

**Williams Syndrome: from genes to clinical features**

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Voor Ger en Mariet, mijn lieve ouders

### ***Whatever Happened to Dr. Williams?***

"Dr. Williams was the Registrar at Greenlane Hospital in Auckland, New Zealand in the 1960s, when he noticed that a number of children coming in for heart surgery (many of them with supravalvular aortic stenosis) shared other features in common with each other. They had similar facial features - they were chatty and outgoing - rather undersized and appeared to suffer from varying degrees of mental retardation. He wondered if there could be a syndrome involved. He spoke to the cardiac consultants, his Chiefs at the time, and was given the go ahead to conduct further investigations and a paper on the subject was printed in Medical journals in 1961"

"Dr. Williams worked at Greenlane until 1964 when he was offered a job at the Mayo Clinic in the USA.

This position was kept open for him, but as he never showed up it was given to someone else.

Dr. Williams then went to London to work, but when the Mayo Clinic offered him another post he again failed to appear. He then disappeared, and his family didn't know of his whereabouts except that a suitcase was left in luggage office in London which was never claimed"

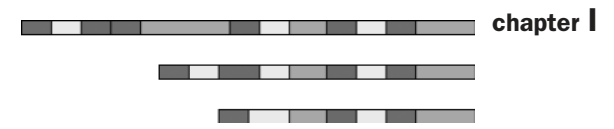
<http://www.wsf.org/family/news/whatever.htm>

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### List of abbreviations

<i>AUTS2</i>	Autism susceptibility candidate 2
<i>BCL7B</i>	B-cell lymphoma 7B
<i>CALN1</i>	Calneuron 1
CGH	Comparative genomic hybridisation
CLIP-115	Cytoplasmic linker protein of 115kDa
<i>CYLN2</i>	Cytoplasmic linker 2
<i>ELN</i>	Elastin
FISH	Fluorescence in situ hybridisation
<i>FKBP6</i>	FK506-binding protein 6
<i>FZD9</i>	Frizzled 9
<i>GTF2I</i>	General transcription factor II i
<i>GTF2IRD1</i>	General transcription factor II i repeat domain-containing protein 1
<i>GTF2IRD2</i>	General transcription factor II i repeat domain-containing protein 2
<i>HIP1</i>	Huntingtin-interacting protein 1
IQ	Intelligence quotient
<i>LIMK1</i>	LIM domain kinase 1
MLPA	Multiplex ligation-dependent probe amplification
<i>NCF1</i>	Neutrophilic cytosolic factor 1
PAS	Peripheral pulmonary artery stenosis
<i>RFC2</i>	Replication factor c subunit 2
<i>STXA1</i>	Syntaxin 1A
SVAS	Supravalvular aortic stenosis
<i>TBL2</i>	$\beta$ -transducin related 2
TSH	Thyroid stimulation hormone
WS	Williams syndrome
WSCP	Williams Syndrome Cognitive Profile



## General Introduction

## 1.1 History

It all started with the discovery of a narrowing of the ascending aorta, beginning at the superior margin of the sinus of Valsalva in 1842 by N. Chevers (Burn, 1986; Chevers, 1842). This narrowing was named supravalvular aortic stenosis (SVAS) in 1930 by L. Mencarelli (Burn, 1986; Beuren, 1972; Mencarelli, 1930). It can vary from slight to severe concentric constriction to hypoplasia of the entire aorta (Beuren, 1972). In 1961 J.C. Williams and colleagues from New Zealand were the first to suggest that SVAS could be part of a previously unrecognized syndrome. They published the findings of four individuals with SVAS in association with mental retardation and a peculiar facial appearance (Williams et al., 1961). A year later Dr. Alois J. Beuren, a pediatric cardiologist from Germany, independently described four individuals with essentially the same findings and expanded the phenotype with peripheral pulmonary artery stenosis and dental anomalies (Beuren et al., 1962). In 1963 J. Black and R. Bonham-Carter noticed that the faces of individuals with infantile hypercalcemia (Fanconi et al., 1952) and the faces of the individuals described by Williams and Beuren had much in common (Black and Carter, 1963). It was Dr. Beuren who presented in 1972 compelling evidence that “Williams-Beuren syndrome” and infantile hypercalcemia are the same disorder (Beuren, 1972). In retrospect, E. Oppenheimer (Oppenheimer, 1938) was probably the first to describe an individual with the features of Williams syndrome: “partial atresia of the main branches of the pulmonary artery occurring in infancy and accompanied by calcification of the pulmonary artery and aorta”. Naming the syndrome has been problematic. The disorder has been known in the past as the idiopathic hypercalcemia-supravalvular aortic stenosis syndrome in spite of the fact that both features are frequently absent. Because the faces in patients with infantile hypercalcemia were called “elfin facies” the term “elfin face” syndrome has been used in the past. But as stated by J. Burn who has never seen an elf this term should be dropped (Burn, 1986). Nowadays most authors use the name Williams syndrome (WS) although some authors prefer the name Williams-Beuren syndrome. We will use the name Williams syndrome. Today Williams syndrome (WS, OMIM #194050) is a well recognized multisystem genetic condition. Hundreds of individuals have been described in the medical literature and the data have been summarised in several reviews (Tassabehji, 2003; Bellugi et al., 1999). WS is caused by a submicroscopic hemizygous deletion of 1.55-1.84 Mb on chromosome 7q11.23, encompassing about 25-30 genes. The exact links between the missing genes and the phenotype of WS is yet to be fully explored.

## Prevalence

WS is present at birth and affects boys and girls equally. The exact prevalence is unknown. Figures vary from 1:20.000 till 1:7.500. Grimm and Wesselhoeft estimated the combined prevalence of SVAS and WS to be 1:10.000 (Grimm and Wesselhoeft, 1980). Morris et al. suggested that at least 1:20.000 individuals has the disorder (Morris et al., 1988). In 2002 Strømme et al. suggested that WS is underdiagnosed. In their epidemiologic study they find a prevalence of 1 in 7500 (Strømme et al., 2002). If this new prevalence figure is accurate, then the occurrence of WS might be almost as common as fragile X syndrome (1/4000-1/8000), one of the most common genetic causes of mental retardation. In this chapter a review of the clinical features of WS will be given. Also the state of the art of making the diagnosis WS will be discussed. Information about genes located in the WS critical region and guidelines for management of patients will be presented.



## 1.2 Clinical features

The clinical features of WS comprise craniofacial features as well as ample characteristics of the organ systems cardiovascular, central nervous system, endocrinology, skeletal, gastro-intestinal and genito-urinary. The incidences of these features are summarized in table 1.

### Craniofacial

#### Facial features

The facial features of individuals with WS change with age. In young children facial features can be subtle and just recognizable by a trained clinical geneticist. The combination of malar flattening, full cheeks and full lips is the most striking visible feature in infants (figure 1, individual 3) (Burn, 1986). Other facial features include periorbital fullness (figure 1, individual 1), short nose with bulbous nasal tip and long philtrum. Blue- and green-eyed children with WS have a prominent "starburst" pattern to their irides (stellate iris). Instead of this flat facial profile with full cheeks, older children and adults have a long narrow face and a long neck (figure 1, individual 6). Some individuals with WS have a hoarse voice and/ or prematurely grey hair.

#### Oral manifestations

Slightly small widely spaced teeth are common in children with WS. They may also have a variety of abnormalities of occlusion (bite), tooth shape or appearance. Abnormal tooth morphology is noted in about 12% of the primary dentitions and 41 % of the permanent dentitions. In individuals with WS over 10 years of age 40.5% have agenesis of one or more permanent teeth and 11.9% have agenesis of six teeth or more (Axelsson, 2005).

**Figure 1:** Six individuals with WS , respectively aged 5, 10,12,14,17 and 28 years, showing the variability in facial features

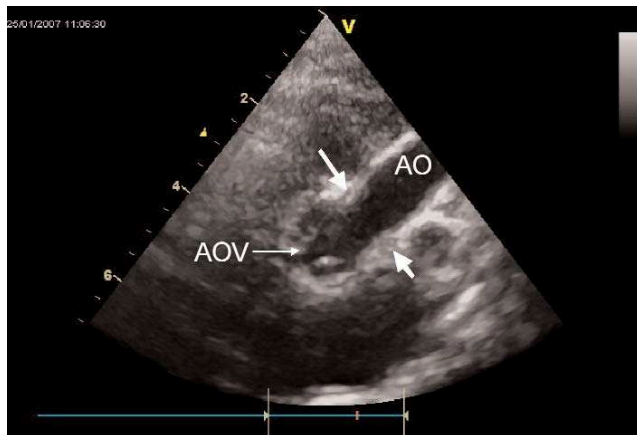
Individual 1, aged 5 years, showing periorbital fullness; individual 2, aged 10 years, showing full lips; individual 3, aged 12 years, showing full cheeks; individual 4, aged 14 years, showing bulbous nasal tip; individual 5, aged 17 years, showing full lips; individual 6, aged 28 years, showing a long narrow face and a long neck.



## Cardiovascular

In 50-80% of individuals with WS a cardiovascular abnormality can be found (Eronen et al., 2002; Committee on Genetics, 2001). Supravalvular aortic stenosis (SVAS) (figure 2) and peripheral pulmonary artery stenosis (PAS) are the most common cardiac manifestations. In clinical studies, it has been shown that with time PAS tends to improve and SVAS to progress. Stenoses of peripheral vessels, including renal, carotid, coronary, subclavian and mesenteric arteries have been reported. More than half of the adults with WS have hypertension (Cherniske et al., 2004). The consequences may include an increased risk of myocardial infarction and stroke (Blanc et al., 2006).

**Figure 2:** Echocardiography showing supravalvular aortic stenosis (arrows). AOV = aortic valve, AO = aorta.



## Central nervous system

### Neuroanatomical characteristics

Individuals with WS have an enlarged cerebellum relative to a small cerebrum (Jones et al., 2002). In addition they show a reduced corpus callosum size that is most pronounced in the area of the splenium and isthmus (Schmitt et al., 2001a). Cortical thickness is significantly increased (by 5-10%) in a broad anatomical region, encompassing perisylvian regions (Thompson et al., 2005). These regions, especially in the right hemisphere, process linguistic and musical syntax and prosody, areas in which WS individuals have relative strengths. There also is a bilateral reduction in sulcal depth in the intraparietal/occipital sulcus as well as in the collateral sulcus and the orbitofrontal region in the left hemisphere (Kippenhan et al., 2005). The intraparietal/occipital sulcus might play a role in the visuo-constructive deficit that is the hallmark neuropsychological feature of WS (Meyer-Lindenberg et al., 2004). Limbic structures of the temporal lobe (including uncus, amygdala, hippocampus and parahippocampal gyrus) are proportionally spared in individuals with WS relative to other cerebral structures (Bellugi et al., 1999).

### Neuropsychology

Most individuals with WS have some degree of mental retardation which can range from severe to mild. Mean full scale IQ is estimated to be about 60 (Bellugi et al., 1999). Older children and adults demonstrate a relative strength in language and auditory memory, with a significant weakness in visuospatial cognition. They have marked difficulty in tasks requiring the use of a pattern or object assembly, for example, following a pattern to build a model or assembling a simple piece of furniture. These characteristics are called the Williams Syndrome Cognitive Profile (WSCP) (Meyer-Lindenberg et al., 2004; Mervis et al., 2000). Remarkably, WS individuals often show a rich, expansive, grammatically complex vocabulary with striking conversation and richly expressive story telling skills. Overfriendliness (61.5-81.8%) and an empathetic nature are also commonly observed (Gosch and Pankau, 1997). In the past this behaviour was sometimes addressed as "cocktail party manners" (Bellugi et al., 1999; Pober and Dykens, 1996). Children with WS are typically unafraid of strangers and show a greater interest in contact with adults than with their peers. Individuals with WS also have behavioral problems including sleep problems (31.4% in children and adolescents), attention-deficit/hyperactivity disorder (64.7% of individuals aged 4-16 years/ 3-7% in general population) and anxiety (generalized anxiety disorder in 12% of WS individuals aged 4-16 years) (Leyfer et al., 2006; Einfeld et al., 1997). It seems that especially adult individuals with WS are very anxious with high rates of obsessions, worries, fears and phobias. As many as 50 different fears are seen with increased frequency in those with WS; a few of these are fears of high places, carnival rides, bee stings, thunderstorms, injections, earthquakes and being teased (Dykens et al., 2005). A functional neuroimaging study reported a reduced activation of the amygdala for threatening faces but an increased activation for threatening scenes (Meyer-Lindenberg et al., 2005). As amygdala signaling is critical for appropriate avoidance behavior, reduced activation to threatening faces may contribute to the diminished fear of strangers. On the other hand, the increased amygdala reactivity to threatening scenes might be associated with the specific fears and phobias found in WS.

Another neurocognitive hallmark of WS is a relative strength in the recognition and discrimination of faces. Additionally people with WS are unusually drawn to faces, seek direct eye gaze and judge unfamiliar faces to be abnormally approachable (Mobbs et al., 2004).

Individuals with WS also appear to have an affinity for music (Dykens et al., 2005). Early observations suggest that children with WS are (relatively) skilled in singing and remembering songs (Udwin et al., 1986). Levitin et al. surveyed a large sample of 118 individuals with WS and found that relative to others with or without disabilities, those with WS displayed greater emotional responses to music, manifested interest in music at an earlier age, and spent more hours per week listening to music (Levitin et al., 2004). Compared to normal age-matched controls, individuals with WS show significantly higher activation levels in the right amygdala, cerebellum (particularly the vermis), pons and brain stem as they listen to music (Levitin and Menon, 2003).

### Neurology

Young affected children often show developmental delays; milestones such as walking, talking and toilet training are often achieved somewhat later than is considered normal. Mean age for sitting is 11 months (normal: 6-9 months), for walking alone 20 months (normal: 12-18 months) and for talking 24 months (normal: 12 months) (Pankau et al., 2000).

