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134

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ANGELMAN AND PRADER-WILLI SYNDROMES IN ESTONIA

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to my patients and their families

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by Roman numerals (I–V), and on some unpublished data:

I Õiglane-Shlik E, Talvik T, Žordania R, Põder H, Kahre T, Raukas E, Ilus T, Tasa G, Bartsch O, Väisänen M-L, Õunap K. Prevalence of Angelman syndrome and Prader-Willi syndrome in Estonian children: sister syndromes not equally represented. American Journal of Medical Genetics Part A 2006;140:1936–1943.

II Õiglane-Shlik E, Rein R, Tillmann V, Talvik T, Õunap K. A female with Angelman syndrome and unusual limb deformities. Pediatric Neurology 2005;33:66–69.

III Õiglane-Shlik E, Žordania R, Varendi H, Antson A, Mägi M-L, Tasa G, Bartsch O, Talvik T, Õunap K. The neonatal phenotype of Prader-Willi syndrome. American Journal of Medical Genetics Part A 2006;140;1241–1244.

IV Eve Õiglane-Šlik, Külli Muug, Kaidi Lunge, Aita Napa, Tiina Talvik, Katrin Õunap. Angelman and Prader-Willi syndromes — short overview and clinical features of Estonian patients. Eesti Arst 2007; in press (in Estonian).

V Õiglane E, Õunap K, Bartsch O, Rein R, Talvik T. Sudden death of a girl with Prader-Willi syndrome. Genetic Counseling 2002;13:459–464.

ABBREVIATIONS

AED	antiepileptic drug
AS	Angelman syndrome
ATP10C	aminophospholipid-transporting ATPase 10C
BP	break point
bp	base pair
CNS	central nervous system
CR	critical region
CSGE	conformation sensitive gel electrophoresis
CT	computed tomography
EEG	electroencephalography
FISH	fluorescence *in situ* hybridization
GH	growth hormone
HC	head circumference
HECT	homologous to the E6-AP carboxyl terminus
HERC2	HECT domain and RCC1 domain protein 2
IC	imprinting centre
ID	imprinting defect
IQ	intelligence quotient
Inv dup	inverted duplication
kb	kilobase pairs
MAGEL2	melanoma-associated antigen (MAGE) — like 2
Mb	megabase pairs
MECP2	methyl CpG binding protein 2
MKRN3	makorin 3
MR	mental retardation
MRI	magnetic resonance imaging
MS-PCR	methylation sensitive polymerase chain reaction
NDN	necdin
PAR (1;5;7)	Prader-Willi/Angelman region
PWS	Prader-Willi syndrome
RFLP	restriction fragment length polymorphism
RT-PCR	reverse transcription — polymerase chain reaction
SD	standard deviation
SNRPN (SmN)	small nuclear ribonucleoprotein polypeptide N
SNURF	SNRPN upstream reading frame
SRO	the shortest region of deletion overlap
SUD	sudden unexpected death
TW-RUS	Tanner-Whitehouse radius, ulna, and short bones score
UBE3A	E6-AP (formely), ubiquitin protein ligase E3A
UPD	uniparental disomy

1. INTRODUCTION

Angelman syndrome (AS) and Prader-Willi syndrome (PWS), so-called sister syndromes, occupy an important place in the recent history of medical genetics and in contemporary research on human genetic disorders. These two clinically distinct disorders were among the first that were recognized to be caused by a small interstitial deletion of chromosomal material (Ledbetter *et al*., 1980; Ledbetter *et al*., 1981) when the technique of high-resolution banding was developed, and they both result from the deletion of the same chromosome region 15q11-13 (Kaplan *et al*., 1987; Magenis *et al*., 1987; Donlon, 1988). In AS the deletion is on the maternally inherited chromosome (Knoll *et al*., 1989), while PWS results from the deletion on the paternally inherited chromosome (Butler *et al*., 1986; Nicholls *et al*., 1989a). This phenomenon of parent-of-origin difference in the expression of genes is presently known to be a consequence of genomic imprinting in this region (Nicholls *et al*., 1989b; Hall, 1990; Williams *et al*., 1990). To date, in addition to the deletion, several different molecular mechanisms causing AS or PWS have been described, such as uniparental disomy (UPD) (Nicholls *et al*., 1989b; Knoll *et al*., 1991), imprinting defects (ID) (Buiting *et al*., 1995) and mutations in a single gene, ubiquitin protein ligase E3A gene (*UBE3A*), in the case of AS (Kishino *et al*., 1997; Matsuura *et al*., 1997; Sutcliffe *et al*., 1997).

In 1965, the first description of AS was reported by Harry Angelman, an English pediatrician. He described three severely mentally retarded children with similar facial features, absence of speech, paroxysms of laughter, abnormal puppet-like gait, epileptic seizures, and an abnormal EEG (Angelman, 1965). The first description of a PWS (-like) patient originates from John Langdon Haydon Down, who in 1864 reported a case that corresponds to the comprehensive diagnostic criteria for PWS (Holm *et al*., 1993), using the term polysarcia, derived from the Greek roots *poly* (much) and *sarca* (flesh) (Down, 1864, cited in Ward, 1997). The syndrome was revived in 1956, when in the "Schweizerische Medizinische Wochenschrift" Andrea Prader, Alexis Labhart and Heinrich Willi from Zürich described nine patients with obesity, short stature, cryptorchidism and oligophrenia, usually preceded by severe amyotonia in infancy (Prader *et al*., 1956). The consensus diagnostic criteria were published in 1993 for PWS (Holm *et al*., 1993) and for AS in 1995 (Williams *et al*., 1995), and now these criteria serve as a basis for the initial clinical diagnosis of AS and PWS. Furthermore, the development of methylation-specific PCR (MS-PCR) assays to detect parent-specific DNA methylation imprint allows more rapid primary confirmation of these syndromes (Kubota *et al*., 1997; Zeschnigk *et al*., 1997).

The occurrence of both syndromes has been considered to be comparable with an approximate frequency of 1 in 10,000 to 15,000 (Nicholls *et al.*, 1998; Cassidy *et al.*, 2000). However, these frequencies were deduced from a few

studies of either AS or PWS only (Burd *et al*., 1990; Åkefeldt *et al*., 1991; Clayton-Smith, 1993a; Ehara *et al*., 1995; Kyllerman, 1995; Petersen *et al*., 1995; Buckley *et al*., 1998; Whittington *et al*., 2001), and any direct comparison between these surveys is weakened by differences in study populations and methods. Additionally, there have been some population-based studies looking for the etiology of mental retardation, with the results indicating that these syndromes are not equally prevalent (Hou *et al.*, 1998; Strømme *et al.*, 2000).

Several studies have emphasized the complexity of the early recognition of both syndromes. Apparently the clinical diagnosis of AS is difficult in the first two or three years of life because the clinical phenotype can be confused with other pathologies or due to the changing clinical features with age (Fryburg *et al*., 1991; Clayton-Smith, 1993c; Williams *et al*., 1995; Smith *et al*., 1996; Williams *et al*., 2006). The suspicion of PWS commonly rises in later childhood with the development of hyperphagia and obesity, while early neonatal/infantile phenotype of PWS have rarely been described in detail (Hoefnagel *et al*., 1967; Greenberg *et al*., 1987; Chitayat *et al*., 1989; Aughton and Cassidy, 1990; Miller *et al*., 1999).

Little is known of the causes of death in AS and only a few autopsy descriptions have been available in the literature (Jay *et al*., 1991; Kyriakides *et al*., 1992; Ishmael *et al*., 2002). In contrast, the sudden unexpected infant death (SUD) in PWS has recently emerged as a subject of discussion (Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Nagai *et al*., 2005). However, it is not known whether children and adults with PWS tend to die suddenly with greater frequency or have decreased life expectancy compared to the general population (Whittington *et al*., 2001; Nagai *et al*., 2005).

The objective of this study was to establish the livebirth and population prevalence of AS and PWS among individuals in Estonia born from 1984–2004, and to compare our results with studies performed in other populations. We also aimed to discover the clinical symptoms that facilitate early recognition of both syndromes, to describe in detail the clinical phenotype of our patients and, when possible, to investigate the causes of early death in patients with AS and PWS.

2. REVIEW OF THE LITERATURE

2.1. History of AS and PWS

AS and PWS are two clinically distinct disorders that result from the loss of expression of imprinted genes in the maternal and paternal chromosome region 15q11-13 respectively. These syndromes have an important place in the contemporary history of human genetic disorders because of their unique and partially shared genetic basis.

AS originates from England, when in 1965 the English pediatrician Harry Angelman (1965) collected three children with flat heads, jerky movements, protruding tongues and bouts of laughter under the term "puppets" because of their superficial resemblance to a marionette. All of these children shared common characteristic features such as horizontal depression in the occipital region of the skull, brachycephaly associated with microcephaly, primary optic atrophy, some degree of cerebral atrophy, frequent fits, a profound degree of mental retardation, paroxysms of laughter, ataxia and an ability to protrude the tongue to an unusual degree. In 1967 Bower and Jeavons described two similar patients and added the adjective "happy" to the "puppets" title (Bower and Jeavons, 1967). The first appearance in the literature under the name "Angelman's syndrome" was in 1972, when Berg and Pakula described one new case and reviewed the previously described cases (Berg and Pakula, 1972). In 1982 Williams and Frias suggested the eponym "Angelman syndrome" instead of "happy puppet syndrome" which was derisive and derogatory to the patients' families (Williams and Frias, 1982).

The first case report of a PWS (-like) patient probably originates from John Langdon Haydon Down, who in 1864 described a mentally subnormal woman of short stature, small hands and feet, extreme obesity and primary amenorrhea and called this condition "polysarcia" (Down, 1864, cited in Ward, 1997). In 1956 Andrea Prader, Alexis Labhart and Heinrich Willi from Zürich described, in the "Schweizerische Medizinische Wochenschrift", nine patients with obesity, short stature, cryptorchidism and oligophrenia, usually preceded by severe amyotonia in infancy (Prader *et al*., 1956). In the same year (1956), together with Guido Fanconi, they presented this syndrome at the 8th International Congress of Pediatrics. Thereafter, Prader and Willi gave an updated overview of 14 cases at the 2nd International Congress on Mental Retardation in 1961, adding to the syndrome's description diminished fetal movements and a predisposition to diabetes in older age (Prader and Willi, 1961). They also suggested that the syndrome might have a genetic background, probably with a recessive mode of inheritance. However, there were no known cases of the occurrence of the syndrome in siblings or in more distant relatives.

Initially, several different chromosomal abnormalities were associated with PWS, including a chromosomal translocation of two autosomes in the 13–15

group in a mentally deficient child with cryptorchidism (Bühler *et al*., 1963). The problem was solved when high-resolution chromosome analysis and multiple banding techniques applied to PWS patients showed *de novo* interstitial deletions of 15q11-13 (Ledbetter *et al*., 1980; Ledbetter *et al*., 1981; Ledbetter *et al*., 1982). The paternal origin of the deleted 15q was shown in all informative cases by Butler *et al*. in 1986, and later this exclusive trait was confirmed by molecular marker analysis (Nicholls *et al.*, 1989a). Surprisingly, in 1987 the same 15q11-12 deletion was verified in three clinically different patients, one of them a patient with suspected Angelman syndrome (Kaplan *et al.*, 1987). In the same year Magenis *et al.* (1987) described two unrelated females with AS having a similar deletion of the proximal long arm of chromosome 15 typical of PWS, suggesting a different chromosomal segment as a cause of the discrepancy. However, the deletions in both syndromes were indistinguishable using comparative molecular analysis (Donlon, 1988). The discrepancy was resolved when Knoll *et al.* (1989) demonstrated the maternal inheritance of the deleted chromosome 15 using restriction fragment length polymorphisms (RFLPs). This phenomenon of parent-of-origin difference in the expression of genes is now known as a consequence of genomic imprinting in this region (Nicholls *et al*., 1989b; Hall, 1990; Williams *et al*., 1990). To date, different molecular mechanisms causing AS or PWS have been described, such as uniparental disomy (UPD) (Nicholls *et al*., 1989b; Knoll *et al*., 1991), imprinting defects (ID) (Buiting *et al*., 1995), and mutations in a single gene, ubiquitin protein ligase E3A gene (*UBE3A*), in the case of AS (Kishino *et al*., 1997; Matsuura *et al*., 1997; Sutcliffe *et al*., 1997).

2.2. Consensus diagnostic criteria for AS and PWS

2.2.1. AS

The diagnosis of AS rests upon a characteristic combination of clinical features and genetic laboratory testing. In 1995, a consensus statement was published summarizing the salient clinical features of AS (Williams *et al.*, 1995). Recently, an updated consensus for diagnostic criteria was released, which takes into consideration the scientific advances made in the last 10 years (Williams *et al.*, 2006) (Table 1, Table 2).

A positive AS genetic test is strong evidence for AS, although a normal result does not rule out the diagnosis. In about 10–15% of individuals whose clinical presentation is characteristic of AS, genetic laboratory studies of chromosome 15 will be normal (no 15q11-13 deletion, UPD, ID or *UBE3A* abnormality) (Williams *et al*., 2006). This group of patients likely represents some combination of (i) as yet undetected lesions in chromosome 15q11-13 that affect the *UBE3A* locus; (ii) other novel genetic lesions that map elsewhere but affect expression of *UBE3A*; and (iii) incorrect diagnoses, representing

genocopies or phenocopies that do not perturb the expression of *UBE3A* (Jiang *et al*., 1999; Lossie *et al*., 2001; Williams *et al*., 2001).

Table 1. Developmental history and laboratory findings in AS (Williams *et al*., 2006)

Normal prenatal and birth history with normal head circumference and absence of major birth defects. Feeding difficulties may be present in the neonate and infant.
Developmental delay evident by 6–12 months of age, sometimes associated with truncal hypotonus. Unsteady limb movements and/or increased smiling may be evident.
Delayed but forward progression of development (no loss of skills).
Normal metabolic, hematologic, and chemical laboratory profiles.
Structurally normal brain using MRI or CT (may have mild cortical atrophy or dysmyelination).

Table 2. Clinical characteristics of AS (Williams *et al*., 2006)

Consistent (100%)	**Frequent (> 80%)**	**Associated (20–80%)**
Developmental delay, functionally severe	Delayed, disproportionate growth in head circumference, usually resulting in microcephaly by age 2	Flat occiput
		Occipital groove
		Protruding tongue
Movement or balance disorder, usually ataxia of gait, and/or tremulous movement of limbs		Tongue thrusting; suck/swallowing disorders
		Feeding problems and/or truncal hypotonia during infancy
	Seizures, onset usually <3 years of age	Prognathia
Behavioral uniqueness: any combination of frequent laughter/ smiling; apparent happy demeanor; easily excitable personality, often with uplifted hand flapping movements; hypermotoric behavior		Wide mouth, wide-spaced teeth
		Frequent drooling
		Excessive chewing/mouthing behaviors
	Abnormal EEG, with a characteristic pattern, can precede clinical features	Strabismus
		Hypopigmented skin, light hair and eye color (compared to family), only in deletion cases
		Hyperactive lower extremity deep tendon reflexes
		Uplifted, flexed arm position especially during ambulation
Speech impairment, none or minimal use of words; receptive and non-verbal communication skills higher than verbal ones		Increased sensitivity to heat
		Wide-based gait with pronated or valgus-positioned ankles
		Abnormal sleep-wake cycle and decreased sleep
		Attraction to/fascination with water and crinkly items
		Abnormal food related behaviors
		Obesity (in the older child)
		Scoliosis
		Constipation

2.2.2. PWS

The diagnosis of Prader-Willi syndrome is based on the characteristic clinical findings and a confirmation through cytogenetic and molecular studies. Consensus diagnostic criteria for PWS were published in 1993 by five physicians, a nurse and a social worker, each of whom had many years of clinical experience with PWS (Holm *et al.*, 1993). The diagnostic criteria are divided into three categories (major, minor and supportive), and are scored on a weighted point system. Major criteria are valued at one point and minor at one half point, whereas supportive criteria serve to increase the confidence, and are not included in the point system (Table 3).

Holm *et al*. (1993) provided two scoring systems: for children aged 0 to 36 months and for children aged 3 years to adults. In children 3 years of age and younger, five points are required for diagnosis, with four points from the major criteria (Table 3). For the older patients, a total score of eight points is required for diagnosis, with the major criteria comprising five or more points of the total score.

Table 3. Clinical characteristics of PWS (Holm *et al*., 1993)

Major criteria	Minor criteria	Supportive criteria
Neonatal and infantile central hypotonia with poor suck	Decreased fetal movements or infantile lethargy or weak cry in infancy	High pain threshold
Feeding problems in infancy with need for special feeding techniques, failure to thrive	Characteristic behavior problems	Decreased vomiting
Excessive or rapid weight gain on weight-for-length charts in a short period of time after 12 months of age, onset of obesity before 6 years of age	Sleep disturbance or sleep apnea	Temperature instability in infancy
Characteristic facial features	Short stature	Scoliosis and/or kyphosis
Hypogonadism	Hypopigmentalism	Early adrenarche
Global developmental delay, learning disabilities	Small hands/feet for height age	Osteoporosis
Hyperphagia/food foraging/ obsession with food	Narrow hands with straight ulnar border	Unusual skill with jigsaw puzzles
Deletion 15q11-13 or any other cytogenetic or molecular abnormality in the PWSCR	Eye abnormalities	Normal neuromuscular studies
	Thick viscous saliva	
	Speech articulation defects	
	Skin picking	

2.3. Epidemiology of AS and PWS

The occurrence of both syndromes has been considered to be similar, with an approximate frequency of 1 in 10,000 to 15,000 (Nicholls *et al.*, 1998; Cassidy *et al.*, 2000). However, these frequencies have been deduced from a few studies of either AS or PWS only (Table 4), and any direct comparison between these surveys is weakened by differences in study populations and methods. Additionally, there have been some population-based studies that examine for the etiology of mental retardation with results leading to a different conclusion — these syndromes are not equally prevalent (Hou *et al.*, 1998; Strømme *et al.*, 2000).

Table 4. Studies on AS and PWS

Author	Estimated character	Study population	Period	Number of cases/ confirmed diagnoses	Data
AS					
Clayton-Smith and Pembrey, 1992	Incidence	Mainly referrals to genetic consultation, all ages		Unknown	1:20,000
Clayton-Smith, 1993a	Population prevalence				1:62,000
Kyllerman, 1995	Prevalence rate	6–13 y, epilepsy, MR	1975–1986	4/2	1:12,000
Petersen *et al.*, 1995	Prevalence rate	Neuropaediatric clinic	1983–1991	5/5	1:10,000
Buckley *et al.*, 1998	Incidence	Institutionalized, severe MR		11/3	1:20,000
Thomson *et al.*, 2006a	Birth prevalence	Disability Services Commission	1953–2003	34/14	1:40,000
AS and PWS					
Jacobsen *et al.*, 1998	Population prevalence	Institutionalized, moderate to profound MR		285/4	1:20,000
Vercesi *et al.*, 1999	% among MR	Boys with MR		256/0	0%
PWS					
Burd *et al*., 1990	Population prevalence	9–30 years old		17/-	1:16,062
Åkefeldt *et al.*, 1991	Population prevalence	0–25 years old		11/5	12:100,000
Ehara *et al.*, 1995	Birth incidence		1980–1989	11/4	1:15,060
	Population prevalence	0–15 years old		15/7	1:17,482

Author	Estimated character	Study population	Period	Number of cases/ confirmed diagnoses	Data
Whittington *et al.*, 2001	Population prevalence	One UK Health Region, all ages		96/68	1:52,000
	Birth incidence				1:29,000
Smith *et al.*, 2003a	Birth prevalence	Australian Paediatric Surveillance Unit	1998–2000	30/30	1:25,000
Vogels *et al.*, 2004a	Population prevalence	Flandria, referrals to four genetic centers, all ages		78/78	1:76,574
	Birth incidence		1993–2000	19/19	1:26,676
Thomson *et al*., 2006b	Birth prevalence	Disability Services Commission	1953–2003	46/30	1:29,500

2.3.1. Prevalence and incidence of AS

The first published epidemiological data (Table 4) on AS estimated the incidence of AS to be around 1:20,000 (Clayton-Smith and Pembrey, 1992). In addition, Clayton-Smith (1993a) provided the minimum prevalence of AS in the UK as 1 in 62,000, mostly considering referrals to genetic consultation. Kyllerman (1995) performed a population-based study on epilepsy in 6- to 13-year-old mentally retarded children born from 1975–86 in Göteborg, Sweden, and found four unrelated children with typical AS, two of whom had a 15q11-13 deletion. The minimum prevalence rate was estimated to be 1:12,000. Petersen *et al.* (1995) reported on five AS children born between 1983 and 1991 with the genetically confirmed 15q11-13 deletion, and calculated the prevalence rate for Denmark to be as high as 1:10,000. Buckley *et al.* (1998) calculated the incidence of AS in the general population to be approximately 1:20,000, considering 11 individuals with a clinical diagnosis of AS who were found in one long-term care facility among 225 mentally challenged residents. However, only three individuals had the deletion confirmed by FISH. The long-term population-based clinical study from Australia (Thomson *et al.*, 2006a) revealed a birth prevalence for AS of 1:40,000 live births on the basis of data collected through the Disability Services Commission. Unfortunately the clinical diagnosis of AS was only confirmed by genetic testing in 14 persons out of 34.

2.3.2. Prevalence and incidence of PWS

There are several studies on the epidemiology of PWS (Table 4). Burd *et al.* (1990) performed an extensive search among the population of the state of North Dakota, USA (population of 638,800 in 1990), and suggested a prevalence rate of 1:16,062 in the age range 9–30 years, equivalent to 1:38,395 in the entire population. This study was based on a clinical phenotype of PWS only and no specific number of cases with confirmed genetic diagnosis was provided. Åkefeldt *et al.* (1991) attempted to identify all cases of PWS among individuals aged up to 25 years in Skaraborg County, Sweden. They collected 11 "definite cases", five of which had the microdeletion 15q11-13. The calculated minimum prevalence for the 7–25 year age range was 13:100,000 (1:7,700). After including borderline cases, the prevalence rose to 20:100,000 (1:5,000) for this age group. In the San-in district in Japan, Ehara *et al.* (1995) collected 19 cases of PWS, and for the years 1980–1989 the birth incidence was 1:15,060 live births, although of eleven patients, only four had the confirmed 15q11-13 deletion. The population prevalence 1:17,482 was calculated considering 15 patients under 15 years old (1976–1990), seven of whom presented the 15q11-13 deletion. Whittington *et al.* (2001) counted all known cases in one UK Health Region and calculated a lower boundary for the population prevalence as 1:52,000, with the lower limit for birth incidence of 1:29,000. Of the 96 individuals who were included, 68 had genetic confirmation of the syndrome. Smith *et al.* (2003a) estimated the birth prevalence of DNA-proven PWS. Thirty infants were reported to the Australian Paediatric Surveillance Unit between 1998 and 2000, indicating a prevalence of 4 per 100,000 live births or approximately 1:25,000 live births per annum. Vogels *et al.* (2004a) estimated the annual incidence of PWS to be 1:26,676 for the period 1993–2000, and a population prevalence 1:76,574 considering all known cases of PWS confirmed by a DNA methylation test. In a more recent study from Australia based on 50 years of observation and 46 patients who were clinically diagnosed as having PWS, 30 had the genetically confirmed diagnosis (Thomson *et al.*, 2006b). The birth prevalence for Western Australia was estimated as being 1:29,500.

2.4. Genetic basis of AS and PWS

2.4.1. General basis of AS and PWS

AS and PWS are disorders linked to abnormalities in the inheritance of chromosome 15q11-13 (Ledbetter *et al*., 1980; Kaplan *et al*., 1987). The 15q11-13 region is considered to have an increased liability toward rearrangement, especially recombination with telomeric and subtelomeric regions of other

autosomes. This may be related to the presence of repeat elements (palindromes) flanking 15q12 that share homology with telomeric regions (Reeve *et al*., 1993). Therefore the chromosome 15q11-13 region is meiotically unstable, with an unusual variety of cytogenetic rearrangements, including the AS and PWS deletions, frequent inverted duplications (Robinson *et al*., 1993a; Robinson *et al*., 1993b; Huang *et al*., 1997; Wandstrat *et al.*, 1998), rare duplications and triplications (Clayton-Smith *et al*., 1993b; Schinzel *et al*., 1994; Cassidy *et al*., 1996; Browne *et al*., 1997; Repetto *et al*., 1998), inversions (Webb *et al*., 1992), and balanced or unbalanced translocations (Butler 1990; Sun *et al*., 1996; Eliez *et al*., 1997; Windpassinger *et al*., 2003; Varela *et al*., 2004).

