotonin reuptake inhibitors have beneficial effects in reducing irritability in children with autism (32, 33). Based on clinical experience, these inhibitors also seem to benefit children with PWS, which is suggestive for a suboptimal serotonin system in PWS. The exact mechanism behind the increased levels of the serotonin precursor and metabolite remains, however, unknown, thus further investigations are warranted.

Limitation and future research

This study was conducted as an exploratory study to determine possible predictors for depression in the dopamine and serotonin system in children with PWS, but we acknowledge some limitations.

We investigated depressive symptoms using a structured interview with the children themselves. It remains challenging to screen for an internalizing disorder as depression in people with an intellectual disability. First, they may present depression with different symptoms such as irritability, either with or without aggression (34). Second, due to the delayed mental development in children with PWS, it might be difficult to be aware of thoughts of guilt, helplessness, and hopelessness and how to describe these. In healthy children, a mental age of 8 years is required before children can reflect on these abstract items. We investigated the presence of depression and depressive symptoms in children with PWS to reveal their inner thoughts. To minimize errors, we only included children who were able to answer the questions in the test example, which was nearly half of the total group. Interestingly, although the excluded group was significantly younger and had a lower IQ, analyses of the dopamine and serotonergic factors were not significantly different in children with and without evaluation of depressive symptoms. Thus, although a smaller group of children with PWS could complete the assessment on depressive symptoms, it is likely that our results on amino acids represent characteristics of the total PWS group.

We used data of plasma amino acids in healthy children originating from another study with a similar method of amino acid analysis (18). This age-matched control group was, however, small. Based on our findings, it seems worthwhile to study dopaminergic and serotonergic factors in a longer follow-up study with a larger PWS and control group. New insights in the functioning of the dopamine and serotonin system are warranted for the early identification and treatment of depression in people with PWS and thus the quality of life of people with PWS.

In conclusion, our study shows that none of the children with PWS fulfilled the criteria for depression, but the feeling of lack of friends, self-devaluation, difficulty in making decisions and being tired were reported. Tryptophan-ratio in children with PWS was higher than in healthy controls, but it was not related to depressive symptoms. However, children with more depressive symptoms had a lower tyrosine-ratio and HVA-level. Aromatic amino acid-levels were higher in children with PWS than in healthy controls, but levels of branched-chain amino acids were similar. Future research is needed to show if these findings could predict the increased incidence of depression in people with Prader-Willi syndrome later in life.

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REFERENCES

1. Cassidy SB 1997 Prader-Willi syndrome. J. Med. Genet. 34:917-923
2. Vogels A, Van Den Ende J, Keymolen K, Mortier G, Devriendt K, Legius E, Fryns JP 2004 Minimum prevalence, birth incidence and cause of death for Prader-Willi syndrome in Flanders. Eur. J. Hum. Genet. 12:238-240
3. Akefeldt A, Gillberg C, Larsson C 1991 Prader-Willi syndrome in a Swedish rural county: Epidemiological aspects. Dev. Med. Child Neurol. 33:715-721
4. Soni S, Whittington J, Holland AJ, Webb T, Maina E, Boer H, Clarke D 2007 The course and outcome of psychiatric illness in people with Prader-Willi syndrome: implications for management and treatment. J. Intellect. Disabil. Res. 51:32-42
5. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM 2011 Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32: 1729-1735
6. Kapur S, Remington G 1996 Serotonin-dopamine interaction and its relevance to schizophrenia. A. J. Psychiatry 153:466-476
7. Shi WX, Nathaniel P, Bunney BS 1995 Ritanserin, a 5-HT2A/2C antagonist, reverses direct dopamine agonist-induced inhibition of midbrain dopamine neurons. J. Pharmacol. Exp. Ther. 274: 735-740
8. Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD 1999 Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th ed. Philadelphia Lippincott-Raven
9. Kendler KS, Heninger GR, Roth RH 1982 Influence of dopamine agonists on plasma and brain levels of homovanillic acid. Life Sci. 30:2063-2069
10. Maes M, Wauters A, Verkerk R, Demedts P, Neels H, Van Gastel A, Cosyns P, Scharpe S, Desnyder R 1996 Lower serum L-tryptophan availability in depression as a marker of a more generalized disorder in protein metabolism. Neuropsychopharmacology 15:243-251
11. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Schroor EJ, Van Alfen AA, Van Leeuwen M, Van Pinxteren-Nagler E, Van Wieringen H, Vreuls RC, Zwaveling-Soonawala N, de Ridder MA, Hokken-Koelega AC 2013 Eight Years of Growth Hormone Treatment in Children With Prader-Willi Syndrome: Maintaining the Positive Effects. J. Clin. Endocrinol. Metab. 98:4013-4022
12. Timbremont B, Braet C, Roelofs J 2008 Children's Depression Inventory Manual. Amsterdam: Pearson Assessment and Information B.V.
13. Kovacs M 1992 Children's Depression Inventory. North Tonawanda, NY: Multi-Health Systems, Inc
14. Beck A, Steer RA, Brown GK 1996 Manual for the Beck Depression Inventory-II. San Antonio, TX: Psychological Corporation
15. Fekkes D 2012 Automated analysis of primary amino acids in plasma by high-performance liquid chromatography. Methods Mol. Biol. 828:183-200
16. Fekkes D, van Dalen A, Edelman M, Voskuilen A 1995 Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated pre-column derivatization with o-phthaldialdehyde. J. Chromatogr. B. Biomed. Appl. 669:177-186
17. Fekkes D, Timmerman L, Pepplinkhuizen L 1997 Effects of clomipramine on plasma amino acids and serotonergic parameters in panic disorder and depression. Eur. Neuropsychopharmacol. 7: 235-239

18. Hammarqvist F, Angsten G, Meurling S, Andersson K, Wernerman J 2010 Age-related changes of muscle and plasma amino acids in healthy children. Amino Acids 39:359-366
19. Lo ST, Siemensma E, Collin P, Hokken-Koelega A 2013 Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. Res. Dev. Disabil. 34:2764-2773
20. Veltman MW, Thompson RJ, Roberts SE, Thomas NS, Whittington J, Bolton PF 2004 Prader-Willi syndrome--a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. Eur. Child Adolesc. Psychiatry 13:42-50
21. Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP 2006 Pervasive developmental disorders in Prader-Willi syndrome: the Leuven experience in 59 subjects and controls. American Journal of Medical Genetics Part A 140:1136-1142
22. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
23. Lo ST, Festen DAM, Tummers-de Lind van Wijngaarden RFA, Collin PJL, Hokken-Koelega ACS 2015 Beneficial effects of long-term growth hormone treatment on adaptive functioning in infants with Prader-Willi syndrome. American Journal on Intellectual and Developmental Disabilities:In press
24. Lo ST, Siemensma EP, Festen DA, Collin PJ, Hokken-Koelega AC 2014 Behavior in children with Prader-Willi syndrome before and during growth hormone treatment: a randomized controlled trial and 8-year longitudinal study. Eur. Child Adolesc. Psychiatry
25. Einfeld SL, Smith A, Durvasula S, Florio T, Tonge BJ 1999 Behavior and emotional disturbance in Prader-Willi syndrome. Am. J. Med. Genet. 82:123-127
26. Festen DA, de Weerd AW, van den Bossche RA, Joosten K, Hoeve H, Hokken-Koelega AC 2006 Sleep-related breathing disorders in prepubertal children with Prader-Willi syndrome and effects of growth hormone treatment. J. Clin. Endocrinol. Metab. 91:4911-4915
27. Haqq AM, Stadler DD, Jackson RH, Rosenfeld RG, Purnell JQ, LaFranchi SH 2003 Effects of growth hormone on pulmonary function, sleep quality, behavior, cognition, growth velocity, body composition, and resting energy expenditure in Prader-Willi syndrome. Journal of Clinical Endocrinoly and Metabolism 88:2206-2212
28. Maas AP, Sinnema M, Didden R, Maaskant MA, Smits MG, Schrander-Stumpel CT, Curfs LM 2010 Sleep disturbances and behavioural problems in adults with Prader-Willi syndrome. J. Intellect. Disabil. Res. 54:906-917
29. Nixon GM, Brouillette RT 2002 Sleep and breathing in Prader-Willi syndrome. Pediatr. Pulmonol. 34:209-217
30. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314
31. Akefeldt A, Ekman R, Gillberg C, Mansson JE 1998 Cerebrospinal fluid monoamines in Prader-Willi syndrome. Biol. Psychiatry 44:1321-1328
32. McCracken JT, McGough J, Shah B, Cronin P, Hong D, Aman MG, Arnold LE, Lindsay R, Nash P, Hollway J, McDougle CJ, Posey D, Swiezy N, Kohn A, Scahill L, Martin A, Koenig K, Volkmar F, Carroll D, Lancor A, Tierney E, Ghuman J, Gonzalez NM, Grados M, Vitiello B, Ritz L, Davies M, Robinson J,