Abnormalities in motor coordination and tone are prevalent, with younger children frequently exhibiting decreased tone (41% of WS individuals aged 2-8 years) and older individuals almost exclusively having increased tone (85%) (Chapman et al., 1996). Although they are not ataxic, individuals with WS have an abnormal gait (figure 3) resembling the gait of cerebellar patients. They also have problems descending stairs and stepping over surface changes (30% of children with WS) (Withers, 1996). These features suggest that the role of the cerebellum in WS deserves further study.

**Figure 3:** WS individuals with characteristic posture

About 50% of WS individuals have strabismus and refractive errors are common (Atkinson et al., 2001; Sadler et al., 1996; Winter et al., 1996; Kapp et al., 1995). Although not confirmed by a systematic ophthalmologic survey, cataract could have an earlier onset in WS individuals compared to individuals in the general population (Cherniske et al., 2004). Hypersensitivity to certain sounds, particularly machines, fireworks and bursting balloons is seen in 85-95% of patients. This "hyperacusis" or the ability to cope with it tends to improve with age. Otitis media occurs frequently in childhood and there is a higher-than-expected prevalence (70-77%) of mild sensorineural hearing loss in children with WS (Marler et al., 2005). Few published studies have analyzed hearing in adults with WS (Johnson et al., 2001; Miani et al., 2001; Plissart et al., 1994; Lopez-Rangel et al., 1992). In a multisystem study of 20 older adults with WS (30-51 years) 16 of 20 subjects underwent standard audiologic testing (Cherniske et al., 2004). In 75% a similar pattern of high frequency sensorineural hearing loss consistent with prebycusis was found. Although this pattern of hearing loss is very common in the older population, it appears to be developing at an earlier than expected age in adults with WS. The findings of Marler and colleagues were consistent with this study (Marler et al., 2005).

## Endocrine

### Growth

Mild prenatal growth deficiency and a postnatal growth rate about 75% of normal are consistently observed and adult stature is slightly smaller than average. Special WS growth charts exist (Committee on Genetics, 2001). Growth hormone deficiency has not been considered a major cause of growth retardation and endocrine studies have failed to reveal abnormalities in the growth hormone - insulin-like growth factor I

axis in the majority of individuals (Partsch et al., 1994). However, this does not rule out the possibility of growth hormone deficiency in a subpopulation of WS individuals (Kuijpers et al., 1999). There are two WS individuals with confirmed growth hormone deficiency who responded well to human growth hormone therapy reported (Xekouki et al., 2005). Several reports indicate that WS adults can become overweight or obese (Cherniske et al., 2004; Davies et al., 1997; Morris et al., 1990).

### Hypercalcaemia

Some young children with WS have increased blood calcium levels. Infantile hypercalcaemia may precipitate symptoms such as extreme irritability, failure to thrive, vomiting and constipation (Morris et al., 1988). Symptomatic hypercalcaemia usually resolves during childhood, but lifelong abnormalities of calcium and vitamin D metabolism may persist. If hypercalcaemia is overt, skeletal changes in adolescence and adulthood include osteosclerosis of the metaphyses of long bones, the skull vault or lamina dura of the alveolar bone (Burn, 1986). The true frequency and cause of this problem is unknown. There is just documented evidence of hypercalcaemia in a minority of individuals. The Committee on Genetics of the American Academy of Pediatrics reported, based on a review of several reports and a database of 315 children and adults with WS, a frequency of hypercalcaemia of about 15% in infants and adults with WS (Committee on Genetics, 2001). However, Sforzini and colleagues reported just one individual with mild hypercalcaemia (<2%) in a study of 57 individuals with WS (1.0-23 years, median 8.5 years) (Sforzini et al., 2002). They speculate that the issue of disturbed calcium homeostasis in WS has been overemphasized in the past. In the past it was stated that hypercalciuria is common in WS and predisposes to nephrocalcinosis. The Committee on Genetics of the American Academy of Pediatrics (Committee on Genetics, 2001) reported hypercalciuria in 30% of individuals with WS. They found nephrocalcinosis in <5% of individuals. Again Sforzini and colleagues (Sforzini et al., 2002) reported a much lower incidence, 3.5%, for hypercalciuria in their group of 57 individuals with WS. They found no cases of nephrocalcinosis. As long as the exact incidence of hypercalcaemia and hypercalciuria is unknown, lifelong monitoring of the calcium level in blood and urine seems indicated.

### Thyroid function and morphology

Subclinical hypothyroidism, that is, high thyroid stimulating hormone (TSH) with normal fT4 and fT3 concentrations, has been described in WS. Cambiaso and colleagues found subclinical hypothyroidism in 31.5% and morpho-volumetric abnormalities of the thyroid gland in 67.5% of WS individuals (Cambiaso et al., 2007). In 2006 Selicorni and colleagues found an elevated TSH in 36 of 95 individuals with WS (38%). Thyroid gland hypoplasia was present in 75% (Selicorni et al., 2006). In 2005 Stagi and colleagues identified in a cohort of 20 WS individuals three cases (15%) with subclinical hypothyroidism and two cases (10%) with overt hypothyroidism. Fourteen individuals (70%) showed thyroid hypoplasia with the left lobe prevalently involved, one individual (5%) showed thyroid hemiagenesis and only five individuals showed a thyroid with normal volume (Stagi et al., 2005). In 2004 Cherniske et al. reported a multisystem study of 20 older adults with WS (age range 30-51 years, mean age 38.8 years) in which they observed a 25% prevalence of TSH elevation (Cherniske et al., 2004). One year earlier Stagi and colleagues reported two girls with WS and thyroid hypoplasia of the left lobe (Stagi et al., 2003). Another WS individual with thyroid hemiagenesis and subclinical hypothyroidism was reported by Cammareri et al. in 1999 (Cammareri et al., 1999). In 2004 Bini and Pela described a child with WS who showed clinical features of severe hypothyroidism and a small sublingual ectopic thyroid. The above cases show that a developmental defect of the thyroid may be associated with WS. Moreover, in both last individuals neonatal TSH screening was normal. This emphasizes the need for an (lifelong) accurate evaluation of thyroid function in individuals with WS. The benefit of treating subclinical hypothyroidism remains a debate.

### Puberty

Another endocrinologic feature of WS is a tendency to early puberty. Although the exact prevalence is unknown, central precocious puberty in girls with WS has been described (Partsch et al., 2002; Cherniske et al., 1999; Douchi et al., 1999; Scothorn and Butler, 1997). In 2002 Partsch and colleagues reported an estimated prevalence of precocious puberty of one in five to six German girls with this disorder (18.3%) (Partsch et al., 2002). They found a mean menarchial age of 11.5 +/- 1.7 (+/-SD) years in 86 German individuals with WS while mean menarchial age in the normal German population was 12.9 +/- 1.1 years. Cherniske and colleagues found Tanner III pubic hair development prior to the age of 12 years in 83% of pubertal males (Cherniske et al., 1999).

### Diabetes Mellitus

An extremely high frequency of abnormal glucose tolerance was observed by Cherniske and colleagues in a cohort of 20 older adults with WS; only two patients had no clinical or laboratory evidence of abnormal glucose metabolism (Cherniske et al., 2004). These findings extend the previous case reports of diabetes mellitus in individual adults with WS (Nakaji et al., 2001; Imashuku et al., 2000; Plissart et al., 1994; Lopez-Rangel et al., 1992; Morris et al., 1988).

### Skeletal/Musculoskeletal

Young children with WS often have low muscle tone and joint laxity. In older children and adults orthopaedic problems are common and include kyphosis, lordosis, scoliosis (Osebold and King, 1994; Morris et al., 1990; Morris et al., 1988) and joint contractures (Kaplan et al., 1989). Another skeletal feature which can be found in WS is radioulnar synostosis (Bzduch, 1994; Bzduch and Spissak, 1989).

### Gastro-intestinal

An infant with WS often has difficulty with feeding and may be brought for medical care because of gastroesophageal reflux, colic or failure to thrive (Morris, 2005b; Morris et al., 1988). In 10 % of children with WS antibody evidence of celiac disease is found (Giannotti et al., 2001). Gastrointestinal symptoms are present at all ages; constipation and rectal prolapse may develop in infancy and childhood and diverticulitis and peptic ulcer later in life (Morris et al., 1988). Partsch and colleagues (Partsch et al., 2005) found sigmoid diverticulitis in 8% of 128 adults with WS. Inguinal and umbilical hernias are more common in WS than in the general population.

### Genito-urinary

According to large studies the frequency of renal tract abnormalities in WS detected by ultrasonography varies between 18% and 41% (Sugayama et al., 2004; Sforzini et al., 2002; Committee on Genetics, 2001; Pankau et al., 1996; Pober et al., 1993). Anomalies which can be found are mild pelvic dilatation, kidney duplication, horseshoe kidney, renal agenesis or hypoplasia, obstructive malformations (e.g., megaureter), renal stone and bladder diverticula.

**Table 1:** Medical problems in individuals with Williams syndrome; in the literature and the present study

Organ system	Incidence (%) in literature (n>315) (Committee on Genetics, 2001)	Incidence (%) in present study (n=59)
<b>Craniofacial</b> - full/sagging cheeks - thick lips - small teeth - long neck/sloped shoulders - hoarse voice	95	37 81 19 75 66
<b>Cardiovascular</b> - cardiac murmur - any abnormality (total) - SVAS - PAS - hypertension	80 75 50 50	80 73 48 15 12
<b>Central nervous system</b> - mental retardation - outgoing/gregarious - sleep problems - preoccupation/obsession - striking musical skills - problems descending stairs < 8 years - reluctance in changing surface <8 years - normal tandem walking - strabismus - "hyperacusis"	75       50 90	100 75 29 51 24 92  46  22 36 93
<b>Endocrine</b> - hypercalcemia - nephrocalcinosis - hypothyroidism - precocious puberty - diabetes mellitus - obesity	15 <5 2  15 30	5 0 5 9 0 12
<b>Musculoskeletal</b> - kyphosis - lordosis - scoliosis - clinodactyly of fifth finger	20 40  	17 25 24 66
<b>Gastro-intestinal</b> - constipation - inguinal hernia - umbilical hernia	40 40 50	9 42 14
<b>Genito-urinary</b> - structural anomaly	20	5

### 1.3 Clinical diagnosis

In chapter 1.2 we described the variety of clinical features which can be found in WS. Not every individual with WS shows all the features of the syndrome and changing of the craniofacial features occurs with increasing age. Even so, none of the symptoms seems to be pathognomonic for WS. Therefore, diagnosing WS may be difficult. In practice a two step approach can be used. First, clinical suspicion can be objectified by a scoring system. Second, when the scoring system confirms the suspicion WS a molecular diagnosis can be pursued.

#### Scoring systems

In the past several scoring systems have been devised to help the diagnostic process. An early attempt of Preus (Preus, 1984) was based on a very detailed questionnaire and was not practical for diagnosis in the clinic. Later Selicorni (Selicorni, 1996) developed an other scoring system. For people familiar with dysmorphology this two step clinical score (Table 2) may provide something to hold on to in order to diagnose WS clinically. However, a few characteristic features of WS are lacking in this scoring system, e.g., hyperacusis, reluctance in changing the surface and difficulties in descending stairs (Table 1). Items which are frequently observed and which can be asked at the parents or caretakers very easily. In 2001 the Committee on Genetics of the American Academy of Pediatrics (Committee on Genetics, 2001) presented a scoring system based on 41 items including hypersensitivity to sound and not otherwise defined visuospatial problems. No other scoring systems have been published since. Because checking 41 items is very timeconsuming during a busy outpatient clinic there's a need for a new scoring system with less items but including critical symptoms such as hyperacusis, reluctance in changing the surface and difficulties in descending stairs.

#### Differential diagnosis

Because facial dysmorphic features and congenital heart malformations can be found in other disorders, the differential diagnosis of WS should include Noonan syndrome, deletion 22q11, Smith-Magenis syndrome, Kabuki syndrome, fetal alcohol syndrome and Coffin-Lowery syndrome (GeneReviews, Williams syndrome, [www.genetests.org](http://www.genetests.org)), (Morris, 2005a)

**Table 2:** Two step clinical score of Selicorni (Selicorni, 1996)

Facial score	
Trait	Score
Coarse facial features	2
Dolichocephaly	1
Bitemporal narrowing	2
Facial Asymmetry	1
Sparse eyelashes	1
Strabismus	1
Stellate pattern of iris	2
Epicanthal folds	2
Periorbital fullness	2
Depressed nasal bridge	1
Broad nasal tip	1
Full/sagging cheeks	2
Malar hypoplasia	2
Long philtrum	1
Large mouth	1
Thick lips	2
Mouth held open	1
Malocclusion/sparse little teeth	2
Facial score	

Total score	
Trait	Score
Facial score <10	0
Facial score 10-15	1
Facial score >15	2
Short stature	1
Inguinal or umbilical hernia	1
Mental retardation	1
Outgoing personality	2
SVAS	2
Congenital heart disease (non SVAS)	1
Total score	

Total score > 4 : WS

Total score = 4 : WS

Total score < 4 : WS

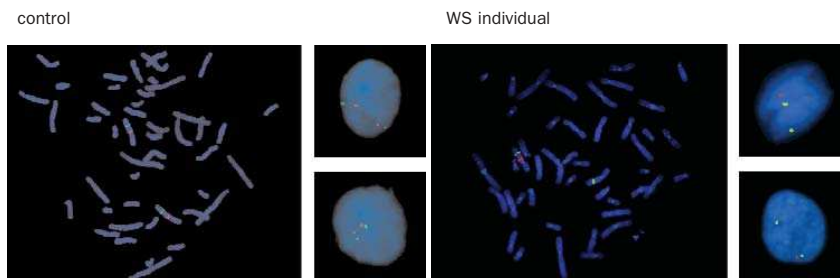


## 1.4 Molecular Diagnosis

Before 1993 several hypotheses about the cause of WS existed. In 1993 Ewart et al. discovered a hemizygous submicroscopic deletion involving the elastin gene on chromosome 7q11.23 in 9 individuals with WS and concluded that WS is probably a contiguous gene syndrome (Ewart et al., 1993). Later in 1995, Lowery and colleagues found a deletion in 96% of individuals with classical WS (Lowery et al., 1995). The deletion appears to encompass both the elastin gene and the Lim kinase 1 gene (*LIMK1*) (Donnai and Karmiloff-Smith, 2000). Therefore, when WS is suspected, based either on clinical suspicion or on a scoring system, the diagnosis should be confirmed by the detection of a submicroscopic deletion in 7q11.23. Most laboratories use fluorescence in situ hybridisation (FISH) on metaphase chromosomes from a lymphocyte culture. The FISH method involves hybridizing a fluorescently labelled DNA probe to fixed cells on a microscope slide. The probe hybridizes to complementary DNA at the site of the WS region. The probe signal can be detected by means of a fluorescence microscope; absence of a signal on one of the chromosomes 7 indicates a microdeletion in the WS region (see figure 4). The most commonly used probe is a commercially available probe which covers approximately 180 kb of the critical region in WS including *ELN*, *LIMK1* and the D7S613 locus (see figure 5). Using the two step clinical score of Selicorni (Selicorni, 1996) a deletion is detected in all individuals with a total score of at least 4. For studies involving large numbers of patients, for instance in an institution for the mentally retarded, interphase FISH on buccal smears can be useful (Nieuwint et al., 2000).