At the molecular level, 15q11-13 represents a very complex chromosomal region, and genes within a 2 Mb domain spanning half of 15q11-13 are imprinted, with expression of these genes dependent on the sex of the parent of origin. AS is associated with loss of expression of the maternal allele and PWS is associated with loss of expression of paternally derived alleles (Nicholls *et al*., 1998). This can happen through different mechanisms, which are described below in greater detail.

2.4.2. Deletions of chromosome region 15q11-13

The majority (65–75%) of AS and PWS cases are due to *de novo* large interstitial deletion (4–4.5 Mb) of 15q11-13 that includes a large cluster of imprinted genes (2–3 Mb) and a nonimprinted domain (1–2 Mb) (Nicholls *et al*., 1998). Interestingly, ≥ 95% of the deletion cases show the common deletion extents with three clustered breakpoints (BP): two alternative proximal clusters and a single distal BP region (Knoll *et al*., 1990; Kuwano *et al*., 1992; Christian *et al*., 1995; Amos-Landgraf *et al*., 1999; Christian *et al*., 1999). The proximal deletion BP commonly lies between D15S18 and the centromere (BP1), or between the D15S18 and D15S9 (BP2) loci, whereas the distal BP (BP3) has been mapped between the D15S12 and D15S24 loci (Knoll *et al*., 1990; Christian *et al*., 1995; Amos-Landgraf *et al*., 1999). AS/PWS deletions are categorized considering the common breakpoints, where the class I deletions extend from BP1 to BP3, and the class II deletions from BP2 to BP3. Analyses of the proximal extent of deletion in a large series of patients with AS or PWS confirmed that ~60% have class II and ~37% have class I deletions (Knoll *et al*., 1990; Christian *et al*., 1995). However, despite sharing common deletion sizes, the critical regions (CR) and the shortest region of deletion overlaps (SRO) are different for AS and PWS (Buiting *et al*., 1995). These deletions define an imprinting centre (IC), which has two critical elements, the AS-SRO and the PWS-SRO. The SRO in patients with AS and an IC deletion (AS-SRO) is 880 bp and maps 35 kb upstream of the exon 1 of *SNURF-SNRPN* (*SNRPN* upstream reading frame — small nuclear ribonucleoprotein polypeptide N) (Bui-

ting *et al*., 1999). The PWS-SRO, described by Ohta *et al*. (1999b), is 4.1 kb and affects the *SNURF-SNRPN* promoter/exon 1 region. However, Bürger *et al*. (2002) reported a patient with a 570 kb deletion of *UBE3A* gene (ubiquitin protein ligase E3A, formely E6-AP) which was familial and was detected through allelic loss at microsatellite loci. The smallest described deletion for PWS was approximately 100–200 kb including genes *SNRPN*, *PAR-5* (Prader-Willi/ Angelman region 5) and *PAR-7* (Prader-Willi/Angelman region 7) (Butler *et al*., 1996).

The common deletion sizes observed in AS and PWS are thought to be connected with regional genomic instability due to the presence of duplicated sequences (duplicons) at the proximal and distal regions at which patients show clustered breakpoints (Amos-Landgraf *et al*., 1999; Christian *et al*., 1999). A large part of these duplicons are derived from duplications of a large ancestral gene *HERC2* (HECT domain and RCC1 domain protein 2) located at the distal PWS/AS breakpoint (Ji *et al*., 1999). A model to explain the generation of common AS and PWS deletions involves homologous misalignment and meiotic recombination between different *HERC2*-duplicons in proximal and distal 15q11-13 (Amos-Landgraf *et al*., 1999; Christian *et al*., 1999).

2.4.3. Uniparental disomy of chromosome 15

Approximately 2% of AS and 25% of PWS cases result from paternal or maternal UPD 15q11-13 respectively (Malcolm *et al*., 1991; Nicholls *et al*., 1989b).

UPD represents the inheritance of both copies of part or an entire chromosome from a single parent, instead of the normal biparental transmission of the chromosome pair (Engel, 1980). UPD may occur both as heterodisomy (sequences from both homologues from the transmitting parent are present) and as isodisomy (two genetically identical segments from one parental homologue are present) (Nicholls and Knepper, 2001). There are multiple mechanisms for developing UPD: i) gamete complementation, when a germ cell by chance has the surplus of what lacks in the other; ii) monosomy duplication, through doubling of the lone chromosome of a monosomy; iii) trisomy rescue, through early mitotic loss of one of the three chromosomes (Engel, 1980; Engel, 2006).

In AS with a causative mechanism of paternal UPD, the likely origin is postzygotic mitotic duplication of the single sperm-derived chromosome, leading to isodisomic paternal UPD (monosomy rescue) (Robinson *et al*., 2000). In PWS most maternal UPD cases show maternal heterodisomy for chromosome 15 and are thought to result mainly from postzygotic correction of a trisomic embryo (Robinson *et al*., 1998; Robinson *et al*., 2000). The relative lack of paternal UPD15 cases compared with maternal UPD15 seems likely to be related to the lower rate of non-disjunction in male meiosis compared with female meiosis (Robinson *et al*., 1993c; Robinson *et al*., 2000). The probability

of maternal UPD due to a maternal non-disjunction event in meiosis I has been shown to increase exponentially with maternal age (Robinson *et al*., 1993d; Robinson *et al*., 1998; Robinson *et al*., 2000). Additionally, postzygotic errors in AS have been associated with maternal age, as the primary event is the maternal meiotic nondisjunction leading to a nullisomic egg fertilized by normal sperm and, later, duplication of the paternal chromosome in the zygote (Robinson *et al*., 1998; Robinson *et al*., 2000; Fridman *et al*., 2000).

2.4.4. Imprinting defects of chromosome region 15q11-13

Mutations in the imprinting process were first recognized in AS (Glenn *et al.*, 1993). However, in only a few patients, 1% in PWS and 2–4% in AS, is the disease caused by aberrant imprinting and gene silencing (Buiting *et al*., 2003). ID can occur without any DNA sequence changes (primary epimutations) or result from mutations in *cis*-regulatory elements or *trans*-acting factors (secondary epimutations) (Horsthemke and Buiting, 2006). The identification of small deletions in a subgroup of patients with an imprinting defect has led to the definition of an IC that in *cis* regulates imprint resetting and maintenance in the whole domain (Sutcliffe *et al.*, 1994; Buiting *et al*., 1995). Maternally inherited microdeletions affecting an 880-bp region 35 kb proximal to *SNURF-SNRPN* exon 1 (AS-SRO) impair the establishment of the maternal imprint and lead to AS. Some of these deletions have occurred *de novo*, but in most cases they have been inherited from the mother (Buiting *et al*., 1999; Ohta *et al*., 1999a). All of the microdeletions found in patients with PWS affect the *SNURF-SNRPN* promoter/exon 1 region (PWS-SRO 4.1 kb), and some of them have occurred *de novo*, but in most cases they have been inherited from the father (Ohta *et al*., 1999b). PWS-SRO is probably not required for the establishment of the paternal imprint, but is necessary for the maintenance of the paternal methylation imprint during early embryonic development (El-Maarri *et al*., 2001). In contrast, the close proximity and/or the correct orientation of the AS-SRO and the PWS-SRO are necessary to establish a maternal imprint (Buiting *et al*., 2001). However, IC deletions only occur in a small fraction of patients — the defect represents a primary epimutation in 92% of patients with AS and 85% of patients with PWS and ID (Buiting *et al*., 2003). Such epimutations can occur during imprint erasure in primordial germ cells, imprint establishment during later stages of gametogenesis, or imprint maintenance after fertilization. If it occurs in the germline, all of the patient's cells are affected. If it occurs after fertilization, it often results in somatic mosaicism (Horsthemke and Buiting, 2006).

2.4.5. *UBE3A* gene mutations

UBE3A gene mutations occur in ~10–14% of AS cases (Lossie *et al*., 2001). Mutations can be identified in 11–44% of sporadic patients with normal methylation and ~75% of familial patients (Malzac *et al*., 1998; Fang *et al*., 1999; Lossie *et al*., 2001; Rapakko *et al*., 2004). The *UBE3A* gene includes 16 exons (coding region from exons 7–16) that span approximately 120 kb, with transcription oriented from telomere to centromere (Yamamoto *et al*., 1997; Kishino and Wagstaff, 1998). Point mutations have been found throughout the entire coding region, with clusters in exons 9 and 16, the latter of which contains a highly conserved domain that has been labeled "homologous to the E6-AP carboxyl terminus" or simply HECT (Malzac *et al*., 1998; Russo *et al*., 2000; Rapakko *et al*., 2004). The catalytic cleft between the two lobes of the HECT domain is the site of many of the reported mutations (Huang *et al*., 1999). Frameshift, nonsense, splice site mutations, and some missense mutations have been identified (Matsuura *et al*., 1997; Fung *et al*., 1998; Fang *et al*., 1999; Lossie *et al*., 2001; Rapakko *et al*., 2004). Several common polymorphisms have also been found within the *UBE3A* gene (Matsuura *et al*., 1997; Malzac *et al*., 1998; Fang *et al*., 1999; Lossie *et al*., 2001; Rapakko *et al*., 2004). The majority of mutations appear to form *de novo*, and only ~ 20% of mothers will carry the same mutation (Clayton-Smith and Laan, 2003). Mosaicism for *UBE3A* mutations has rarely been detected (Malzac *et al*., 1998; Rapakko *et al*., 2004), however, there have as yet been no patients with AS mosaic for maternal allele *UBE3A* mutations. With the exception of a few mutations that have been found in more than one patient, most mutations have been unique (Fung *et al*., 1998; Malzac *et al*., 1998; Baumer *et al*., 1999; Laan *et al*., 1999; Lossie *et al*., 2001; Rapakko *et al*., 2004).

2.4.6. Structural chromosomal rearrangements involving 15q11-13

Structural abnormalities of the chromosome region 15q11-13, such as inversions and translocations, account for less than 1% of PWS and AS cases (Nicholls *et al*., 1998). Most of these rearrangements have been associated with a deletion of 15q11-13, uniparental disomy or an imprinting defect. Only a few translocations in PWS (Schulze *et al*., 1996; Sun *et al*., 1996; Conroy *et al*., 1997; Kuslich *et al*., 1999; Wirth *et al*., 2001) and two inversions in AS without these changes have been described (Greger *et al*., 1997; Buiting *et al*., 2001). In all PWS cases the translocation was located within the *SNURF-SNRPN* locus, two of them had typical PWS phenotypes when the translocation disturbed the *SNURF* (Sun *et al*., 1996; Kuslich *et al*., 1999), yet three of them with atypical PWS phenotype had a balanced translocation with a BP distal to *SNURF-SNRPN*, probably disrupting a cluster of multiple snoRNA genes (Schulze *et*

al., 1996; Conroy *et al*., 1997; Wirth *et al*., 2001). In the case of AS, a maternally inherited paracentric inversion had one breakpoint within the *UBE3A* gene, inactivating the maternally active allele leading to the AS phenotype (Greger *et al*., 1997), and a familial inversion spanning approximately 1.5 Mb with one breakpoint inside the IC disrupting the IC and removing the AS-SRO from the PWS-SRO in the centre of the imprinted domain leading to the AS through the female germline (Buiting *et al*., 2001).

2.4.7. Candidate genes for AS and PWS

2.4.7.1. Candidate genes for AS

In AS, the lesions of 15q11-13 are associated with the maternal chromosome, and therefore it was assumed that AS involves a maternally expressed gene(s). Based on the findings in patients with AS, an ubiquitin-protein ligase E3A gene, *UBE3A*, has been identified as the gene affected in AS (Kishino *et al*., 1997; Matsuura *et al*., 1997; Sutcliffe *et al*., 1997). There is another gene in 15q11-13 that is preferentially expressed from the maternal chromosome in the brain and fibroblasts — aminophospholipid-transporting ATPase 10C, *ATP10C* (Herzing *et al*., 2001, Meguro *et al*., 2001).

However, while the *UBE3A* gene was mapped to the AS/PWS region in as early as 1994, preliminary studies failed to show an imprinted expression using RT-PCR from fibroblasts and lymphoblasts of deletion PWS and AS patients, leading to the suggestion that *UBE3A* was an unlikely candidate locus for AS (Nakao *et al*., 1994). Only after the discovery of *UBE3A* mutations (Kishino *et al*., 1997; Matsuura *et al*., 1997) the gene was found to exhibit tissue-specific imprinting with maternal allele expression in the brain but not in the lymphocytes or fibroblasts (Rougeulle *et al*., 1997). In mice the imprinting was shown to be not only tissue specific, but also cell-type specific — the imprinted expression of *UBE3A* was preferentially restricted to the hippocampal and Purkinje neurons (Albrecht *et al*., 1997).

UBE3A belongs to the family of proteins known as E3 ubiquitin-protein ligases, which are thought to play a key role in defining the substrate specificity of the ubiqutin-proteasome degradation system (Scheffner *et al*., 1993; Ciechanover, 2001). In the ubiquitin-proteasome system, the intracellular short-lived regulatory proteins are marked by the attachment of multiubiquitin chains, which targets the selected proteins to the 26S proteasome for destruction (Vu and Sakamoto, 2000; Ciechanover, 2001). Several *UBE3A* mRNA subtypes encoding three protein isoforms differing at their N termini were reported (Rougeulle *et al*., 1997; Vu and Hoffman, 1997; Yamamoto *et al*., 1997; Kishino and Wagstaff, 1998). In addition to UBE3A function as an ubiquitin-protein ligase, Nawaz *et al.* (1999) showed that the protein directly interacts

with and coactivates the transcriptional activity of the human progesterone receptor in a hormone-dependent manner. However, in the majority of AS patients examined, the ubiquitin-protein ligase function of UBE3A was defective, but the co-activation function was intact (Nawaz *et al*., 1999).

Defective or absent E6-AP may result in the accumulation of substrate proteins, which are responsible for the disorder. However, in the case of AS it is not yet known what the targets of E6-AP are. Studies in a mouse model have shown that maternal deficiency heterozygotes (m-/p+) had a deficiency of context dependent learning (hippocampal-dependent learning deficit) and exhibited a deficit in long-term synaptic potentiation in the hippocampus in the absence of observable defects in baseline synaptic responses (Jiang *et al*., 1998). Recently a misregulation of phospho-calcium/calmodulin-dependent protein kinase II (CaMKII) in a maternal knockout *Ube3a* mouse model confirmed the role of hippocampal LTP involvement in the neurobehavioral deficits in a human learning disorder, AS (Weeber *et al*., 2003).

In 2001, *ATP10C*, showing preferential maternal expression in brain and fibroblasts, was mapped within ASCR 200 kb distal to the *UBE3A* locus (Herzing *et al*., 2001). ATP10C putatively functions as an aminophospholipid translocase likely to be involved in the asymmetric distribution of proteins in the cell membrane and therefore to be an important link in the maintenance of cell membrane integrity (Halleck *et al*., 1998; Meguro *et al*., 2001). Specific localization of the *Atp10c* transcript to mouse cerebellar granule cells, the hippocampus, and cells surrounding the corpus callosum (Halleck *et al*., 1999; Kashiwagi *et al*., 2003) is especially intriguing, since all of these areas have been suggested as being preferentially involved in autism (Lord *et al*., 2000) and overlap regions of imprinted *Ube3a* expression in mice (Albrecht *et al*., 1997).

2.4.7.2. Candidate genes for PWS

PWS arises from the lack of expression of imprinted genes that are active only on the paternal chromosome (Nicholls *et al*., 1998). In contrast to AS, several such genes are known at the present time: *SNURF-SNRPN*, *NDN*, *MAGEL2*, *MKRN3*, and more than seventy C/D box snoRNA genes (Horsthemke and Buiting, 2006). Despite their similar expression patterns in the central nervous system (Lee *et al*., 2003) the contribution to PWS of any of these genes is unknown, and it is still a matter of debate whether PWS is caused by the loss of function of a single gene or of several genes (Horsthemke and Buiting, 2006).

Genetic evidence based on microdeletions in ID in humans and balanced translocation patients has implicated the central *SNURF-SNRPN* locus as a strong candidate for a major role in PWS (Buiting *et al*., 1995; Glenn *et al*., 1996; Nicholls and Knepper, 2001). This exceedingly complex locus encodes at

least four functions: i) a *cis*-acting regulatory region (promoter/exon1) that is referred to as an IC (Sutcliffe *et al*., 1994; Buiting *et al*., 1995), ii) SNRPN (Buiting *et al*., 1993; Glenn *et al*., 1996), iii) SNURF (Gray *et al*., 1999), iv) a long (~460 kb) alternatively spliced RNA transcript that contains several families of small nucleolar RNAs (snoRNAs) (Gray *et al*., 1999; Cavaillé *et al*., 2000; Horsthemke and Buiting, 2006). SmN is encoded by exons 4–10 of *SNRPN* and is a core spliceosomal protein involved in mRNA splicing in the brain, although mice carrying the specific gene ablation of the SmN-coding sequence (exons 5–7) were viable with no obvious phenotypical or histopathological defects (Yang *et al*., 1998). *SNURF* encodes a 71 amino acid protein that localizes in the nucleus. The potential functions of SNURF include regulation of SmN or of the imprinting process, the latter as a consequence of *SNURF* being the only protein-coding locus to overlap IC (Nicholls and Knepper, 2001). Nevertheless, ablation of the *Snurf* coding sequence (exon 2) in the absence of an IC defect has not revealed a phenotypic effect (Tsai *et al*., 1999). Nevertheless, because *SNURF* is disrupted by balanced translocations in only two patients with a classical PWS phenotype (Sun *et al*., 1996; Kuslich *et al*., 1999), the role of this gene still needs further elucidation.

The *NDN*, encoding the MAGE family necdin protein, has been a particular focus of study, and it is proposed that nectin may act as a transcriptional repressor (Muscatelli *et al*., 2000; Lee *et al*., 2003; Lee *et al*., 2005). The related *MAGE* gene, *MAGEL2*, an intronless gene in proximity to the *NDN* locus, encodes a protein that is widely expressed in the central nervous system with unknown function (Boccaccio *et al*., 1999; Lee *et al*., 2000). The *MKRN3* intronless gene encodes a 507-amino acid protein with a RING (C3HC4) zinc finger motif and multiple C3H zinc finger motifs predicting a ribonucleoprotein function for the MKRN3 polypeptide (Jong *et al*., 1999).

Small nucleolar RNAs are a group of noncoding RNAs that function mainly as guides for the modification of ribosomal RNAs and small nuclear RNAs (Rogelj, 2006). Three families of human C/D box snoRNAs map to chromosome 15q11-13; they are brain specific and expressed only from the paternal allele (Cavaillé *et al*., 2000; de los Santos *et al*., 2000). However, the role of snoRNAs in PWS remains enigmatic. It was initially proposed that they could be crucial in developing PWS phenotype (Runte *et al*., 2001; Gallagher *et al*., 2002). Further it was shown that the complete loss of the *HBII-52* genes in one family members who carried the deletion on their paternal chromosome was not associated with an obvious clinical phenotype (Runte *et al*., 2005). In addition, Schule *et al*. (2005) described a 22-year-old male with a milder PWS phenotype and a *de novo* translocation t(4;15)(q27;q11.2), concluding that PWCR1/HBII-85 snoRNAs probably play a major role in the PWS- phenotype. Also, based on their studies in mice carrying the large radiation-induced p(30PUb) deletion that includes the *p* locus, Ding *et al*. (2005) proposed that the lack of Pwcr1/MBII-

85 snoRNA expression is the most likely cause for neonatal lethality in PWS model mice.

2.4.8. Inheritance and risks of recurrence of AS and PWS

The causal heterogeneity of AS and PWS complicates the evaluating of the risks of recurrence, and the correct genetic diagnosis plays a crucial role in counseling these families.

The most common genetic mechanism giving rise to AS and PWS (70–75% of patients) is an interstitial deletion of chromosome 15q11-13. Most deletions appear *de novo* and the risk of recurrence in these families is less than 1% (Horsthemke *et al*., 1996; Stalker and Williams, 1998). The incidence of complex structural chromosome abnormalities resulting in AS and PWS is relatively low, accounting for only 0.1% of cases (Chan *et al*., 1993; Nicholls *et al*., 1998). If the deletion identified in a proband is due to unbalanced translocation or chromosome rearrangement, the risk to the family depends on whether the rearrangement is inherited or arose *de novo*. Familial translocations involving 15q11-q13 can give rise to interstitial deletions causing PWS or AS, and these families need a case-specific evaluation of recurrence risks (Horsthemke *et al*., 1996; Stalker and Williams, 1998). Several patients have been reported in whom a supernumerary inverted duplication (15) was present in addition to an alteration leading to AS or PWS, and it was hypothesized that the presence of such a marker could increase the risk for other chromosome abnormalities (microdeletions, UPD) involving 15q11-q13 (Cheng *et al*., 1994; Spinner *et al*., 1995; Liehr *et al*., 2005).

The occurrence of UPD is sporadic, and the risk of recurrence in families where AS or PWS are due to paternal or maternal UPD without any evidence of translocation is less than 1% (Stalker and Williams, 1998). In some cases UPD(15) arises in association with a Robertsonian translocation or a reciprocal translocation involving chromosome 15, and in families where the parent(s) of the affected child carries a balanced translocation, the recurrence risk is case-specific (Smith *et al*., 1994; Tonk *et al*., 1996; Flori *et al*., 2004).

To date, 32 deletions and one inversion in patients with AS or PWS and an ID on human chromosome 15 have been reported (Horsthemke and Buiting, 2006). Some of the deletions have occurred *de novo*, but in most cases they have been inherited from the mother or father. Familial IC deletions are associated with a 50% recurrence risk. Other female (in AS) or male (in PWS) relatives who carry a known deletion in IC are also at a 50% risk of having an affected child, whereas male (AS) or female (PWS) relatives who carry the mutation are at risk of having affected grandchildren through their daughters (AS) and sons (PWS), who could inherit the mutation (Stalker and Williams, 1998; Buiting *et al*., 2000; Horsthemke and Buiting, 2006). Since all ID without

IC deletions have been sporadic, they may represent a *de novo* defect in the imprinting process in 15q11-13 (Bürger *et al*., 1997; Buiting *et al*., 1998, Buiting *et al*., 2003). However, in 18 AS patients with a primary epimutation, the maternal chromosome carrying an incorrect paternal imprint was inherited from the maternal grandfather in 11 patients and from the paternal grandmother in seven patients. In contrast, in 19 informative PWS patients with a primary epimutation, the paternal chromosome carrying an incorrect maternal imprint was always derived from the paternal grandmother (Buiting *et al*., 2003). This bias was found to be highly significant (*p=0.000002*) and suggests that the incorrect imprint in the patients results from a failure of the paternal germline to erase the grandmaternal imprint (epigenetic inheritance). Although epigenetic inheritance of endogenous genes has not been reported in mammals, it is well known in other species (Chong and Whitelaw, 2004). Horsthemke and Buiting (2006) propose that it occurs at a low frequency in 15q11-13 and possibly also at other human loci.

UBE3A mutations can be identified in 11–44% of sporadic patients and in ~ 75% of familial patients with normal methylation (Malzac *et al*., 1998; Fang *et al*., 1999; Lossie *et al*., 2001; Rapakko *et al*., 2004). The majority of mutations occur *de novo*, and most of them have been unique (Malzac *et al*., 1998; Fang *et al*., 1999; Lossie *et al*., 2001; Rappako *et al*., 2004). If the mother carries the same mutation, the risk of recurrence is 50%, and female relatives of the mother who carry the same mutation also have a 50% risk of having a child with AS. The mother's male relatives with the same mutation are not at risk of having an affected child, but are at risk of having affected grandchildren through their daughters, who could inherit the same defect from them. There are, however, several families where mothers have had more than one affected child despite having had a negative *UBE3A* analysis, and these mothers are likely to be gonadal mosaics (Clayton-Smith and Laan, 2003). In addition, Bürger *et al*. (2002) described a patient with a 570 bp deletion of *UBE3A* that was familial and detected through allelic loss at microsatellite loci only.