McMahon D 2002 Risperidone in children with autism and serious behavioral problems. N. Engl. J. Med. 347:314-321

33. Owen R, Sikich L, Marcus RN, Corey-Lisle P, Manos G, McQuade RD, Carson WH, Findling RL 2009 Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. Pediatrics 124:1533-1540
34. Fletcher R 2007 Diagnostic Manual- Intellectual Disability: A Textbook of Diagnosis of Mental Disorders in Persons with Intellectual Disability. New York: NADD Press/National Association for the Dually Diagnosed

CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

GENERAL DISCUSSION

In November 2010, the study resulting in this thesis was started to investigate psychiatric disorders (PD) in children with Prader Willi syndrome (PWS), also called PD-study. The participating children originated from the Dutch randomized controlled growth hormone trial and the ongoing Dutch PWS Cohort study. The PD-study was started due to increasing number of questions by parents and health professionals about behavioral problems and psychiatric symptoms in children with PWS. Although knowledge about these two topics was increasing, most studies were conducted in adults or in a wide age-range and growth hormone (GH) status was often unknown.
This thesis describes the prevalence of psychiatric disorders, the behavioral phenotype and level of adaptive functioning in children with PWS. In addition, we investigated the effect of long-term growth hormone treatment on behavior and adaptive functioning.
The present chapter discusses the findings of our studies in view of the current literature. Additionally, clinical implications of our results are presented and recommendations for future research are provided.

8.1 Psychiatric disorders

People with PWS have an increased risk for psychiatric disorders. Those with mUPD are more prone to a psychosis than those with a deletion (1-4). However, studies about psychiatric disorders in children with PWS were very rare (5, 6). We therefore conducted a study to investigate the prevalence of psychiatric disorders in 61 children with PWS. We found that none of the children (median age 11.8 years) developed schizophrenia or other psychotic symptoms during 2 years of follow-up, and that only 2 boys had psychotic symptoms prior to the study, of which one had a relapse of psychotic symptoms during the study. The most common behavioral disorder was oppositional defiant disorder, found in 20% of our study group.
In contrast to earlier reports in adults, we did not find psychotic symptoms in children with PWS, except in the abovementioned boy with the relapsing symptoms. One explanation could be that our study group is relatively young. On the other hand, the age at onset of psychosis has been reported between 13 and 19 years (4). Another explanation could be the structured multidisciplinary care for our study group. All children were 3-monthly seen by their pediatricians and the Dutch PWS team (7). The guidance by a multidisciplinary team, early start of physiotherapy, speech therapy, family and social support might have improved the mental health of these children and prevented psychiatric illness. Also, children were referred to a child-psychiatrist when any sign of psychopathology was suspected. Three children were treated with psychotropic medication prior to this study due to increased irritability, aggression and compulsive behavior. Early treatment might have prevented the first signs or deterioration of

psychiatric disorders. An alternative explanation could be that GH contributed to the neurodevelopment, leading to a lower risk for psychiatric disorders as we also found that long-term GH treatment improved cognitive functioning (8).
The prevalence of oppositional defiant disorder (ODD) in children with PWS aged 7 to 17 years was 20%. The presence of ODD was not associated with age, gender, genetic subtype or IQ. A child with ODD increases stress in the family (9, 10). It is therefore important to be aware of ODD in children with PWS, in order to prevent overestimation of the child's capacity with regard to language skills, emotional skills and social functioning. It also means that families require practical advices how to approach their child. Overestimation could be decreased by breaking down larger tasks into clearly defined small steps, and allowing more time to rest. Communication with the child with PWS should be clear and concise by describing tasks simply, using short sentences, and avoiding abstract language (11).
As reported earlier, we also found a high rate of hoarding/collecting objects and insisting on (hygienic) routines in children with PWS (12, 13), which was not different between children with an mUPD or DEL. Parents mentioned that their child with PWS often resumed compulsive activity after interruption, and often waited with resuming until the parent had left the immediate area. The majority of children with PWS would not hit or kick the parent who intervened and would not hurt themselves after interruption of a compulsion.

Our study shows the prevalence of psychiatric and behavioral disorders in children with PWS. Schizophrenia and other psychotic symptoms were rare in our large group of children with PWS who were all treated with long-term GH. Oppositional defiant disorder was the most common behavioral disorder which requires practical advices and guidance for parents and carers of children with PWS.

8.2 Theory of Mind and Autism Spectrum Disorder

There was very limited information about the Theory of Mind (ToM) development and prevalence of autism spectrum disorder (ASD) in children with PWS. The Theory of Mind describes the level of emotion recognition, awareness of ones own thoughts and of others, abstract language usage, and the ability to feel empathy for others. In this study, we investigated the ToM and the prevalence and symptoms of ASD. Our results demonstrated that children with PWS are severely impaired in their ToM-development. More delay in ToM was associated with an older age and lower total IQ, but not with genetic subtype. Additionally, we found that 36% of children with PWS fulfills the criteria for ASD. This was also not associated with genetic subtype. Within ASD, symptoms of maladaptive behavior were most prominent.

Our finding of a higher ToM in those with a higher IQ is in line with previous studies in people with an intellectual disability (14-16). This raises the question if the impaired ToM in our study group is affected by PWS specifically, or by the intellectual disability due to PWS. In contrast to our findings, an earlier study found more problems in ToM in those with an mUPD (17). The marked delay in ToM requires an adjusted approach of children with PWS. Children with PWS are impaired in feeling empathy for others and have therefore difficulties in social reciprocity. Family and carers should be informed about the delayed ToM development and be aware of the risk to overestimate the empathetic abilities of their child with PWS.
The prevalence of 36% having ASD was in line with earlier studies (18, 19). Children with PWS show, however, an atypical profile of ASD, i.e. they especially have difficulties in maladaptive behavior and not in being obsessed about light and sound or making contact with others. Although 36% of our participants fulfilled the criteria for ASD, even more children had autistic symptoms but did not meet all criteria for ASD. Thus, many more than one-third of children with PWS would benefit from a structured approach as used in children with autism. We could not confirm that ASD is more common in those with an mUPD (17). It might be that the long-term GH treatment in our study group had also a beneficial effect on social cognitive functioning, especially for those with more social impairments. This would be in accordance with our previous study showing that long-term GH treatment improves cognition in children with PWS (8).

Our study demonstrates that children with PWS have a markedly impaired Theory of Mind. In addition, 36% of children fulfills the criteria for Autism Spectrum Disorder, in which maladaptive behavior is the most prominent impairment. These findings highlight the need for caretakers to adjust to the child's level of social cognitive functioning to prevent overestimation of the empathetic and abstract language abilities in the child with PWS.

8.3 Behavior

Information about 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 at least 8 years of continuous GH treatment.
We found that children with PWS display similar problem behavior as a reference population with a comparable intellectual disability. However, they showed particularly problems with social abilities as problem behavior, indicating that there is a more socially-related problem behavior. In contrast to our expectations, which were based on parental reports and our clinical experience, our study did not show improvement

but also no deterioration during long-term GH treatment on behavioral problems in children with PWS.

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 (8). Similar findings were found in short children born small for gestational age (SGA). In short children born SGA, long-term GH treatment had beneficial effect on cognition, while attention deficits, especially accurateness and impulsiveness did not change during GH treatment (20, 21). The authors concluded that attention deficits were related to being born SGA, and we might draw a comparable conclusion for the behavioral problems in 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 children with PWS on a 3-point scale ('0=not true', '1=sometimes or somewhat true', '2=often true or very true') in both questionnaires. On this sort of scale, it is only possible for parents to indicate if certain behaviors occur 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 (6), 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 disappear completely. 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 prior to start of treatment. Remarkably, most parents did notice subtle changes, which they reported during their visits to our hospital. Our data are in line with those of a 2-year GH-controlled study in children with PWS (22), in which parents reported improved behavior during GH treatment during open interviews, while this was not the case in the untreated controls. Also in their study, structured behavioral questionnaires with a 3-point scale did not show differences in behavior between GH treated and untreated patients.