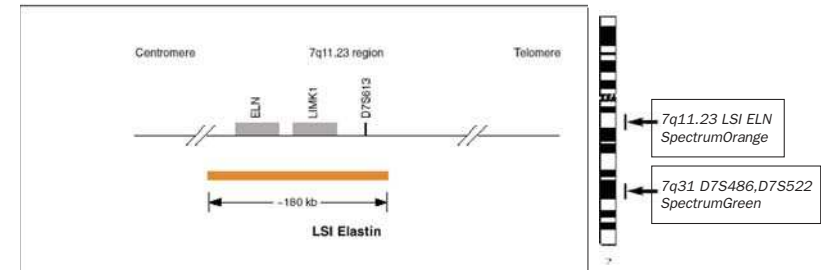
During the last years more and more genes in the deleted region have been mapped. Also some individuals with atypical deletions have been described. We think this new knowledge warrants a new strategy for molecular diagnosis.

**Figure 4:** Example of the FISH technique applied for the identification of a WS individual. In the metaphases of an individual without WS (left) there are 2 green signals indicating 2 chromosomes 7, and 2 red signals indicating that the elastin gene is present on both chromosomes 7. The same can be seen in cells that are not dividing, i.e. cells that are in the interphase of the cell cycle. A WS individual (right) shows 2 green signals, but only 1 red signal, indicating a deletion of the elastin gene on 7q11.23, in metaphase and interphase cells. DNA counter stain is DAPI.



**Figure 5:** Structure of the FISH probe from Vysis inc. (Abbott Laboratories).

The probe consists of two parts: one for the 7q11.23 WS region, labelled with Spectrum Orange which produces a red signal. The other hybridizes to band 7q31, a control region labelled with Spectrum Green, which produces a green signal, for the identification of chromosome 7.



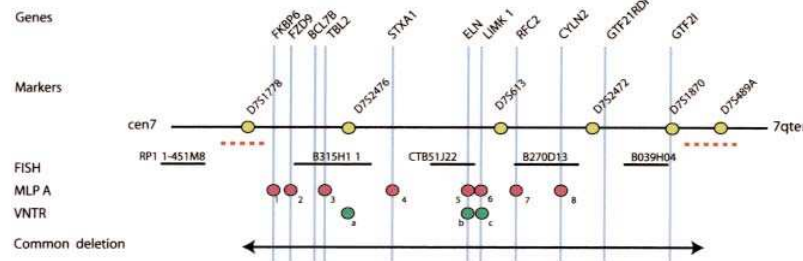
## 1.5 Williams syndrome critical region

### Submicroscopic deletion

Today we know that most WS individuals have a 1.55 Mb submicroscopic deletion of 25-30 genes in chromosome band 7q11.23. These deletions arise as a consequence of unequal crossing over during meiosis. A small proportion of WS individuals have a larger deletion of 1.8 Mb (Bayes et al., 2003). Deletions on the maternally and paternally inherited chromosomes occur probably with equal frequency, and there appears to be no clear parental age effect, although a correlation of shorter stature and microcephaly with maternally inherited deletions has been suggested (DelCampo et al., 2006; Donnai and Karmiloff-Smith, 2000; Perez Jurado et al., 1996).

The common deletion encompasses, among others *FZD9*, *STX1A*, the elastin gene (*ELN*), the LIM-kinase 1 gene (*LIMK1*), the gene for Replication Factor C Subunit 2 (*RFC2*), *CYL2* the gene encoding a cytoplasmatic linker protein (*CLIP-115*), *GTF2IRD1* and *GTF2I* (figure 6).

**Figure 6:** Map of the Williams syndrome critical region: five FISH probes are represented by black lines, MLPA probes by red dots (1: FKBP6; 2: FZD9; 3:TBL2; 4:STXA1; 5:ELN; 6:LIMK1; 7:RFC2; 8:CYLN2) and VNTR markers by green dots (a:D7S2476; b:ELN; c:LIMK1GT). The common deleted region is presented in a black dual arrowhead line.



#### Genotype-phenotype correlations

The clinical phenotype is probably a consequence of abnormal gene dosage due to the hemizygous deletion, although it is also possible that decreased relative levels of expression of normal-copy neighboring genes contribute to the phenotype (Merla et al., 2006).

The only firm genotype-phenotype correlation is between vascular problems (SVAS and PAS) and haploinsufficiency for the elastin gene. As the main component of elastic fibers, elastin provides strength and elasticity to extensible tissues that require resiliency, such as the heart, skin, lung and major blood vessels. *ELN* hemizygosity is probably also responsible for the hernias and the striking hoarseness which can be present (Tassabehji, 2003; Vaux et al., 2003). Elastin may also have a function in maintaining hearing sensitivity. Reduced elastin deposition could lead to cochlear dysfunction through different mechanisms: reduced perfusion of the cochlea caused by vascular stenoses resulting in hypoxia and cell death, an increase in the rigidity of the basilar membrane, misregulation of cochlear cell proliferation, and/or changes in signal transduction functions of the cochlear hair cells (Marler et al., 2005).

Another gene located in the common deletion is *RFC2*. This gene encodes a 40-kD protein which is one of five subunits of the replication factor C complex that together with proliferating cell nuclear antigen (PCNA) is required for elongation of primed DNA templates by DNA polymerase  $\delta$  and  $\epsilon$ . Reduced efficiency of DNA replication could account for growth deficiency as well as other developmental disturbances (Peoples et al., 1996).

Until now it is not clear which genes are responsible for the neurological phenotype in WS (Meyer-Lindenberg et al., 2006; Eckert et al., 2006). In this respect the few exceptional individuals with smaller deletions and either a full or partial phenotype are of great interest (Howald et al., 2006; Tassabehji et al., 2005; Doyle et al., 2004; Heller et al., 2003; Gagliardi et al., 2003; Hirota et al., 2003; Karmiloff-Smith et al., 2003; DelCampo et al., 2002; Korenberg et al., 2000; Tassabehji et al., 1999; Botta et al., 1999a). Based on these studies in individuals with smaller deletions, presumed neurological function of several genes out of the WS critical region have been proposed. These are summarized in table 3 and will be discussed in more detail.

**Table 3:** Presumed neurological function of several genes from the WS region

Gene	Presumed effect of haploinsufficiency
<i>FZD9</i>	Impairment in visuospatial skills
<i>STXA1</i>	No neurological effect
<i>LIMK1</i>	Impairment in visuospatial skills
<i>CYLN2</i>	Deficits in motor coordination
<i>GTF2IRD1</i>	Impairment in cognitive development
<i>GTF2I</i>	Mental retardation

*FZD9* is selectively expressed in the hippocampus. Frizzled 9-null mice, in which *FZD9* has been knocked out, have generally normal gross anatomical hippocampal organization but show large increases in apoptotic cell death in the developing dentate gyrus. These mice have severe deficits on tests of visuospatial learning memory. So it has been suggested that *FZD9* could be a determinant of hippocampal development and a contributing factor to the neurodevelopmental and behavioral phenotype of patients with WS (Zhao et al., 2005).

*STXA1* encodes syntaxin1A which is a component of the synaptic apparatus. Synaptic vesicles store neurotransmitters that are released during calcium-regulated exocytosis. The specificity of neurotransmitter release requires the localization of both synaptic vesicles and calcium channels to the presynaptic zone. Syntaxins function in the vesicle fusion process. However, analysis of individuals with atypical deletions suggests that the neurological symptoms in WS do not result from a hemizygous deletion of *STXA1*, even though this gene is prominently expressed in the brain (Botta et al., 1999a).

*LIMK1* encodes a protein kinase that is strongly expressed in the developing brain, especially the cerebral cortex (Frangiskakis et al., 1996). The protein interacts with the transmembrane receptor neuregulin (Wang et al., 1998) and also phosphorylates cofilin, a regulator of the actin cytoskeleton important in cell movement and axonal growth of neurons (Arber et al., 1998). *LIMK1* regulates Golgi dynamics in developing neurons and is important for promoting axon outgrowth and the delivery of proteins to growth cones involved in the development of neuronal polarity (Rosso et al., 2004). The effect of hemizygosity of *LIMK1* is not clear yet. It has been suggested that *LIMK1* is linked to the striking impairment in visuospatial skills but this has not been confirmed in later studies (Gray et al., 2006).

*CYLN2* is abundantly expressed in dendrites and cell bodies of many neurons in the brain and encodes a highly conserved protein, CLIP-115. CLIP-115 is a member of a family of cytoplasmic linker proteins (CLIPs) that specifically associate with the ends of growing microtubules. They are involved in regulating microtubule dynamics and establishing interactions between microtubule tips and various cellular structures, including cargoes destined for transport by dynein. CLIP-115 is thought to play a role in the formation and/or turnover of gap junctions (Hoogenraad et al., 2000; De Zeeuw et al., 1997). Mice with haploinsufficiency for *CYLN2* have features reminiscent of WS, including mild growth deficiency, brain abnormalities, cognitive dysfunctions and particular deficits in motor coordination (Hoogenraad et al., 2002). These data suggest that hemizygosity of *CYLN2* might contribute significantly to the neurological features of WS.

**GTF2IRD1** can bind regulatory elements upstream of genes involved in tissue development and differentiation. *GTF2IRD1*-null mice exhibit phenotypic abnormalities reminiscent of WS. *GTF2IRD1* could be a genetic determinant of mammalian craniofacial and cognitive development (Tassabehji et al., 2005). However, probably no single gene is responsible for the craniofacial or cognitive features of WS.

**GTF2I** is expressed in both fetal and adult tissues, most notably in the brain. This gene encodes two proteins: BAP-135, which is a target for Bruton's tyrosine kinase, and TFI-I. TFI-I is a transcription factor that shuttles between the cell nucleus and the cytoplasm, activating other genes. This protein has also a role in transcriptional repression. Hemizyosity of *GTF2I* is likely associated with the mental retardation found in most WS individuals, although some authors hypothesize that hemizyosity of both *GTF2I* and *GTF2IRD1* produce the visuospatial deficits seen in WS (Edelmann et al., 2006; Morris et al., 2003).

## 1.6 Medical Guidance and Genetic Counselling

### General

In individuals with WS various medical problems may occur (Table 1). Therefore, it is recommended to document some basic values at the time of (clinical) diagnosis (Table 4) (<http://www.williams-syndrome.org/>, (Committee on Genetics, 2001)). Because some problems are age-dependent, it is necessary to check individuals with WS regularly (Morris, 2005b; Cherniske et al., 2004; Committee on Genetics, 2001). Especially cardiovascular problems and hypertension can manifest later in life, although there is proof that in WS individuals who have a deletion that includes NCF1 hypertension is significantly less prevalent (DelCampo et al., 2006). The Dutch federation of parents organisations (Federatie van Ouderverenigingen, FvO) and the Dutch association of doctors working with mentally handicapped persons (Nederlandse Vereniging van Artsen voor Verstandelijk Gehandicapten, NVAVG) have developed medical guidelines in dutch for the general practitioner and dentist. These dutch guidelines can be found on the internet ([www.fvo.nl](http://www.fvo.nl), [www.williams-syndroom.nl](http://www.williams-syndroom.nl), [www.nvavg.nl](http://www.nvavg.nl)) and are summarized in table 5. The time interval of periodic reviews can be adjusted to the need of the individual patient.

### Dental care

Due to their poor visual-spatial motor skills dental hygiene in WS is a greater problem than normally observed in individuals with mental retardation. Supervised brushing/flossing and more frequent dental cleanings are recommended to maintain adequate dental hygiene (Cherniske et al., 2004). In children with WS, orthodontic evaluation is generally recommended as soon as the patient's behavior allows treatment (Hertzberg et al., 1994). Sedation can be helpful in the younger age group to reduce anxiety and uncooperative behavior during minimal dental treatment. Treatment under general anesthesia seems more appropriate for children and adolescents (Moskovitz et al., 2005). Special attention should be given to evaluating individuals prior to treatment under general anesthesia, especially because aortic stenosis tends to intensify with age.

### Coping with hyperacusis and hearing loss

Medical procedures accompanied by noises, like squirting out ears or the use of a dentist drill, may be problematic for individuals that are hypersensitive for specific sounds. Explaining the origin of the sound to the person involved can sometimes significantly reduce the problem (Hoff et al., 1998; Van Borsel et al., 1997). Early and regular hearing testing is important for WS patients (Marler et al., 2005).

### Treatment of hypercalcemia

Hypercalcaemia may present for the first time in adolescents or adults or presents itself again at those ages. Occasionally, dietary or medical treatment (intravenous fluid administration, furosemide, calcitonin) is needed. In 2004 Cagle and colleagues (Cagle et al., 2004) described two male individuals with WS (11 months and 13 months of age) and severe symptomatic hypercalcemia who were treated with intravenously administered pamidronate (a biphosphonate) after traditional measures proved only partially successful.