There remain some patients (10–15%) with a clinical phenotype of AS with no identified chromosome 15 abnormality. This group is likely to be heterogeneous, and may include patients with other disorders (Williams *et al*., 2001; Clayton-Smith and Laan, 2003). Recently, Horsthemke and Buiting (2006) proposed that in some of these patients an ID cell line may have contributed to the brain and other tissues, but not to the blood system.

2.4.9. Laboratory diagnostics of AS and PWS

The diagnosis of AS and PWS rests upon a combination of clinical features and molecular genetic testing and/or cytogenetic analysis.

2.4.9.1. DNA methylation test

Paternal-only expression of *MKRN3*, *NDN* and *SNURF-SNRPN* is associated with differential DNA methylation (Dittrich *et al*., 1992; Driscoll *et al*., 1992; Glenn *et al*., 1993). Whereas the promoter/exon 1 regions of these genes are unmethylated on the expressing paternal chromosome, the silent maternal alleles are methylated. Using restriction digests with the methylation-sensitive enzymes *Hpa*II and *Hha*I and probing Southern blots with several genomic and complementary DNA probes, Driscoll *et al*. (1992) systematically scanned segments of 15q11-13 for DNA methylation differences between patients with AS and those with PWS. With the complementary DNA DN34, which maps in the proximal or central part of the AS/PWS CR (D15S9), they observed a marked difference in the methylation patterns of AS and PWS patients with deletions or UPD, and proposed that this phenomenon could serve as a molecular test for the diagnosis of these syndromes. If the DNA methylation test demonstrates only the methylated maternal allele, PWS is diagnosed; but when only unmethylated paternal allele is present, AS is diagnosed. Nowadays the development of methylation-specific PCR (MS-PCR) assays of the *SNRPN* region allows more rapid analysis of these syndromes (Kubota *et al*., 1997; Zeschnigk *et al*., 1997). The DNA methylation test could be the first step, but it does not distinguish patients with deletion, UPD or ID, and further testing of patients with abnormal parent-specific methylation imprint is essential.

2.4.9.2. Genetic testing strategies for AS and PWS

Table 5. Testing strategies for AS and PWS, proportions of diagnosed individuals

Parent specific DNA methylation imprint	Test method	Abnormality detected	Proportion of abnormality
Abnormal	High resolution cytogenetics; FISH	*de novo* **deletion** 15q11-13	70% of AS & PWS
		Chromosomal **rearrangement**	< 1% of AS & PWS
	RFLP analysis; microsatellite or STR marker analysis	**UPD**	2% of AS
			25% of PWS
	Sequence analysis	**IC mutation**	<1% of AS & PWS
		Negative for IC mutation (**ID**)	~ 4% of AS & PWS
Normal	Sequence analysis (AS)	**Mutations in *UBE3A* gene**	10–15% of AS

Modified from Nicholls *et al*. (1998), Clayton-Smith and Laan (2003), and Horsthemke and Buiting (2006).

2.5. Clinical aspects of AS and PWS

2.5.1. AS

2.5.1.1. Early manifestations

AS (Table 2) is characterized by four consistent (100%) clinical features: profound developmental delay, movement or balance disorder, severe speech impairment, and behavioral uniqueness with an inappropriate happy demeanor (Angelman, 1965; Williams *et al*., 1995; Williams *et al*., 2006). Unfortunately, AS is usually not suspected during the first year of life, but becomes a more frequent diagnostic consideration between 1 and 4 years of age (Fryburg *et al*., 1991; Zori *et al*., 1992; Buntinx *et al*., 1995; Williams *et al*., 2006). There is, however, a growing experience in the early phenotype of AS if the diagnosis is given due consideration (Van Lierde *et al*., 1990; Yamada and Volpe, 1990; Fryburg *et al*., 1991; Bottani *et al*., 1994; Casara *et al*., 1995; Smith *et al*., 1996; Gillessen-Kaesbach *et al*., 1999; Moncla *et al*., 1999a; Moncla *et al*., 1999b; Williams *et al*., 2006).

In general, individuals with AS have an uneventful prenatal and birth history with a birth weight, height and head circumference (HC) within the normal range (Fryburg *et al*., 1991; Clayton-Smith, 1993c; Williams *et al*., 1995; Smith *et al*., 1996; Williams *et al*., 2006). However, Clayton-Smith (1993c) found that babies with AS tended to be 200 g lighter in birth weight than their sibs. Fryburg *et al*. (1991) and Smith *et al*. (1996) noticed that birth weight and HC showed clustering below the 50^{th} percentile in the case of AS newborns with 15q11-13 deletion. Among 7 children with AS and an imprinting defect, HC at birth was average or between +3SD in 5 children (Gillessen-Kaesbach *et al*., 1999). In spite of normal HC at birth, the growth of HC tends to stagnate and does not follow somatic growth, being remarkably microcephalic by 2 years of age (Fryburg *et al*., 1991; Smith *et al*., 1996). Babies with AS frequently demonstrate feeding difficulties, restlessness and poor sleep (Van Lierde *et al*., 1990; Yamada and Volpe, 1990; Clayton-Smith, 1993c; Smith *et al*., 1996; Schulze *at al*., 2001). In addition, they seem to be hypotonic at birth (Smith *et al*., 1996; Gillessen-Kaesbach *et al*., 1999); however, mostly truncal hypotonia with brisk deep tendon reflexes has been described since 6 months of age, with uncoordinated movements involving the upper limbs in particular (Van Lierde *et al*., 1990; Yamada and Volpe, 1990; Fryburg *et al*., 1991; Clayton-Smith, 1993c; Casara *et al*., 1995; Gillessen-Kaesbach *et al*., 1999; Schulze *et al*., 2001). Excessive tongue thrusting and drooling with mainly happy disposition seem to be present by the same age (Van Lierde *et al*., 1990; Yamada and Volpe, 1990; Fryburg *et al*., 1991; Bottani *et al*., 1994; Schulze *et al*., 2001). Further motor development delays considerably — children with AS start to sit unsupported at on average 12 months of age, crawling begins at 22 months of

age, and most children are walking by the age of 5 years (Clayton-Smith, 1993c; Buntinx *et al*., 1995; Smith *et al*., 1996).

2.5.1.2. EEG findings

The EEG features of AS appear to be sufficiently characteristic to help identify patients at an early age, before the clinical features become obvious (Williams *et al*., 1995; Williams *et al*., 2006). An abnormal EEG is often seen in patients with AS from at least 1 year of age, being uncommon before the age of 6 months (Boyd *et al*., 1988; Casara *et al*., 1995; Boyd *et al*., 1997). The prevalence of EEG abnormalities in children and adults with AS is about 90% (Boyd *et al*., 1997; Laan *et al*., 1997). One or more of the following EEG abnormalities were seen in almost all patients with AS, irrespective of genotype: 1) persistent rhythmic 4–6/s theta activity reaching more than 200 μV, often generalized and not associated with drowsiness, can be seen in AS patients under the age of 12 years (Boyd *et al*., 1988; Matsumoto *et al*., 1992; Boyd *et al*., 1997; Laan *et al*., 1997); 2) prolonged runs of rhythmic 2–3/s delta activity (200–500 μV) often more prominent over the frontal regions and sometimes associated with ill-defined spike/wave complexes (Boyd *et al*., 1988; Matsumoto *et al*., 1992; Casara *et al*., 1995; Laan *et al*., 1997; Minassian *et al*., 1998); 3) bursts or runs of high amplitude (>200 μV) 3–4(5)/s rhythmic activity, maximal over the occipital region, sometimes containing spikes and sharp waves, and facilitated by, or only seen on eye closure, can be seen in young children (Boyd *et al*., 1988; Casara *et al*., 1995; Dan and Boyd, 2003). The latter could be the most reliable feature, but it is not easy to obtain voluntary eye closure in severely retarded and frequently hypermotoric AS patients (Laan *et al*., 1997). EEG abnormalities are much more prominent in AS patients with a deletion (97–100%), and not so pronounced in those with other genetic disturbances (Matsumoto *et al*., 1992; Laan *et al*., 1997; Minassian *et al*., 1998; Valente *et al*., 2005). A normal awake EEG background rhythm was reported in 72.2% of AS patients with UPD, ID and *UBE3A* gene mutations (Minassian *et al*., 1998). Nevertheless, Laan *et al*. (1999) found abnormal EEG patterns in 6 of 8 patients with *UBE3A* gene mutations, and Valente *et al*. (2005) observed suggestive EEG pattern for AS in 75% patients with UPD.

2.5.1.3. Epilepsy

The knowledge of features of epilepsy in AS is predominantly based on studies in patients determined by deletion, since this subgroup comprises ~ 70% of AS individuals (Matsumoto *et al*., 1992; Viani *et al*., 1995; Buoni *et al*., 1999). Epilepsy in AS is described as generalized, with mostly atypical absences and

myoclonic seizures, early onset and age-related refractoriness (Williams *et al*., 1995; Laan *et al*., 1997; Valente *et al*., 2003; Williams *et al*., 2006). However, patients with AS may have different seizure types including epileptic spasms, atonic, astatic, tonic, tonic-clonic and even partial seizures (Matsumoto *et al*., 1992; Clayton-Smith, 1993c; Casara *et al*., 1995; Viani *et al*., 1995; Guerrini *et al*., 1996; Laan *et al*., 1997). Seizures mostly start in infancy or early childhood, and the first seizure episode is frequently diagnosed during a febrile period (Casara *et al*., 1995; Viani *et al*., 1995; Laan *et al*., 1997). Non-convulsive *status epilepticus* seems relatively common and is probably underdiagnosed in AS (Matsumoto *et al*., 1992; Laan *et al*., 1997), though more overt myoclonic *status epilepticus* was recorded in nine out of 18 patients by Viani *et al*. (1995). In adulthood, a significant decrease in the incidence of epileptic seizures has been reported, and atypical absences, myoclonic seizures or a combination of the two were the most prominent seizure types (Matsumoto *et al*., 1992; Clayton-Smith, 1993c; Viani *et al*., 1995; Laan *et al*., 1997). The most effective AEDs in AS are valproic acid alone or in combination with clonazepam or other benzodiazepines, whereas carbamazepine sometimes had an adverse effect (Viani *et al*., 1995; Laan *et al*., 1997; Buoni *et al*., 1999). As a subgroup, patients with AS and UPD seem to have milder epilepsy and overall good response to AEDs (Fridman *et al*., 2000; Valente *et al*., 2005). In addition, the absence of EEG changes associated with stereotyped behaviors, particularly bursts of laughter, and even during head drops, argues against an epileptic origin of these specific manifestations of AS (Williams and Frias, 1982; Bjerre *et al*., 1984; Boyd *et al*., 1988; Sugimoto *et al*., 1992). Nevertheless, ventricular asystole during outbursts of laughter followed by syncope was observed in one AS patient (Vanagt *et al*., 2005), and vagal hypertonia was described in three children with AS, and one of them also had a ventricular asystole associated with bouts of laughter (Douchin *et al*., 2000). These findings emphasize the possibility of cerebral hypoxia induced non-epileptic convulsions in subjects with AS.

2.5.1.4. CT/MRI findings

Brain images using computed tomography (CT) or magnetic resonance imaging (MRI) show a structurally normal brain in most patients, but a mild cortical atrophy or dysmyelination may sometimes be present (Williams *et al*., 1995; Williams *et al*., 2006). Nevertheless, several case reports (Van Lierde *et al*., 1990; Yamada and Volpe, 1990) and series of patients with AS (Fryburg *et al*., 1991; Casara *et al*., 1995; Guerrini *et al*., 1996; Rubin *et al*., 1997; Saitoh *et al*., 1997; Buoni *et al*., 1999) have frequently pointed out CT and/or MRI abnormalities, such as enlargement of the peri- and intracerebral spaces and, in particular, of the sylvian fissures, asymmetry of the lateral ventricules,

hypoplasia of the corpus callosum, and concomitant abnormalities in myelination. Furthermore, various rare brain malformations have been reported in patients with AS, including a vermian cyst (Incorpora *et al*., 1994), a split-cord malformation (Mastroyianni and Kontopoulos, 2002), and an intracranial aneurysm (Meyer Witte *et al*., 2005). When compared with PWS children, AS children had a significantly larger proportion (75%) of anomalous fissures than the PWS children (12%) (Leonard *et al*., 1993).

2.5.1.5. Causes of death/autopsies

Little is known of the causes of death in AS, and only few autopsy descriptions were available in the literature (Jay *et al*., 1991; Kyriakides *et al*., 1992; Ishmael *et al*., 2002). Jay *et al*. (1991) presented findings of a 21-year-old woman with clinical AS, who died from a recurrent pneumonia complicated by pulmonary abscess. Her autopsy revealed an overall small brain and marked cerebellar atrophy with reduced γ-aminobutyric acid content in the cerebellar cortex, and a decreased dendritic arborization of the visual cortex. Another neuropathological study of a 34-month-old boy who died suddenly during sleep showed small temporal and frontal lobes with disorganized and irregular gyri; the occipital lobes appeared flattened but otherwise normal, as were the parietal lobes (Kyriakides *et al*., 1992). Microscopically, there was irregular distribution of neurons in layer 3, a few subcortical ectopic neurons in the cerebral white matter of the temporal and frontal lobes, and a single Purkinje cell heterotopia in the cerebellum. Ishmael *et al*. (2002) reported on a 9.6-year-old boy previously diagnosed with AS, whose fascination with water became deadly when he drown in a backyard wading pool.

2.5.1.6. Phenotype-genotype correlation

All of the genetic mechanisms in AS lead to a somewhat uniform clinical picture of severe to profound mental retardation, characteristic behaviors, and severe limitations in speech and language (Lossie *et al*., 2001; Clayton-Smith and Laan, 2003; Williams *et al*., 2006). While there are some clinical differences in genotype-phenotype correlation, great variability within each group has been described (Moncla *et al*., 1999a; Moncla *et al*., 1999b; Lossie *et al*., 2001; Peters *et al*., 2004; Varela *et al*., 2004):

i) Patients with AS resulting from large chromosome deletions appear to be more severely affected than patients from other genetic classes (Moncla *et al*., 1999b; Lossie *et al*., 2001; Varela *et al*., 2004; Williams *et al*., 2006), and the most striking difference was the observation that 50% of patients with deletion were non-ambulatory at 5 years of age (Lossie *et al*., 2001).

Also, the deletion group is the most severely affected by microcephaly, seizures, relative hypopigmentation and language impairment (Moncla *et al*., 1999b; Lossie *et al*., 2001; Williams *et al*., 2006). In addition, patients with a large deletion (BP1→BP3) tend to present the most severe phenotype considering motor and language development as compared to patients with a slightly smaller deletion (BP2→BP3) (Varela *et al*., 2004).

ii) Patients with AS and UPD have a better physical growth (less likely to have microcephaly, frequently with weight > 75th percentile), fewer or no seizures (late onset, infrequent seizures, good response to AEDs), less ataxia and hypopigmentation, less obvious dysmorphic facial features, and better cognitive skills (Bottani *et al*., 1994; Fridman *et al*., 2000; Varela *et al*., 2004; Valente *et al*., 2005).

iii) Patients with ID are less likely to have microcephaly, hypopigmentation or seizures, and show better growth, motor milestones and communication skills. Early childhood obesity is relatively common in this group (Saitoh *et al*., 1997; Gillessen-Kaesbach *et al*., 1999; Ohta *et al*., 1999a). An exceptionally mild phenotype is associated with an incomplete ID (Brockmann *et al*., 2002).

iv) The abilities of patients with *UBE3A* mutations fall somewhere in between those of the deletion group and the UPD group. They frequently have seizures and microcephaly, but no hypopigmentation, and their motor and communication skills are better than that of patients in the deletion group. Notably, this group shows a high incidence of early onset obesity (Moncla *et al*., 1999a; Lossie *et al*., 2001).

2.5.1.7. Differential diagnosis

A positive AS genetic test is strong evidence for AS, although a normal result does not exclude the diagnosis (Williams *et al*., 2006). In about 10–15% of individuals whose clinical presentation is characteristic of AS, genetic laboratory studies of chromosome 15 will be normal (no 15q11-13 deletion, UPD, ID, or *UBE3A* abnormality), indicating either yet undetected lesions in 15q11-13 affecting function of the *UBE3A* gene, novel genetic lesions mapping elsewhere but affecting the expression of *UBE3A*, mutations in other genes with regulating effects (Jiang *et al*., 1999; Lossie *et al*., 2001) or a misdiagnosis of AS (Williams *et al*., 2001).

Infants with AS initially display global psychomotor delay and/or seizures, and therefore the differential diagnosis is broad and encompasses the chromosomal anomalies such as 22qter deletion (Precht *et al*., 1998), 15q11-13 duplication (Clayton-Smith *et al*., 1993b; Repetto *et al*., 1998; Schroer *et al*., 1998) and single gene disorders such as Rett syndrome with mutations in *MECP2* (Ellaway *et al*., 1998; Watson *et al*., 2001), Mowat-Wilson syndrome

(Mowat *et al*., 2003), homocystinuria (Arn *et al*., 1998), and mutations in *CDKL5*, cyclin-dependent kinase-like 5, gene (Tao *et al*., 2004). In most cases, a group of heterogeneous disorders including cerebral palsy (Williams *et al*., 2001), static encephalopathies (Williams *et al*., 2001), West and Lennox-Gastaut syndrome (Boyd *et al*., 1988; Laan *et al*., 1997), pervasive developmental disorders (Steffenburg *et al*., 1996; Williams *et al*., 2001), and mitochondrial encephalopathy (Nissenkorn *et al*., 2000) constitute the list for provisional investigations.

2.5.2. PWS

2.5.2.1. Early manifestations

The diagnosis of PWS (Table 3) is difficult to establish in infancy. Usually the suspicion of PWS rises later, due to appearance of hyperphagia and obesity (Greenberg *et al*., 1987; Chitayat *et al*., 1989; Aughton and Cassidy, 1990).

The major clinical features in newborns include neonatal central hypotonia with poor suck, feeding problems, characteristic facial features and hypogonadism (Holm *et al*., 1993). A variety of less frequent findings (minor and supportive criteria) (Table 3) include decreased fetal movements, infantile lethargy, weak cry, sleep disturbance or sleep apnea, and temperature instability in infancy (Holm *et al*., 1993). Several investigators have emphasized the complexity of recognizing a discrete facial dysmorphism and hypogenitalism, especially in female PWS newborns, and admit only the uniqueness of marked muscular hypotonia (Wharton and Bresnan, 1989; Miller *et al*., 1999; Gunay-Aygun *et al*., 2001; Richer *et al*., 2001). Aughton and Cassidy (1990) have confirmed that in some cases dysmorphism may be observed in the neonatal period, but dysmorphic features are more commonly subtle or absent. In contrast, Chitayat *et al*. (1989) described four newborn patients with microdeletion, and stressed the importance of typical facial features, including a high forehead, frontal bossing, narrow bifrontal diameter, almond-shaped eyes, epicanthal folds, hypoplasia of the malar area, a high arched palate and minimal to no facial expression. Recent survey of 21 patients by Trifirò *et al*. (2003) demonstrated the same — more than half of patients displayed at least three different craniofacial features. In his short description of eight PWS patients, Stephenson (1980) found their faces sufficiently dysmorphic to order chromosome examination within the first weeks of life. In addition to faces, Chitayat *et al*. (1989) described patients' fingers as very long, and Aughton and Cassidy (1990) also pointed out long and/or tapering fingers in two of their five patients. There are also several descriptions of hands in PWS newborns with flexed wrists and paralyzed distal parts (Aughton and Cassidy, 1990), a case report of distal arthrogryposis in PWS newborn (Denizot *et al*., 2004), and another case

where the patient tended to hold both thumbs adducted (Klinge *et al*., 2001). For newborns, Chitayat *et al*. (1989) also added cold extremities and intense skin mottling to the list of symptoms. Attention should be paid to the probable higher incidence of hip dysplasia in patients with PWS, as severe muscular hypotonia could disguise this problem, which requires early correction (Klinge *et al*., 2001; West and Ballock, 2004; Stevenson *et al*., 2004).

In 1989, Wharton and Bresnan described birth asphyxia in 23% of patients, but recently published survey by Trifirò *et al*. (2003) found neither perinatal asphyxia nor severe respiratory distress after birth in their group of patients with PWS. A number of studies have suggested a disposition to respiratory distress or apnoic spells in PWS (Chitayat *et al.*, 1989; Saitoh *et al*., 1997; Yoshii *et al*., 2002; Horsthemke *et al*., 2003; Denizot *et al*., 2004).

Feeding problems with subsequent failure to thrive, temperature instability and lethargy are common for PWS infants (Prader *et al*., 1956; Hoefnagel *et al*., 1966; Chitayat *et al.*, 1989; Butler, 1990; Holm *et al*., 1993). The psychomotor development delay causes the average onset of crawling to take place at 16 months, walking at 28 months and talking at 39 months (Butler, 1990). Later, mostly between 2–4 years of age, an insatiable appetite develops, leading to excessive weight gain and the onset of obesity before 6 years of age (Butler, 1990; Dimitropoulos *et al*., 2000). Early diagnosis of PWS could serve to prevent obesity if the child's diet can be strictly controlled. However, children on an early diet tend to become shorter than children without diet regulations (Schmidt *et al*., 2001). Children with PWS frequently develop learning disabilities and speech articulation defects (Curfs *et al*., 1991; Roof *et al*., 2000). Hypogonadism as a manifestation of genital hypoplasia is diagnosed soon after birth, especially in males, while an incomplete puberty and infertility develop in both sexes later in life (Holm *et al*., 1993).

2.5.2.2. CT/MRI findings

PWS has been viewed as a genetic human obesity syndrome with characteristic phenotypes, including gross hyperphagia, hypogonadism and growth hormone (GH) deficiency that indicate hypothalamic dysfunction (Prader *et al*., 1956; Holm *et al*., 1993; Swaab, 1997). However, there are only very few reports documenting peculiarities in the brain of PWS in respect to presumable hypothalamic dysfunction/deficiency or impaired central nervous system (CNS) development (Miller *et al*., 1996; Hashimoto *et al*., 1998; Klinge *et al*., 2001; Yoshii *et al*., 2002; Miller *et al*., 2006; Yamada *et al*., 2006). Miller *et al*. (1996) hypothesized that an MRI of the pituitary gland in PWS patients might reveal a measurable decrease in the size of the anterior pituitary gland, and that there might be an alteration in the posterior pituitary bright spot, which is a reflection of hypothalamic function and has been found to be absent in other

groups of patients (diabetes insipidus). All of their 15 study subjects with PWS had smaller average gland sizes, but the differences were too small to reach statistical significance. However, three patients (20%) demonstrated total absence of the posterior pituitary bright spot, and one patient showed an unusually small posterior pituitary bright spot. No data about the patients' genetic background was provided (Miller *et al*., 1996). Hashimoto *et al*. (1998), using proton magnetic resonance spectroscopy (^{1}H-MRS) in five patients with PWS, found that in the right parietal region the amount of N-acetylaspartate (an indicator of neuron function) was decreased. This finding could suggest a regional brain damage causative in the loss of cognitive functions. Yoshii *et al*. (2002) reported on a preterm girl (gestational age of 35 weeks) with PWS and deletion, whose MRI, performed at a corrected age of 40 weeks, showed a diffusely abnormal gyral folding pattern and multiple regions where the gray-white matter junction was markedly irregular involving all lobes. Recently, Yamada *et al*. (2006), using diffusion tensor imaging based on a high-field MRI system, observed eight patients with PWS and altered diffusivity characteristics in the frontal white matter, the left dorsomedial thalamus, the posterior limb of the internal capsule, and the splenium of the corpus callosum, indicating probable developmental abnormalities in these brain areas. Also, three dimensional MRI scans of twenty PWS individuals revealed ventriculomegaly in all, decreased volume of brain tissue in the parietal-occipital lobe in 50%, sylvian fissure polymicrogyria in 60%, and incomplete insular closure in 65% of PWS subjects (Miller *et al*., 2006).