Behavioral problems in children with PWS were comparable to children with a similar intellectual disability, but they had relatively more socially-related problems. Children with PWS experienced particularly more problems in being tuned to the social situation, and understanding social information and had more stereotyped behavior. This social impairment is typically found in people 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) (11, 23, 24).

In conclusion, our study shows that problem behavior in children with PWS is comparable with a reference population with a comparable intellectual disability. Within problem behavior, socially-related problems were the most prominent ones. Parents

noticed improvements in behavior during GH treatment, but tests did not confirm an improvement of behavioral problems in children with PWS, albeit also no deterioration.

8.4 Adaptive functioning

Only few studies investigated the adaptive functioning in people with PWS (17, 25). Adaptive functioning describes the ability to deal with daily activities which are required in the personal and sociocultural environment and forms an essential part of the behavioral phenotype (26). It includes skills in communication (receptive, expressive, and written), daily living (personal care, domestic tasks and within the community), social functioning (interpersonal relationships, play and leisure time, and coping skills), and motor functioning (gross and fine). We investigated the level of adaptive functioning and the effect of short-term and long-term GH treatment on adaptive functioning in children with PWS aged 7 to 17 years. Our results showed that children with PWS are impaired in their adaptive skills. The developmental delay was larger when children were older or had a lower IQ. Although no effect of short-term GH treatment was found on adaptive functioning, we showed that an earlier age at start of GH treatment during infancy was associated with improved adaptive functioning after long-term GH treatment.

Our data are in line with earlier findings that children with PWS have a marked delay in adaptive functioning, including social functioning (5, 27). Additionally, social disabilities in PWS are more impaired than in other children with intellectual disability, indicating that the impaired social functioning is specific for PWS (10, 23). In our large group of children with PWS, we did not find a difference in daily living skills between those with a deletion and mUPD, which is in contrast to an earlier study (17).

The maladaptive functioning has a major impact on the daily life of children with PWS and their families. Not only does it highlight the need to adjust daily tasks to the level of the child, it has also consequences for the care by parents and carers. Although some children were able to perform some adaptive skills themselves, they needed supervision and it was difficult to perform the tasks within a certain time limit. Our finding that an earlier start of GH treatment during the infancy improves the adaptive skills on the long-term is therefore important to improve the quality of life of the children with PWS and their families.

Our study demonstrates that children have a marked delay in adaptive functioning, and supervision and adjustments are usually required. The earlier the GH treatment was started during infancy, the better the adaptive skills on the long-term.

8.5 Visual-motor Integration

We also investigated the visual-motor integration in 73 children with PWS. Our data showed that the level of visual-motor integration was 3 standard deviations lower compared to healthy references. Older children, those with a DEL or a higher IQ were less impaired in visual-motor integration. Simplifying visual-motor tasks by dividing tasks into a visual task and a motor task resulted in better scores of approximately 1 standard deviation lower compared to healthy references for both tasks, indicating that children with PWS have problems with combining these tasks at the same moment. A follow-up of 2 years did not show changes in visual-motor integration in children with PWS.

Impairment in visual-motor integration has implications in daily life. Most tasks at school require visual-motor integration, such as writing, mathematic formulations, and drawing. Our data show that it is important to simplify tasks by breaking them into visual tasks and motor tasks, and allow the child to acquire these separate tasks first. After obtaining these individual skills, tasks requiring both visual and motor integration, should be introduced as the final step.

VMI performance was strongly associated with total IQ, which is in line with an earlier report in children with intellectual disability (28), suggesting that intelligence might be particularly related to the planning stage of the VMI task and not so much to the motor component of the task. Children with DEL scored higher on VMI and visual perception than those with mUPD, which is in line with an earlier finding that children with DEL have stronger visual skills (19, 29)

This study shows that the visual-motor integration in children with PWS is very impaired. It is therefore important to simplify tasks into purely visual and motor tasks as it will improve performance. We suggest to implement this in the learning process of the child with PWS, as it will improve their daily functioning.

8.6 Depressive symptoms and the dopamine and serotonin system

People with PWS have an increased risk in developing depression compared to a healthy population. It has been described that changes in the dopamine and serotonin system are related to depression. In this explorative study, we investigated depressive symptoms and precursors and metabolites of dopamine (i.e. tyrosine and HVA) and serotonin (i.e. tryptophan) in plasma of in children with PWS. We found that none of the children with PWS fulfilled all criteria for a depression. However, approximately half of the children reported the feeling of lack of friends. Weaknesses in social skills are part of the behavioral phenotype of PWS and reported by parents as well (17, 19, 30). Our assessment shows that children with PWS were aware of their limited number of friends, but their social deficits challenged them to make friends.

Plasma tyrosine was higher in children with PWS than in healthy controls, but tyrosine-ratio, an indicator of tyrosine availability for central dopamine synthesis, and HVA-levels were normal. A lower tyrosine-ratio was related with more depressive symptoms. Plasma tryptophan and tryptophan-ratio, an indicator of tryptophan availability for central serotonin synthesis, were higher in children with PWS than in healthy controls. Our finding that the tryptophan-ratio is increased in children with PWS suggests a possible dysfunction in the serotonin system in children with PWS. One explanation could be that the serotonin system in children with PWS is compensating for a serotonin deficiency in the brain, resulting in increased levels of the serotonin precursor. However, a serotonin deficiency seems unlikely as higher serotonin metabolites (5-HIAA) in cerebrospinal fluid were found by Akefeldt et al. (31). Remarkably, the aromatic amino acid-levels (tyrosine, tryptophan, and phenylalanine) were higher than in healthy children, but this was not the case for branched-chain amino acids. The exact mechanism behind the increased levels of the aromatic amino acids, tryptophan-ratio and serotonin metabolite in PWS remain, however, unknown. Further investigation is warranted as it might lead to early identification and treatment of depression in people with PWS and thus improve the quality of life of people with PWS.

Our exploratory study did not find depression in children with PWS, but we found depressive symptoms. We also found changes in the plasma precursors and metabolites of the dopamine and serotonin system which might contribute to the increased risk for a depression in people with PWS later in life.

8.7 General conclusions

In this thesis, we described several aspects of the psychiatric and behavioral phenotype in children with PWS, such as social functioning, psychiatric disorders, behavior, adaptive functioning, visual-motor integration and depressive symptoms and its relation to the dopamine and serotonin system, using specific tools in a large cohort of children with PWS participating in the Dutch PWS studies.

We found more problems and delay in social development than we expected. Our study showed that the Theory of Mind-development in children with PWS was 3 standard deviation scores lower compared with healthy peers and autism spectrum disorder was found in 36% of children with PWS. These social disabilities increase the risk of overestimation of the child by its environment, leading to more stress with a higher susceptibility to behavioral problems or even psychiatric disorders. Our findings have therefore important implications for the guidance of the child and adolescent with PWS. First, it is important to inform parents, family, and other caretakers about the delay in social and adaptive skills in PWS, and how to approach the child. Our findings of the Theory of Mind assessment showed that children with PWS have difficulties in feeling

empathy for others and have problems in social reciprocity. Most children were able to recognize emotions and aware of their own thinking (e.g. 'I think it rains'), but it was difficult for them to be aware of the thoughts of others (e.g. 'I think that John thinks it is raining'). Carers should therefore be aware of these social limitations and be concise in their communication. Second, one third of children with PWS fulfilled the criteria for autism spectrum disorder, but an even larger percentage showed autistic traits without fulfilling the diagnosis. A structured approach in communication and daily life, as being used in children with autism, could improve the daily life of children with PWS. Third, we found that an earlier start of GH treatment was associated with less delay in adaptive functioning on the long-term. GH treatment appears thus not only beneficial for body composition, bone mineral density, motor skills and cognition, but also for adaptive functioning.