### Monitoring of thyroid function

Individuals with WS should be monitored for thyroid function and a thyroid ultrasound screening should be considered, especially in those individuals with changes in thyroid function (Cambiaso et al., 2007; Stagi et al., 2005). Treatment should probably be reserved for the individuals with overt hypothyroidism or those whose thyroid function shows signs of progressive deterioration (Cambiaso et al., 2007).

### Postponing puberty

Sometimes individuals with WS and precocious puberty are treated with GnRH agonists because the mental retardation together with the specific personality and character traits can be risk factors for early sexual activity, potential sexual abuse and aggressive behavior; especially in boys. Moreover, young girls with WS can be unable to handle self-care of menses and can have psychosocial problems due to a menstruation at a young age (Partsch et al., 2002).

### Treatment of constipation

Constipation needs to be aggressively managed as it appears to be a risk factor for rectal prolapse, hemorrhoids, diverticulosis and diverticulitis. Recurrent abdominal pain warrants a complete examination as it may have identifiable and treatable medical causes.

### Surgery

When an individual with WS needs surgery, preoperative evaluation of the cardiovascular status is recommended and the need for endocarditis prophylaxis should be established. Because sudden death and anaesthetic complications have been reported in young individuals with WS, these individuals should be monitored by an anaesthetist specialised in children (Wessel et al., 2004; Bird et al., 1996).

**Table 4:** Medical evaluations at the time of diagnosing WS (<http://www.williams-syndrome.org/>, (Committee on Genetics, 2001))

Medical evaluations at the time of diagnosing Williams syndrome
Complete physical and neurologic examination
Growth parameters plotted on WS growth charts
Cardiologic evaluation <ul style="list-style-type: none"> <li>• full clinical evaluation by cardiologist with experience in pediatric patients that includes 4-limb blood pressure measurements and echocardiography</li> </ul>
Genito-urinary system evaluation <ul style="list-style-type: none"> <li>• Ultrasonography of bladder and kidneys</li> <li>• Renal function studies (serum urea nitrogen and creatinine levels)</li> <li>• Urinalysis</li> </ul>
Calcium determinations (serum calcium, spot urine calcium and creatinine levels)
Thyroid function tests
Ophthalmologic evaluation
Multidisciplinary development evaluation (older than 2 years)
FISH to determine <i>ELN</i> deletion



**Table 5:** Medical guidelines for follow-up in individuals with WS (www.fvo.nl,www.williams-syndroom.nl,www.nvavg.nl)

Age	0-2 years	2-13 years	>13 years
Interval	Every 3-6 months	Every 1-2 years	Every 2-4 years
Development	X	X	X
Growth (L/W/HC)	X	X	X
Feeding problems	X	X	X
Cardiovascular (1)	X	X	X
Blood pressure (2)	X	X	X
Endocrine:			
• calcium (3)	X	X	X
• thyroid (4)	X	X	X
Gastro-intestinal	X	X	X
Genito-urinary			
• bladder/kidneys (5)	X	X	X
• urinary tract infections (6)	X	X	X
Orthopedic/musculoskeletal/ neurologic	X	X	X
Herniae	X	P	P
Otitis media/Hearing assesment	X	X	P
Eyes/Vision	X	X	X
Dental	P	X	P
Anesthesia consultation	X	X	X

X= to be performed, P= to be performed in case of problem

(1) = cardiology evaluation by cardiologist with pediatric expertise and experience.

Perform periodic cardiovascular evaluations even after a baseline examination with normal findings

(2) = blood pressure measurements (both arms and legs) annually

(3) = serum calcium and calcium-creatinine ratio in urine every 2 years

(4) = serum TSH and free T4 every 4 years

(5) = bladder and renal ultrasonography at puberty and every 5 years thereafter, serum creatinine and urea nitrogen every 2 years

(6) = urinalysis every year and with unexplained fever

Special consideration: Do not give multivitamin preparations because of the potential deleterious effects of vitamin D

### Genetic counselling

Most individuals with WS are the only one in the family with this syndrome. Unambiguous autosomal dominant inheritance has been described in nine families, living respectively in Bulgaria (Metcalf et al., 2005), Estonia (Ounap et al., 1998), Germany (Pankau et al., 2001), the United Kingdom (Mulik et al., 2004) and the USA (Morris et al., 1993; Sadler et al., 1993). In six of these families, a deletion of the elastin-gene was found in one of the parents and its child (Metcalf et al., 2005; Mulik et al., 2004; Pankau et al., 2001; Morris et al., 1993). In all nine families the affected parent (two fathers, seven mothers) showed mild mental retardation and facial features consistent with WS. In one case this parent had a mitral valve insufficiency. In another case a SVAS was documented in childhood but not verified on echocardiographic reexamination at age 24 years. No congenital heart malformations were described in the other parents. Kara-Mostefa described two brothers with WS and a proven deletion of *ELN*. No deletion could be found in either of the parents (Kara-Mostefa et al., 1999). Based on these prior publications we can conclude that in most

individuals WS is caused by a de novo deletion. In only a few individuals WS is due to vertical transmission. In one case gonadal mosaicism was suggested (Kara-Mostefa et al., 1999; Baumer et al., 1998). Therefore the recurrence risk in the sibship of a proband with WS with unaffected parents is expected to be negligible, close to the population risk. Recent studies have shown that almost 30% of the parents transmitting the WS chromosome are heterozygous for a paracentric inversion estimated to be 1.5 Mb and encompassing the entire WS region (Bayes et al., 2003; Osborne et al., 2001). Since WS is almost always sporadic the significance of this finding is not clear.

### Prenatal testing

Parents of a child with WS can be offered testing for the deletion to determine the recurrence risk. In case neither member of a couple has a deletion the recurrence risk is low. In that case prenatal diagnosis is not strictly indicated but can be considered because of the risk for gonadal mosaicism. Prenatal diagnosis can also be considered in cases in which due to maternal age or other risk factors a chorionic villus sampling or amniocentesis is performed.

## 1.8 Scope of this thesis

The preceding paragraphs discussed the history, clinical features, diagnosis, neurogenetics and medical management of WS, a genetic disorder resulting in medical complications, cognitive impairment and brain morphologic changes. However, several issues remain to be elucidated. Can we improve the clinical and molecular diagnosis of WS? Can we quantify the deficits in motor behaviour in WS? Can we establish a link between the deleted genes and the phenotype in WS?

### Diagnosis and genes (Chapter II)

As discussed, due to the variable phenotypical expression establishing a clinical WS diagnosis can be difficult. Clinical scoring systems can be useful. In the past several scoring systems were devised (Committee on Genetics, 2001; Selicorni, 1996; Lowery et al., 1995; Preus, 1984). However, these scoring systems contain either the rather subjective item "typical facial features" (Lowery et al., 1995) or too many items to be of practical use during a busy outpatient clinic (Selicorni et al., 2006; Committee on Genetics, 2001; Preus, 1984). Besides that, the diagnostic scoring system devised by Selicorni (Selicorni, 1996) is also lacking important features such as hyperacusis and difficulty descending stairs. In chapter IIa we address the question if it is possible to devise a scoring system with fewer items, which are easy to determine and score. In 1993 Ewart and colleagues discovered that WS is caused by a deletion of *ELN* (Ewart et al., 1993). Most diagnostic laboratories use Fluorescence In Situ Hybridisation (FISH) with a probe for *ELN* as a (initial) diagnostic test (Osborne et al., 2006; Brondum-Nielsen et al., 1997). During the last years it has become clear that most WS individuals do not just lack *ELN* but 20-25 other genes as well. Also individuals with smaller, atypical deletions have been described. Atypical individuals can be missed with the routine FISH tests. In chapter IIb we investigate if Multiplex Ligation-dependent Probe Amplification (MLPA) is a reliable alternative for FISH. As can be concluded from part 1.6 of this introduction (medical guidance and genetic counselling), it is recommended to check WS individuals regularly. Therefore, diagnosing WS is important, but blood sampling, necessary for FISH or MLPA analysis, is considered stressful for people with intellectual disability. In chapter IIc we address the question if MLPA on buccal smears is a feasible non-invasive screening method for (WS) microdeletions.

*Dysfunction in motor behaviour in Williams syndrome (Chapter III)*

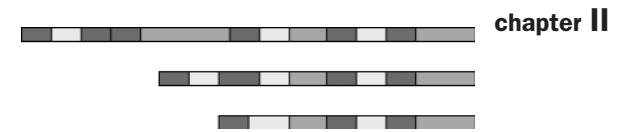
Neurological problems of WS include coordination difficulties such as trouble walking down a staircase or on non-uniform surfaces (Withers, 1996). Numerous studies have described the poor visuo-spatial processing capacities of subjects with WS (Atkinson et al., 2001; Mervis et al., 2000; Mervis and Klein-Tasman, 2000; Wang et al., 1995). We hypothesize that the coordination difficulties may be related to the poor visuo-spatial processing capacities either by an impairment in the control of the accuracy of saccadic eye-movements or by a deficit in the perception of visual depth. Saccades are very fast goal-directed movements of the eyeball that serve to project objects onto the fovea. Since the fovea is dedicated to detailed inspection of objects, proper use of saccadic eye movements allows the detailed but also rapid inspection of the whole visual environment by orienting the eyes in sequence to the objects of interest. It is imperative that these saccadic eye movements are accurate. Inaccurate saccades call for secondary corrective eye movements that may severely slow down the processing of the visual environment. In chapter IIIa we address the question if WS patients have abnormal saccadic eye movements. In WS a suboptimal perception of binocular depth is likely to be found (Atkinson et al., 2001; Olitsky et al., 1997; Sadler et al., 1996). However, typically developed individuals with no or suboptimal binocularity can still function well using only monocular depth cues such as perspective to judge distance. In chapter IIIb of the presented thesis we address the question if in WS problems in motor activities requiring visuo-motor integration are related to an inability to judge distance.

*Linking the genes to the cognitive and motor coordination functions (Chapter IV)*

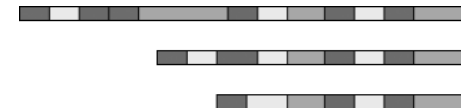
Knowing the results of the saccadic eye movement and visual depth processing tests, the next challenge is to delineate the individual and combined contribution of the ~28 genes in the WS region to these results. Two complementary strategies will be pursued. *CYLN2* and *GTF2IRD1* mouse mutants allow us the investigation of two single gene effects in animals. The detection and study of an individual with an atypical (smaller) deletion allows us to make inferences about single genes or groups of genes not deleted in this individual.

*Discussion (Chapter V)*

In the final chapter of the presented thesis we will discuss in more depth the (future) strategies which can be followed to establish a clinical and molecular WS diagnosis. We will also address to more extent the presumed function of brain related genes in the WS region and make recommendations for future genetic counselling



## Diagnosis and genes



## Chapter IIa

### The Dutch WS Questionnaire: a quick and easy tool for diagnosing Williams Syndrome

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Manuscript in preparation

## Abstract

Williams syndrome (WS) is a rare complex multisystem disorder characterised by mental retardation and various congenital and neurological abnormalities, caused by a 1.55-1.84 Mb deletion on chromosome 7q11.23. As genetic deletion detection is invasive, time consuming and relatively expensive, clinical diagnosis is important. However, existing scoring systems are rather elaborative. We evaluated two existing scoring systems in a Dutch population of 69 subjects and aimed to develop a new questionnaire for the clinical diagnosis of WS. The Dutch WS questionnaire indicated deletion detection for all subjects with a WS deletion, whereas both other scoring systems missed WS subjects. We conclude that the Dutch WS Questionnaire may provide a simple and easy tool in diagnosing Williams Syndrome using 10 easy questions.

## Introduction

Williams syndrome (WS, also known as Williams-Beuren syndrome, MIM 194050) is a multiple congenital anomalies-mental retardation (MCA/MR) syndrome characterised by distinct facial features, strabismus, congenital heart disease, growth retardation, intermittent hypercalcaemia, glucose intolerance, hyperacusis, sensorineural hearing loss, mental retardation and an unique cognitive and personality profile (Cherniske et al., 2004; Tassabehji, 2003; Bellugi et al., 1999). The prevalence is estimated at between 1 in 7.500 people and 1 in 20.000 people (Stromme et al., 2002; Morris et al., 1988). Characteristic facial features are periorbital fullness, stellate pattern of iris, short nose with bulbous nasal tip, long philtrum, large mouth, thick lips, small teeth and malocclusion (Burn, 1986). The facial features of patients with WS change with age. The combination of malar flattening and full cheeks is the most striking visible feature in infants (figure 1). Instead of this flat facial profile with full cheeks, older children and adults have a long narrow face and a long neck (figure 1). The typical vasculopathy of WS involves supravalvular aortic stenosis (SVAS) and pulmonary arterial stenosis (PAS) (Eronen et al., 2002). Overfriendliness and an empathetic nature are commonly observed (Gosch and Pankau, 1997). One of the relatively neglected phenotypical characteristics in WS are the commonly observed deficits in motor behaviour, such as an abnormal gait and problems descending stairs and stepping over surface changes (Van Der Geest et al., 2005; Withers, 1996). WS is caused by a hemizygous 1.55 - 1.84 Mb submicroscopic deletion of 25-30 genes, including the elastin gene (*ELN*), in chromosome band 7q11.23 (Bayes et al., 2003; Peoples et al., 2000). This submicroscopic deletion can be detected using commercial Fluorescent In Situ Hybridisation (FISH) probes containing the elastin gene. Ideally one would prefer to perform molecular analysis in all patients, but this is both time-consuming and expensive. Therefore, clinical diagnosis is important.

At present two clinical scoring systems are available: the two step clinical score of Selicorni (Selicorni, 1996) and the Williams syndrome diagnostic scoring table of the Committee on Genetics of the American Academy of Pediatrics (Committee on Genetics, 2001). However, both scoring systems have several drawbacks. First of all, they both contain too many items to be of practical use during a busy clinic and both demand much expertise in dysmorphology. Furthermore, the Selicorni score takes not into account the neurological characteristics often observed in Williams Syndrome (van der Geest et al., 2005; van der Geest et al., 2004; Atkinson et al., 2001; Chapman et al., 1996; Trauner et al., 1989).