2.5.2.3. Causes of death/autopsies

Individuals with PWS often develop life-threatening abnormalities such as severe hypotonia and feeding difficulties in infancy, dysregulation of temperature in young children, and massive obesity, diabetes mellitus, respiratory failure, and *cor pulmonale* in adolescents and/or adults (Holm *et al*., 1993; Cassidy, 1997a; Smith *et al*., 2003b; Stevenson *et al*., 2004; Vogels *et al*., 2004a; Nagai *et al*., 2005). However, it is not known whether children and adults with PWS tend to die suddenly with greater frequency and have decreased life expectancy than the general population (Whittington *et al*., 2001; Nagai *et al*., 2005). Recently introduced GH treatment and successively reported cases of sudden unexpected death (SUD) during a few months of treatment have raised the questions of whether the GH treatment is related to the SUD, and on the relative importance of extreme critical illnesses in PWS (Clericuzio *et al*., 1997; Schrander-Stumpel *et al*., 1998; Eiholzer *et al*., 2002; Nordmann *et al*., 2002; Smith *et al*., 2003b; Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Van Vliet *et al*., 2004; Nagai *et al*., 2005). Infants under 1 year of age without GH treatment tended to die suddenly during feeding

(hypoventilation and aspiration) or after a short febrile illness due to infection (bronchopneumonia, acute diarrhea, pneumonia, sepsis) (Hayashi *et al*., 1992; Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Nagai *et al*., 2005). However, one 8-month-old boy receiving GH died during feeding, but the postmortem examination revealed bronchopneumonia (Nordmann *et al*., 2002). Infants up to 3 years of age without GH therapy mostly had a febrile illness associated with respiratory symptoms, diarrhea, aspiration or convulsion, although, when seen by a physician, the symptoms were not considered to be life-threatening and the death was unexpected; none of these children was obese (Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Nagai *et al*., 2005). One 3-year-old with ongoing GH therapy died suddenly during sleep, although concomitant pneumonia was revealed on autopsy (Nagai *et al*., 2005). SUD among older children and adolescents not on GH therapy was seldom described, but all reported deceased patients receiving GH therapy were boys and had massive obesity (Nagai *et al*., 2005).

So far only a few autopsies with a special interest in brain abnormalities have been reported. Swaab (1997) found a markedly decreased number of oxytocin neurons in the hypothalamic paraventricular nucleus. Hattori *et al*. (1985) reported a shortness of the frontal lobe, partial micropolygyria of the nucleus dentatus, heterotopia of the inferior olivary nucleus, ectopia of Purkinje cells in the molecular layer, heterotopia of the middle-sized neurons in the deep white matter of the cerebellum, and a large number of residual nerve cells in the cerebral subcortical white matter among the autopsy findings of a 23-year-old man who died due to acute renal failure. Reske-Nielsen and Lund (1992) described extensive calcifications of the central nervous system, mostly in the leptomeninges, the first and second layer of the cerebral and cerebellar cortex, and along the ventricular system in the autopsy of a 16-year-old boy who died from a cardiac arrest. An autopsy case by Hayashi *et al*. (1992) of a 6-month-old girl with PWS and a deletion, who died from bronchopneumonia, showed disturbed undulating structures, resembling micropolygyria and pachygyria in the dentate nucleus and the inferior olivary nucleus, grumose degeneration of the nerve cells in the dentate nucleus, and heterotopia of the middle-sized neurons in the cerebellar white matter. Stevenson *et al*. (2004) reported on a deceased 9-month-old boy with PWS and deletion, whose brain autopsy revealed a mildly reduced neuronal population in the cerebral white matter, and concomitant neuronal heterotopia. Another child — a girl (PWS confirmed by methylation study, FISH negative) who died at the age of 3.5 years after an acute gastrointestinal illness, was reported to have had an abnormal gyrification, particularly of the occipital lobes, resembling polymicrogyria, an unusually deep third ventricle with a thin corpus callosum, and neuronal loss in the hippocampus (Stevenson *et al*., 2004).

2.5.2.4. Phenotype-genotype correlation

All of the genetic mechanisms of PWS lead to a somewhat uniform clinical picture of neonatal hypotonia, with poor sucking and failure to thrive in the postnatal period, delayed psychomotor development, hyperphagia in early childhood resulting in obesity, short stature, hypogonadism, and peculiar behavioral problems (Table 3). Comparisons between the subgroups of PWS individuals indicate some subtle differences:

i) Hypopigmentation is the most distinctive feature in subjects with PWS and deletion. In addition, individuals with a larger type I deletion (BP1→BP3) tend to have a more severe phenotype than those with type II deletion (BP2→BP3), including greater self-harming behavior, deficits in adaptive behavior, obsessive compulsive behaviors, and difficulties with reading, mathematical skills, and visual-motor integration (Butler *et al*., 2004). Varela *et al*. (2005) pointed out that type I individuals acquired speech later than type II, and that seizures (especially febrile seizures) were six times more common in patients with PWS and deletion than in those with UPD.
ii) Subjects with UPD tend to have lower mean percentiles of birth weight and birth length compared to those with deletion (Gunay-Aygun *et al*., 1997a), and have a shorter course of gavage feeding and later onset of hyperphagia in females (Mitchell *et al*., 1996). They are less likely to have a typical facial appearance, and less frequently show some of the minor manifestations, such as skin picking, skill with jigsaw puzzles, a high pain threshold and articulation problems (Cassidy *et al*., 1997b). After adolescence, recurrent affective psychoses are relatively frequent in UPD individuals: these psychotic states have a subacute onset, polymorphous, shifting symptomatology with an increase in psychomotor symptoms reminiscent of catatonia, and a need for psychiatric hospitalization with consecutive complete recovery (Vogels *et al*., 2004b; Verhoeven and Tuinier, 2006).
iii) Little is known about the subjects with PWS and ID, but several case reports indicate that they display the typical PWS phenotype and age-appropriate spectrum of symptoms, except hypopigmentation (Saitoh *et al*., 1997; Ohta *et al*., 1999b; Buiting *et al*., 2000; Ming *et al*., 2000). They tend to have frequent respiratory problems and therefore increased risk of sudden infant death (Saitoh *et al*., 1997; Buiting *et al*., 2000; Ming *et al*., 2000); however, there may be an ascertainment bias because of the small number of described individuals with ID.

2.5.2.5. Differential diagnosis

The list of disorders that may present with neonatal/infantile hypotonia, developmental delay/mental retardation, obesity and hypogonadism is long, and

the diagnostic process is often very complex. Hypotonia in infancy is seen in the following conditions: hypoxic-ischemic encephalopathy, neonatal sepsis, metabolic encephalopathies, CNS congenital structural defects, and acquired hypothalamic insufficiency (Richer *et al*., 2001). A PWS-like phenotype has been described in patients with chromosome abnormalities involving the different chromosome regions: Xq23–25 duplication (Monaghan *et al*., 1998), 1p36 deletion (D'Angelo *et al*., 2006), 4p deletion and duplication (Wieczorek *et al*., 2000; Richer *et al*., 2001), 6q deletion (Gilhuis *et al*., 2000; Varela *et al*., 2006). Several disorders caused by single gene mutations can contribute to the floppy infant syndrome, such as spinal muscular atrophy (Richer *et al*., 2001), Albright hereditary osteodystrophy and Bardet-Biedl syndrome (Gunay-Aygun *et al*., 1997b), as well as Cohen syndrome (Kolehmainen *et al*., 2003), Börjeson-Forssman-Lehmann syndrome in males (Turner *et al*., 2004), fragile X syndrome (de Vries *et al*., 1993) and congenital myotonic dystrophy type I (Richer *et al*., 2001).

3. AIMS OF THE PRESENT STUDY

The aims of the present study were:

1. To establish the birth prevalence and population prevalence of AS and PWS in Estonia.

2. To investigate the genetic etiology of patients with AS and PWS.

3. To ascertain the clinical symptoms facilitating early recognition of both syndromes.

4. To characterize the clinical phenotype of patients with AS and PWS.

5. To investigate the causes of early death in patients with AS and PWS.

4. MATERIAL AND METHODS

4.1. Study subjects

The population-based epidemiological study was performed involving the whole of Estonia. From 2000 to 2004, we conducted a focused search for individuals with AS and PWS among children and adolescents aged 1 day to 18 years born between 1984 and 2004. On the prevalence day, January 1, 2005, the total population of Estonia was 1,347,510 (620,600 males; 726,910 females), of whom 336,669 were 0–20 years old (172,496 males; 164,173 females) (Statistical Office of Estonia, final update on December 22, 2005).

In the year before the onset of the study, the information about the study and detailed descriptions of AS and PWS were provided in the national pediatric meetings and in the continuous medical education courses at the Department of Pediatrics of the University of Tartu, Faculty of Medicine. According to a good medical practice guideline for Estonian family physicians, all children younger than 19 years of age with developmental problems should be referred to one of the two tertiary children's hospitals for evaluation: in Tallinn for northern Estonia and in Tartu for southern Estonia. At the beginning of the study, introductory meetings were held at both hospitals. Study subjects were selected from the patients with developmental problems referred to these two hospitals. In addition, persons who had either been diagnosed with or were suspected to have AS or PWS in the past, but did not complete the diagnostic procedures, were invited for genetic re-evaluation, diagnostics and counseling. In order to find the undiagnosed individuals, in 2002 we visited all long-term-care and special educational facilities for disabled children in Estonia (28 sites with 1298 residents). In all of the selected individuals we collected a brief case history, performed a clinical examination and took a blood sample (2–5 ml) for the DNA methylation test. All children with a positive DNA methylation test were subsequently seen at the Children's Clinic of the University of Tartu. In all patients, a detailed medical and family history was collected using available medical documents and the questioning of parents or caregivers. Each patient with suspected AS underwent an awake and/or sleep EEG, and each patient with suspected PWS underwent electroneuromyography. In all cases an electrocardiogram (ECG), CT or MRI, X-ray for bone age evaluation, and abdominal ultrasonography were performed. All of these children were examined by a team consisting of a child neurologist, psychologist, physiotherapist, otorhinolaryngologist and an ophthalmologist. In addition, all parents of DNA methylation test positive but FISH negative patients gave a blood sample (5 ml) for further investigations.

The total study sample included two subgroups: i) the retrospective group (born between 1984–1999) of subjects, who were selected using the consensus diagnostic criteria for AS (Williams *et al*., 1995) and PWS (Holm *et al*., 1993)

and ii) the prospective group (born between 2000–2004), which was selected using less stringent criteria in infants aged under 1 year; all infants with psychomotor developmental delay, marked congenital hypotonia or dystonia and seizure disorder of unknown cause were included. Children who were born in 2004 were followed up to the end of 2005.

4.2. Methods

In all selected persons with a clinical suspicion of AS or PWS, a blood sample (2–5 ml) was taken for the DNA methylation test. Patients with a positive test result were further investigated using cytogenetic and FISH analysis. Blood samples obtained from FISH negative patients and their parents were studied for UPD using polymorphic DNA markers. Blood samples of individuals with strong clinical evidence of AS but a negative methylation test were further investigated for mutations in the *UBE3A* gene.

4.2.1. Methylation specific PCR

DNA was extracted from whole blood with a genomic DNA purification kit (Gentra Systems, Minneapolis, USA), followed by sodium bisulfite treatment of the DNA (Kubota *et al.*, 1997). The DNA was purified using the Wizard^{R} DNA clean-up system (Promega, Madison, USA). Methylation specific PCR of the *SNRPN* exon 1 region was carried out with primers SNRPN-common 5′-CTC CAA AAC AAA AAA CTT TAA AAC CCA AAT TCC-3′, SNRPN-Mat 5′-TAT TGC GGT AAA TAA GTA CGT TTG CGC GGT C-3′, and SNRPN-Pat 5′- GTG AGT TTG GTG TAG AGT GGA GTG GTT GTT G-3′ (Zeschnigk *et al.*, 1997). PCR products (313 bp for maternal product, 221 bp for paternal product) were analyzed using electrophoresis, 2.5% agarose gel, and a tris-borate buffer.

4.2.2. Cytogenetic analysis and FISH

Chromosomes were analyzed using peripheral blood and standard GTG banding (G bands by trypsin using Giemsa). Translocation of chromosomes 14 and 15 was studied using FISH and probes CP5032 (D14Z1/D22Z1, chromosome 14cen) (Oncor, Gaithersburg, MD), CEP 15 SG (D15Z1 classical satellite, chromosome 15p11.2), CEP 15 SO (D15Z alphoid DNA, chromosome 15cen), LSI SNRPN (*SNRPN*, chromosome 15q11.2-q12), and LSI PML (*PML*, chromosome 15q22) (Vysis Inc., Downers Grove, IL), BAC RP11–463i22 (spanning from 25,725,185 to 25,892,792 Mb on chromosome 15q13.1, BAC

ends AZ515863 and AQ634845), and YAC 895h10 (D15S207, chromosome 15q26). All other methylation test positive patients were studied using a DNA probe for the Prader-Willi/Angelman region (D15S63, *SNRPN*/imprinting center; chromosome 15q11-13) with a 15q telomere specific control probe (154P1) (Cytocell Ltd., Oxfordshire, UK). Usually 15 metaphases were analyzed per patient and per test.

4.2.3. UPD analysis

To trace the transmission of chromosome 15 from each parent to the child, the following set of microsatellites from outside the PWS/AS critical region was used: D15S123, D15S153, D15S125, D15S131, D15S100, and D15S211. PCR was performed using fluorescently labeled oligonucleotides and electrophoresis performed in an ABI 310 Genetic Sequencer (Applied Biosystems, Foster City, CA).

4.2.4. *UBE3A* mutation analysis

Ten protein encoding exons of the *UBE3A* gene (exons 7–16) were screened for sequence alterations, using PCR and conformation sensitive gel electrophoresis (CSGE) and direct sequencing. Primers for each exon were as described by Rapakko *et al.* (2004). CSGE was performed according to Körkkö *et al.* (1998). All of the band shifts detected by CSGE were further characterized by sequencing with the LI-COR IR4200 DNA Analysis System (LI-COR, Inc., Lincoln, NE).

4.2.5. Statistical analysis

Livebirth prevalence was estimated via the Generalized Linear Model Analysis using GENMOD procedure of the SAS system, Release 8.2 (SAS Institute, 1999). The distribution of the prevalence cases was assumed to be binomial, and the default logit link function was used. The only variable factor in the model was the observation year. The mean (expected) prevalence rate for a given year and a corresponding 95% confidence interval were predicted with the OUTPUT statement of the GENMOD procedure. Differences were considered statistically significant if the p-value was less than 0.05. The study only included individuals with a genetically proven diagnosis of AS or PWS.

4.2.6. Ethical considerations

The study was approved by the Ethics Committee on Human Research at the University of Tartu. Informed consent was obtained from the parents or legal guardians of the children, and none refused to participate in the study.

5. RESULTS AND DISCUSSION

5.1. Birth prevalence and population prevalence of AS and PWS in Estonia (publication I)

The study included a total of 188 persons: in the retrospective study group, four patients (2 with AS, 2 with PWS) had a genetically confirmed diagnosis, and over the period 2000–2004, 184 new index cases (93 with suspected AS, 91 with suspected PWS) were identified and studied using MS-PCR. Twenty one of the index cases, 5 with suspected AS and 16 with suspected PWS, were included in the prospective study group, using the broadened selection criteria for infants less than 1 year old. In total, DNA MS-PCR test results were positive for 15 index cases, among them 7 infants from the prospective subgroup. Therefore the study comprised a total of 19 patients with a DNA proven diagnosis, seven of them had AS, and twelve PWS (Table 6).

Table 6. Numbers of index cases and genetically confirmed cases

	AS	**PWS**
Total number of subjects in this survey	95	93
Number of index cases studied by MS-PCR (2000–2004)	93	91
Number of index cases in retrospective subgroup	88	75
Number of index cases in prospective subgroup	5	16
Number of patients diagnosed in 1999 and earlier	2	2
Number of MS-PCR positive cases in retrospective subgroup	3	5
Number of MS-PCR positive cases in prospective subgroup	2	5
Number of genetically confirmed study cases	**7 (4M/3F)**	**12 (4M/8F)**

Seven persons (two of them aged <1 year) had AS, and six of them (85.7%; two females, four males) showed a deletion of chromosome 15q11-13 (Table 7). The remaining female patient demonstrated paternal UPD (paternal age at birth was 30 years). Twelve persons had PWS (five of them aged <1 year), of whom four (33%; three females, one male) had 15q11-13 deletion and six (50%; four females, two males) had maternal UPD. Three out of the six mothers of children with PWS and UPD were older than 35 years (others were 24-, 32- and 33-year-old). One child with PWS and UPD was conceived through assisted reproduction technologies (*in vitro* fertilization). Two persons with PWS had different chromosome rearrangements (Table 7). A boy with a positive methylation test showed a Robertsonian translocation of chromosomes 15q;15q,

but he moved abroad, and material for FISH and UPD studies was not available. One girl had an unbalanced translocation of chromosomes 14 and 15 resulting in a deletion of chromosome 15p13-q13. Using FISH, the deletion included the PWS critical region and BAC clone RP11–463i22, karyotype 45,XX,der(14)t(14;15)(p11;q13).ish der(14)t(14;15)(D14Z1/D22Z1+,D15Z1–, SNRPN–,bac463i22/AQ634845–,PML+,yac895h10+). Clone RP11–463i22 corresponds to the segment from 25,725 to 25,892 Mb on chromosome 15 (UCSC Genome Browser, May 2004 assembly). The distal end of the typical 15q11-13 deletions in the PWS and AS is defined by BAC RP11–48j4 (http://www.sanger.ac.uk/PostGenomics/decipher), which spans from 24,346 to 24,490 Mb on chromosome 15 (UCSC Genome Browser). Hence this girl had a deletion at least 1.4 Mb larger than typical 15q11-13 deletions.

Table 7. Results of genetic studies

Genetic diagnosis	AS	PWS
15q11-13 deletion	6	5[a]
Chromosomal translocation	-	2[b]
UPD15	1	6
UBE3A mutation	1[c]	-
Total of genetically confirmed cases	7	12

[a] Including an unbalanced 14;15 translocation resulting in deletion of the PWSCR
[b] Including the unbalanced 14;15 translocation and a Robertsonian translocation 15q;15q
[c] Familial polymorphism, most likely not the causal mutation

UBE3A mutation screening was performed in six individuals with a normal methylation test result, who were selected based on a strong clinical suspicion of AS (Moncla *et al.*, 1999a) (Table 8). One person showed an aberrant fragment by CSGE in a large exon 9 of the *UBE3A* gene, and a missense mutation of codon 243 (nt1315C>A, Asn243Lys) was detected through sequencing. However, the father of the patient had the same sequence variation, supporting the phenotypically silent nature of this polymorphism.

From 1984 to 2004, the annual number of births in Estonia decreased from 24,234 (peak in 1987–1988 with 25,086 and 25,060 births, respectively) to 13,992 newborns (Statistical Office of Estonia, www.stat.ee) (Figure 1).

Table 8. Clinical findings of patients investigated for *UBE3A* mutations

Clinical finding	KH	JK	JuK	BP	KMS	RT
Severe developmental delay	+	+	+	+	+	+
Speech impairment	+	+	+	+	+	+
Movement/balance disorder	+	+	+	+	+	+
Relevant behavior	+	+	+	+	+	+
Microcephaly	+		+	+	+	+
Seizures	+	+	+	+	+	
Flat occiput		+	+	+	+	
Protruding tongue		+		+	+	
Prognathia		+	+	+	+	
Wide mouth, widespaced teeth	+	+	+	+	+	
Strabismus	+	+	+	+	+	
Sleep disturbance		+	+	+	+	
Fascination with water			+	+		

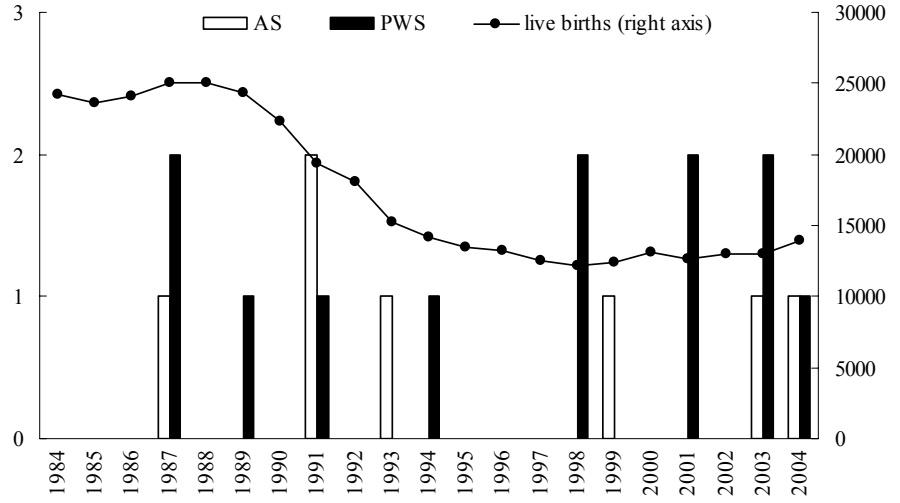


Figure 1. Numbers of live births, AS and PWS cases in Estonia from 1984 to 2004

A total of 365,266 live births were recorded, indicating a minimum livebirth prevalence of 1:52,181 (95% CI 1:25,326–1:129,785) for AS and 1:30,439 (95% CI 1:17,425–1:58,908) for PWS. On the prevalence day, January 1, 2005, the total number of persons aged 20 years and under was 336,669. Among AS and PWS persons, a girl with AS and a deletion and a girl with PWS and the deletion died before the prevalence day. Thus, among children and adolescents in Estonia, the prevalence of AS (six prevalent cases) was 1:56,112 (95% CI 1:1:25,780–1:152,899), and the prevalence of PWS (11 prevalent cases) was 1:30,606 (95% CI 1:17,105–1:61,311).

Statistical analysis showed an increase in the livebirth prevalence of AS during the years 1984–2004, from 0.88 cases per 100,000 livebirths or 1:113,636 (95% CI 1:23,923–1:526,316) to 4.23 cases or 1:23,640 (95% CI 1:6,658–1:84,034). However, this trend was not statistically significant (p=0.2) (Figure 2). For PWS we noticed a more substantial, statistically significant increase (p=0.032) in the livebirth prevalence, from 1.13 cases per 100,000 livebirths or 1:88,495 (95% CI 1:24,390–1:320,580) to 7.97 cases or 1:12,574 (95% CI 1:540 — 1:29,154) (Figure 3).

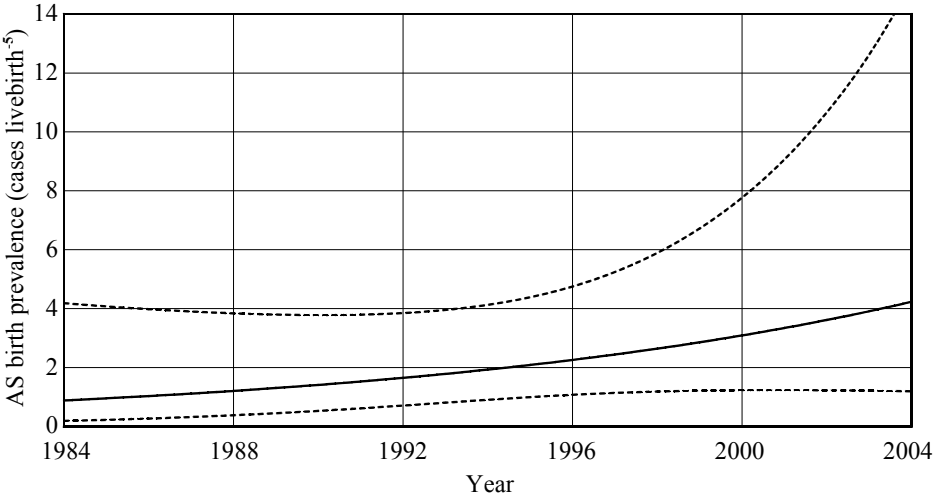


Figure 2. Distribution of AS birth prevalence in Estonia from 1984 to 2004 by statistical logit analysis. The dashed lines indicate the 95% confidence interval.