The present study also showed that psychotic symptoms or depression were rare in children with PWS, but the prevalence of oppositional defiant disorder was unexpectedly high. This finding stresses once again that raising a child with PWS is challenging, especially if the child is showing oppositional defiant behavior. Families should be aware of the high prevalence of this behavior and receive practical advices how to approach the child.

At last but not least, we found increased levels of the serotonin precursor tryptophan and its ratio in children with PWS. An earlier study also found increased levels of the serotonin metabolite 5-HIAA in cerebrospinal fluid (31). These findings suggest that the serotonin system might function suboptimally in children with PWS, although the exact mechanism remains unknown. Further investigations are warranted to study if these changes could explain the increased risk for a depression in people with PWS later in life.

8.8 Practical consequences for parents and carers

Our findings on ToM are essential in the approach of these children. Both children with DEL and mUPD are delayed in ToM development, which might result in an increased risk to overestimate their empathetic abilities. Due to the relatively stronger verbal development of children with mUPD compared to those with DEL, children with mUPD are often overestimated in their ability to feel empathy for others. Most children with PWS did not have any problems in recognizing emotions, but false belief (the individual's comprehension of another person's mistaken belief) was more difficult. The most difficult was second belief, which is the inference of someone's belief about another's belief such as 'I think that you think…'. As major impairments were found in the ToM, carers should adjust their communication and mention why and what they are thinking. For example, instead of saying "Clean up your toys" to the child with PWS, the caretaker should say: "John, put your toys in the basket in the coming five minute". The latter communication will be much clearer for children with PWS because the caretaker's thinking is said

aloud which is much easier for the child to understand. The theory of this way of communication is explained in the Dutch book called 'Geef me de Vijf' ('Give me the Five') by Colette de Bruin, who introduced to use the five elements: who, what, where, when and how for structured communication as used in children with autism. When children show increased irritability or more behavioral problems, we suggest to determine the developmental level of the child, including social cognition. These developmental assessments are useful to inform parents and carers about the child's developmental age and how to approach their child. This will contribute in understanding the child to prevent overestimation which could result in frustration and bursts of emotional disturbance such as temper tantrums.

Approximately one-third of children with PWS had ASD, but even a greater percentage showed autistic traits. We therefore advise parents to implement structure in the child's daily living, such as in communication (using the five elements of who, what, where, when and how), in daily routines and tasks.

Children with intellectual disability can experience their daily life in another perspective than their healthy peers and events or changes which will be unnoticed by people with normal intelligence, can be highly stressful for children with PWS (such as holidays, changing of seasons, Sinterklaas, and fireworks, but also unplanned visits or changes during the day). Due to the intellectual disability and the hypotonia in children with PWS, adaptive functioning is also delayed. The early start of GH treatment should be considered to improve adaptive functioning on the long-term, besides the beneficial effects of growth hormone on body composition, bone mineral density, motor abilities and cognition

It remains challenging to find the right balance between stimulating adaptive skills in children with PWS and protecting them from danger. Certain 'risky' daily livings skills such as cooking, being part of traffic by walking or cycling alone, or using money are often activities that are avoided by parents to prevent their children from dangerous situations or troubles.

Visual-motor integration is an important skill in daily life. We found that combining visual skills and motor skills at the same time is very difficult for the children. In the learning process of writing and daily skills, we suggest to allow the children to acquire the visual and motor skills first and to allow them sufficient time to learn and later on to complete their visual-motor tasks.

8.8 Directions for future research

In this thesis, we presented the impaired Theory of Mind (ToM)-development in children with PWS. As the ToM is fundamental in social functioning, it may be worthwhile to investigate if ToM-development could be improved by providing a ToM-training to children with PWS. Some children are already introduced to ToM-training by family or

school. Studying the effect of a ToM-training in children with PWS may result in new insights how to guide children with PWS in their social impairments.

We investigated behavioral problems in children with PWS and found that oppositional defiant disorder was present in one of five children. Further follow-up of these children is needed to investigate if they are more prone to psychotic disorders later in life. Psychotic disorders were rare in our study group aged 7 to 17 years. Future studies should include more adolescents and young adults to investigate at what age psychiatric problems do occur, and if GH treatment can prevent or limit the severity of psychiatric disorders in young adults with PWS. Clinically, it is also important to investigate the effect of testosterone replacement therapy on behavior, as parents and health professionals may unnecessarily limit the prescription of testosterone replacement due to concerns about deterioration of behavior. Selective serotonin reuptake inhibitors (SSRIs) are prescribed to children with PWS who experience mood disorders, but also in case of increased irritability or compulsive behavior. The effect of SSRIs on these disorders has, however, not yet been studied in children with PWS.

Our previous study showed beneficial effects of long-term GH treatment on cognitive functioning (8). This might be due to a beneficial effect of GH on brain structures and networks involved in cognition or behavior. Brain imaging studies in patients with PWS will provide more insight in the relation between brain abnormalities and cognitive dysfunctions or behavioral problems and how GH affects the brain and behavior.

PD-STUDY

Psychiatric disorders

- Psychotic symptoms and depression are uncommon
- 20% of children with PWS fulfills criteria for oppositional defiant disorders
- ► Be aware of overestimation of mental and physical skills
- ► Guidance for families how to approach child

Behavior

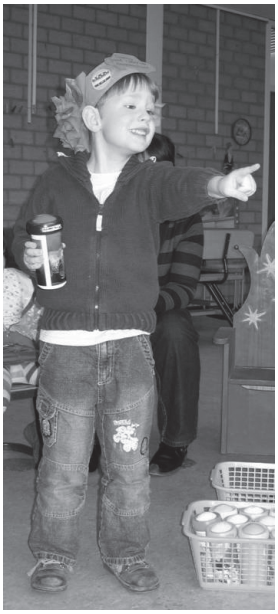
- Similar problem behavior as children with comparable intellectual disability
- Problems with social abilities most pronounced
- No effect during short- or long-term GH treatment
- ► No deterioration of behavior during long-term GH treatment

Visual-motor integration (VMI)

- VMI is severely impaired
- Simplifying into pure visual or motor tasks improves VMI
- ► Allow more time for VMI-tasks
- ► Allow to acquire visual and motor skills first

Adaptive functioning

- Severe delay in adaptive functioning
- More delay with older age and lower IQ
- No effect during short-term GH treatment
- Less delay when GH treatment was started at earlier age
- ► Beneficial effect of early start of GH on adaptive skills



Theory of Mind (ToM) and Autism Spectrum Disorder (ASD)

- Severe delay in ToM
- More delay in ToM with older age and lower IQ
- 36% have ASD
- ASD mainly due to maladaptive behavior
- ► Be aware of overestimation in social cognition
- ► Adjusted approach including
  - structured communication
  - adjustments to the child's level at home and school
  - being clear and consistent

Depression and dopamine and serotonin system

- No depression found in children with PWS
- Aromatic amino acid-levels are higher than in healthy controls
- Dopamine: more depressive symptoms correlated with lower plasma tyrosine-ratio
- Serotonin: tryptophan-ratio significantly higher than in healthy controls