The aims of the present study were 1) to evaluate the two existing WS scoring systems on a population of Dutch subjects with a tentative diagnosis of WS; and 2) to develop a simple scoring system for the clinical diagnosis of WS. We hypothesized that the sensitivity of the clinical diagnosis can be improved if several neurological characteristics are taken into account.

**Figure 1:** Six WS individuals, respectively aged 6,11,16,16,23 and 25 years, showing characteristic facial features: thick lips and large mouth are visible in individuals 2-6.



## Methods

### Subjects

The study described here was part of a larger Dutch study on Williams Syndrome, and was approved by the Medical Ethical Committee of the Erasmus MC and the VU University Medical Center. In this larger study, 69 people with the tentative diagnosis of WS were recruited through the Dutch “Netwerk Williams syndroom” and several departments of Clinical Genetics in the Netherlands. Written informed consent for the study was obtained from (the parents of) all subjects. All 69 subjects were mentally retarded and were clinically diagnosed in the past by others as having Williams Syndrome. In 15 subjects the clinical diagnosis was not yet confirmed by genetic analysis at the onset of the study.

### Genetic Diagnosis

In all 69 subjects genetic analysis of chromosome 7q11.23 was performed. In 62 subjects deletion analysis was performed in the present study, using both an experimental Fluorescent in Situ Hybridisation (FISH) assay with four probes that cover the whole critical region (B315H11, CITB5J22, B270D13, B39H04) and multiplex ligation-dependent probe amplification (MLPA) with 8 genes out of this region: *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2* (van Hagen et al., 2007). The remaining seven subjects did not agree to collect a new blood sample for the present study. For these subjects the laboratory results of tests performed in the past (e.g. FISH with Vysis or Oncor probe, or marker analysis in proband and parents) were collected.

### Clinical examinations

We performed systematic medical assessment on the 69 subjects, who participated in a multidisciplinary research protocol involving a variety of medical, genetic, cognitive and behavioral evaluations (Montfoort et al., 2007; van Hagen et al., 2007; van der Geest et al., 2006; van Strien et al., 2005; van der Geest et al., 2005; van der Geest et al., 2004). Medical history was obtained by direct interview with subjects and parents supplemented by a questionnaire designed by the authors and a review of the collected medical records.

The presence or absence of SVAS was determined based on the medical records. Each participant was clinically and medically examined (JMvH). The presence or absence of a heart murmur was checked. Special attention was paid to the dysmorphic and neurological features. Based on these clinical examinations diagnoses were made according to three scoring systems: the two-step score of Selicorni, the WS diagnostic scoring table of the Committee on Genetics (American Academy of Pediatrics), and the new Dutch WS Questionnaire.

### The Selicorni scoring system

In each participant the clinical diagnosis according to the two-step scoring system of Selicorni (Selicorni, 1996) was determined (table 1). In step 1 the presence or absence of 18 minor facial anomalies was scored, and lumped into one “facial score”. In step 2 of this scoring system the “facial score” was converted into the value 0 when the facial score was less than 10, into 1 when the facial score was between 10 and 15, and into 2 when the facial score was larger than 15. After that a Selicorni total score based on the combination of the “converted facial score” and six extrafacial traits was made. The Selicorni scoring system yields the clinical diagnosis of “WS” if the total score exceeds 4, “doubtful” if the total score equals 4, and “no WS” if the score is less than 4. The clinical score developed by Selicorni and colleagues is based on a study of 27 individuals (20 WS individuals).

**Table 1:** Two step clinical score of Selicorni

Facial score	
Trait	Score
Coarse facial features	2
Dolichocephaly	1
Bitemporal narrowing	2
Facial Asymmetry	1
Sparse eyelashes	1
Strabismus	1
Stellate pattern of iris	2
Epicanthal folds	2
Periorbital fullness	2
Depressed nasal bridge	1
Broad nasal tip	1
Full/sagging cheeks	2
Malar hypoplasia	2
Long philtrum	1
Large mouth	1
Thick lips	2
Mouth held open	1
Malocclusion/sparse little teeth	2
Facial score	
Total score	
Trait	Score
Facial score <10	0
Facial score 10-15	1
Facial score >15	2
Short stature	1
Inguinal or umbilical hernia	1
Mental retardation	1
Outgoing personality	2
SVAS	2
Congenital heart disease (non SVAS)	1
Total score	

Williams syndrome diagnostic scoring table, Committee on Genetics, American Academy of Pediatrics

In each participant the WS diagnostic score according to the Committee on Genetics of the American Academy of Pediatrics (Committee on Genetics, 2001) was assessed (table 2). According to the committee a diagnosis of WS is unlikely if the score is less than 3, and WS deletion detection should be considered otherwise. This scoring system is based on a study of 107 WS individuals (mean score for WS individuals 9, standard deviation 2.86).

**Table 2:** Williams syndrome diagnostic scoring table, Committee on Genetics, American Academy of Pediatrics

Growth (Past or Present Evidence of)	If 3 of 5 items are checked, score 1 point ____
Post-term birth > 41 wk gestation	
Failure to thrive/height and weight < 5th percentile	
Vomiting or gastroesophageal reflux	
Prolonged colic > 4m irritability	
Chronic constipation	
Behaviour and Development	If 3 of 6 items are checked, score 1 point ____
Overly friendly personality	
Hypersensitivity to sound	
Anxiety	
Developmental delay or mental retardation	
Visuospatial problems	
Delayed speech acquisition, followed by excessive talking	
Facial Features	If 8 of 17 items are checked, score 3 points ____
Bitemporal narrowing	
Epicanthal folds or flat nasal bridge	
Strabismus (present or past)	
Short nose or anteversion of nares	
Full cheeks	
Long philtrum	
Small, widely spaced teeth	
Wide mouth	
Prominent ear lobes	
Broad brow	
Periorbital fullness	
Stellate lacy iris pattern	
Bulbous or full nasal tip	
Malar hypoplasia (flat cheek bones)	
Full prominent lips	
Malocclusion	
Small jaw	

**Cardiovascular Problems****(by Echocardiography) (a)****If 1 of 2 items are checked score 5 points \_\_\_\_**

SVAS
Peripheral pulmonary artery stenosis

**Cardiovascular Problems (b)****If 1 of 3 items are checked, score 1 point \_\_\_\_**

Other congenital heart disease
Cardiac murmur
Hypertension

**Connective Tissue Abnormality****If 2 of 6 items are checked, score 2 points \_\_\_\_**

Hoarse voice
Inguinal hernia
Bowel or bladder diverticula
Long neck or sloped shoulders
Joint limitation or laxity
Rectal prolapse

**Calcium Studies****If 1 of 2 items are checked, score 2 points \_\_\_\_**

Hypercalcemia
Hypercalciuria

**Total Points: \_\_\_\_****Dutch WS Questionnaire**

Based on the clinical information obtained from the 69 mentally retarded subjects a two step scoring system "Dutch WS Questionnaire" was developed (table 3). Starting-point of this Questionnaire is mental retardation. The first step of this questionnaire focuses on the appearance of the subject and is based on the first step of the Selicorni score. Dysmorphic features like stellate pattern of iris, large mouth, thick lips and dental abnormalities (sparse little teeth and/or malocclusion) and short stature (length < -2SD) are scored. If the sum score of this first step is 0 the diagnosis WS is "very unlikely", and if it is larger than 2, the diagnosis WS is "very likely". When the sum score of the first step is 1 or 2, the second step of the "Dutch WS Questionnaire" is applied. The second step focuses on neurological items of WS: hyperacusis (past or present), problems descending stairs and reluctance in changing the surface (both before 8 years of age), outgoing personality and preoccupations/obsessions (van der Geest et al., 2005; Mervis and Klein-Tasman, 2000; van Borsel et al., 1997; Withers, 1996; Klein et al., 1990).

When an characteristic is present that item is adjudged 1 point. Only for those subjects with a score of 1 or 2 in the first step of the "Dutch WS Questionnaire", the total sum score of the second step is calculated. If the total score of the second step is 0 or 1, the diagnosis of WS is unlikely, and if the second score exceeds 2 the diagnosis of WS is likely. In the end four outcomes for the diagnosis of WS are possible: "very unlikely", "unlikely", "likely" and "very likely" (table 3).

**Table 3.** The Dutch WS Questionnaire

Step 1: Appearance (five yes-no questions)

<b>Appearance</b>	<b>Present = 1, Absent = 0</b>
Stellate pattern of iris	
Large mouth	
Thick lips	
Dental abnormalities (sparse little teeth and/or malocclusion)	
Short stature (length <-2SD)	
<b>Sum score Appearance</b>	

Step 2: Neurological characteristics (if Sum Score Appearance is 1 or 2)

<b>Neurological Items</b>	<b>Characterized by</b>	<b>Present = 1, Absent = 0</b>
Hyperacusis (past or present)	frightened reaction to or bothered by certain sounds (e.g., power saw, electric drill, fire-works, airplane, motorcycle, thunder, vacuum cleaner); covering ears with hands, crying or cringing in response to sounds that are neither intrinsically threatening nor unusually loud to a normal individual	
Problems descending stairs < 8 years of age	Putting two feet on the same step, using two hands to hold onto the banister; looking down constantly to the steps ahead; less problems in ascending stairs	
Reluctance in changing surface < 8 years of age	Stopping or refusing to proceed when changing one surface (e.g., tiles) to another (e.g., carpet); feeling out new surfaces (probing with foot, touching with hand)	
Outgoing personality (gregarious and people-oriented)	Highly sociable interactions with others; overly friendly; highly empathic; less reservation toward strangers	
Preoccupation/obsession	Collecting objects in extreme manner; obsessive fascination in, e.g., rotating objects (washer)	
<b>Sum score Neurological Items</b>		

Diagnosis

<b>Appearance Score</b>	<b>Neurological Score</b>	<b>Clinical Diagnosis</b>
0		WS very unlikely
1-2	0-1	WS unlikely
	2+	WS likely
3+		WS very likely



### Analysis

For each subject the three scoring systems were applied to make three (possibly different) diagnoses. These diagnoses were correlated with the results of the molecular tests. The sensitivity, specificity, and the positive (PPP) and negative predictive power (NPP) of the three clinical scoring systems were calculated using the number of true positives (hits), true negatives (correct rejections), false negatives (misses) and false positives (false alarms). The diagnosis “WS doubtful” of the Selicornì scoring system was not used in for this analysis.

### Results

The study cohort of 69 subjects consisted of 59 subjects with a deletion of the WS region: 30 males and 29 females, average age at time of the study 17.1 years (range: 3 - 39 years) and 10 patients without a WS deletion: 5 males and 5 females, average age at time of the study 15.3 years (range: 5 - 22 years).

#### Two step clinical score of Selicornì

Of the 59 subjects with a deletion only 41 were diagnosed as having WS using the Selicornì scoring system, 9 subjects were doubtful to have WS, and 9 subjects were missed. Of the 10 subjects without a deletion 4 were correctly diagnosed as not having WS. So, in 45 out of 69 subjects in total the clinical diagnosis was correct, and in 11 the clinical diagnosis was doubtful (table 4).

**Table 4:** Correlation between Selicornì scores and WS deletion. Presented are the number of subjects. The bold cases in grayed cells represent misdiagnoses

Selicornì Diagnosis	WS Deletion	
	Yes	No
No WS (< 4)	9	4
WS doubtful (= 4)	9	2
WS (> 4)	41	4

#### Diagnostic scoring table of Committee on Genetics

Using the scoring table of the committee in the 59 subjects with a deletion of the WS critical region, 54 subjects scored three points or higher, suggesting that deletion detection should be considered, but 5 subjects scored below three points, suggesting that in these subjects WS is unlikely (table 5). All 10 subjects without the deletion were scored with the diagnosis WS unlikely

**Table 5** Correlation between Committee on Genetics scores and WS deletion. Presented are the number of subjects. The bold cases in grayed cells represent misdiagnoses.

Committee on Genetics Score	WS Deletion	
	Yes	No
WS unlikely (<3)	5	10
Indication WS deletion detection (≥3)	54	0

#### Dutch WS Questionnaire

Using the Dutch WS Questionnaire the diagnosis WS was “very likely” in 38 (64%) and “likely” in the remaining 21 subjects (36%) of the 59 individuals with the deletion. No subjects with the deletion were missed (table 6). Of the 10 subjects without the deletion, the diagnosis WS was “very unlikely” in 4, “unlikely” in 1 subject and “likely” in the remaining 5 subjects. In none of these 10 subjects the diagnosis WS was very likely. Note that not all individual items of the Dutch WS Questionnaire correlated with the absence or presence of the deletion (table 7)

**Table 6:** Results after step 1 and 2 of the Dutch WS Questionnaire. The total score of the Neurological Items are only used when the Appearance Score is 1 or 2. Presented are the number of subjects. The bold cases in grayed cells represent misdiagnoses.

Total Scores			WS deletion	
Appearance (step 1)	Neurological (step 2)	Diagnosis of WS	Yes	No
0		very unlikely	0	4
1	0-1	unlikely	0	1
2	0-1	unlikely	0	0
1	1+	likely	7	2
2	1+	likely	14	3
2+		very likely	38	0

**Table 7:** Correlation between several characteristics and the WS deletion. Presented are the number of subjects. (\*) Heart murmur was examined in a more elaborate medical examination; (\*\*) the presence of SVAS was based on medical records (but echocardiography was not performed in 12 subjects).

Characteristic		N	WS Deletion	
			Yes	No
Mental retardation	Yes	69	59	10
	No	0	0	0
Stellate pattern of iris	Yes	32	31	1
	No	37	28	9
Large mouth	Yes	41	40	1
	No	28	19	9
Thick lips	Yes	50	48	2
	No	19	11	8
Dental abnormalities (sparse little teeth and/or malocclusion)	Yes	35	33	9
	No	34	26	1
Short stature (<2SD)	Yes	17	13	4
	No	52	46	6
Hyperacusis (past or present)	Yes	62	55	7
	No	7	4	3
Problems descending stairs before 8 years of age	Yes	59	54	5
	No	10	5	5
Reluctance in changing surface before 8 years of age	Yes	29	27	2
	No	40	32	8
Outgoing personality	Yes	50	45	6
	No	18	14	4
Preoccupation/obsession	Yes	36	30	6
	No	33	29	4
Heart murmur *	Yes	45	44	1
	No	24	15	9
SVAS **	Yes	28	28	0
	No	29	12	24
	Unknown	7	5	5

### Comparison

The sensitivity of the Dutch WS questionnaire (1.0) was higher than those of the Selicorni scoring system (0.82) or the scoring table of the Committee of Genetics (0.92) (table 8).