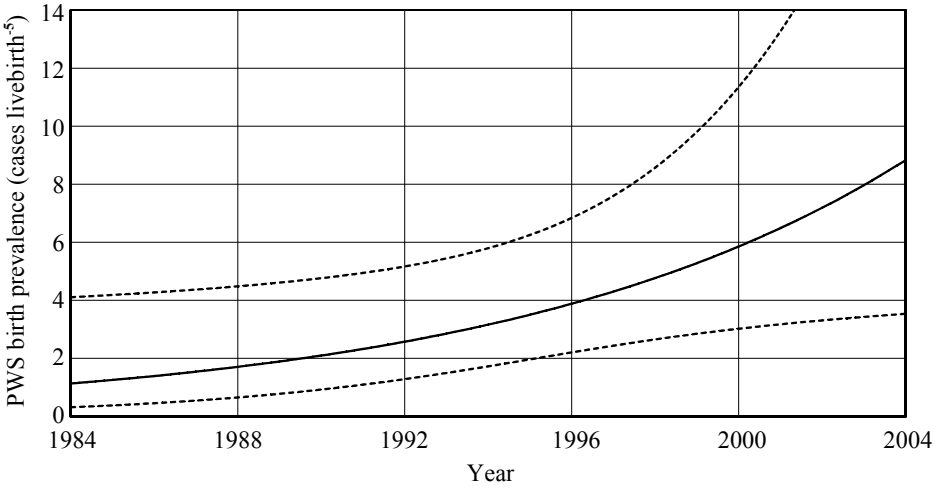


Figure 3. Distribution of PWS birth prevalence in Estonia from 1984 to 2004 by statistical logit analysis. The dashed lines indicate 95% confidence interval.

To our knowledge, this is the first epidemiological study to estimate the birth prevalence and population prevalence of genetically proven AS and PWS in the same population at the same time. The birth prevalence and population prevalence data presented by us for AS and PWS from 1984–2004 are somewhat lower than those previously reported (Table 4), where the calculations were based on selected populations, short study period or, in earlier studies, used patients without a genetically confirmed diagnosis.

The first population-based study by Clayton-Smith (1993a) estimated the minimum population prevalence in the UK at 1:62,000, considering clinically and genetically confirmed AS individuals, mainly among referrals to genetic consultation. Other previous studies on AS ascertained patients based on their disabilities (mental retardation, epilepsy) (Kyllerman, 1995; Jacobsen *et al.*, 1998; Vercesi *et al.*, 1999), used a shorter study period (Kyllerman, 1995; Petersen *et al.*, 1995), and/or included ≥50% persons without a genetically confirmed diagnosis (Kyllerman, 1995; Buckley *et al.*, 1998; Thomson *et al.*, 2006). Our data for AS showing a 1:52,181 prevalence in liveborns and a 1:56,112 population prevalence in persons under 20 years of age are based on a relatively large and well-defined data set and may be the most reliable. However, the expected birth prevalence at the end of this study period was 1:23,640 (2 times higher, p=0.2). Over the period 2001–2004 when the broadened inclusion criteria were used, the actual live birth prevalence was 1:26,331, indicating that the expected and achieved results did not differ significantly, and we probably did not miss any AS cases. The increased incidence could possibly be explained by either a true incidence increase in the

syndrome, improved diagnostic possibilities, or a focused search for the syndrome.

We included in our AS group only persons with a confirmed genetic diagnosis, considering the variety of mimicking conditions and the debate over yet undetermined mechanisms causing AS in 10–15% of "clinically definite" cases (Rougeulle *et al.*, 1998; Herzing *et al.*, 2001; Lossie *et al.*, 2001; Landers *et al.*, 2005). A phenotypic overlap has been reported between AS and *MECP2* mutations, and *MECP2* mutation screening has been proposed for AS patients without a demonstrable genetic lesion of chromosome 15q11-13 (Watson *et al.*, 2001). We collaborated in a study ascertaining the incidence of *MECP2* mutations among patients with an Angelman-like phenotype but no detectable lesion of chromosome 15. However, none of these persons, five boys and four girls, showed a relevant *MECP2* mutation (Ylisaukko-Oja *et al.*, 2005).

Our data for PWS indicate a mean livebirth prevalence of 1:30,439. To date, there have been two previous DNA methylation test-based studies providing similar data on incidence at birth, 1:25,000 from Australia for a three year period (Smith *et al.*, 2003a), and 1:26,676 from Flanders, Belgium, considering eight consecutive years (Vogels *et al.*, 2004a). In our 21-year-study (1984–2004), the mean livebirth prevalence of 1:30,439 is lower, but not significantly, which may be explained by the considerably longer study period. Nevertheless, the statistically expected prevalence of 1:12,547 (2.4 times higher and statistically significant ($p=0.032$) increase) and the observed livebirth prevalence of 1:10,532 at the end of the study period and during the years 2001–2004 are comparable, indicating that we probably did not miss any cases of PWS. These results could indicate a true increase in PWS prevalence or, alternatively, may be explained by early testing and an improvement in neonatal care. The age of women giving birth has increased over the study period from 25.9 in 1984 to 27.9 years in 2004, although the proportion of those older than 30 years of age has remained the same. Therefore, a change in maternal age did not seem to be of significance for the increased occurrence of PWS in Estonia in recent years.

It is important to emphasize that our findings contradict the widespread opinion that AS and PWS are equally frequent. The livebirth prevalence and population prevalence of AS was lower than that of PWS by a factor of 1.7 and 1.8, respectively. We observed some periodic fluctuations in the birthrates of both syndromes (Figure 1). There were several years with frequent or infrequent AS and PWS cases that could easily influence the prevalence data over short periods of time. We assume that we missed no AS and PWS patients during the last 4 year period (2001–2004). There is, however, a possibility that we lost patients before the study period due to death. Different medical complications may develop in individuals with AS or PWS (Clayton-Smith, 2001; Butler *et al.*, 2002), and during our study a 3.5-year-old girl with AS died of Reye syndrome. However, early death appears to be more common in PWS (Hayashi

et al., 1992; Clericuzio *et al*., 1997; Schrander-Stumpel *et al.*, 1998; Schrander-Stumpel *et al.*, 2004; Stevenson *et al*., 2004; Nagai *et al*., 2005). Even with an added 10–15% "clinically definite", but not genetically confirmed cases of AS (10%=0.7 patient or maximum 1 patient in our study), the livebirth prevalence of AS remains 1.5 times lower than that of PWS.

We identified no cases of *UBE3A* gene mutations causative for AS or imprinting mutations. These mutation types are rare. Imprinting mutations are primarily detected by the DNA methylation test and as the core clinical features are not different, we probably missed none of these patients (Buiting *et al*., 1995; Saitoh *et al.*, 1997; Buiting *et al*., 1999; Gillessen-Kaesbach *et al.*, 1999; Ohta *et al*., 1999a; Ohta *et al*., 1999b; Buiting *et al*., 2001; Buiting *et al*., 2003). On the other hand, the 50% rate of UPD among individuals with PWS was surprisingly high, and three (50%) of these six mothers were older than 35 years. Recently, Whittington *et al*. (2006) reported a greater proportion (50%) of UPD in children with PWS under 5 years of age living in the UK. They explained the changed rates of genetic subtypes by increasing maternal age at conception in this generation of mothers, but they did not provide data concerning increased/changed prevalence of PWS. In Estonia from 1984 to 2004, the proportion of women giving birth when older than 30 years of age has remained the same, and the subjects with UPD were evenly distributed during these years. Therefore we cannot associate the markedly different proportions in genetic mechanisms of PWS with recently increased maternal age at conception. One child with PWS and UPD was born after *in vitro* fertilization (IVF), although at present IVF is thought to be a causative for epigenetic modifications which may play an important role in the increased rate of imprinted gene abnormalities (Lidegaard *et al*., 2005; Allen *et al*., 2006). Nevertheless, the role of assisted reproductive technologies in adverse outcomes needs further clarification and population-based comparative studies will be needed before definitive conclusions can be drawn. Furthermore, we identified two new cases of PWS caused by chromosomal translocations, which is usually a very rare cause of PWS (<1%). All these differences might just be coincidental, as our group of patients was small, but taken together they provide some additional argument that we most likely missed very few if any PWS or AS cases.

5.2. Clinical phenotype of AS and PWS patients (publications II, III, IV)

5.2.1. Clinical phenotype of AS

5.2.1.1. Phenotype of patients with AS

In total, seven children (3 girls, 4 boys) born between 1984 and 2004 were diagnosed with AS. During the study period (2000–2004) we identified five patients, two girls and three boys. The overall diagnostic frequency was 5.4% (5 cases in 93 suspected patients), although considering the patients born from 2000–2004 (prospective subgroup), the diagnostic frequency was 40% (2 cases in 5 suspected patients). This difference partly shows the importance of clinical experience — all 88 patients born between 1984 and 1999, who were studied using MS-PCR for AS (the diagnostic test available in Estonia since 1998), showed marked developmental delay with speech impairment, movement or balance disorder, and behavioral disturbances characteristic of AS. The experience gained greatly facilitated the much earlier recognition of the two most recent patients (the clinical suspicion arose during the first year of life and confirmed diagnosis was made at 13 and 14 months respectively). The mean age of diagnosis considering all seven patients was 6.1 years. Recent studies on a series of patients with AS have reported the mean age at diagnosis of 10.2 y (Laan *et al*., 1997), 7y 7m (Molfetta *et al*., 2003), and 5y 8m in patients with deletion and 9y in those with UPD (Varela *et al*., 2004b). These data show that AS is being recognized at an earlier age.

All subjects with AS in our study were born at term with normal (< +/- 1SD) birth weights (3100–3994 g), lengths (49–52 cm), and Apgar scores (available for five subjects; > 7 in the first minute of life). Head circumference (HC) measurements at birth were available in the case of three subjects — two were between mean and +1 SD, and one subject (UPD) showed HC <50th percentile. There was normal prenatal and birth history for four subjects, and three subjects (43%) were born after a complicated pregnancy: one was in a forehead position, one was born from a pregnancy with abnormal double-test result (amniocentesis showed normal karyotype 46,XX), and one pregnancy was with twins, where the second fetus died at 5–6 weeks of pregnancy. All newborns were discharged from the maternity hospital as usual, and none of them was hospitalized during the first six months of life. During the first months of life, however, one child had feeding difficulties with frequent regurgitation of feeds, and three children were irritable and had sleep disturbance. When the children reached the age of six months, all mothers and also family physicians began to seek consultation with a child neurologist due to developmental problems and deceleration in head growth. The problems noticed by neurologist were truncal hypotonia, dystonia, and spasticity of the lower limbs. All children were passive but smiled

frequently; tongue thrusting was noticed in only one subject. In those with HC -2 SD (three subjects), the head growth retardation began after five months of age and reached the point of -2 SD at 10 months of age in one subject and at the age of 2 years in the other subject. The corresponding data were not available for the third subject.

The number of patients with AS was small (Table 9), and only two subjects were younger than 1 year old when the suspicion of AS arouse, and therefore it is difficult to determine the seminal symptoms that may facilitate early diagnosis. In spite of the generally supported observations that normal prenatal and birth history are typical of children with AS (Fryburg *et al*., 1991; Clayton-Smith, 1993c; Williams *et al*., 1995; Smith *et al*., 1996; Williams *et al*., 2006), we found various complications during pregnancy and birth in three out of seven (43%) children. However, they all showed normal growth parameters at birth. A recent update of the diagnostic criteria and some previous reports have pointed out frequent feeding difficulties at neonatal and infantile age (Van Lierde *et al*., 1990; Clayton-Smith, 1993c; Smith *et al*., 1996; Williams *et al*., 2006), but only one child in our study group had problems with feeding. Three out of seven (43%) in our study group were irritable and had decreased sleep during the first six months of life, features that are rarely described within early infantile development (Schulze *et al*., 2001). Nevertheless, it is remarkable that the parents and family physicians of all of our patients with AS were seeking specialized neurological consultation even before the children were six months old. In all children the neurologist noticed delayed motor development, truncal hypotonia, and brisk deep tendon reflexes in association with small HC. It has been noticed by previous investigators (Fryburg *et al*., 1991; Smith *et al*., 1996) that the growth of HC tends to stagnate, and children with AS and deletion appear to be remarkably microcephalic by two years of age. Two of our patients had normal HC (one with UPD), two had HC that deviated by -1 SD, and three (43%) were markedly microcephalic by the age of two years. Even if the stagnation of head growth cannot serve as a universal symptom, it is alarming, especially in conjunction with marked developmental delay. Excessive tongue thrusting and drooling with a mainly happy disposition has previously been described in babies with AS (Van Lierde *et al*., 1990; Yamada and Volpe, 1990; Fryburg *et al*., 1991; Clayton-Smith, 1993c; Bottani *et al*., 1994; Schulze *at al*., 2001). Only one child in our study group was noticed to have remarkable tongue thrusting at the age of eight months, while all others were described as passive with an apparent happy demeanor. The most constant finding within the first neurological examination was marked truncal hypotonia, which was noticed in six children. Considering previous observations (Fryburg *et al*., 1991; Clayton-Smith, 1993c; Smith *et al*., 1996), updated consensus concerning diagnostic criteria (Williams *et al*., 2006) and our own experience, the global developmental delay with overt truncal hypotonia, in association with happy demeanor and/or disproportionate growth in HC, could serve as the guiding

symptom complex for early diagnosis of AS. Our experience with children under 1 year of age proved: (i) the usefulness of broadened selection criteria (psychomotor developmental delay, marked congenital hypotonia or dystonia, and seizure disorder of unknown origin) — early diagnosis of AS considerably diminished pressure on medical staff and facilitated appropriate management; (ii) our family physicians are well-trained to recognize developmental problems and seek consultation from a child neurologist at an early stage; (iii) the parents had marked difficulty accepting the diagnosis of AS in early age, showing hopelessness and denial.

Four patients (57%) with AS had epilepsy. Two patients (NL, MM) demonstrated infantile spasms at 6 and 10 months of age respectively, which gave the indication for the first EEG. In both cases the EEG revealed a hypsarrhytmia-like pattern — irregular generalized high-voltage slow activity with multifocal spikes and sharp waves. Antiepileptic treatment with valproic acid was successful in both patients. One patient (MV) had two consequent febrile seizures at the age of 2 years, after that epilepsy with myoclonic absances was diagnosed, and subsequent treatment with valproic acid was effective. He demonstrated an EEG pattern typical of young children with AS — runs of high amplitude (>200μV) 3–4/s rhythmic activity, maximal over the occipital region and containing spike waves. The EEG findings facilitated the diagnosis of AS, which was made at the age of 2y 7m. The fourth subject (HE) was 1y 8m old when generalized tonic-clonic seizures began. In his case the treatment with carbamazepine and valproic acid was ineffective, clonazepam worked well, but he still has 1 or 2 short partial seizure attacks per year. His EEG at the age of 11y3m showed persistent rhythmic 5 Hz theta activity intermittently with high-voltage spike and slow wave complexes over the frontoparietal regions, with bursts of generalized slow high-voltage delta activity. For three patients without epilepsy (1y 1m; 8y; 13y), EEG showed a slow and disorganized background rhythm.

Specific EEG findings and the early onset of epilepsy facilitated the diagnosis of AS in three patients (MV, MM, NL). The fourth patient (HE) was strongly suspected of having a metabolic disorder and the diagnosis of AS was made at the age of 10y 7m, when he demonstrated the entire phenotype of AS. The EEG abnormalities found in patients with AS are not pathognomonic to AS and have to be seen in the appropriate clinical context (Laan and Vein, 2005). Moreover, the changes in EEG alter with age, being more prominent in younger patients and less apparent after the age of 10–12 years (Boyd *et al*., 1988; Matsumoto *et al*., 1992; Clayton-Smith, 1993c; Casara *et al*., 1995; Laan *et al*., 1997; Buoni *et al*., 1999).

Table 9. Clinical characteristics of patients with AS

	KK	MV	HE	MM	RS	NL	YK
Sex	female	male	male	female	male	male	female
Age at diagnosis	13y 8m	2y 9m	10y 7m	2y 6m	1y 2m	1y 1m	8y
Age of sitting	9m	1y	na	1y 4m	-	-	6–7m
Age of walking	NA	NA	3y 4m	NA	-	-	1y 2m
Developmental delay (Griffiths)	10–11m	10–11m	10–11m	8m	7m	7–8m	na
Microcephaly	-2 SD	-1 SD	0 SD	-2 SD	-2 SD	-1 SD	0 SD
Absence of speech	+	+	3–4 words	+	+/?	+/?	few words
Hyperactivity	-	-	+	-	+	-	+/-
Tremor	severe	-	-	-	-	+	-
Truncal ataxia	+	+	+	+	+	+	-
Hyperkineses	-	-	-	+	-	-	-
Bottom-shuffling	+	+	+	+	-/?	-/?	na
Seizures	-	+	+	+	-	+	-
Onset of seizures		2y	1y 8m	10m		6m	
Characteristic EEG	+	+	+	+	+	+	-
MRI/CT	asymmetrical lateral ventricles	mild ventriculo-megaly, mild cortical atrophy	normal	mild ventriculo-megaly, mild frontal atrophy	mild ventriculo-megaly	normal	normal
Bone age	delayed	na	delayed	accelerated	accelerated	normal	na
Etiology	deletion	deletion	deletion	deletion	deletion	deletion	UPD

na — not available, NA — not achieved

Despite the fact that EEG abnormalities are usually manifested in the first 2 years of life, the abnormal EEG indicating possible AS is uncommon before the age of six months (Boyd *et al*., 1988; Casara *et al*., 1995). Therefore the EEG as a tool for early recognition of AS is not very helpful within the first half-year of life (when all of our patients were consulted by child neurologists), and several EEG recordings are often needed to find the typical pattern (Laan and Vein, 2005). The early onset of seizures and repeated EEGs have facilitated the diagnosis of AS in our patients. Surprisingly, none of the patients with AS in our study group showed severe epilepsy that was difficult to control as it has been described in the literature (Viani *et al*., 1995; Guerrini *et al*., 1996; Laan *et al*., 1997; Valente *et al*., 2005). All our patients with epilepsy received monotherapy: one with clonazepam and two with valproic acid. The early recognition of seizure disorder and careful AED adjustment with concomitant monitoring of serum concentration of AED may have played a role in adequate seizure control in the children in our study. The 57% rate of epilepsy (4 patients out of 7) in our AS group was lower than the previously reported 80% (Matsumoto *et al*., 1992; Williams *et al*., 1995; Laan *et al*., 1997; Buoni *et al*., 1999; Williams *et al*., 2006).

Three patients (43%) were suspected of having metabolic disturbances before the diagnosis of AS was made: two of them (MM, RS) demonstrated generalized organic aciduria (MM was treated with vitamins B_1 and B_6, and biotin), and one (HE) had primary lactic acidosis and was also treated with vitamins. In general, children with AS lack any significant biochemical/ metabolic abnormalities (Williams *et al*., 1995; Williams *et al*., 2001; Williams *et al*., 2006). Nevertheless, Williams *et al*. (2001) have described two children with mild lactic acidosis and encephalomyopathy who subsequently had resolution of their lactic acidemia and had positive genetic testing for AS.

MRI/CT showed a various degree of brain atrophy in four patients (57%): mild ventriculomegaly in three patients, with concurrent mild cortical atrophy in two cases, and an asymmetrical enlargement of the lateral ventricles in one patient. The neuroradiological findings of our patients were nonspecific and similar to those described in the literature: symmetrical or asymmetrical enlargement of the lateral ventricles, mild cortical atrophy, and dysmyelination in approximately 50% of patients with AS (Casara *et al*., 1995; Guerrini *et al*., 1996; Buoni *et al*., 1999). We were unable to identify any structural anomaly that originated from the early stage of brain development in our patients with AS. This concurs with the prevailing view that the CNS defects responsible for cognitive deficits and balance and/or movement disorder in AS may be too subtle to affect gross brain morphology (Leonard *et al*., 1993; Williams *et al*., 1995; Williams *et al*., 2006) or evolve in the course of time, and therefore further investigations are needed in order to acquire a better understanding of the pathological mechanisms of AS.

Interestingly, all children with the deletion of 15q11-13 who were older than 2 years developed the bottom-shuffling, quite unusual form of motion among children with other causes of mental retardation. The reason for this behavior could be a compensation for truncal ataxia and hypotonia. Moreover, one patient demonstrated severe generalized resting tremulousness (KK), a poorly understood and not universal clinical manifestation of AS. Another patient (NL) was 6 months old when a marked tremor in the upper extremities was noticed, and the third patient (MM) developed athetotic involuntary movements, which mostly involved the lower extremities. It has been shown that one third of older AS individuals suffer from worsened tremor compromising their ability to be ambulant and to feed themselves (Clayton-Smith, 2001). Moreover, two adults with a deletion (Harbord, 2001) and one with an UPD (Jacobsen *et al*., 1998) were described as presenting typical Parkinson disease, and the deletion patients demonstrated a good response to levodopa medication. Long-term observations are needed to clarify the connection between childhood movements/balance disorders and the development of Parkinsonism in older age.

In conclusion, according to our study and considering the data from the literature (Van Lierde *et al*., 1990; Yamada and Volpe, 1990; Fryburg *et al*., 1991; Clayton-Smith, 1993c; Williams *et al*., 1995; Smith *et al*., 1996; Schulze *et al*., 2001; Williams *et al*., 2006), testing for AS in the first year of life is indicated when a combination of the following symptoms are present: irritability, sleep disturbance, feeding difficulties, developmental delay with slow progression of head growth, truncal hypotonia with pyramidal signs, specific EEG pattern and/or seizures.

5.2.1.2. Bone abnormalities and AS (publication II)

Bone age was available for five patients, and this corresponded to chronological age in only one patient. Two patients had an accelerated bone age: MM at the age of 3y 2m demonstrated a bone age of 3y 9m and RS at the age of 1y 1m had a bone age of 1y 6m according to Tanner-Whitehouse radius, ulna, and short bones score (TW-RUS). Two patients showed delayed bone age: HE had a bone age corresponding to the age of 10 years when he was 11 years old and KK showed a bone age of 8.1 years when she was 13 years old. KK also demonstrated unusual marked limb deformities, generalized osteoporosis, and brachydactyly type B.

Case report of KK. She was born at term after an uneventful pregnancy and delivery with normal birth weight, length, HC and Apgar scores. During the first months of life she was irritable and had difficulty sleeping. She started to sit at 9 months, pulled herself to a standing position at 13 months, but she never began to walk independently. The patient was evaluated at the age of 21 months at Tartu Children's Hospital due to developmental delay and lack of speech. She

underwent full clinical investigation without significantly abnormal results, only her bone age was 7 months behind the chronological age. Her karyotype was normal (46,XX). The cause of her developmental delay at that time remained unclear. She was next hospitalized at the age of 13.3 years due to a marked developmental delay and severe limb deformities. On examination, her weight was 22 kg (-3 SD) and HC 51.5 cm (-2 SD). Her height was not measurable due to severe limb deformities. There was a pigmented spongy formation with a diameter of 3 cm on her forehead. She still had milk teeth. There was no breast development and she had few pubic hairs. Her fingers were short and wide, especially the thumb and first toe. She had a marked generalized resting tremor, an intentional tremor and truncal ataxia, and she moved by bottom-shuffling or on her hands and knees. Her tendon reflexes were slightly exaggerated with no pathological reflexes, and her muscle tone was considered normal.