REFERENCES

1. Boer H, Holland A, Whittington J, Butler J, Webb T, Clarke D 2002 Psychotic illness in people with Prader Willi syndrome due to chromosome 15 maternal uniparental disomy. Lancet 359:135-136
2. Sinnema M, Boer H, Collin P, Maaskant MA, van Roozendaal KE, Schrander-Stumpel CT, Curfs LM 2011 Psychiatric illness in a cohort of adults with Prader-Willi syndrome. Res. Dev. Disabil. 32: 1729-1735
3. Soni S, Whittington J, Holland AJ, Webb T, Maina E, Boer H, Clarke D 2007 The course and outcome of psychiatric illness in people with Prader-Willi syndrome: implications for management and treatment. J. Intellect. Disabil. Res. 51:32-42
4. Vogels A, Matthijs G, Legius E, Devriendt K, Fryns JP 2003 Chromosome 15 maternal uniparental disomy and psychosis in Prader-Willi syndrome. J. Med. Genet. 40:72-73
5. Skokauskas N, Sweeny E, Meehan J, Gallagher L 2012 Mental health problems in children with prader-willi syndrome. J Can Acad Child Adolesc Psychiatry 21:194-203
6. Descheemaeker MJ, Vogels A, Govers V, Borghgraef M, Willekens D, Swillen A, Verhoeven W, Fryns JP 2002 Prader-Willi syndrome: new insights in the behavioural and psychiatric spectrum. J. Intellect. Disabil. Res. 46:41-50
7. Bakker NE, Kuppens RJ, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Bindels-de Heus GC, Bocca G, Haring DA, Hoorweg-Nijman JJ, Houdijk EC, Jira PE, Lunshof L, Odink RJ, Oostdijk W, Rotteveel J, Schroor EJ, Van Alfen AA, Van Leeuwen M, Van Pinxteren-Nagler E, Van Wieringen H, Vreuls RC, Zwaveling-Soonawala N, de Ridder MA, Hokken-Koelega AC 2013 Eight Years of Growth Hormone Treatment in Children With Prader-Willi Syndrome: Maintaining the Positive Effects. J. Clin. Endocrinol. Metab. 98:4013-4022
8. Siemensma EP, Tummers-de Lind van Wijngaarden RF, Festen DA, Troeman ZC, van Alfen-van der Velden AA, Otten BJ, Rotteveel J, Odink RJ, Bindels-de Heus GC, van Leeuwen M, Haring DA, Oostdijk W, Bocca G, Mieke Houdijk EC, van Trotsenburg AS, Hoorweg-Nijman JJ, van Wieringen H, Vreuls RC, Jira PE, Schroor EJ, van Pinxteren-Nagler E, Pilon JW, Lunshof LB, Hokken-Koelega AC 2012 Beneficial Effects of Growth Hormone Treatment on Cognition in Children with Prader-Willi Syndrome: A Randomized Controlled Trial and Longitudinal Study. J. Clin. Endocrinol. Metab. 97: 2307-2314
9. Mazaheri MM 2010 Quality of life and caregiving in families of children diagnosed with prader-willi syndrome. Dissertation Abstracts International: Section B: The Sciences and Engineering 71: 2029
10. Wulffaert J, Scholte EM, Van Berckelaer-Onnes IA 2010 Maternal parenting stress in families with a child with Angelman syndrome or Prader-Willi syndrome. J Intellect Dev Disabil 35:165-174
11. Lo ST, Siemensma E, Collin P, Hokken-Koelega A 2013 Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. Res. Dev. Disabil. 34:2764-2773
12. Clarke DJ, Boer H, Whittington J, Holland A, Butler J, Webb T 2002 Prader-Willi syndrome, compulsive and ritualistic behaviours: the first population-based survey. Br. J. Psychiatry 180:358-362
13. Dimitropoulos A, Blackford J, Walden T, Thompson T 2006 Compulsive behavior in Prader-Willi syndrome: examining severity in early childhood. Res. Dev. Disabil. 27:190-202
14. Abbeduto L, Short-Meyerson K, Benson G, Dolish J 2004 Relationship between theory of mind and language ability in children and adolescents with intellectual disability. J. Intellect. Disabil. Res. 48:150-159

15. Lorusso ML, Galli R, Libera L, Gagliardi C, Borgatti R, Hollebrandse B 2007 Indicators of theory of mind in narrative production: a comparison between individuals with genetic syndromes and typically developing children. Clin Linguist Phon 21:37-53
16. Muris P, Steerneman P, Meesters C, Merckelbach H, Horselenberg R, van den Hogen T, van Dongen L 1999 The TOM test: a new instrument for assessing theory of mind in normal children and children with pervasive developmental disorders. J. Autism Dev. Disord. 29:67-80
17. Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, Bellamy M, Curran SR, Sporikou CM, Bolton PF 2005 Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry 46:1089-1096
18. Veltman MW, Craig EE, Bolton PF 2005 Autism spectrum disorders in Prader-Willi and Angelman syndromes: a systematic review. Psychiatr. Genet. 15:243-254
19. Veltman MW, Thompson RJ, Roberts SE, Thomas NS, Whittington J, Bolton PF 2004 Prader-Willi syndrome--a study comparing deletion and uniparental disomy cases with reference to autism spectrum disorders. Eur. Child Adolesc. Psychiatry 13:42-50
20. van der Reijden-Lakeman IE, de Sonneville LM, Swaab-Barneveld HJ, Slijper FM, Verhulst FC 1997 Evaluation of attention before and after 2 years of growth hormone treatment in intrauterine growth retarded children. J. Clin. Exp. Neuropsychol. 19:101-118
21. van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC 2004 Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. Journal of Clinical Endocrinoly and Metabolism 89:5295-5302
22. Whitman BY, Myers S, Carrel A, Allen D 2002 The behavioral impact of growth hormone treatment for children and adolescents with Prader-Willi syndrome: a 2-year, controlled study. Pediatrics 109:E35
23. Koenig K, Klin A, Schultz R 2004 Deficits in social attribution ability in Prader-Willi syndrome. J. Autism Dev. Disord. 34:573-582
24. Clarke DJ, Boer H, Chung MC, Sturmey P, Webb T 1996 Maladaptive behaviour in Prader-Willi syndrome in adult life. J. Intellect. Disabil. Res. 40 (Pt 2):159-165
25. Di Nuovo S, Buono S 2011 Behavioral phenotypes of genetic syndromes with intellectual disability: comparison of adaptive profiles. Psychiatry Res. 189:440-445
26. Sparrow SS, Balla DA, Cicchetti D 1984 Vineland Adaptive Behavior Scales-Revised. Circle Pines, Minnesota: American Guidance Service
27. Dimitropoulos A, Ho A, Feldman B 2012 Social Responsiveness and Competence in Prader-Willi Syndrome: Direct Comparison to Autism Spectrum Disorder. J. Autism Dev. Disord.
28. Memisevic H, Sinanovic O 2012 Predictors of visual-motor integration in children with intellectual disability. Int. J. Rehabil. Res.
29. Dykens EM 2002 Are jigsaw puzzle skills 'spared' in persons with Prader-Willi syndrome? J. Child Psychol. Psychiatry 43:343-352
30. Descheemaeker MJ, Govers V, Vermeulen P, Fryns JP 2006 Pervasive developmental disorders in Prader-Willi syndrome: the Leuven experience in 59 subjects and controls. American Journal of Medical Genetics Part A 140:1136-1142
31. Akefeldt A, Ekman R, Gillberg C, Mansson JE 1998 Cerebrospinal fluid monoamines in Prader-Willi syndrome. Biol. Psychiatry 44:1321-1328

CHAPTER 9

SUMMARY / SAMENVATTING

SUMMARY

Chapter 1

This chapter provides information on the genetic cause, symptoms, behavioral phenotype and psychiatric disorders in children and adolescents with Prader-Willi syndrome (PWS).

Chapter 2

Reports about psychiatric disorders in children with PWS are very limited. We therefore studied 61 children with PWS aged 7 to 17 years using the Diagnostic Interview Schedule for Children (DISC) and Compulsive Behaviour Checklist (CBC). Thirty-eight of 61 children were retested after 2 years. Prior to the study, only two boys were known with psychotic symptoms and treated with antipsychotics. At baseline, none of the other children fulfilled the criteria for schizophrenia or other psychotic disorders. After 2 years, none of the children had schizophrenia or other psychotic disorders. Oppositional defiant disorder (ODD) was the most common diagnosis and present in 20% of children with PWS, and was not associated with age, gender, genetic subtype, or total IQ. The most common compulsions were hoarding and fixed hygiene sequences.
In conclusion, in our large group of 61 children with PWS, 59 had no psychotic disorder. Oppositional defiant disorder was the most common disorder. The prevalence of the psychiatric disorders did not change during the 2-year follow-up.

Chapter 3

Social cognitive functioning was studied by testing the Theory of Mind (ToM) and symptoms of Autism Spectrum Disorder (ASD) in children with Prader-Willi syndrome (PWS). The ToM describes the cognitive capacity to infer in the mental state of oneself and others, i.e. the ability to be conscious of 'I think that …', 'I think that you think…' and 'I think that you think that he thinks ….'. We studied 66 children with PWS aged 7 to 17 years with growth hormone treatment and used the Theory of Mind test-R and the Diagnostic Interview Social Communication disorders. Median Total ToM score was 3 standard deviations lower than healthy peers. The ToM score was positively associated with total IQ, in particular verbal IQ. We found no difference in Total ToM SDS between children with a deletion and those with an mUPD. One-third of children with PWS scored positive for ASD. The prevalence of ASD was not associated with total IQ and did not differ between genetic subtypes, or boys and girls. Most prominent aberration in ASD was maladaptive behavior.
In conclusion, our findings demonstrate a markedly reduced level of social cognitive functioning, which has consequences for the approach of children with PWS. Parents

and caretakers are advised to test the developmental age of children with PWS and to adjust to the child's level of social functioning in communication and social demands.