**Table 8:** The sensitivity, specificity, and the positive (PPP) and negative predicative power (NPP) of the three clinical scoring systems. Presented are number of true positives (hits), true negatives (correct rejections), false negatives (misses) and false positives (false alarms). The diagnosis "WS doubtful" of the Selicorni scoring system was not used in this table.

System	Hits	Correct Rejections	False Alarms	Misses	Sensitivity	Specificity	PPP	NPP
Selicorni	41	4	4	9	0.82	0.50	0.91	0.31
Committee	54	10	0	5	0.92	1.00	1.00	0.67
Dutch WS Questionnaire	59	5	5	0	1.00	0.50	0.92	1.00

### Medical examination

In 44 of the 59 subjects (75%) with a deletion, and in 1 of the 10 subjects without the deletion a heart murmur was heard. In 57 subjects a echocardiography was performed in the past. In 28 of the 52 subjects (47%) with the deletion, and in none of the 5 subjects without the deletion, SVAS was detected (table 7).

In 12 subjects the presence or absence of SVAS could not be determined.

### Discussion

The present study was set out to evaluate three scoring systems for the clinical diagnosis of WS. The new Dutch WS Questionnaire successfully diagnosed all subjects with the deletion, whereas the other two scoring systems missed several subjects with the deletion (9 subjects using the Selicorni system, and 5 using the system of the Genetic Committee of the American Academy of Pediatrics). As for our 10 subjects without the deletion, the Dutch WS Questionnaire falsely identified 5 subjects as having likely WS, which is comparable to the 6 subjects identified by the Selicorni scoring system. The Genetic Committee scoring correctly excluded all of our 10 subjects without the deletion.

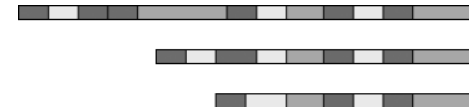
These results suggest that the Dutch WS Questionnaire may provide an easy and reliable diagnostic tool. It has several advantages over the two existing clinical diagnostic instruments. First of all it contains only ten scoring items (as opposed to the 24 items of Selicorni and the 41 items of the Committee score) which makes it suitable for use in a busy outpatient clinic. Moreover, these ten questions are quite easy to score. Photographs of a subject can be used to score the items large mouth and thick lips and subject height can be obtained from the parents and plotted on a growthchart for easy evaluation. The age dependent items full cheeks, long face and long neck are not included. Secondly, there is no direct need for any physical examination, which may frighten the subject. Thirdly one has not to wait for the results of an echocardiography. Finally, as opposed to the Selicorni system, it also takes neurological items of WS, such as hyperacusis and motoric problems, into account.

Extending the Dutch WS Questionnaire with a physical examination or echocardiography can give complementary information. Hearing a heart murmur or determination of the presence of SVAS might strengthen the likelihood of a WS diagnosis. In our research group all subjects with a SVAS had a heart murmur. One could argue that not in all subjects of our research group an ultrasound of the heart was made and that this could have negatively influenced the Selicorni score and the score of the Committee on Genetics. However, in seven of the nine deletion subjects that were missed by the Selicorni score and in four of the

five deletion subjects that were missed by the score of the Committee on Genetics (three subjects were missed by both scoring systems) echocardiography was performed and no SVAS was detected. In total, in two subjects no ultrasound was made. In one subject the total Selicorni score was 2 and could have been 4 (WS doubtful) in case a SVAS has been missed. In the other patient the total Selicorni score was 3 and the Committee score was 1. These scores could have been 5 (WS) and 6 (indication for deletion detection) respectively in case a SVAS has been missed. This would lower the missed deletion subjects from 9 to 7 for the Selicorni score and from 5 to 4 for the Committee score. With the Dutch WS Questionnaire no subjects with the deletion were missed. We conclude that the Dutch WS Questionnaire may provide a simple and easy tool in diagnosing Williams Syndrome using 10 easy questions. Deletion detection is indicated for those subjects in which the diagnosis of WS is "likely" and "very likely"

### Acknowledgements

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## Chapter IIb

### FISH or MLPA as a diagnostic test for Williams syndrome?

#### Comparing two diagnostic laboratory tests for Williams syndrome: fluorescent in situ hybridisation versus multiplex ligation-dependent probe amplification

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## Abstract

Most people with Williams syndrome (WS) have a heterozygous 1.55 Mb deletion on chromosome 7q11.23. For diagnostic purposes, Fluorescence In Situ Hybridisation (FISH) with commercial FISH probes is commonly used to detect this deletion. We investigated whether Multiplex Ligation-dependent Probe Amplification (MLPA) is a reliable alternative for FISH. The MLPA kit (SALSA P029) contains probes for 8 genes in the WS critical region: *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYL2*. The experimental FISH assay that was used consist of 4 probes covering the WS critical region. A total number of 63 patients was tested; in 53 patients, a deletion was detected both with FISH and MLPA(P029), in 10 patients both techniques failed to demonstrate a deletion. In only 1 patient, a deletion was detected which was not previously detected by two commercial FISH probes. This patient appeared to carry a small, atypical deletion. We conclude that MLPA is a reliable technique to detect WS. Compared with FISH, MLPA is less time-consuming and has the possibility to detect also smaller, atypical deletions and duplications in the WS critical region.

## Introduction

Williams syndrome (WS, MIM 194050) is a developmental disorder characterised by dysmorphic features (figure 1), congenital heart malformations, intermittent hypercalcemia and mental retardation (Bellugi et al., 1999;Burn, 1986).

**Figure 1:** Child with WS showing typical facial features with full lips



The majority of patients have a 1.55 Mb deletion of the WS critical region on chromosome 7q11.23. Interestingly, inversion of the same segment has been found as a polymorphic variant in parents of WS patients and in some atypical patients (Osborne et al., 2001). A small proportion of WS patients have a larger deletion of 1.84 Mb (Bayes et al., 2003). A few exceptional patients with a smaller deletion have been described in the literature (Howald et al., 2006;Tassabehji et al., 2005;Morris et al., 2003;Heller et al., 2003;Gagliardi et al., 2003;Hirota et al., 2003;Karmiloff-Smith et al., 2003;Korenberg et al., 2000; Tassabehji et al., 1999;Botta et al., 1999a). Until now most diagnostic laboratories use Fluorescence In Situ Hybridisation (FISH) with a probe for the WS critical region as a (initial) diagnostic test

(Osborne et al., 2006; Brondum-Nielsen et al., 1997). Commonly used probes in a standard FISH assay are from Vysis and Cytocell. In the past also a probe from Oncor (Ewart et al., 1993) has been widely used, but this probe is no longer available. As an alternative for FISH, analysis of polymorphic VNTR markers can be used to detect WS patients. The disadvantage of this approach is that also DNA of the parents of the patient must be available and not all markers will be informative in all cases. Recently the set up and validation of paralogous sequence quantification (PSQ) and quantitative real time PCR (QPCR) for diagnosing the recurrent DNA deletion present in WS patients has been described (Howald et al., 2006; Schubert and Laccone, 2006). Multiplex ligation-dependent probe amplification has been introduced in the DNA diagnostic laboratories for the detection of deletions in various disease genes (Vorstman et al., 2006; Koolen et al., 2004; Hogervorst et al., 2003; Schouten et al., 2002; Gille et al., 2002). Also for WS a MLPA kit is commercially available. This kit contains probes for 8 genes in the WS critical region: *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*. Using this MLPA kit a more precise mapping of a deletion in the WS critical region is possible. This is of interest because patients with smaller, atypical deletions have been described and not in all patients with a clinical diagnosis of WS a deletion can be detected by a standard commercial FISH assay. In this study we have used MLPA as a diagnostic test in 63 WS patients. The results obtained with MLPA were compared with those obtained with an experimental FISH assay containing four probes covering the commonly deleted WS region.

## Materials and Methods

### Patients

73 patients in which in the past the (tentative) diagnosis WS was made by a clinical geneticist participated in the study. They were recruited from the Dutch "Netwerk Williams syndroom" and the departments of Clinical Genetics in the Netherlands. Most patients were re-examined by a clinical geneticist (JvH) and medical data were obtained from the hospital case notes, parental accounts, clinical photographs and specific investigations. In 57 of the 73 patients deletion analysis by standard FISH or VNTR DNA-analysis was performed previously and of those 50 patients were known to carry a deletion (table 1). 63 of the 73 patients (or their parents) gave permission to take a blood sample for both experimental FISH and MLPA analysis. Written informed consent prior to the study was obtained in all cases. The study was approved by the Medical Ethical Committee of the Erasmus MC and the VU University Medical Center.

### FISH analysis

If the results of a previously obtained karyogram were not already available, routine karyotyping was performed according to standard methods. From a previous study (Meng et al., 1998) the probes B315H11, CTB51J22, B270D13 and B39H04 were selected and alternated labelled with Bio-16-dUTP/Dig-11-dUTP. The chromosome slides and probes were denatured simultaneously and incubated overnight at 37°C. The detection was performed in one single layer containing streptavidine-Alexa 594 (Invitrogen) and anti-digoxigenine-FITC (Roche). Hybridisations were analysed and captured with an epifluorescence microscope (Zeiss Imaging II) suited with ISIS software (MetaSystems). To determine whether a given probe was deleted, more than 50 metaphase cells were evaluated and scored for the presence or absence of a signal from the test probes.

### DNA analysis

Genomic DNA was extracted from peripheral blood samples using standard procedures. The MLPA kit SALSA P029, LOT 0403, (MRC Holland, The Netherlands) containing probes for 8 genes out of the Williams syndrome critical region (*FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*) was used. For *ELN* and *CYLN2* probes for various exons are present in the kit. The MLPA reaction was performed according to the instructions of the supplier. MLPA products were separated by capillary electrophoresis on ABI310 Genetic Analyzer using POP4 polymer and TAMRA-500 (Applied Biosystems) as a standard. Data analysis was performed using GeneScan Software.

## Results

In 63 (suspected) WS patients experimental FISH and MLPA analysis was performed (table 1, figure 2). In 52 patients a deletion of all four FISH probes as well as a deletion of the 8 genes tested with MLPA was detected. All these patients proved to have a normal karyotype.

**Table 1:** Results of experimental FISH and MLPA analysis in 63 patients with (suspected) Williams Syndrome

		Results in this Study	
		FISH <sup>1</sup>	MLPA <sup>2</sup>
Previous results*	deletion n = 50	deletion n = 41	deletion n = 41
		no deletion n = 0	no deletion n = 0
		not performed n = 9	not performed n = 9
	no deletion n = 7	deletion n = 1	deletion n = 1
		no deletion n = 6	no deletion n = 6
		not performed n = 0	not performed n = 0
	not performed n = 16	deletion n = 11	deletion n = 11
		no deletion n = 4	no deletion n = 4
		not performed n = 1	not performed n = 1

\* Deletion analysis was performed with standard FISH or VNTR DNA-analysis

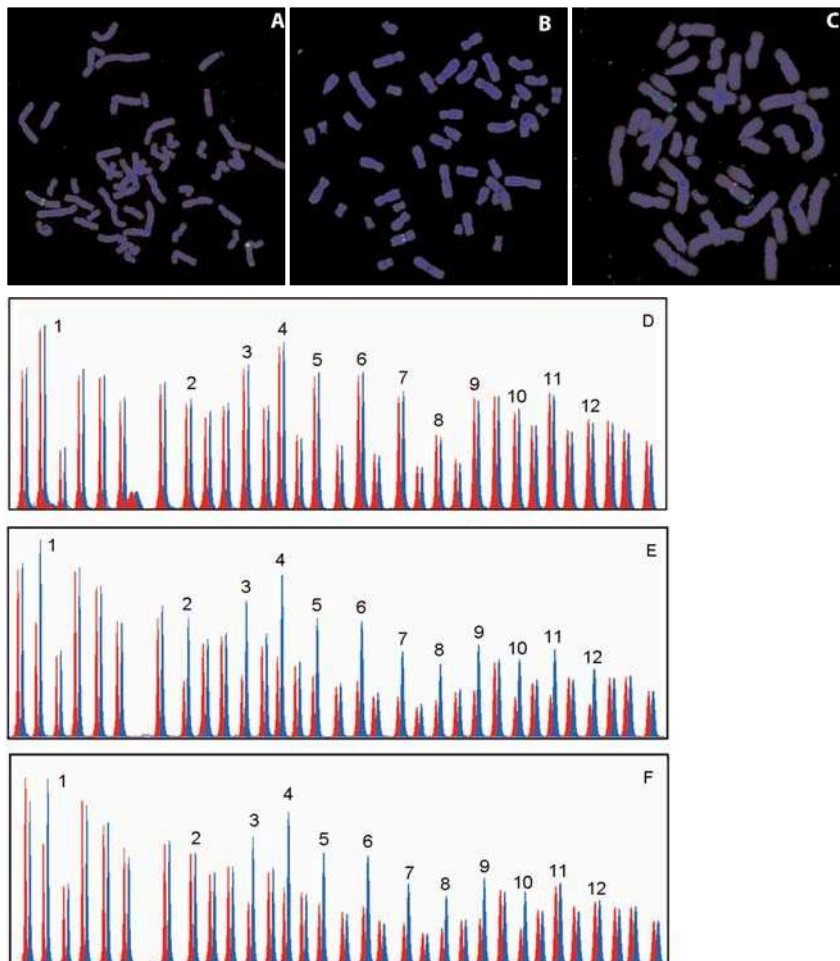
1 The FISH assay contains the probes B315H11, CTB51J22, B270D13 and B39H04

2 The MLPA kit SALSA P029 (MRC Holland, The Netherlands) contains probes for *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*

One patient proved to have a smaller (atypical) deletion including the B315H11 and CTB51J22 FISH probes and the *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN* and *LIMK1* MLPA probes (van Hagen et al., 2007). This deletion was not detected by using the commercial Cytocell probe and Vysis probe (figure 3). A normal karyotype was found in this patient. In 10 patients both the experimental FISH and the MLPA failed to show a deletion of the WS critical region. A normal karyotype was found in 9 of these patients and a 46XY/XXY mosaicism was observed in one patient. Using the score described by the American Academy of Pediatrics (Committee on Genetics, 2001) all these 10 patients had a score of less than 3 meaning that WS is unlikely. Using the score described by Selicorni (maximum score is 10) (Selicorni, 1996) 4 patients had a score of less than 4 (no WS), 2 patients had a score of 4 (WS doubtful) and 4 patients had a score that exceeds 4 (WS). After re-evaluation by 3 medical doctors who participated in the study the diagnosis of WS was rejected in these 10 patients without a deletion. No duplication of the WS critical region was detected in the 63 patients analysed with MLPA.