X-ray investigations demonstrated generalized osteoporosis. The femur, tibia, fibula, ulna and radius showed curved deformities in the distal diaphysis-metaphysis areas. The compact layer possessed marked changes of rebuilding, the epiphyses were dysmorphic, and the epiphyseal lines were porous. Her bone age was 8.1 years according to TW-RUS. The distal phalanges of all her fingers were short. The left hand lacked the epiphyses of the distal phalanges of the II, III and V fingers, and the right hand lacked the epiphyses of the distal phalanges of the III and V fingers. On both hands the III metacarpal bone was hypertrophic with a curve to the palmar side. The biochemical results were as follows: normal ionized calcium 1.18 mmol/l (normal range 1.17–1.29), mildly decreased phosphorus 1.01 mmol/l (normal range 1.1–2.0) and slightly elevated parathyroid hormone 8.72 pmol/l (normal range 1.5–8.1). Alkaline phosphatase (ALP) was elevated 1179.0 U/L (normal range 70–300). Markers of bone formation (bone ALP iso-enzymes 888.3 U/L (normal range < 140) and osteocalcin 149.8 ng/ml (normal range 11–43)) and markers of bone resorption (desoxypyridinolin creatinin ratio 40 nmol/mmol (normal range 4.2–35.7) and C-telopeptide 1.93 ng/mmol (normal range 0,025–0,573)) were all elevated, indicating rapid bone turnover. The serum 25-hydroxyvitamin D_3 level of 2.4 μg/l (normal range 9.0–37.6) was significantly reduced. The excretion of phosphorus and calcium in urine were within normal limits. The serum quantitative amino acids analysis was normal, and there was generalized hyperaminoaciduria in the urine. The syphilis RPR latex test was negative.

To our knowledge the association of AS and marked limb deformities as well as brachydactyly has not previously been reported. The scabbard-like lower limb deformities in our patient resembled the changes associated with congenital syphilis, but the syphilis RPR latex test was negative. The generalized osteoporosis can be explained by the patient's severe disability, delayed puberty and presumable nutritional and environmental deficits (vitamin-D-deficient rickets). Considering that patients with AS have problems with bone maturation, delayed dentition, microcephaly, scoliosis and kyphosis,

it is possible that gene(s) causing AS play a role not only in the development and maturation of the brain, but also in the process of bone formation.

The clinical manifestations of scoliosis and/or kyphosis are consistently described in the independent series of AS patients and appear to become more pronounced or visible with advancing age (Buntinx *et al*., 1995; Sandanam *et al*., 1997; Clayton-Smith, 2001). These features could be nonspecific and are relatively common in adults with other severe developmental disabilities, related mostly to decreased mobility and wheelchair dependence. However, the fact that these features were present in several independent series of AS patients in different age groups indicates the possibility that scoliosis and/or kyphosis are clinical features of AS that are probably explainable by genetic factors.

The shortening of the distal phalanges of all of the fingers and the absence of some epiphyses of the distal phalanges in our patient can be classified as brachydactyly type B, typically characterized by the absent or hypoplastic terminal portions of digits II–V of the hands and/or feet (Winter *et al*., 1993). Brachydactyly may be either sporadic, part of an isolated familial brachydactyly or a feature of various skeletal dysplasias or syndromes: at least 345 syndromes and skeletal dysplasias with brachydactyly have been described, including PWS (Winter, 2001). Our patient had hypertrophic curved III metacarpal bones, probably caused by asymmetric shortening of other metacarpal bones typical to brachydactyly type B. Small hands and feet are often cited as a manifestation of PWS, but the shortening of hands and feet have not been specified (Hudgins and Cassidy, 1991). In AS, brachydactyly or shortening of the hands and feet have not to our knowledge been described before. The brachydactyly in our patient is probably a sporadic finding, although as our group of patients with AS was small, we cannot rule out the possibility that this feature is more frequent in AS.

Absolute or relative microcephaly is another frequent symptom of AS (Fryfurg *et al*., 1991; Buntinx *et al*., 1995; Williams *et al*., 1995; Smith *et al*., 1996). Head circumference is usually normal at birth, but head growth decelerates during the early years of life (the first year), and many individuals with AS become microcephalic. Two of our patients had normal HC (one with UPD), two had HC measurements that deviated by -1 SD, and three were markedly microcephalic by the age of two years. In addition, several typical AS features such as mid-face hypoplasia, prognathism and delayed and disturbed dentition had been explained by brain development abnormality or behavioral phenotype (Clayton-Smith, 1993c), although these features could just as likely be consequences of abnormal bone maturation, as four children (57%) in our study group demonstrated abnormalities in bone growth.

The *UBE3A* gene, which is known to play an important role in the development of the characteristic clinical phenotype of AS (Kishino *et al*., 1997; Matsuura *et al*., 1997; Sutcliffe *et al*., 1997), encodes the protein with two separable independent functions — a ligase and a coactivator for the nuclear

hormone receptor superfamily (Nawaz *et al*., 1999). Coactivators with additional enzymatic activity are thought to play an important role in regulating the magnitude of the biological response to steroids, vitamin D and retinoids in different tissues. Nawaz *et al*. (1999) found that in the majority of the examined patients with AS, the UBE3A ligase function was defective, but the coactivator function was intact. It is, however, possible that deletions of *UBE3A* can result in defective steroid receptor coactivation in tissues where *UBE3A* is expressed in an imprinted manner. To our knowledge, there are no available data of *UBE3A* expression in bone tissue. It is likely that the defective function of *UBE3A* influences organ growth, including bone formation and mineralization, or the severity of the manifestation of rickets.

Patients with PWS frequently develop osteoporosis and scoliosis at any age and kyphosis mostly in early adulthood (Cassidy *et al*., 2000). Osteoporosis in PWS is thought to originate from the combination of decreased production of sex or growth hormones and/or long-standing hypotonia. Interestingly, in PWS both bone mineral content and bone mineral density tend to be reduced, especially in the limbs (Brambilla *et al*., 1997; van Mil *et al*., 2001). It is plausible that in AS and PWS a certain gene or genes are responsible for bone formation and/or mineralization, as our patient KK's deformities and severe osteoporosis mostly involved the extremities, and the proportion of children with deviated bone age was almost the same (57% AS, 67% PWS) in both group of patients.

5.2.2. Sudden death and autopsy in the case of AS

Interestingly, three patients (43%) in our series of AS children were at first suspected of having metabolic disturbances: two of them (MM, RS) demonstrated generalized organic aciduria and one patient (HE) was diagnosed with primary lactic acidosis. Unfortunately, patient MM died of cardiac arrest one months and three weeks after falling into a coma due to putative Reye-like syndrome.

Case report of MM. MM (Table 9) was born after an uncomplicated full term pregnancy as the fourth child in the family (birth weight 3120 g, length 47 cm, HC 36 cm, Apgar score 8/8). On the 16th week of pregnancy an amniocentesis was performed due to advanced maternal age (37y), which revealed normal fetal karyotype (46,XX). However, since the first month of her life the mother was worried because of her extremely slow psychomotor development. At the age of nine months MM was referred to Tallinn's Children Hospital due to marked developmental delay. At that time her HC was 43.5 cm (-1 SD), she could not sit, crawl or stand, and her muscle tone was dystonic with marked truncal ataxia. Brain US showed slight ventriculomegaly (sin>dex), amino acid analysis of the serum revealed slightly elevated cysteine,

and alkaline phospatase (ALP) was 488 U/L (normal range <462 U/L). She was re-admitted and thoroughly investigated at the age of 16 months. The mother complained about the jerks that had been present during the last 5–6 months and a periodic appearance of tonic posture. On examination, she was very pale with a slight rash of diathesis on her cheeks. Her tongue was relatively large. Her muscle tone was hypotonic, with slight spasticity in the lower extremities, and bilaterally spontaneous Babinski reflex. HC was 45 cm (-1 SD), and she had no speech. She was able to sit when seated, and crawl. Her ENMG was normal, but her EEG showed hypsarrhythmia. Laboratory findings showed metabolic acidosis (ABE –7.5 mmol/l), but the anion gap was normal (19.0 mmol/l). Amino acid analysis of the serum and urine was normal; lactate was 2.0 mmol/l. Ammonia in plasma was initially mildly elevated — 85 μmol/l (normal range <50 μmol/l), but when repeated one month later it was normal. Urinary organic acid gas-chromatography/mass-spectrometry (GC/MS) showed mildly increased excretion of adipic acid (82 mmol/mol crea, normal <34.3), sebacic acid (2.1 mmol/mol crea, normal <1.4), 3-OH-isobutyric acid and 3-OH-isovaleric acid. The acyl-carnitine profile was normal by tandem mass-spectrometry, which excluded fatty acid oxidation defects in this patient. The origin of her developmental delay remained unclear, but epilepsy was suspected and treatment with valproic acid and vitamins B_1, B_6 and biotin was begun. Later she was repeatedly seen by a pediatric neurologist. Her ammonia was elevated (120 μmol/l) again after 5 months of valproic acid treatment, and metabolic acidosis persisted. The level of ammonia remained elevated — 90 μmol/l, 52 μmol/l, 73 μmol/l (ALP 621 U/L, normal range <462 U/L). A brain CT was performed at the age of 23 months and showed mild ventriculomegaly and mild frontal atrophy. When 2y 5m old, she had normal growth, with height 90 cm (0 SD), weight 15.5 kg (0 SD), but microcephaly (HC 46 cm, –2 SD). She was severely mentally retarded with a hypomimic face, fair hair, a small and upturned nose, everted lower lip, mild prognathia, autistic behavior, frequent smiling and absent speech. There were marked axial hypotonia and truncal ataxia, slight spasticity in her lower limbs, exaggerated deep tendon reflexes with pathological reflexes (Babinski), and episodic athetotic movements. She could walk only with support. AS was clinically suspected and confirmed by a positive DNA methylation test, which revealed that the maternal allele was missing in AS/PWSCR. Later on, FISH analysis showed micro-deletion in region 15q11-13. At the age of 3y 3m the brain MRI did not show any focal abnormalities in her brain tissue.

At the age of 3y 5m she was hospitalized to the intensive care unit due to persisting high fever in the past three days, recurrent tonic seizures and presumed unconsciousness. Acetaminophen was repeatedly administered at home, but to no avail. She was immediately intubated, and assisted ventilation was begun. She had mild hepatomegaly and a maculose skin rash around her mouth, which was suspected to be a herpes virus (HV) infection, as her mother

had an HV infection on her lips. Cerebrospinal fluid (CSF) analysis was normal (pleocytosis $1x10^6$/l, glucose 4.6 mmol/l, lactate 2.3 mmol/l, and protein 0.16 g/l). Laboratory investigations showed increased liver transaminases (ALAT 118 U/l, ASAT 286 U/l). EEG showed generalized slow and high amplitude activity, epileptic slow spike-wave complexes and also triphasic waves, which are thought to be characteristic of herpes encephalitis. Brain MRI showed a diffuse high signal of white matter in periventricular regions, as well as foci of high intensity in the basal ganglia (capsula interna, globus pallidus). This finding did not affirm HV encephalitis, but Reye syndrome was possible. Concurrent brain CT was considered normal. Both Reye syndrome and viral infection (HV), however, remained clinically probable. Therefore treatment with acyclovir and antibiotics was begun. Treatment of her seizures was performed with phenobarbital instead of valproic acid, considering increased liver transaminases and hepatomegaly.

On the 3rd day of hospitalization, liver transaminases were maximally increased (ALAT 2232 U/l, ASAT 1594 U/l), but later reduced to the normal level. LDH was also increased 1846 (normal range <615 U/l) and ammonia was 67 (normal range <48μmol/l). There was a reversible coagulation problem — prothrombine activity was low: 29% (normal range 70–130%), prothrombine INR was high: 1.89 (normal range 0.85–1.25) and antithrombine factor III was low: 52% (normal range 80–120%).

On the 4th day of hospitalization, brain CT showed prevalent diminution of the intensity of white matter and diffuse brain edema. The PCR reaction of CSF for HV 1 and 2 was negative, as also were immunoglobulins G and M for HV1 and HV2. No other microbes or fungi had not been found in the blood or CSF. Therefore HV encephalitis and the possibility of any other infectious disease was excluded. Reye syndrome with unknown origin remained the main/only clinical diagnosis.

After 10 days her condition worsened — the cough reflex as well as reaction to pain and aspiration disappeared. The pupil reflex was very weak. Repeated brain MRI showed foci with high signal intensity in the basal ganglia region and in the occipital area as a subject of the ischemic damage due to persistent brain edema. She remained in a deep coma and died one month and three weeks later due to cardiac arrest.

On the 2nd week of hospitalization a liver biopsy was performed and the material for electron microscopic investigation was collected. In hepatocytes, electron microscopic investigation showed extensive glycogen storage in the cytosol. In the lysosomes, heterogeneous and various size vacuoles filled with glycogen granules were found. The endoplasmatic reticulum only had free space for functioning that amounted to 10–15% of the total cytosol. The structure of mitochondrions was changed; there were giant mitochondrions and paracristalline structures in mitochondrions. No cholestasis and lipid accumulation were found in hepatocytes, but there were lipid granules and

hemosiderine inclusions in Kupffer cells. A lot of neutrophils were between hepatocytes and in capillaries, and perivascular tissue showed some fibrotic changes. These findings are typical of energy production failure in either the primary or secondary respiratory chain, or to the glycogenoses. These findings are not typical to Reye syndrome, as in the case of Reye syndrome there is usually liver steatosis or swallowing of mitochondrions.

At autopsy the only significant findings in the systemic examination were petechias on the upper side of the diaphragm, under the capsule of both kidneys, and on the mucosa of the larynx, trachea and bronchi. The kidneys and the liver were flaccid, and the section surface of the liver was fawn-colored. The most striking finding on gross inspection of the brain was its size and consistency — the brain was small (896g (normal 1150g)) and flaccid. The gyri were flattened, the sulci narrow, the blood vessels of the meninges congested, and on the surface of the cerebellum there was only a mild impression from the strangulation. Sections from various locations of the brain were frozen, and the remainder was fixated in formalin.

Pathohistological specification affirmed an acute purulent bronchitis, a chronic interstitial nephritis, a polycystic ovarian degeneration and a disseminated fatty degeneration of the liver in our patient.

Macroscopically, there were clots in the venous sinuses of the dura (taken for histological specification), and the blood vessels of the leptomeninges were slightly congested. On the basal surface of both temporal lobes and the left occipital lobe, there were extensive indrawn flaccid areas of collapsed brain tissue. All lateral ventricles were dilated; the entorhinal cortex area was shrunken and yellow. The medial part of the temporal lobes was softened and grizzle-brown in color. In these areas the cortex was very thin with microgyri, and the white matter underneath the cortex was friable and easily breakable. An unusually large epiphysis with cystic degeneration was noticed.

Histological investigation verified the thrombosis of the sinus sagittalis. Sections from the frontal cortex showed normal neuronal layering and lamination, but extensive areas of ruined neuronal layering with only solitary preserved neurons and widespread astrogliosis throughout the whole frontal cortex without clear demyelination of white matter was stated. Unharmed areas from temporal lobes had a subjectively normal architecture and number of neurons, but previously macroscopically described dorsal and medial parts showed extensive areas of necrosis with intensive macrophagous infiltration, gliosis, and Alzheimer type II astrocytosis. On the right, there was a softened area at the border of the temporal and occipital lobes where, in addition to encephalomalacia, extensive deposition of calcifications throughout the cortex was found. The basal ganglia showed intensive necrosis bilaterally, and extensive gliosis was found in both hippocampi, with almost vanished pyramidal cells beginning from the CA 2 region. There were old and recent necrotic areas in the right thalamus, as well as abundantly Alzheimer type II

astrocytes, although the epiphysis seemed to be of normal structure. The mediodorsal parts of the occipital lobes revealed almost vanished cortex tissue with proliferous macrophages and gliotic callus, as white matter showed gliotic changes and a spongiform constitution. Small softened areas with macrophages and gliosis were found in the ventral mesencephalon and also around the aqueduct. The substantia nigra was preserved and of normal structure. In the brainstem, the grey and white matter were intermittently laminated, and ischemic lesions of neurons were found. Cerebellar neurons had ischemic changes with Bergmann's gliosis, and some lessening in the amount of neurons and Purkinje cells in the dental nucleus was noticed. Extensive ischemic alterations were found in otherwise normally structured olivary nuclei.

Valproic acid, the most effective AED in AS seizure disorder (Viani *et al*., 1995; Laan *et al*., 1997; Buoni *et al*., 1999; Valente *et al*., 2006), seems to be well-tolerated and is widely used alone or in combination with the other(s) AED(s) in epilepsy patients with AS. However, in 2004 Deda *et al*. described a case of reversible toxic hepatitis in a 2-year-old boy with AS and Lennox-Gastaut syndrome after valproic acid and lamotrigine comedication. Unfortunately, no pathohistological findings were presented in this case. Our patient had slight metabolic acidosis, elevated ammonia and ALP even before the treatment with valproic acid, and showed a fluctuating persistence of these laboratory abnormalities during the treatment period. It is possible that valproic acid treatment could lead to subacute hepatic failure, and concomitant fever management with acetaminophen, one of the most common medications causing drug-induced hepatic toxicity in children (Pineiro-Carrero and Pineiro, 2004), aggravated the situation to the critical Reye-like illness. Her clinical problems on admission most closely resemble a valproate-induced hyperammonemic encephalopathy (VHE) — a decreased level of consciousness, low-grade fever, and an increase in the frequency of seizures. On admission she demonstrated EEG findings characteristic not only to herpes-encephalitis, but also to VHE — triphasic waves and, moreover, the brain MRI showed a diffuse high white matter signal of the periventricular regions, foci of high intensity in the basal ganglia (capsula interna and globus pallidus), the findings described in VHE (Ziyeh *et al*., 2002). The function of the *UBE3A* in the pathogenesis of AS is poorly understood. Ubiquitin-mediated proteolysis is regarded as the major pathway by which most intracellular proteins are destroyed (Ciechanover, 2001). From the toxicological standpoint it is equally important that the ubiquitin-proteasome system is also widely considered to be a cellular defense mechanism, since it is involved in the removal of damaged proteins generated by adduct formation and oxidative stress (Donohue, 2002). Our case emphasizes the possibility that patients with AS who lack maternally imprinted *UBE3A* tissue specific expression, may be more prone to hepatic problems. Our patient likely died of a combined idiosyncratic valproic acid and acetaminophen

hepatotoxicity with Reye-like syndrome, which was complicated by malignant brain edema and edema-induced ischemic lesions in the brain.

To our knowledge, only two AS autopsy cases have been published (Jay *et al*., 1991; Kyriakides *et al*., 1992) to date: a 21-year-old female and a 2y 10m old boy. In these cases the diagnosis of AS was based on clinical findings alone. The young adult died from recurrent pneumonia and pulmonary abscess, and the child experienced SUD (probable asphyxia, secondary to a seizure). The most striking findings from the autopsy of the female were: a small brain, marked cerebellar atrophy with loss of Purkinje and granule cells and extensive Bergmann's gliosis, as well as a marked decrease in the dendritic arborization of layer 3 and 5 pyramidal cells of the visual cortex (Jay *et al*., 1991). The major neuropathological findings in the boy were macroscopic and consisted of a small brain, truncated temporal lobes and relatively small frontal lobes with poorly delineated gyri (Kyriakides *et al*., 1992). Our patient, 3y 7m, had a small brain, extensive edematous-ischemic lesions with necrosis and gliosis, Alzheimer type II astrocytosis of temporal lobes, hippocampal gliosis with almost completely destroyed hippocampal pyramidal cells, and also cerebellar atrophy with concomitant unobtrusive lessening in the amount of Purkinje cells and Bergmann's gliosis. The autopsy findings of MM indicate three different pathological changes: chronic edematous-ischemic, chronic hepatic failure, and also alterations of AS. In the case of chronic liver failure, the astrocytes, the neural cells most vulnerable to hepatic failure, undergo characteristic changes known as Alzheimer type II astrocytosis (Hazell and Butterworth, 1999), and these changes were present in our case in the thalamus and in the medial parts of the temporal lobes. However, the only similarity connecting these three deceased patients with AS is microcephaly (typical for AS?), whereas the patient described by Jay *et al*. (1991) and our patient showed cerebellar atrophy with a decreased number of Purkinje cells (long term AED?/link to truncal ataxia?) and Bergmann's gliosis (a regular accompaniment of anoxic/ischemic injury and other conditions associated with the death of Purkinje cells). More morphological studies are necessary to elucidate the enigmatic pathogenesis and the involvement and susceptibility of different organ systems in AS.

5.2.3. Clinical phenotype of PWS

5.2.3.1. Phenotype of patients with PWS (publication III, IV)

A total of 12 patients with PWS were diagnosed among children born between 1984 and 2004; one girl (del 15q11-13) died before the study period, and one boy (Robertsonian translocation 15q;15q) was lost for follow-up before molecular genetic studies were completed. Ten patients (3 boys, 7 girls) were diagnosed during the study period (2000–2004). The overall diagnostic

frequency was 11% (10 cases out of 91 suspected subjects), but considering only the subjects born between 2001–2004 (prospective group), the diagnostic frequency was 33% — of 15 suspected patients, five were positive for PWS. The mean age at diagnosis was 4.3 years, but since 2000 all children were diagnosed in infancy, and the last three patients in their first week of life. Two recent studies reported the mean age at diagnosis for PWS being between 5y 4m (del) and 6y 2m (UPD) (Varela *et al*., 2005), or between 3.86 y (del) and 3.89 y (UPD) (Torrado *et al*., 2006), indicating that new and reliable diagnostic possibilities could play an important role in much earlier diagnosis than was the case years ago. Moreover, a systematic search for certain patients and quick diagnostic work-up can serve as a good training tool for neonatologists/ physicians to recognize relevant patients, and our low mean age at diagnosis of PWS is a good example of this.

After birth, all patients were hospitalized from maternity hospital to the children's hospital due to insufficient arousal and muscular hypotonia of unknown etiology. Early diagnosis of five consecutive patients with PWS allowed us to study in detail the peculiarities of the neonatal period in PWS. All neonates showed marked central muscular hypotonia (Figure 4), insufficient arousal, almost absent or weak crying, no interest in food, transient bradycardia (110–70 beats per minute) and thermolability (incubator care for hours/days), pale and/or intensely mottling skin with acrocyanosis, and needed aided feeding for several weeks/months (Table 10). During the first two or three days of life, congenital and deep tendon reflexes were difficult to elicit. In addition, all five had a distinctive facial appearance — a high prominent forehead, a narrow bifrontal diameter, downturned corners of the mouth, micrognathia and dysplastic ears (Figure 5). Three neonates had a high-arched palate and four out of five demonstrated arachnodactyly. We also noticed a peculiar position of the thumbs during the first days of life — patients 2–5 constantly held their thumbs adducted over the index and middle finger, and sometimes even over the ring finger (Figure 6). Two of our five newborns had signs of hypogenitalism — the male patient had bilateral cryptic testes and one female had hypoplastic labiae majora. Two patients with UPD demonstrated a delay in passing meconium, and spontaneous defecation occurred on the 6^{th} and 3^{rd} day respectively. Two patients with deletion revealed cephalhematomas after birth.

The first major consensus diagnostic criterion of PWS is neonatal central hypotonia with poor suck, which gradually improves with age (Holm *et al*., 1993). All of our patients had central hypotonia, poor suck during the first few weeks/months of life and feeding difficulties. It is not entirely clear whether the poor suck (inability to suck and swallow) is due to an immaturity of the sucking reflex, insufficient arousal, muscular hypotonia, lack of interest in food or a combination of all of these.

The facial appearance of neonates with PWS has been subject to debate for many years. Some authors reported subtle or absent facial dysmorphy (Aughton

and Cassidy, 1990; Miller *et al*., 1999), whilst others considered facial features to be relevant for the diagnostic process (Stephenson, 1980; Chitayat *et al*., 1989; Trifirò *et al*., 2003). Recently, Trifirò *et al*. (2003) reported that 12 out of 21 infants with PWS presented at least three different craniofacial features (almond-shaped eyes, small palpebral fissures, narrow bifrontal diameter, micrognathia, low-set ears, a long philtrum, a thin upper lip, dolichocephaly, a downturned mouth), and that seven infants were clinically diagnosed with PWS as neonates. All of our newborns with PWS were photographed to document and investigate the facial features. The comparison of the photographs reveals striking similarities and a recognizable facial phenotype — a high prominent forehead, a narrow bifrontal diameter, downturned corners of the mouth, micrognathia and dysplastic ears, regardless of the genetic etiology of the syndrome (Figure 4A-E). To our knowledge there have to date been only a few reports documenting the facial features of newborns with PWS using photographs (Stephenson, 1980; Matsumura *et al*., 2003; Klein *et al*., 2004). We found that photographs were extremely helpful in recognizing facial phenotype. Furthermore, our patients have demonstrated a similarity in facial gestalt to the previously described cases of newborns with PWS.