Chapter 4

Based on clinical experience and parents' reports, we expected that behavior would improve after long-term growth hormone treatment in children with PWS. In this study, we investigated behavior in children with PWS and the effects of growth hormone treatment in 44 prepubertal children aged 3.5 to 14 years in a randomized controlled trial during 2 years (RCT), followed by a longitudinal study during 8 years of growth hormone treatment. Behavior was measured annually by the Developmental Behavior Checklist for children with intellectual 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 children with a comparable intellectual disability. However, they showed particularly problems with social abilities, indicating that there is a more socially-related problem behavior. A lower IQ was associated with more self-absorbed behavior, more communication problems and more problem behavior in general. Problem behavior measured by the CSBQ was similar compared to children with a comparable intellectual disability, but children with PWS scored significantly higher on the 'Not tuned', 'Understanding' (understanding social information such as jokes or main conclusion from stories), and 'Stereotyped' subscales than the CSBQ total score.

In contrast to our experience and parents reports, our study shows no improvement but also no deterioration of behavioral problems in children with PWS during long-term GH treatment.

In conclusion, children with PWS showed similar problem behavior as a reference population with a comparable intellectual disability. Social problems were the most pronounced in children with PWS. In contrast to our expectations and parents' reports, our study showed no improvement during long-term growth hormone treatment, but also no deterioration of their behavioral problems. It might be that the questionnaires were not sufficiently sensitive to find the subtle improvements of behavior during growth hormone treatment as was reported by parents.

Chapter 5

The aim of this study was to investigate the effect of growth hormone treatment on adaptive functioning in children with PWS. Adaptive functioning describes the ability to deal with daily activities including skills in communication (receptive, expressive, and written), daily life (personal care, domestic tasks and within the community), social functioning (interpersonal relationships, play and leisure time, and coping skills), and motor functioning (gross and fine). We used the Vineland Adaptive Behavior Scale (VABS) to

assess adaptive functioning during a randomized controlled trial and after 7 years of growth hormone treatment. In the RCT, 75 children (42 infants and 33 prepubertal children) with PWS were included. Subsequently, 53 children were treated with long-term growth hormone. Our study demonstrates a marked delay in adaptive functioning in infants and children with Prader-Willi syndrome, which was associated with older age and lower intelligence. Results show that the earlier growth hormone treatment was started during infancy, the better the adaptive skills were on the long-term.
In conclusion, adaptive functioning in children with PWS is impaired, but an earlier start of growth hormone treatment during infancy results in better adaptive skills on the long-term.

Chapter 6

Information about visual-motor integration (VMI) in children with PWS was scarce. We determined VMI in 73 children with PWS aged 7 to 17 years who were all treated with growth hormone. Beery Visual-motor Integration test, which consisted of a visual-motor integration test (no time-limit), a visual integration subtest and a motor perception subtest (both limited in time) were assessed at start of the study and after 2 years. Children with PWS scored 3 standard deviations lower in visual-motor integration and 1 standard deviation lower in visual perception and motor coordination compared to healthy children. Scores on visual-motor integration were higher in children with a deletion, in older children, and in those with a higher total IQ. Scores on visual perception were higher in children with a deletion and higher total IQ, but scores on motor coordination were only higher in those with a higher total IQ. Visual perception and motor coordination were not associated with age or gender. Neither visual perception and motor coordination, nor visual-motor integration had improved in the second assessment after 2 years.
In conclusion, the VMI is poor in children with PWS. Children scored higher on the time-limited subtests for visual perception and motor coordination than on the combined test for visual-motor integration, indicating that children with PWS have problems combining these tasks at the same moment. Separation of visual and motor tasks prior to the integration of visual and motor tasks and allowing sufficient time to learn and perform these tasks will improve daily activities, both at home and at school.

Chapter 7

No studies had investigated whether depressive symptoms correlate with biomarkers of the dopamine and serotonin system in children with PWS. In this exploratory study, we investigated depressive symptoms using the Dutch version of the Children's Depression Inventory (n=30), and determined precursors and a metabolite of the dopamine and serotonin system during 2 years in children with PWS aged 7 to 17 years (n=68) compared to healthy controls (n=10). None of the children fulfilled the criteria for the

diagnosis of depression. The four most rated depressive symptoms were lack of friends (57% of the children), self-devaluation compared to peers (27%), difficulty in making decisions (20%), and being tired (23%).

Our study showed that the plasma precursor availability and metabolite of dopamine were decreased in case more depressive symptoms were present which suggests a decreased dopamine synthesis. The level of these biomarkers of dopamine also decreased during 2 years of follow-up. The plasma precursor availability of serotonin was significantly higher in children with PWS than in healthy controls, but it was not related with depressive symptoms. During 2 years of follow-up, the plasma precursor availability of serotonin increased. Interestingly, plasma levels of the aromatic amino acids (i.e. tyrosine, tryptophan, and phenylalanine) were increased compared to healthy controls, but levels of branched-chain amino acids were similar.

In conclusion, depression is uncommon in children with PWS. We found differences in the biomarkers of the dopamine and serotonin system compared to healthy controls and changes over time. Remarkably, aromatic amino acid-levels were increased in children with PWS, but the exact mechanism is unclear. Further research is needed to show if the changes in precursors and metabolites of the dopamine and serotonin system in plasma could predict the increased incidence of depression in people with PWS later in life.

Chapter 8

In the general discussion, we discuss our findings in a broader context. We present our general conclusions in relation to current literature. We give practical implications for parents and caregivers and provide directions for future research.

SAMENVATTING

Hoofdstuk 1

Dit hoofdstuk geeft een korte introductie over het Prader-Willi syndroom (PWS) en beschrijft de genetische oorzaken van het syndroom, de symptomen, het gedragsfenotype en de weinige literatuur over psychiatrische stoornissen bij mensen met PWS.

Hoofdstuk 2

Er waren tot de start van huidige studies weinig studies bekend die de psychiatrische stoornissen bij kinderen met PWS beschreven. We hebben daarom 61 kinderen met PWS in de leeftijd van 7 tot 18 jaar onderzocht met de Diagnostic Interview Schedule for Children (DISC) en Compulsive Behaviour Checklist (CBC). Na 2 jaar werden 38 van de 61 kinderen opnieuw getest. Voor start van de studie waren 2 jongens bekend met psychotische symptomen en behandeld met antipsychotica. Bij start van de studie scoorde geen van de andere kinderen positief voor schizofrenie of andere psychotische stoornissen. Geen van de kinderen had een psychotische stoornis tijdens de studie. Oppositioneel opstandig gedrag was de meest voorkomende diagnose met een prevalentie van 20% bij kinderen met PWS. Dit was niet geassocieerd met leeftijd, geslacht, genetisch subtype of IQ. De meest voorkomende compulsies waren het verzamelen van spullen en een vaste routine in hygiëne-activiteiten.
Concluderend, in onze groep van 61 kinderen met PWS had 59 geen psychotische stoornis. Oppositioneel opstandig gedrag was de meest voorkomende stoornis. Wij vonden geen verslechtering in het psychisch welzijn gedurende de vervolgstudie van 2 jaar.