**Figure 2:** Results of FISH and MLPA analyses in (suspected) WS patients. In figures 2a-2c the results of 2 of the 4 probes of the the experimental FISH assay, B315H1 (red) and B270D13 (green) are shown. 7qter RP1-3K23 (green) is used as control probe. (a) no deletion. (b) (common) deletion (c) atypical deletion: B315H1 deleted, B270D13 not deleted.

In figures 2d-2f the results of MLPA analyses are depicted: normalized MLPA peak patterns of patients in red and of controls in blue. Peaks from genes in the Williams syndrome critical region are labeled with numbers: 1: *FKBP6*; 2: *CYLN2*; 3: *FZD9*; 4: *TBL2*; 5: *STX1A*; 6: *ELN*; 7: *ELN*; 8: *ELN*; 9: *ELN*; 10: *LIMK1*; 11: *RFC2*; 12: *CYLN2* (for *ELN* and for *CYLN2* respectively 4 and 2 probes for various exons are present). Unlabeled peaks represent control genes. (d) patient without a deletion. (e) WS patient with a deletion. (f) patient with an atypical deletion.



**Figure 3:** Map of the Williams syndrome critical region: VNTR markers are represented by green dots, SALSA P029 MLPA (LOT 0403) probes, used in this study, by red dots and SALSA P064 MLPA (LOT 0704/0305) probes, used by Kirchoff et al.<sup>5</sup>, by white dots. The four experimental FISH probes (this study) and the commercial FISH probes Vysis and CytoCell are represented by blue lines. The Vysis FISH probe consists of an approximately 180 kb probe including *ELN*, *LIMK1* and the D7S613 locus. The probe also contains a control probe for the region containing loci D7S486 and D7S522 (7q31). The CytoCell FISH probe consists out of a directly labelled probe mixture containing three non-overlapping clones covering most of the 1.55 Mb deletion. The first clone contains the genes *FZD9*, *BAZ1B*, and *TBL2*. The second clone contains *LIMK1* and *RFC2*. The third clone contains *CYLN2*. Together the clones are approximately 450 kb. As a control probe the 7 centromere alpha-satellite probe D7Z1 is used.

The common deletion of 1.55 Mb and the large deletion of 1.84 Mb are presented in a black dual arrowhead lines. The atypical deletion is depicted in a red box flanked by the not deleted area in black. Duplications from the literature (including a patient with supernumerary ring chromosome 7 mosaicism) are depicted in green boxes, flanked by the not duplicated area in black. Uncertain boundaries of deletions or duplications are depicted as a shaded area.

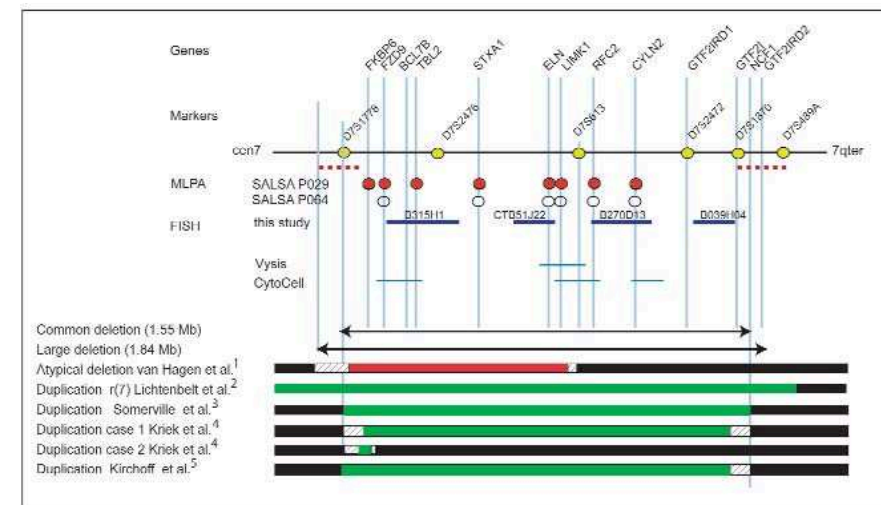
<sup>1</sup> Van Hagen et al, 2007

<sup>2</sup> Lichtenbelt et al, 2005

<sup>3</sup> Somerville et al, 2005

<sup>4</sup> Kriek et al, 2006

<sup>5</sup> Kirchoff et al, 2006



## Discussion

By comparing experimental FISH and MLPA for the detection of deletions in the WS critical region we did not find any discrepancies. One of the deletions was not detected using two commercial FISH assays (Vysis and Cytocell).

In accordance with data from the literature, most patients with WS carry a heterozygous deletion of 1.55 Mb at 7q11.23: the common deletion (figure 3). This deletion is thought to arise from recombination between misaligned repeat sequences flanking this region during meiosis. At the centromeric end of the common deletion *FKBP6* is located; at the telomeric end *GTF2I* (Bayes et al., 2003). A small proportion of WS patients have a larger deletion of 1.84 MB (Bayes et al., 2003).

The common deletion can be detected using commercially available FISH probes, the set of four FISH probes we used or the SALSA P029 Williams-Beuren syndrome MLPA kit (figure 3). With the Vysis FISH probe deletions outside the 180 kb region of the probe will not be detected and atypical deletions can be missed. Using the Cytocell FISH probe probably all common deletions will be found. However, patients with an atypical deletion can be missed. Due to the mixture of three clones, in case of a deletion of one of the clones the two other clones will provide a signal which can not be differentiated from a signal of the three clones together. The experimental FISH assay and the MLPA kit we used seem to have an advantage over the commercially available FISH probes for detecting atypical deletions.

Several other microdeletion disorders, including the 22q11 deletion (velocardiofacial) syndrome, the Smith-Magenis syndrome, the Prader-Willi and Angelman syndromes and the hereditary neuropathy with liability to pressure palsies, are also thought to be the result of non-allelic homologous recombination between low-copy repeats found in the region (Lupski and Stankiewicz, 2005; Stankiewicz and Lupski, 2002; Inoue and Lupski, 2002). For each of these microdeletions, a matching duplication has been identified: dup 22q11, dup17p11.2, dup15q11-q13, and Charcot-Marie-Tooth type 1A, respectively (Inoue and Lupski, 2002). Recently patients with a duplication of the WS region have also been described (Kriek et al., 2006; Kirchhoff et al., 2006; Somerville et al., 2005). Somerville et al. discovered the duplication in a boy who was initially referred for velocardiofacial syndrome and was screened with the use of a real-time method on the basis of a polymerase-chain-reaction assay. Kriek et al. screened 105 patients with developmental delay and/or congenital malformations using a Multiplex Amplifiable Probe Hybridisation (MAPH) assay containing 63 exon-specific single-copy sequences and found one patient with a duplication of *FKBP6*, *TBL2*, *ELN* and *CYLN2*. They confirmed their findings with a MLPA kit containing probes for *GTF2IRD1* and *GTF2I*. By using both MAPH and MLPA they also detected a duplication of *FKBP6* in a patient. Kirchhoff et al., using the MLPA kit SALSA P064 containing probes for the genes *FZD9*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*, found one patient with a 7q11 duplication reciprocal of the WS common deletion in a retrospective study in 258 mentally retarded and dysmorphic patients with normal conventional karyotypes. The phenotype of the duplication of the WS critical region is still unclear although severe expressive language delay could be part of it (Kriek et al., 2006; Kirchhoff et al., 2006; Tassabehji and Donnai, 2006; Somerville et al., 2005; Lichtenbelt et al., 2005). In the 10 patients of our study, who were originally suspected to have WS but in the end proved to have neither the common deletion nor the WS phenotype, no duplication of 7q11.23 was found. So, knowing very little about the phenotype of a duplication of the WS locus, it might be worthwhile to screen for duplications in patients referred for WS until more knowledge is available. In contrast to FISH the MLPA technique has the advantage that both (atypical) deletions and duplications can be detected.

In comparison with FISH, the rather limited hands-on time needed renders MLPA a very efficient and cheap technique. MLPA has also a rather low turn around time. Problems with the signal, as can be discovered using FISH, are not present using MLPA. A potential problem with the MLPA technique is that polymorphisms

that could be present in the probe annealing site might be interpreted as a deletion (Wehner et al., 2005). However, in our 63 cases such MLPA interfering polymorphisms were not encountered.

The current MLPA kit Salsa P029 can be improved by adding the probes *GTF2IRD1*, *GTF2I*, *NCF1* and *GTF2IRD2* to the kit, because haploinsufficiency of the first three genes seems to be important for the WS phenotype and a deletion of *GTF2IRD2* can be found in rare WS cases with a 1.84 Mb deletion. A recent publication shows that the involvement of *NCF1* in the common deletion is variable and that hypertension is significantly less prevalent in WS patients with a deletion that includes *NCF1* (DelCampo et al., 2006). So, knowing the status of *NCF1* could influence medical management of an individual WS patient. *GTF2IRD1* could be a genetic determinant of craniofacial and cognitive development (Tassabehji et al., 2005), hemizygosity of *GTF2I* is likely associated with the mental retardation found in most WS patients, although some authors hypothesize that hemizygosity of both *GTF2I* and *GTF2IRD1* produce the visuospatial deficits seen in WS (Edelmann et al., 2006; Morris et al., 2003; Hirota et al., 2003). Adding *GTF2IRD1* and *GTF2I* to the MLPA kit probably improves the chance of finding atypical deletions in mentally retarded patients with a partial WS phenotype. Detecting a deletion of *GTF2IRD2* is suggestive for the presence of a 1.84 Mb deletion, although an inversion mediated 1.55 Mb deletion can not be excluded (Bayes et al., 2003). At the moment new techniques like high resolution SNP or oligonucleotide array analysis are developed. For example, the common WS deletion will take away 60 to 80 SNP's from the Affymetrix 250K or Illumina 317K array. In the near future it probably will be much more efficient to test all patients with mental retardation on an array first, detecting all possible microdeletions/duplications in one go. In the mean time a cheap and simple test to detect WS is preferred if a clinical diagnosis is suspected. MLPA appears to be a cheap and reliable technique to detect (atypical) deletions and duplications of the WS region and seems a good choice.

## Acknowledgements

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## Chapter IIc

**MLPA on buccal smears as a screening method for microdeletions in adult institutionalised patients**

**Feasibility and outcomes of Multiplex Ligation Dependent Probe Amplification on buccal smears as a screening method for microdeletions and duplications among 300 adults with an intellectual disability of unknown aetiology**

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## Abstract

**Background:** Determining the aetiology of intellectual disability enables anticipation of specific co-morbidity and can thus be beneficial. Blood sampling however is considered stressful for people with intellectual disability. Our aims were to evaluate the feasibility and outcomes of a non-invasive screening technique of nine microdeletions/duplications among adults with intellectual disability of unknown aetiology.

**Methods:** In a random sample of 300 adult clients of Dutch intellectual disability services without an aetiological diagnosis, DNA was collected on site using oral swabs. Multiplex Ligation Dependent Probe Amplification was applied to screen for nine microdeletions/duplications related to intellectual disability syndromes (Williams-Beuren, 22q11-deletion, 1p-deletion, Miller-Dieker, Smith-Magenis, Prader-Willi, Alagille, Saethre-Chotzen and Sotos syndrome).

**Results:** Feasibility: Prior to the consent procedure, for 2.1% (10/471 eligible participants) the method was considered undesirable. In 0.7% (2/300 participants) oral swabs failed due to resistant behaviour, while in 16.1% (48/298 swabs) analysis was unsuccessful due to insufficient amounts of DNA. A repeated attempt yielded an equal success rate. Microdeletions were diagnosed in 4 participants: 22q11 deletion (n=2), 5q35 deletion (Sotos-syndrome) (n=1) and 1p deletion (n=1). One participant had a duplication of the Prader Willi-region (15q11-13) due to mosaicism of a supernumerary marker chromosome (15).

**Conclusions:** Oral swabs are a feasible method for DNA sampling in adults with intellectual disabilities.

A diagnosis could be made in 5 out of 275 people with intellectual disability of unknown aetiology.

After screening, in the total population sample (N=620), the prevalence of syndromes associated with the microdeletions/duplications studied was at least 2.3% (95% CI 1.1-3.4%)

## Introduction

The aetiology of intellectual disability (ID) among adults in the Netherlands is mostly unknown. In a recent epidemiological study of sensory impairments in 1130 Dutch adults with intellectual disability by other causes than Down syndrome, 87% (980/1130) did not have an etiological diagnosis, confirmed by laboratory assessments (Van Splunder et al., 2003). A diagnosis can be useful for various reasons. In recent years knowledge about the specific morbidity and mortality patterns related to causes of intellectual disability has increased. This has resulted in the development of health watch programs (Cassidy SB and Allanson, 2005). The description in literature of behavioural phenotypes can give carers and psychologists more insight into the treatment of possible problematic behaviour. Obtaining a diagnosis is also important for genetic counselling and determination of the recurrence risk.