Denizot *et al*. (2004) reported on a case of distal arthrogryposis in a newborn with PWS, and Klinge *et al*. (2001) described a newborn who tended to hold both thumbs adducted, although they did straighten on passive movements. In our study, four of five newborns with PWS constantly held their thumbs adducted over the I and II fingers, and sometimes even III finger (Figure 6). The same phenomenon was seen on two photographs of newborns with PWS at the age of 3–4 weeks published by Stephenson (1980). In addition, there were several nonspecific descriptions of PWS newborns' hands as flexed wrists and paralyzed distal parts of the hands (Aughton and Cassidy, 1990). The specificity of this finding for PWS needs further clarification. In addition, the acceptance of small hands and feet as the criteria for PWS (Holm *et al*., 1993) might be confusing in neonatal age. In contrast, long and/or tapering fingers have been described (Chitayat *et al*., 1989; Aughton and Cassidy, 1990), and in our study group four out of five newborns had arachnodactyly as a remarkable symptom.

Another controversial issue in PWS is the presence of neonatal respiratory distress. Some authors have described birth asphyxia in 23% of patients with PWS (Wharton and Bresnan, 1989), but others found neither perinatal asphyxia nor severe respiratory distress after birth (Trifirò *et al*., 2003). All of our patients were born with an Apgar score of 7 or more immediately after birth and also at 5 minutes. There may be a predisposition to mild birth asphyxia, respiratory distress or apnoic spells in PWS (Chitayat *et al*., 1989; Yoshii *et al*., 2002; Horsthemke *et al*., 2003; Denizot *et al*., 2004). Wharton and Bresnan (1989) have pointed out that asphyxiated babies did not differ from their peers in terms of pregnancy duration, position *in utero* or fetal movements, but others noted that respiratory distress or apnoic spells were related to prematurity or

were present in babies who were small for their gestational age (Chitayat *et al*., 1989; Yoshii *et al*., 2002; Horsthemke *et al*., 2003; Denizot *et al*., 2004). A similar correlation was seen in our patient 2 (Table 10), who was small for her gestational age (40 wks/2316 g) and demonstrated apnoic spells. Due to severe hypotonia and often abnormal presentation in utero, babies with PWS could be predisposed to birth asphyxia and may also have hypoxic-ischemic brain damage. Recognizing postnatal signs not related to hypoxic birth-insult could assist in establishing an accurate diagnosis of PWS.

All of our patients (5/5) demonstrated thermolability and acrocyanosis, and three of them also intense skin mottling. Williams *et al*. (1994) proved that abnormal temperature regulation in patients with PWS, compared with other neurodevelopmentally handicapped individuals, is a perceptual bias, but during the neonatal period, the coexistence of profound hypotonia, bradycardia and thermolability is a valuable marker for the identification of patients with probable PWS. Chitayat *et al*. (1989) has also pointed out cold extremities and skin mottling, and all of the newborns in our study group were pale and/or demonstrated intensely mottling skin.

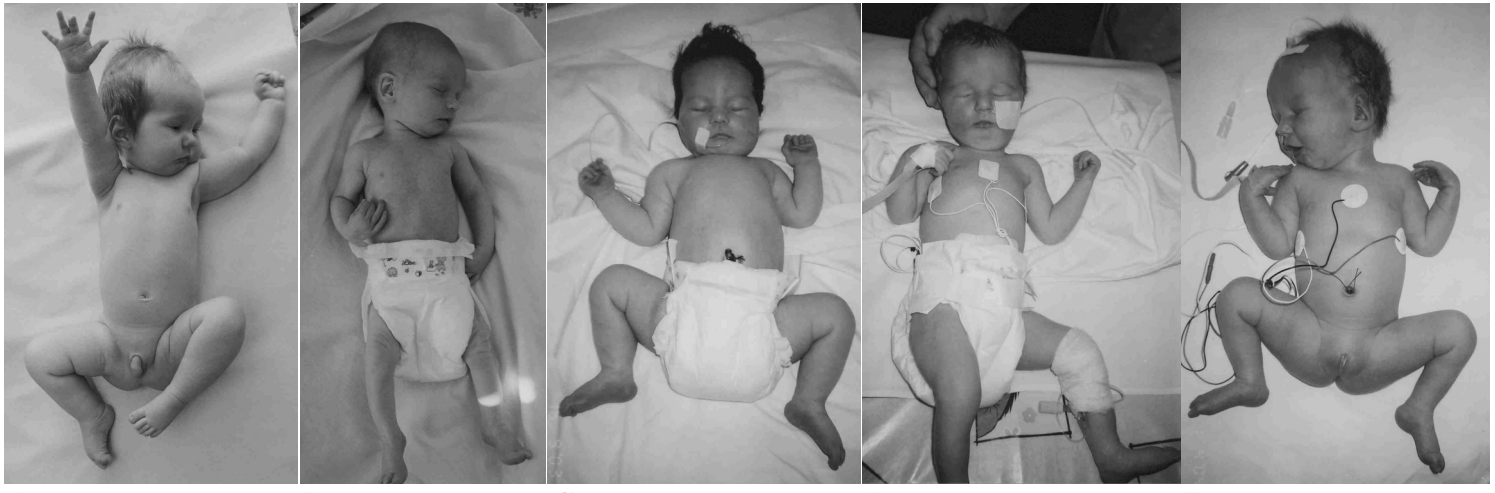
The minor criteria of PWS include decreased fetal movements or infantile lethargy or weak cry in infancy, improving with age (Holm *et al*., 1993). The vast majority of investigators have dealt with absent or weak abnormal cry in neonates with PWS (Chitayat *et al*., 1989; Wharton and Bresnan, 1989; Aughton and Cassidy, 1990; Holm *et al*., 1993; Miller *et al*., 1999), while lethargy, insufficient arousal or hypersomnolence have been only sparsely mentioned (Hoefnagel *et al*., 1967; Wharton and Bresnan, 1989; Yoshii *et al*., 2002). Nevertheless, weak or absent cry seems to be a consequence of insufficient arousal. In addition, the controversy in assessing congenital reflexes may be explained by the different time of examination (Hoefnagel *et al*., 1967; Stephenson, 1980; Miller *et al*., 1999; Klinge *et al*., 2001). Our experience with neonates showed that all of the babies were severely depressed after birth, so congenital and deep tendon reflexes during the first two or three days of life were released with latency or were difficult to elicit, but later were normal or even slightly exaggerated.

Table 10. Neonatal phenotype and etiology of five newborn children with PWS

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**
Age at clinical suspicion of PWS	4 days	21 days	11 days	4 days	3 days
Sex	male	female	female	female	female
Maternal age (yrs)	33	22	32	18	41
Diminished fetal movements	-	-	+	-	+
Gestation (weeks)	40	40	41	41	41
Birthweight (g)/length (cm)	3220/50	2316/49	3528/51	2700/53	2870/48
Clinical features					
Lethargy or apathy	lethargy	apathy	lethargy	apathy	apathy
Muscular hypotonia	+	+	+	+	+
Cry	weak	weak	weak	almost absent	weak
Aided feeding	+	+	+	+	+
Duration of tube feeding	17 days	16 days	3 months	3 months	2 months
First spontaneous defecation	6 days	unknown	3 days	before 12 hours	before 12 hours
Acrocyanosis	+	+	+	-	+
Thermolability	+	+	+	+	+
Bradycardia	+	+	+	+	+
Cephalhematoma	-	+	-	+	-
Pale appearance	?	+	+	+	-
Other findings		apnoic spells			

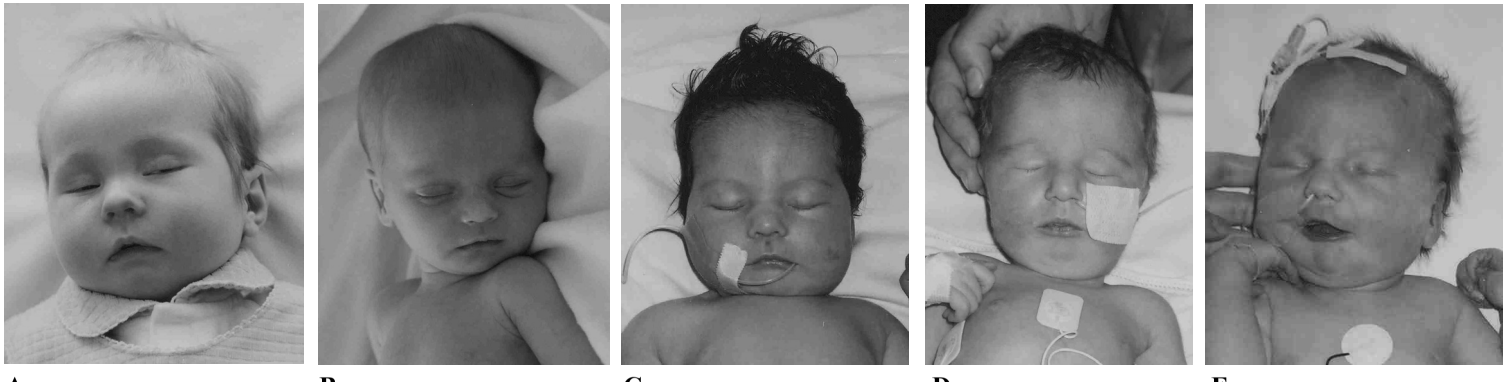
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Facial anomalies					
High prominent forehead	+	+	+	+	+
Dolichocephaly	-	-	-	+	-
Small anterior fontanelle	-	+	-	+	-
Narrow bifrontal diameter	+	+	+	+	+
Almond-shaped eyes	+	+	+	+	+
Nose			small	beaked	small
Downturned corners of the mouth	+	+	+	+	+
High-arched palate	-	-	-	+	+
Micrognathia	+	+	+	+	+
Inward obliquity of the upper gums	-	-	-	+	-
Dysplastic ears	+	+	+	+	+
Long neck	-	-	-	+	-
Extrafacial anomalies					
Thumbs overlapping II and/or III fingers	not mentioned	+	+	+	+
Arachnodactyly	-	+	+	+	+
Camptodactyly	-	-	-	+	-
Clinodactyly of 5th fingers	-	+	-	-	+
Heart anomaly	-	-	-	FOA, PDA	FOA, PDA
Other anomalies	bilateral cryptic testes	hypoplastic labiae majora	-	-	horseshoe kidney, hip dysplasia
Etiology	UPD	deletion	UPD	15p13-q13 deletion	UPD

UPD — uniparental disomy; FOA — *foramen ovale apertum*; PDA — patent *ductus arteriosus*



A B C D E

Figure 4.



A B C D E

Figure 5.

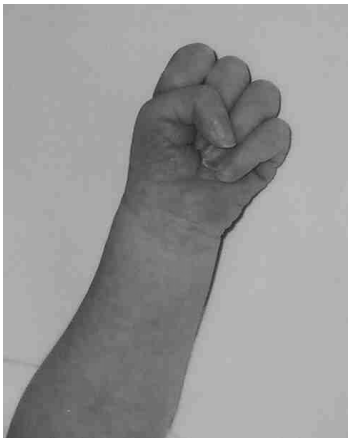


Figure 6.

According to our results and the previously published data, the main diagnostic criterion of PWS is neonatal hypotonia, but it's differential diagnosis comprises a broad spectrum of disorders. According to Paro-Panjan and Neubauer (2004), the diagnostic approach to floppy infant consists of a detailed history and clinical evaluation as the first step, neuroimaging techniques as the second step, Oxford Medical Data as the third step, karyotyping and FISH for PWS as the fourth step, biochemical tests as the fifth step and specific investigations of muscle and nerve as the sixth step. However, Richer *et al*. (2001) suggested a more flexible schedule of investigations, based on family history, case evaluation and clinical findings, and the next step consisting of neuroimaging, EMG/NCS and/or specific genetic tests. They also recommended that a profoundly hypotonic neonate without electrophysiological abnormalities could be an object for DNA methylation testing for PWS. The results of our study suggest using the DNA methylation test even before EMG/NCS if the full described complex of symptoms in a term neonate is present. The DNA methylation analysis is a reliable primary and time-saving test covering the most frequent causes of PWS (up to 99%) and by using its advantages we were able to make the diagnosis as early as in the first week of the patient's life. For a patient and his/her family, this is undoubtedly the best solution, and saves them from unnecessary/painful tests and investigations, but it is also cost effective. Three of five newborns of our study group had UPD, and using the schedule proposed by Paro-Panjan and Neubauer (2004) and the FISH analysis as the primary test, we may have lost 60% of diagnoses, at least for some time.

All six patients with PWS who were born before 2000 were hospitalized in the neonatal care unit due to marked muscular hypotonia, feeding difficulties and apparent arousal insufficiency (Table 11). Two mothers out of six suffered

imminent miscarriage, two children were born in breech presentation, one mother suffered from hyperthyroidism during pregnancy; one mother with twins showed an increased level of 17-OH progesterone and was treated with prednisolone. Only one pregnancy was uncomplicated. Two children were preterm (34 wks; 33wks) and one was small for gestational age (38wks/1900g). Brain US showed different problems in three out of six neonates, one child had neonatal seizures and one demonstrated a marked neonatal tremor. They all needed assisted feeding, and the shortest duration of tube feeding was 17 days. Independent walking appeared after 18 months of age in all children. The mean age of unsupported walking was 20 months, while independent walking normally begins between 13 and 15 months. One subject (TP) had febrile seizures, and two others had abnormal EEG. Four out of six (67%) showed mild brain atrophy on CT/MRI: two had mild frontal atrophy and two mild ventriculomegaly. Bone age by TW-RUS deviated in four children: in one case it was accelerated by one year, whereas three others showed a delay of six months to 1 year. The onset of excessive weight gain was observed relatively late, at mean age of 3 years, which may be explained by the fact that two children were on a low protein diet during their first three years of life. Two older subjects were severely obese (BMI >40), three slightly overweight (BMI 25.0–29.9), and the youngest girl had a normal BMI. Adenoidectomia was performed in three patients. Four subjects showed various cardiovascular problems including decreased myocardial contractility, prolonged PQ time, suspected incipient cardiomyopathy, and FOA with mitral valve prolapse. Vision problems were noticed in four patients.

Using consensus diagnostic criteria, all patients collected 13 and more points; the mean score for six subjects was 13.6 points. None of our patients demonstrated thick viscous saliva, skin picking was a problem for only one individual, and there was no evident scoliosis or kyphosis. We observed frequent abnormalities in brain US in the neonatal period, and later the brain MRI/CT was abnormal in 67% of cases (4 patients out of 6), with two children having an abnormal EEG. The incidence of cardiovascular problems was surprisingly high (67%; 4 patients out of 6). The same percentage of patients showed deviations from normal bone maturation/bone age.

The neuropathologic defects associated with PWS are still largely unknown, and there have only been a few studies on brain abnormalities in PWS (Miller *et al*., 1996; Hashimoto *et al*., 1998; Klinge *et al*., 2001; Yoshii *et al*., 2002). Only recently Miller *et al*. (2006), using three dimensional MRI scans, showed ventriculomegaly in all, decreased volume of brain tissue in the parietal-occipital lobe in 50%, sylvian fissure polymicrogyria in 60%, and incomplete insular closure in 65% of subjects with PWS. We found mild ventriculomegaly or mild frontal atrophy in four out of six patients (67%) with PWS, although the origin of these abnormalities remained unknown — they might be a

consequence of dysontogenesis or complication of birth asphyxia induced hypoxic-ischemic changes.

Interestingly, no one in our study group had epilepsy, but two patients demonstrated abnormal EEG; slow background activity was found in one and bursts of spike waves in the other. It has been shown that patients with PWS and deletion tend to have epileptic seizures (Varela *et al*., 2005; Wang *et al*., 2005), but Wang *et al*. (2005) also detected EEG abnormalities in patients with PWS without epilepsy. Moreover, Wang *et al*. (2005) found that 20% of study subjects (both with and without seizures) had EEG features of persistent high-amplitude 4–6 Hz activities resembling a pattern typical of AS.

The ongoing discussion about SUD has raised questions about the still unknown or poorly understood features of PWS posing a vital risk. Disturbance of central respiratory control and hypoventilation probably are involved in the genesis of SUD, but the time of death being early in the morning (a normally high level of cortisol) and some autopsy cases showing adrenal hypoplasia (Stevenson *et al*., 2004) also suggest a role for adrenal insufficiency in PWS. Cardiomyopathy has been reported in individuals with larger than usual chromosome 15 deletion (Ahmad *et al*., 2001), cardiac alpha actin gene (*ACTC*) disruption at 15q14, but also in patients with common deletions in PWSCR and UPD (Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Nagai *et al*., 2005). As noted above, four out of six patients in our study group had different cardiovascular problems. Excess secretion of GH has been connected with increased soft tissue thickness and with sleep apnea (Gerard *et al*., 1997), but hypertrophy of the tonsils and adenoids have been reported in patients with PWS with and without GH treatment (Grugni *et al*., 2005). Three patients (50%) in our study group had adenoidectomia, two of them had UPD, and one had deletion. Therefore we would like to emphasize that all children with PWS need careful medical supervision that should include cardiovascular monitoring and otorhinolaryngological examination. We suggest that all children with PWS should undergo an interdisciplinary medical observation at least once a year and always before and during GH treatment. More detailed studies are needed to elucidate the role of different organ systems in critical illness and early death in PWS.

Several correlation studies between genotypic variants and different manifestations have been performed (Robinson *et al*., 1991; Mitchell *et al*., 1996; Cassidy *et al*., 1997b; Gunay-Aygun *et al*., 1997a; Webb *et al*., 2002; Varela *et al*., 2005). Most of these authors agree that individuals with deletion or UPD do not present significant differences in anthropometric measurements, neonatal hypotonia, hypogonadism and hyperphagia, but data for certain traits such as characteristic faces, IQ scores and behavioral problems are inconclusive. As our study group was small and five out of the twelve children were younger than 3 years old, we cannot give a reliable comparison between different subgroups.

Table 11. Clinical data and etiology of the remaining patients with PWS

	HV	KG	TP	MK	SK	AT
Age of diagnosis	14y	12y	11y	3y 3m	6y	7m
Sex	female	female	male	female	male	female
Maternal age	23y	39y	29y	22y	36y	18y
Complications	-	mother's hyperthyroidism	*abortus imminens* 24/25 wks	breech presentation	17-OH-progesterone↑, *gemelli*	*abortus imminens* breech presentation
Labor	term	preterm 34/35wks	term/cesarean section	term	preterm 33 wks cesarean section	term cesarean section
Birth weight	2810g	2000g	3450g	3000g	1404g	1900g
Neonatal care unit	+	+	+	+	+	+
Neonatal brain US/CT	subarchnoidal haemorrhagia	-	subependymal haemorrhagia in left lateral ventricle	N	N	periventricular cysts
Duration of tube feeding	?	?	1 m	17 days	2 m	1 ½ m
Independent walking	2 y	1 ½ y	1 ½ y	1 ½ y	2y 2m	1y 8m
Heart anomaly	-	decreased myocardial contractility	prolonged PQ		incipient cardiomyopathy	FOA mitral valve prolaps
Seizures	neonatal seizures	neonatal tremor	febrile seizures	-	-	-

	HV	KG	TP	MK	SK	AT
EEG	-	slow background activity	na	bursts of spike waves	-	-
Brain MRI/CT	-	mild frontal atrophy	mild frontal atrophy	mild ventri-culomegaly	-	mild ventriculomegaly
Bone age	accelerated (1y)	delayed (1y)	delayed (6m)	N	-	delayed (6m)
Mental development	moderate MR	moderate MR	moderate MR	-	mild MR	mild MR
BMI	44.6 (14y 10m)	40.4 (14y 7m)	26.1 (10y 3m)	27.1 (2y 10m)	26.5 (8y)	22.5 (6y 2m)
Excessive weight gain	?	>3y	5–6y	>3y	>2y	>2y
Adenoidectomia	+	-	-	-	+	+
Other	myopia	astigmatism	X-linked ichthyosis (steroid sulfatase deficiency), myopia	narcolepsy-like attacks, *synechii vulvae*	*hypospadia penoscrotalis*, astigmatism	*dysplasia coxae congenita*
Etiology	UPD	UPD	deletion	deletion	UPD	deletion

5.2.3.2. Severe PWS phenotype and unusual additional problems

Most deletions causing PWS are interstitial, but approximately 1% of the patients have *de novo* unbalanced structural rearrangements involving the proximal 15q region (Nicholls *et al*., 1998).

Case report of IB. Patient IB (Table 10, patient 4), the first daughter of a healthy 18-year-old woman, was born at 42 weeks of gestation. Her birthweight was 2700 g (-1.5 SD), length 53 cm (+1 SD), HC 35 cm (0 SD) and Apgar scores 7/8. She showed insufficient arousal, severe muscular hypotonia, bradycardia and thermolability. At 16 hours of life she started to keck and was admitted to the neonatal intensive care unit. Dolichocephaly, a small anterior fontanelle, a prominent nasal bridge, a beaked prominent nose, inward obliquity and slight hypertrophy of the alveolar ridges, microretrognathia, low set dysplastic ears, a long neck, arachnodactyly, slight camptodactyly of the II, III and IV fingers, a particular position of the thumbs overlapping the third fingers and long nails were noted (Figure 4D, Figure 5D, Figure 6). Cardiovascular examination revealed FOA and DAP. Tube feeding was discontinued at the age of three months. At the age of 8 months her psychomotor development was markedly delayed — she had no proper head control, and she was only able to turn to the side when in the spine position. Her psychomotor development corresponded to the age of 3 months. Brain MRI at the age of 8 months showed mild brain atrophy and some patchy gray matter areas close to the lateral ventricles, and subependymal heterotopia was diagnosed. The girl had an unbalanced translocation of chromosomes 14 and 15 resulting in a deletion of chromosome 15p13-q13. Using FISH, the deletion included the PWS critical region and BAC clone RP11–463i22, karyotype 45,XX,der(14)t(14;15)(p11;q13).ish der(14)t(14;15)(D14Z1/D22Z1+,D15Z1–,SNRPN–,bac463i22/AQ634845–,PML+,yac895h10+). Clone RP11–463i22 corresponds to the segment from 25,725 to 25,892 Mb on chromosome 15 (UCSC Genome Browser, May 2004 assembly). The distal end of the typical 15q11-13 deletions in PWS and AS is defined by BAC RP11–48j4 (http://www.sanger.ac.uk/PostGenomics/decipher), which spans from 24,346 to 24,490 Mb on chromosome 15 (UCSC Genome Browser). Hence this girl had a deletion at least 1.4 Mb larger than typical 15q11-13 deletions (Butler *et al*., 2004).