Hoofdstuk 3

In deze studie werd het sociaal cognitief functioneren onderzocht bij kinderen met PWS door de Theory of Mind (ToM) en symptomen passend bij Autisme Spectrum Stoornissen (ASS) te bestuderen. De ToM is de cognitieve vaardigheid om je bewust te zijn van je eigen gedachten en die van een ander, dat wil zeggen gedachten als 'Ik denk dat…', 'Ik denk dat jij denkt …' en 'Ik denk dat jij denkt dat hij denkt …'. We onderzochten 66 kinderen met PWS in de leeftijd van 7 tot 18 jaar en gebruikten hiervoor de testen Theory of Mind test-R en de Diagnostic Interview Social Communication disorders. De mediaan van de totale ToM-score was 3 standaard deviaties lager dan leeftijdsgenoten. De ToM score was positief geassocieerd met IQ, met name het verbale IQ. We vonden geen verschil in totale ToM score tussen kinderen met een deletie en mUPD. Een op de drie kinderen scoorde positief voor ASS en dit was niet geassocieerd met totaal IQ. Er was geen verschil in de prevalentie van ASS tussen kinderen met een deletie of

mUPD, en tussen jongens en meisjes. Binnen de ASS hadden zij vooral symptomen van maladaptief gedrag.
Concluderend, onze bevindingen wijzen naar een aanzienlijk verminderd sociaal functioneren, dat consequenties heeft voor de manier waarop kinderen met PWS benaderd moeten worden. Wij adviseren ouders en verzorgers om de ontwikkelingsleeftijd van kinderen met PWS te testen en de communicatie en de sociale verwachtingen aan te passen aan de sociale ontwikkelingsleeftijd van het kind met PWS.

Hoofdstuk 4

Gedragsproblemen komen veel voor bij kinderen met PWS, maar er was nog weinig bekend over het effect van groeihormoonbehandeling op het gedrag van de kinderen, ondanks het feit dat ouders gedragsverbeteringen rapporteerden tijdens de groeihormoonbehandeling. We hebben daarom het effect van groeihormoonbehandeling op het gedrag onderzocht in een gerandomiseerde en gecontroleerde groeihormoonstudie gedurende 2 jaar bij 42 kinderen met PWS in de leeftijd 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 8 jaar groeihormoonbehandeling op het gedrag hebben onderzocht. Het gedrag van de kinderen werd jaarlijks geëvalueerd met behulp van 2 oudervragenlijsten, namelijk 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 het probleemgedrag bij kinderen met PWS gemeten met de VOG overeenkwam met kinderen met een vergelijkbare verstandelijke beperking. Echter, kinderen met PWS hadden relatief meer sociale problemen. Probleemgedrag gemeten met de VISK wees uit dat sociale problematiek bij kinderen met PWS vergelijkbaar is met die van kinderen met een vergelijkbare verstandelijke beperking, maar meer voorkomt vergeleken met gezonde leeftijdsgenoten. Kinderen met PWS laten relatief meer problemen zien op de onderdelen onaangepast gedrag, niet begrijpen van sociale informatie zoals grapjes of de kern van een gesprek en zij hebben meer stereotiep gedrag. In tegenstelling tot onze verwachtingen gaf langdurig groeihormoonbehandeling geen verbetering op het gedrag, maar ook geen verslechtering.
Concluderend, kinderen met PWS hadden dezelfde mate van probleemgedrag als andere kinderen met een verstandelijke beperking, maar lieten vooral sociale problemen zien. In tegenstelling tot onze verwachting lieten onze resultaten geen veranderingen zien op gedrag tijdens langdurige groeihormoonbehandeling. Mogelijk waren de vragenlijsten niet gevoelig genoeg om subtiele verbeteringen in het gedrag te objectiveren.

Hoofdstuk 5

In deze studie werd het effect van groeihormoonbehandeling op het adaptief functioneren bij kinderen met PWS onderzocht. Adaptief functioneren omschrijft in hoeverre

een individu functioneert in het dagelijkse leven op het gebied van communicatie (begrijpen, luisteren en spreken, schrijven), dagelijks leven (zelfverzorging, huishoudelijke taken, maatschappelijk), sociaal functioneren (interpersoonlijke relaties, spelen en vrije tijd, sociale vaardigheden) en motoriek (grof en fijn). Hiervoor gebruikten wij de Vineland Adaptive Behavior Scales (VABS) tijdens de gerandomiseerde en gecontroleerde studie en na 7 jaar groeihormoonbehandeling. In de gerandomiseerde studie werden 75 kinderen (42 peuters en 33 prepubertaire kinderen) met PWS geïncludeerd. Ouders van 53 kinderen werden opnieuw benaderd voor de VABS-afname na 7 jaar groeihormoonbehandeling bij het kind. Wij vonden een opmerkelijke vertraging in de ontwikkeling van het adaptief functioneren bij kinderen met PWS. Kinderen die ouder waren of een lagere intelligentie hadden, hadden een grotere achterstand van het adaptief functioneren dan hun gezonde leeftijdsgenoten. Wij vonden tevens dat kinderen beter scoorden op het adaptief functioneren indien zij als peuter eerder waren behandeld met groeihormoon.
Concluderend, het adaptief functioneren is vertraagd bij kinderen met PWS. Een eerdere start van groeihormoonbehandeling op de peuterleeftijd leidt tot betere adaptieve vaardigheden op de lange termijn.

Hoofdstuk 6

Er was nog weinig bekend over de visueel-motorische integratie (VMI) bij kinderen met PWS. Wij hebben de VMI onderzocht in 73 kinderen met PWS in de leeftijd van 7 tot 18 jaar die allen werden behandeld met groeihormoon. Hiervoor werden de Beery Visual Motor Integration test, welke bestond uit de visueel-motorische integratie test (zonder tijdslimiet), alsmede de visuele perceptie subtest en de motorische coördinatie subtest (beide met tijdslimiet) gebruikt, bij start en na 2 jaar studie. Kinderen met PWS scoorden 3 standaard deviaties lager in visueel-motorische integratie, en 1 standaard deviatie lager in visuele perceptie en motorische coördinatie in vergelijking met gezonde leeftijdsgenoten. Kinderen met een deletie en een hoger IQ hadden een betere visueel-motorische integratie. Motorische coördinatie was alleen beter in geval van een hoger IQ. Visuele perceptie en motorische coördinatie waren beide niet geassocieerd met leeftijd of geslacht. Na 2 jaar studie werd geen verbetering gezien in de visuele perceptie, motorische coördinatie, en visueel-motorische coördinatie,.
Concluderend, laten onze data zien dat kinderen met PWS zwak scoren op de visueel-motorische integratie, waarbij de visuele perceptie en de motorische coördinatie tegelijkertijd getest worden. Zij scoorden beter indien de visuele perceptie en motorische coördinatie apart werden getest. Dit geeft aan dat het voor kinderen met PWS moeilijker is om tegelijkertijd visuele en motorische taken uit te voeren. In de praktijk betekent dit dat dagelijkse activiteiten thuis of op school verbeterd kunnen worden door taken met visueel-motorische integratie eerst op te delen in alleen een visuele of motorische

taak, voldoende tijd hiervoor te geven en dan pas de visueel-motorische integratie te oefenen.

Hoofdstuk 7

Er waren nog geen studies verricht naar het voorkomen van depressieve symptomen bij kinderen met PWS en naar een eventuele relatie tussen depressieve symptomen en biomarkers van het dopamine- en serotoninesysteem. Daarom hebben we de aanwezigheid van depressieve symptomen onderzocht in 30 kinderen met PWS tussen de 7 en 17 jaar met de Nederlandse versie van de Children's Depression Inventory. Tevens hebben wij in het plasma de beschikbaarheid van de precursor en de concentratie van de metaboliet van het dopamine- en serotoninesysteem bepaald bij 68 kinderen met PWS gedurende 2 jaar en vergeleken met 10 gezonde leeftijdsgenoten. Geen van de kinderen met PWS voldeed aan de criteria van de diagnose depressie. De 4 meest voorkomende depressieve symptomen waren gebrek aan vrienden (57% van de kinderen), gevoelens van minderwaardigheid ten opzichte van leeftijdsgenoten (27%), moeite hebben om beslissingen te nemen (20%), en moe voelen (23%).

Onze studie laat zien dat de beschikbaarheid van de precursor en concentratie van de metaboliet van dopamine verlaagd zijn indien er meer depressieve symptomen bestaan, welke kan wijzen op een verminderde dopaminesynthese. De concentratie van deze biomarkers van dopamine verminderde in de loop van 2 jaar. De beschikbaarheid van de precursor van serotonine was significant hoger bij kinderen met PWS dan bij gezonde leeftijdsgenoten, maar dit was niet gerelateerd aan depressieve symptomen. Gedurende 2 jaar nam de beschikbaarheid van de precursor van serotonine toe. Tevens vonden wij dat plasma concentraties van de gearomatiseerde aminozuren (tyrosine, tryptofaan en phenylalanine) verhoogd waren vergeleken met gezonde controles, maar de concentraties van vertakte keten aminozuren was normaal. Het is nog onbekend hoe deze verschillen kunnen worden verklaard.

Concluderend, depressie is zeldzaam bij kinderen met PWS. Wij vonden verschillen in de biomarkers van het dopamine- en serotoninesysteem vergeleken met gezonde controles en veranderingen van deze biomarkers gedurende 2 jaar. Het was opvallend dat de concentraties van de gearomatiseerde aminozuren verhoogd waren in vergelijking met gezonde controles. Verdere studies zijn nodig om te onderzoeken of plasma precursors en metabolieten van het dopamine- en serotoninesysteem kunnen voorspellen of een persoon met PWS een hogere kans heeft op het ontwikkelen van depressie op oudere leeftijd.

Hoofdstuk 8

In de algemene discussie bespreken we onze bevindingen in een bredere context. We presenteren onze algemene conclusies in relatie tot de huidige literatuur. Wij sluiten het

hoofdstuk af met de praktische adviezen voor ouders en verzorgers en suggesties voor toekomstig onderzoek.

CHAPTER 10

LIST OF ABBREVIATIONS

LIST OF CO-AUTHORS AND AFFILIATIONS

LIST OF PUBLICATIONS

PhD PORTFOLIO

CURRICULUM VITAE

DANKWOORD

LIST OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
ASD	Autism Spectrum Disorder
BMI	Body Mass Index
CAAs	Competing Amino Acids
CBC	Compulsive Behavior Checklist
CDI	Children's Depression Inventory
CSBQ	Children's Social Behavior Questionnaire
DBC	Developmental Behavior Checklist
DEL	Deletion
DISC	Diagnostic Interview Schedule for Children
DISCO	Diagnostic Interview Social and Communication disorders
DNA	Deoxyribonucleic adic
GH	Growth Hormone
HVA	Homovanillic Acid
ICD	Imprinting Center Defect
IQ	Intelligence Quotient
IQR	Interquartile Range
mUPD	maternal Uniparental Disomy
ODD	Oppositional Defiant Disorder
PDD	Pervasive Developmental Disorders
PIQ	Performal IQ
PWS	Prader-Willi Syndrome
RCT	Randomized Controlled Trial
SDS	Standard Deviation Score
SGA	Small for Gestational Age
SSRI	Selective Serotonin Reuptake Inhibitors
Trp-ratio	Tryptophan-ratio
ToM	Theory of Mind
Tyr-ratio	Tyrosine-ratio
VIQ	Verbal IQ
VABS	Vineland Adaptive Behavior Scale
VMI	Visual-Motor Integration
WIPPSI-R	Wechsler Preschool and Primary Scale of Intelligence-Revised
WISC-R	Wechsler Intelligence Scale for Children-Revised

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LIST OF PUBLICATIONS

1. Lo ST, Collin P, Hokken-Koelega A. Psychiatric disorders in children with Prader-Willi syndrome. *American Journal of Medical Genetics Part A 2015, In press*

2. Lo ST, Siemensma E, Collin P, Hokken-Koelega A. Impaired theory of mind and symptoms of Autism Spectrum Disorder in children with Prader-Willi syndrome. *Research in Developmental Disabilities 2013 Sep; 34(9): 2764-2773*

3. Lo ST, Siemensma EPC, Festen DAM, Collin P, Hokken-Koelega ACS. Behavior in children with Prader-Willi syndrome before and during growth hormone treatment: A randomized controlled trial and 8-year longitudinal study. *European Child & Adolescent Psychiatry 2015, In press*

4. Lo ST, Festen DAM, Tummers-de Lind van Wijngaarden RFA, Collin PJL, Hokken-Koelega ACS. Beneficial effects of long-term growth hormone treatment on adaptive functioning in infants with Prader-Willi syndrome. *American Journal on Intellectual and Developmental Disabilities 2015, In press*

5. Lo ST, Collin PJL, Hokken-Koelega ACS. Visual-motor integration in children with Prader-Willi syndrome. *Submitted*

6. Lo ST, Fekkes D, Collin PJL, Verhoeven WMA, Hokken-Koelega ACS. Depressive symptoms and the dopamine and serotonin system in children with Prader-Willi syndrome: An exploratory study. *Submitted*

PHD PORTFOLIO

Summary of PhD training

General Courses	Year	Workload (ECTS)
Good clinical practice, Erasmus MC	2011	1.0
Introduction to Clinical Research, NIHES, Erasmus MC	2012	0.9
Biostatistics for Clinicians, NIHES, Erasmus MC	2012	1.0
Regression Analysis for Clinicians, NIHES, Erasmus MC	2012	1.9
Courses for the Quantitative Researcher, NIHES, Erasmus MC	2012	1.4
Biomedical English Writing and Communication, MolMed, Erasmus MC	2012	4.0
Integrity in Medical Research, Medical Ethics and Philosophy, Erasmus MC	2013	2.0
Specific Courses		
Psychiatric Epidemiology, NIHES, Erasmus MC	2011	1.4
Diagnostic Interview Social Communication, University of Leiden	2011	1
Basic Principles of Genetics, MolMed, Erasmus MC	2011	0.5
PubMed and Endnote, Medical Library, Erasmus MC	2011	0.3
Photoshop & Illustrator CS5 , Molmed, Erasmus MC	2012	0.3
Follow-up Photoshop and Illustrator CS5, Molmed, Erasmus MC	2012	0.3
InDesign CS5, MolMed, Erasmus MC	2012	0.3
Seminars and workshops		
Weekly research meeting, department of Pediatric Endocrinology	2011-2014	4
National Autism Congress, Rotterdam	2011	0.2
Annual PhD day	2012	0.3
PWS Patient Care Day, Diegem/Brussel, Belgium	2013	0.2
Minisymposium 'Gewetensbezwaren op de werkvloer: waar ligt de grens?', Commissie Medisch Ethische Vraagstukken, Erasmus MC, Rotterdam	2014	0.2
Research Day, Sophia Children's Hospital	2014	0.2
International and national conferences		
National Autism Congress, Rotterdam (poster presentation)	2013	1
IPWSO 8th International PWS conference, Cambridge, UK (poster presentation)	2013	1
47th Gatlinburg Conference, Chicago, US (poster presentation)	2014	1
PWS expert meeting, Toulouse, France (oral presentation)	2014	1
53rd Annual Meeting of the European Society of Paediatric Endocrinogy (ESPE), Dublin, Ireland (poster presentation)	2014	1

Lecturing

PWS Parents Information Day, Utrecht (oral presentation)	2011	0.5
PWS Parents Information Day, Capelle a/d IJssel (oral presentation)	2014	1
Annual IMC Weekendschool "Growth and Development", Rotterdam	2011, 2013, 2014	2.5

Miscellaneous

Co-author "Wegwijzer PWS"	4

Awards

47th Gatlinburg Conference, Chicago: Dissertation Travel Award	2014
53rd Annual Meeting of the European Society of Paediatric Endocrinology (ESPE), Dublin: Nomination for Presidential Poster Award	2014

CURRICULUM VITAE

Sin Ting (Sinddie) Lo was born in Utrecht, the Netherlands, on September 6, 1984. She passed her secondary school exam (gymnasium) at the Christelijk Gymnasium Utrecht in 2002. In that same year, she started medical school at Leiden University Medical Center (LUMC). She finished the theoretical part of her medical training in 2006, after completing graduate research in Australia at the Department of Obstetrics and Gynaecology, University of Sydney. In 2007, she began her intership and obtained her medical degree in 2009. Upon completion of her degree she took up a post as a pediatric resident at the Sint Franciscus Gasthuis in Rotterdam. In 2010, she started working on a clinical research project (PWS-study) at the Dutch Growth Research Foundation and the department of Pediatric Endocrinology at the Erasmus University Medical Center – Sophia's Children's Hospital, Rotterdam (supervisor Prof. dr. A.C.S. Hokken-Koelega). This thesis is the result of this research project. In March 2015, she commenced her training in General Practice at the University Medical Center Utrecht, Utrecht.

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