Several cases of familial and *de novo* balanced or unbalanced translocations have been reported, but only two case reports describe translocations involving chromosomes 14 and 15. Smith and Noël (1980) reported on a Robertsonian translocation t(14;15) in a mother and three of her seven children. One of them, a girl with the karyotype of 45,XX,t(14;15)(p11;q11), had the classical phenotype of PWS. Hasegawa *et al*. (1984) described a family with the reciprocal translocation of the chromosomes 14 and 15, which was detected in three family members: the mother, the maternal grandmother, and a maternal uncle of the proband. The proband and one of the first cousins had an

unbalanced translocation as 46,XY or XX,-15, + der(14),rcp(14;15)(q11.2;q13). They had a partial trisomy of the 14pter leads to the q11.2 segment and a partial monosomy of the 15pter leads to the q13 segment. The clinical features observed in both cousins were pale complexion, oxycephaly, a flat occiput, a very small forehead with narrow bifrontal diameter, thick hair and eyebrows, a beaked nose with a broad and flaring nasal bridge, poorly modeled ears, a hypoplastic maxilla, prognathism, a triangular mouth, a thin upper lip, a high-arced palate, abnormal dentition, small hands and feet, clinodactyly of the fifth fingers, hypoplastic genitalia, severe hypotonia and profound mental retardation. The boy (10y) was overweight and the girl (5y) was proportionally small for her age. Brain CTs were unremarkable; the boy had myoclonic seizures and abnormal EEG. However, since no DNA analysis was performed in these two cases, it is unknown whether the clinical phenotype was a consequence of maternal or paternal monosomy of the PWS/ASCR, as they both showed the clinical phenotype that overlaps with AS. An unbalanced translocation, including the deletion of the centromere of chromosome 15 and the proximal part of the short arm together with the deletion of PWSCR, seems to be extremely rare, as we did not find any similar cases in the literature. Also, we are not aware of previously published cases of a larger deletion involving the centromere and 15p13. Nevertheless, there is no clear evidence of functional genes proximal to the common PWS deletion breakpoints, and therefore it has been assumed that genes involved in the additional symptoms of the reported cases are located within the common BP3 and the distal end of the described deletions (Krajewska Walasek *et al*., 1998; Smith *et al*., 2000; Matsumura *et al*., 2003; Windpassinger *et al*., 2003; Varela *et al*., 2004a). However, our patient IB showed disproportionate intra-uterine growth, a prominent nasal bridge, a beaked prominent nose, inward obliquity and slight hypertrophy of the alveolar ridges, microretrognathia, low-set dysplastic ears, a long neck, slight camptodactyly of the II, III and IV fingers, FOA, DAP, marked psychomotor developmental delay, and subependymal heterotopia not ascertained as a classical PWS phenotype. Nevertheless, the origin of her "severe" PWS phenotype remains unclear, as in translocation cases, the imbalance of the derivative chromosome involved in the translocation could be another cause of the different/complicated phenotype.

In one patient with PWS (Table 11, TP), the X-linked ichthyosis due to steroid sulfatase (SS) deficiency was also diagnosed. He has a positive family history for X-linked ichthyosis: his older brother had the same problem. It has been shown that approximately 90% of patients with SS deficiency have a 1.9 Mb deletion on Xp22.3, the region susceptible to chromosomal rearrangements mediated by VNTR-like repeats flanking the deletion interval and resulting in complete deletion of the *SS* gene (Ballabio *et al*., 1989; Yen *et al*., 1990).

5.2.3.3. Sudden death (publication V)

Patient MK suffered a cardiac arrest during an episode of recurrent bronchitis at the age of 3 years and 6 months

Case report of MK. She was the first child of non-consanguineous parents and was born at term (Table 11). The labor was complicated: there was a period of 15 hours without amniotic fluid, and the umbilical cord was found to be around the neck and body. Her birth weight was 3000 g, length 52 cm and Apgar score 7/8. From the first hours of life she demonstrated severe hypotonia, lethargy, a weak cry and feeding problems, and needed treatment in a neonatal care unit. Her initial diagnosis was hypoxic-ischemic encephalopathy.

Feeding difficulties, failure to thrive, hypersalivation and marked hypotonia persisted. At seven months of age thorough clinical investigations were performed, but did not yield helpful results. An unspecified metabolic disorder was suspected, and a strict protein limitation (1.5 g/kg) in daily food intake was started *ex iuvantibus*. Her motor milestones delayed: head control was achieved at 4 months, crawling at 13 months and independent steps at 19 months. Brain CT at 17 months of age indicated mild ventriculomegaly with no focal abnormalities in the brain tissue. Upon clinical and metabolic evaluation at the age of 2 years, the low protein diet was discontinued. Shortly after that she developed an insatiable appetite and rapidly gained weight. At the age of 2 years 10 months she was seen by a clinical geneticist. She was severely obese (weight 25.5 kg, +5.5 SD) with normal height (97 cm, +1 SD) and HC (49.5 cm, 0 SD). Facial features included almond-shaped palpebral fissures, epicanthal folds, narrow bifrontal diameter, a small nose and a thin upper lip. Her hands and feet were small. Moderate muscular hypotonia and mild developmental delay were noticed. PWS was suspected, and FISH revealed the 15q11-q13 deletion.

At 3 years of age she developed excessive daytime sleepiness with frequent narcolepsy-like attacks, breathing disturbances and recurrent bronchitis. At 3 years 6 months, during an episode of bronchitis, she suffered a cardiac arrest during early morning sleep. After resuscitation she remained in a chronic vegetative state. CT imaging showed extensive brain tissue hypodensities in the frontal and temporal lobes, especially in the basal areas, moderate hyperdensity of the thalamus and middle structures, and bilateral marked symmetrical hyperdensities in the globus pallidus. The latter findings were initially interpreted as calcifications of the basal ganglia, but retrospectively were thought to represent hypoxic-ischemic brain lesions with bilateral symmetrical hemorrhages in the basal ganglia. She died two months later. The parents refused an autopsy.

This was the first report of a chronic severe breathing disturbance in a child with PWS, which in combination with acute recurrent bronchitis preceded a cardiac arrest. We were aware of only two previous reports on early deaths in PWS, and most of these patients had a short history of a febrile infection that took a very rapid course (Clericuzio *et al*., 1997; Schrander-Stumpel *et al*.,

1998). In our case, the history of bronchitis was in line with the previous reports, but we wanted to emphasize the pre-existing marked breathing disorder. Apnea and chronic hypoventilation are well-known causes of hypoxia, hypercapnia and *cor pulmonale*. In PWS, the severity of nocturnal oxygen desaturation correlates with the degree of obesity (Hertz *et al*., 1995). Ventilatory failure and cardiorespiratory distress may develop after massive weight gain, and can progress rapidly (Cadle and Hall, 1989). Our patient also became extremely obese within one year, with narcolepsy-like attacks and breathing disturbance.

Based on the recurring CT findings, her brain lesions most likely evolved between the age of 1y 5m and 3y 6m. The lesions may be explained by the chronic hypoventilation or the cardiac arrest. Their bilateral and symmetrical spatial distribution could possibly indicate that in PWS the basal ganglia may be especially vulnerable to hypoxemia. Few reports have focused on specific brain abnormalities in deceased patients with PWS, and reported findings have been heterogeneous (Hattori *et al*., 1985; Hayashi *et al*., 1992; Reske-Nielsen and Lund, 1992; Swaab, 1997; Stevenson *et al*., 2004). Hattori *et al*. (1985) reported on the disturbed undulating structures in the dentate nucleus, ectopia of Purkinje cells in the molecular layer and the heterotopia of middle-sized neurons in the cerebellar white matter in a 23-year-old male. Hayashi *et al*. (1992) described almost the same abnormality in a 6-month-old girl — disturbance of the undulating structures in the dentate nucleus and the inferior olivary nucleus, as well as a grumose degeneration of the nerve cells in the dentate nucleus. In an autopsy of 16-year-old boy, Reske-Nielsen and Lund (1992) found extensive calcifications in the leptomeninges, the first and second layer of the cerebral and cerebellar cortex, and along the ventricular system. Examination of the basal ganglia revealed calcified nerve cells and lumps of encrustations and lime salts in the vessel walls (Reske-Nielsen and Lund, 1992). These latter observations (Reske-Nielsen and Lund, 1992) are in line with our hypothesis that the basal ganglia may be especially susceptible to hypoxic stress in PWS.

Until 2000, the early death of PWS subjects had only been reported twice (Clericuzio *et al*., 1997; Schrander-Stumpel *et al*., 1998). Recently, widely introduced GH treatment and successively reported cases of SUD during the first months of treatment have raised the issues of the relationship between GH treatment and SUD and the importance of extreme critical illnesses in PWS (Eiholzer *et al*., 2002; Nordmann *et al*., 2002; Smith *et al*., 2003b; Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Van Vliet *et al*., 2004; Nagai *et al*., 2005). The same causes of death were, however, found in patients with and without GH treatment (Schrander-Stumpel *et al*., 2004; Stevenson *et al*., 2004; Nagai *et al*., 2005). Our patient never received GH, and her case follows the typical pattern of SUD in PWS — central respiratory dysregulation with recurrent respiratory infections followed by a cardiac arrest during early morning sleep.

6. CONCLUSIONS

1. The minimum livebirth prevalence of AS in Estonia is 1:52,181 (95% CI 1:25,326–1:129,785), and the population prevalence 1:56,112 (95% CI 1:25,780–1:152,899).

2. The minimum livebirth prevalence of PWS in Estonia is 1:30,439 (95% CI 1:17,425–1:58,908), and the population prevalence 1:30,606 (95% CI 1:17,105–1:61,311).

3. Our results demonstrate that the livebirth prevalence of AS is 1.7 times less than the livebirth prevalence of PWS. This finding differs from previous studies showing the seemingly similar prevalence of these syndromes.

4. We observed a nominal increase in the livebirth prevalence of AS in the years 1984–2004, from 0.88 to 4.23 per 100,000 livebirths (p=0.2). The increase in the livebirth prevalence of PWS over the same period was more pronounced, from 1.13 to 7.97 per 100,000 livebirths (p=0.032).

5. Genetic investigations of patients with AS confirmed 15q11-13 deletion in 6 patients and UPD15 in one patient. Six patients with PWS had UPD15, a surprisingly high proportion (50%), four patients had 15q11-13 deletion, and two had chromosomal rearrangements — a Robertsonian translocation 15;15, and an unbalanced translocation with karyotype 45,XX,der(14)t(14;15)(p11;q13).ish der(14)t(14;15)(D14Z1/D22Z1+,D15Z1-,SNRPN-,bac463i22/AQ634845-,PML+,yac895h10+).

6. We found that the coexistence of profound central muscular hypotonia, insufficient arousal, no interest in food, transient bradycardia and thermolability, peculiar skin appearance, the characteristic facial phenotype, and a peculiar position of the thumbs was indicative of PWS in a term neonate. The presence of this symptom complex appears to be sufficient to justify the DNA methylation-based test as a primary step in diagnostic investigations.

7. The patients with AS were diagnosed later (mean age at diagnosis 6.1 years) than patients with PWS (mean age at diagnosis 4.3 years). Early diagnosis of AS is complicated by the lack of seminal clinical symptoms in the infantile period.

8. The childhood clinical phenotype of patients with AS and PWS was generally similar to previously described patients. The exceptions were frequent brain abnormalities and cardiac problems in patients with PWS

(67%), and abnormal bone maturation in AS (57%) and in PWS (67%) in patients older than 1 year.

9. In 21% of our study patients we identified health problems not reported previously in AS and PWS: a girl with AS demonstrated unusual limb deformities, a boy with PWS had X-linked ichthyosis, a girl with unbalanced translocation 14;15 resulting in a large deletion of 15p13-q13 showed severe PWS with additional symptoms, and a girl with AS died because of a Reye–like syndrome.

10. We observed two cases of early death in patients with AS and PWS: a girl with PWS died suddenly from cardiac arrest during a respiratory infection, and a girl with AS died from Reye-like syndrome.

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SUMMARY IN ESTONIAN

ANGELMANI JA PRADER-WILLI SÜNDROOM EESTIS

Angelmani sündroom (AS) ja Prader-Willi sündroom (PWS) on tänapäeval geneetikas tuntud kui õed-sündroomid, sest mõlema sündroomi tekkepõhjuseks on muutused 15. kromosoomi pika õla regioonis q11-13. Kõigepealt avastati, et PWS on põhjustatud väikesest interstitsiaalsest deletsioonist 15q11-13 regioonis (Ledbetter jt., 1980; Ledbetter jt., 1981). Segadus saabus siis, kui selgus, et ka AS puhul on patsientide kromosoomianalüüsil võimalik avastada samasugune deletsioon (Kaplan jt., 1987; Magenis jt., 1987; Donlon, 1988). Alles RFLP metoodika kasutuselevõtt võimaldas eristada kahte deletsiooni — AS põhjustav deletsioon asus alati emalt päritud ja PWS deletsioon isalt päritud 15. kromosoomis (Butler jt., 1986; Knoll jt., 1989; Nicholls jt., 1989a). AS ja PWS esindavad geneetilisi haigusi, mille tekke aluseks on genoomne imprinting (vermimine) ehk nähtus, kus geeni(de) avaldumine (ekspressiivsus) sõltub geeni(de) ema- või isapoolsest pärinemisest (Nicholls jt., 1989b; Hall, 1990; Williams jt., 1990). Molekulaargeneetilisi mehhanisme, mis põhjustavad AS-i ja PWS-i on mitu: 15q11-13 piirkonna deletsioon, uniparentaalne disoomia (UPD) (mõlemad 15. kromosoomid ühelt vanemalt) (Nicholls jt., 1989b; Knoll jt., 1991), defektne vermimine (ID) (Buiting jt., 1995) ning AS puhul ka mutatsioonid *UBE3A* geenis (Kishino jt., 1997; Matsuura jt., 1997; Sutcliffe jt., 1997).

AS kirjeldas esmakordselt inglise pediaater Harry Angelman 1965. aastal kui ta artiklis ““Puppet children”: a report on three cases” võttis kokku kolme sarnase patsiendi kliinilise leiu ja uuringute tulemused. Kolme patsienti iseloomustasid sügav vaimupuue, kõne puudumine, sarnased näojooned, naeruhood, (hüpik)nukulikud liigutused, krambid ja EEG leiu iseärasused. PWS esmakirjeldus pärineb John Langdon Downilt, kes 1864. aastal avaldas ühe oma patsiendi põhjaliku kirjelduse ning nimetas haigust, mille all patsient kannatas, “polysarca” (Down JL, 1864, tsiteering Ward, 1997). Haigus taasavastati kui 1956. aastal Zürichi arstid Andrea Prader, Alexis Labhart ja Heinrich Willi kirjeldasid üheksat ülekaalulist, lühikesekasvulist, krüptorhismi ja oligofreeniaga patsienti, kes kõik olid olnud varases lapsepõlves hüpotoonilise lihaskonnaga (Prader jt., 1956). 1993. aastast pärineb konsensus diagnostilistele kriteeriumitele diagnoosimaks PWS (Holm jt., 1993) ning 1995. aastast konsensus AS kohta (Williams jt., 1995). Mõlema sündroomi geneetilist diagnostikat on oluliselt lihtsustanud DNA metülatsioonispetsiifilise PCR metoodika juurutamine (Driscoll jt., 1992; Kubota jt., 1997; Zeschnigk jt., 1997). DNA metülatsioonitest sobib patsientide esmaseks uuringuks, kuid ei erista erinevaid geneetilisi tekkepõhjusi (deletsioon, UPD, ID). Täiendavad geneetilised analüüsid on vajalikud korrektseks diagnoosiks, millel põhineb perekondade nõustamine ja sündroomi kordusriski hindamine.

Mõlema sündroomi levimust on pikka aega hinnatud sarnaseks, umbes 1:10 000 kuni 1:15 000 kohta (Nicholls jt., 1998; Cassidy jt., 2000), kuid siiani on nende sündroomide levimust uuritud eraldi ja erinevatel rahvastikel (Burd jt., 1990; Åkefeldt jt., 1991; Clayton-Smith, 1993a; Ehara jt., 1995; Kyllerman, 1995; Petersen jt., 1995; Buckley jt., 1998; Whittington jt., 2001). Tänapäevaseid molekulaargeneetilisi diagnoosimismeetodeid kasutades on uuritud üksnes PWS levimust (Smith jt., 2003; Vogels jt. 2004). AS ja PWS klassikaline kliiniline pilt kujuneb välja teiseks-kolmandaks eluaastaks (Fryburg jt., 1991; Clayton-Smith, 1993c; Williams jt., 1995; Smith jt., 1996), harva on õnnestunud jõuda õige diagnoosini esimesel eluaastal (Hoefnagel jt., 1967; Greenberg jt., 1987; Chitayat jt., 1989; Aughton ja Cassidy, 1990; Miller jt., 1999). Samas on mõlema sündroomi korral hilisem elukvaliteet oluliselt mõjutatud varakult rakendatud arendusravist ja PWS korral dieedist. Hiljuti ulatuslikku rakendust leidnud kasvuhormoonravi ja järjestikku avaldatud surmajuhtude kirjeldused on tõstatanud küsimuse PWS-ga laste äkksurmadest ning surmade seotusest kasvuhormooni kasutamisega (Schrander-Stumpel jt., 2004; Stevenson jt., 2004; Nagai jt., 2005).

Levimuse ja haigusega/sündroomiga kaasuvate tervise- ja arenguprobleemide uurimine võimaldab hinnata meditsiini- ja sotsiaalsüsteemi vajadusi, planeerida ja koordineerida kahe süsteemi omavahelist koostööd ning seeläbi parandada konkreetsete haigete elukvaliteeti ja vähendada perekondade stressi.

Uuringu eesmärgid

1. Uurida Eestis AS ja PWS levimust aastatel 1984–2004 elusalt sündinud laste hulgas.
2. Määrata kõigil AS ja PWS patsientidel sündroomi geneetiline tekkepõhjus.
3. Leida kliinilisi sümptomeid, mis võimaldaksid mõlema sündroomi varajast avastamist.
4. Kirjeldada mõlema sündroomiga patsientide kliinilist pilti.
5. Võimalusel uurida mõlema sündroomiga patsientide surma põhjusi.

Uurimisgrupid ja uurimismeetodid

Aastatel 2000–2004 toimus kogu Eestit hõlmav retrospektiivne ja prospektiivne epidemioloogiline uuring, mille käigus uuriti 1 päeva kuni 18 aasta vanuseid Tartu ja Tallinna lastehaiglasse ning Tartusse ja Tallinna geneetilisele konsultatsioonile pöördunud AS ja PWS kahtlusega patsiente. Retrospektiivse grupi (1984.–1999.a. sündinud) moodustasid patsiendid, kes olid selekteeritud vastavalt AS (Williams jt., 1995) ja PWS (Holm jt., 1993) diagnostilistele konsensuskriteeriumidele. Lisaks külastasime 2002.a. jooksul kõiki Eesti puuetega laste

lastekodusid ja õppeasutusi (28 asutust 1298 elanikuga). Prospektiivse grupi (2000.–2004.a. sündinud) moodustasid lapsed, kellel kasutati lihtsustatud valikukriteeriume: uuringusse arvati kõik lapsed ebaselge arengupeetuse, lihastoonuse muutuste ja/või krambisündroomiga. Kõigilt selekteeritud uuringualustelt võeti 2–5 ml verd DNA metülatsioonitestiks. Kõik positiivse DNA metülatsioonitestiga patsiendid läbisid kliinilise uurimise TÜH Lastekliinikus, kus igal AS kahtlusega patsiendil tehti EEG ja igal PWS kahtlusega patsiendil ENMG, kõigile DNA positiivse metülatsioonitestiga patsientidele tehti vere- ja uriinianalüüs, EKG, MRI/CT, kõhuorganite ultraheliuuring ja karpogramm. Kõik selekteeritud patsiendid läbisid lasteneuroloogi, oftalmoloogi ja otorinolarüngoloogi konsultatsiooni, füsioterapeutilise ning psühholoogilise testimise. Sündroomi geneetilise etioloogia väljaselgitamiseks teostati kõigil positiivse DNA metülatsioonitestiga patsientidel järgnevalt FISH ja kromosoomianalüüs, FISH negatiivseid patsiente uuriti edasi uniparentaalse disoomia suhtes. Negatiivse DNA metülatsioonitestiga, kuid kliiniliselt AS-ga patsientide DNA saadeti *UBE3A* geeni mutatsiooni uuringule. Levimusandmete arvutamiseks kasutati Statistikaameti andmeid seisuga 1. jaanuar 2005.a.

Uuringu peamised tulemused

1. AS minimaalne levimusmäär elussündide hulgas aastatel 1984–2004 oli 1:52,181 (95% CI 1:25,326–1:129,785) ja levimuspäeval, 1. jaanuaril 2005.a. oli AS levimusmäär (6 levijuhtu) kuni kahekümneaastaste isikute hulgas 1:56,112 (95% CI 1:25,780–1:152,899).

2. PWS minimaalne levimusmäär elussündide hulgas aastatel 1984–2004 oli 1:30,439 (95% CI 1:17,425–1:58,908) ja levimuspäeval, 1. jaanuaril 2005.a. oli PWS levimusmäär (11 levijuhtu) kuni kahekümneaastaste isikute hulgas 1:30,606 (95% CI 1:17,105–1:61,311).

3. Meie uuring näitas, et elussündide hulgas oli AS levimusmäär 1,7 korda väiksem kui PWS. Eelnevalt läbi viidud uuringute põhjal arvati, et mõlemad sündroomid on võrdselt levinud.

4. AS statistiliselt eeldatav levimus tõusis 0,88 juhult 100 000 elussünni kohta 1984. aastal 4,23 juhuni 2004. aastal (muutus on statistiliselt mitteoluline, p=0,2). PWS eeldatav levimus tõusis 1,13 juhult 100 000 elussünni kohta 1984. aastal 7,97 juhuni 2004. aastal (statistiliselt oluline muutus, p=0,032).

5. Geneetiliste uuringutega kinnitus seitsmest AS-ga patsiendist 15q11-13 deletsioon kuuel patsiendil (86%) ja UPD15 ühel patsiendil (14%). Kuuel PWS-ga patsiendil oli tegemist UPD15 (50%), neljal patsiendil (33%) oli

15q11-13 deletsioon sündroomi põhjuseks ja kahel patsiendil (17%) oli põhjuseks kromosomaalne translokatsioon –ühel patsiendil oli Robertsoni translokatsioon 15;15 ja teisel patsiendil oli tasakaalustamata translokatsioon karüotüübiga 45,XX,der(14)t(14;15)(p11;q13).ish der(14)t(14;15) (D14Z1/ D22Z1+,D15Z1-,SNRPN-,bac463i22/AQ634845-,PML+,yac895h10+).

6. PWS-ga vastsündinute kliiniline uurimine näitas, et väljendunud tsentraalse lihashüpotoonia, häirunud ärkveloleku, söömise vastu huvi puudumise, mööduva bradükardia ja termolabiilsuse, teatud nahanähtude, iseloomulike näojoonte ja omapärase pöidla hoiaku koosesinemine ajalisel vastsündinul on sümptomkompleks, mille puhul on näidustatud esmase diagnostilise uuringuna DNA metülatsioonitest PWS-le.

7. Meie uuringus oli laste keskmine vanus AS-i diagnoosimisel 6,1 aastat ja PWS-i diagnoosimisel 4,3 aastat. AS varane diagnoosimine on komplitseeritud spetsiifiliste, imikueas avalduvate sümptomite puudumise tõttu.

8. Meie patsientide kliiniline fenotüüp lapseeas ei erinenud oluliselt eelnevalt kirjeldatud AS ja PWS patsientide omast. Üllatuslik oli sage (67%) ajumuutuste ja kardiaalsete probleemide esinemine PWS-ga patsientidel. Üle ühe aasta vanuste patsientide hulgas täheldasime häirunud luusüsteemi küpsemist 57% AS-ga ja 67% PWS-ga lastel.

9. Meie uuringugrupis esines 21% patsientidest terviseprobleeme, mida pole seostatuna AS-ga või PWS-ga eelnevalt kirjeldatud. Ühel AS-ga tüdrukul esinesid väga väljendunud luumuutused, ühel PWS-ga poisil diagnoosisime X-liitelise ihtüoosi, ühel tüdrukul tasakaalustamata 14;15 translokatsiooniga ning 15p13-q13 deletsiooniga oli PWS sümptomatoloogia eriti väljendunud ning esinesid ka mitmed lisasümptomid, üks tüdruk AS-ga suri Reye sündroomile sarnaselt kulgeva ägeda haigestumise tõttu.

10. Lapseea surma esines meie AS ja PWS patsientide grupis kahel juhul: üks tüdruk PWS-ga elas läbi ootamatu varahommikuse südameseiskuse tavaliselt kulgeva ägeda respiratoorse haigestumise foonil ning suri hiljem teadvusele tulemata; üks tüdruk AS-ga suri Reye sündroomile sarnase ägeda haigestumise tõttu.

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Teadustöö

Peamised uurimisvaldkonnad on Angelmani ja Prader-Willi sündroomi epidemioloogia, sümptomid, mis võimaldaksid AS-i ja PWS-i varasemat äratundmist ning sündroomide neuroloogiline aspekt.

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