A well known cause of intellectual disability is a microdeletion: a hemizygous, submicroscopic chromosomal deletion resulting from unequal crossing-over during meiosis. The hemizygosity leads to a quantitative shortage of gene product, which is known as haploinsufficiency (Dallapiccola et al., 1995). This causes a specific, yet variable phenotype: a microdeletion syndrome. Variability of clinical features depends on the size of the underlying deletion and the influence of the remainder of the genotype (Tassabehji, 2003). Current knowledge of the prevalence of microdeletions is mostly based on rough estimates, not on population-based genetic screening and therefore has a high level of uncertainty. Since the associated phenotypes are variable, it is possible that individuals with mild features are not recognized. In a Swedish prevalence study the number of diagnoses of 22q11 deletion syndrome - probably the most common microdeletion syndrome - varied significantly with the region studied, which could be related to the experience and awareness of the specialists who were involved with these children (Oskarsdottir et al., 2004). This underlines the necessity for genetic screening to establish the incidence and prevalence of the 22q11-deletion syndrome accurately. A fast and low-cost genetic test for the detection of microdeletions is Multiplex Ligation-dependent Probe Amplification (MLPA). With this technique both microdeletions and duplications of chromosomal

regions can be detected (Vorstman et al., 2006). The population with an intellectual disability is obviously a risk population for microdeletion syndromes, but has - to our knowledge - never been subjected to unselected large-scale screening for microdeletions. Kirchhoff has recently applied MLPA retrospectively in a selected population: 258 dysmorphic patients with normal conventional karyotypes, who were initially referred for HR-CHG analysis. MLPA revealed 10 deletions, 5 duplications (5.8% imbalances). In the same study the yield of MLPA in patients with dysmorphism referred specifically for this test - many with a clinical suspicion of a specific microdeletion syndrome - was reviewed (Kirchhoff et al., 2006).

The MLPA technique is potentially suitable for screening purposes (Rooms et al., 2004). The benefits of a diagnosis mentioned above must however be weighed against the burden of screening for participants. Parents of individuals with intellectual disability who did not consent to a screening for fragile X syndrome in the Netherlands, most often mentioned the stress of the necessary blood sample as the prevailing reason (De Vries et al., 1997). Therefore a less stressful alternative seems desirable.

As the MLPA technique requires only small amounts of DNA it can also be applied on buccal smears, a non-invasive procedure compared to venapuncture. The Mental Retardation 1 MLPA kit (www.mrc-holland.com) contains probes for 7q11 (Williams syndrome) and 22q11 (22q11-deletion syndrome), as well as for 1p (1p-deletion syndrome), 17p13 (Miller-Dieker syndrome), 17p11 (Smith-Magenis syndrome), 15q11-13 (Prader-Willi syndrome/Angelman syndrome), 20p12 (Alagille syndrome), 7p21 (Saethre-Chotzen syndrome) and 5q35 deletion (Sotos syndrome). These syndromes are all related to intellectual disability. Most of them can also be caused by other genetic aberrations than deletions - e.g. point mutations - which can not be detected by MLPA. Using MLPA on oral swabs we performed a screening for microdeletions among adults with intellectual disability. Our main focus was to determine the feasibility of this method for screening purposes in this population and to obtain a first impression of the prevalence of undiagnosed microdeletions/duplications among adults with intellectual disability.

## Methods

### *Study design*

This descriptive study was conducted in a multi-centred setting. The study was approved by the boards of directors of the participating care organizations and the Medical Ethical Committee of the VU University Medical Centre.

### *Study population*

The participants were recruited from four care organizations providing residential care to people with intellectual disabilities in the Netherlands. Two categories of participants were distinguished: those living in centralized locations receiving medical care by intellectual disability physicians versus those living in community-based homes receiving medical care by general practitioners. A random sample of 408 adults was drawn from a population of 2700 residing in centralized locations. To limit the number of visits to residencies, the sampling of individuals living in community-based homes was conducted on a residence level: 257 adults were selected out of the 2400 individuals receiving residential care in the community from these four organizations. This resulted in a total sample of 665 adults with intellectual disability drawn from a population of 5100. Medical files of all selected individuals living in centralized locations were analysed for available information on the aetiology of the intellectual disability. For persons residing in community-based homes, psychological files were used.

For the estimation of the prevalence we included all individuals about whom reliable information could be obtained. A total of 45 selected individuals were excluded from our study because they had either moved or died before the start of the study or we could not obtain any information due to uncooperative staff of their residency. Individuals with an established genetic or metabolic diagnosis, confirmed with a laboratory test, were included for prevalence estimation but excluded from DNA sampling (table 1). In case of Down syndrome, diagnoses based on clinical characteristics were also accepted as exclusion criteria for DNA sampling. Individuals with a history of brain injury after their second birthday were excluded from DNA sampling if development before the second birthday had been reported normal. Individuals with a history of brain injury prior to their second birthday and those who had possibly suffered from perinatal asphyxia were not excluded for sampling. In the first case too little was known about previous cognitive development and in the second case it was considered that the underlying mechanism of perinatal complications can be a genetic aetiology (Wharton and Bresnan, 1989).

## *Methods*

Before starting the informed consent procedure, psychologists and/or intellectual disability physicians of eligible participants were asked to determine whether an individual could be considered competent to consent and/or if there were any objections to approaching them.

Carers were asked to help the individuals decide and to answer questions on the ethnic background and severity of intellectual disability. Participants were visited in their home or work environment and DNA was collected using a swab on buccal mucosa. Carers were asked to observe if participants showed resistant behaviour. Our definition of resistance implied that the participant showed more resistance or displeasure than when receiving assistance from their carers brushing their teeth. Noticing resistance the attempt was stopped. This was interpreted as failure of the screening method.

In case not enough DNA could be extracted from the swab to yield a reliable test result, the participants were offered a second buccal smear. If a microdeletion/duplication was diagnosed the participants and their legal representatives were offered genetic counselling. The buccal smear was repeated to confirm the diagnosis and - if possible - a blood sample was taken for further genetic evaluation.

### *DNA-analysis*

DNA was isolated from the swabs with the QIAGEN DNA extraction kit. DNA was analysed with the multiplex ligation-dependent probe amplification (MLPA)-technique (Vorstman et al., 2006) using the Mental Retardation 1 MLPA kit produced by MRC Holland.

**Table 1:** Known genetic/metabolic aetiological diagnoses before DNA collection in a randomly selected sample of 620 adults with intellectual disability

Known diagnosis	Centralized location (n= 401)	Community-based home (n=219)	Total (n=620)
Chromosomal aberrations			93
Down syndrome	48	40	88
Turner syndrome (mosaic)	1	0	1
Translocation (Partial monosomy chr. 14 with partial trisomy chr. 18)	1	0	1
ringchromosome 18	1	0	1
Cat Eye syndrome	1	0	1
46,XX 9p+	1	0	1
Cri du Chat syndrome (5p-)	1	0	1
Microdeletion syndromes			5
Williams syndrome	2	2	4
22q11 deletion syndrome	1	0	1
Craniosynostosis syndromes			2
Crouzon syndrome	0	1	1
Saethre Chotzen syndrome	0	1	1
Other syndromes			15
Fragile X syndrome	5	3	8
Cornelia de Lange syndrome	1	1	2
Rett syndrome	1	0	1
Prader Willi syndrome	1	1	2
Angelman syndrome	2	0	2
Neurocutaneous disorders			3
Neurofibromatosis type 1	1	0	1
Tuberous sclerosis	2	0	2
Metabolic diseases			2
Homocystinuria	1	0	1
Sanfilippo syndrome type A	1	0	1
Other			5
Myotonic dystrophy	1	1	2
Kernicterus	1	0	1
OPCA	0	1	1
Waardenburg syndrome	1	0	1
Total	75	51	126
(%)	(18.7%)	(23.2%)	(20.3%)

(Chr=chromosome)

### Analysis

The feasibility of the MLPA technique on buccal smears as a screening method among adults with intellectual disabilities was operationalized using three criteria: 1. the percentage of individuals for whom the method was considered undesirable in advance, 2. the proportion of buccal smears, failed due to resistance by the participant and 3. the proportion of samples yielding insufficient amounts of DNA for a reliable diagnosis. Feasibility and prevalence data were analysed with SPSS version 12.0 for Windows. The data are presented as proportions or percentages with 95% confidence intervals. To analyse the representativity of our results for the total study sample, characteristics of participants were compared with those of remaining eligible participants. Secondly we analysed whether the participants with reliable test results were representative for all eligible participants. Finally characteristics of individuals whose first or second samples yielded insufficient amounts of DNA for a reliable diagnosis were compared with those of individuals with a DNA result. Differences between groups were assessed with the unpaired T-test (for age) and Chi-square test (for other variables) and considered significant in case of p-values lower than 0.05. A prevalence estimate of the syndromes associated with the 9 microdeletions/duplications was calculated by dividing all known diagnoses after screening by the 620 individuals with intellectual disability in the study sample.

### Results

#### Study population

Inclusion and participation are shown in table 2. Regarding 10 individuals with a mild intellectual disability we were strongly advised by their psychologists or physicians not to approach them for DNA sampling. The main reason was the stress this test would possibly induce and fear of provoking psychotic reactions. Other objections were not related to the method of screening. Informed consent was obtained for 310 of the 436 (70.9%) approached persons (table 2). Their characteristics are shown in table 3. 10 persons for whom informed consent was obtained did not participate due to repeated unintentional absence at visits; this was not related to the screening method, but to communication errors. The characteristics of participants and non-participants did not differ significantly, except for age. Mean age of participants was significantly higher (48.4 yrs) as compared to non-participants (44.1 yrs,  $p=0.004$ ) (table 3). Likewise, those with a reliable test-result were significantly older (mean age: 48.4 yrs) as compared to the remaining eligible participants (44.6 yrs,  $p=0.008$ ). None of the characteristics of individuals whose first samples yielded insufficient amounts of DNA for a reliable diagnosis ( $n=48$ ) differed significantly from the individuals with a DNA result during the first round ( $n=250$ ). This was also the case for the second round of buccal smears (25/30 successful). We conclude that the results are representative for our study population without an aetiological diagnosis.

#### Feasibility of the MLPA-technique on buccal smears

Prior to the consent procedure, for or 10 of 471 (2.1%, 95% CI 0.8-3.4) individuals the method was considered undesirable. In two out of 300 participants (0.7%, 95% CI 0.0-1.6), both with a severe intellectual disability, buccal smears failed due to resistance. In 250/298 samples (83.9%, 95% CI 79.7-88.1%) the MLPA technique was successful at the first attempt. The other 48 (16.1%, 95% CI 11.9-20.3%) contained insufficient amounts of DNA to yield a reliable test result. A second buccal smear was performed in 30 participants (success rate 83.3%, 95% CI 70.0-96.7%). We did not obtain a second buccal smear for the remaining 18 participants due to non-consent ( $n=8$ ) or organisational problems ( $n=10$ ).

**Table 2:** Study population: the (informed consent) procedure leading towards a DNA-result

Study population		Loss before DNA-result	
665	Sample		
		Died before start	4
		Moved before start	8
		Uncooperative staff of residency / home	33
620 (inclusion for prevalence estimate)	Chart analyses		
		Genetic / metabolic diagnosis	126
		Brain injury > 2 years	23
471 (inclusion for DNA sampling)	Eligible participants		
		Method unfit, too stressful	10
		No psychologists / physicians approachable	5
		Not competent to consent, no legal representative	8
		Advice to not approach family for scientific purposes	12
436	Approached for consent		
		No informed consent	114
		No response received	12
310	Informed consent		
		Resistance	2
		No buccal smear obtained (repeated absence participant)	10
298	Buccal smear		
		Insufficient DNA first time and no second buccal smear	18
		Insufficient DNA both first en second buccal smear	5
275	DNA analysis	DNA result	
		No microdeletion / duplication	270
		Microdeletion / duplication*	5

\* One participant had a duplication of the Prader Willi-region (15q11-13) due to mosaicism of a supernumerary marker chromosome (15).

### Diagnoses

In 4 of 275 samples (1.5%, 95% CI 0.04-2.9%) a microdeletion was found: 22q11 deletion (n=2), 5q25 deletion (Sotos syndrome) (n=1) and 1p deletion (n=1). One participant had a duplication of the Prader Willi-region (15q11-13) due to mosaicism of a supernumerary marker chromosome (15). Characteristics of these five participants are shown in table 4. Four out of five individuals with a newly diagnosed microdeletion duplication were living in a community-based home vs. 35.3% (219/620, table 1) of the study population.

**Table 3:** Characteristics of participants

Characteristics of participants (n=300) and non-participants (n=171)			
		Participants	Non-participants
Gender	Male	175 (58.3%)	109 (63.8%)
	Female	125 (41.7%)	62 (36.3%)
Ethnicity	Caucasian	280 (93.3%)	120 (70.2%)
	Non-Caucasian	20 (6.7%)	13 (7.6%)
Mean age		48.4 years (18-84, SD 14.5)	44.1 years (18-96 SD 16.6)
Severity of intellectual disability	Mild (IQ $\pm$ 50-80)	95 (31.7%)	46 (26.9%)
	Moderate (IQ $\pm$ 30-50)	118 (39.3%)	44 (25.7%)
	Severe (IQ $\pm$ <30)	78 (26.0%)	35 (20.5%)
	Unknown	9 (3.0%)	46 (26.9%)
Type of residency	Centralized location	201 (67.0%)	116 (67.8%)
	Community-based home	99 (33.0%)	55 (32.2%)

**Table 4:** Characteristics of individuals with a microdeletion/duplication diagnosed by screening

Case	Diagnosis	Age	M/F	Severity ID	Ethnicity	Residency
a	5q35 deletion Sotos syndrome	41	M	moderate	Caucasian	Community-based home
b	Duplication Prader Willi Region	56	F	severe	Caucasian	Community-based home
c	1p-deletion syndrome	20	F	moderate	Caucasian	Community-based home
d	22q11 deletion syndrome	28	F	mild	Caucasian	Community-based home
e	22q11deletion syndrome	46	F	severe	Caucasian	Centralized location

### Prevalence estimate

In the sample of 620 individuals, 126 (20.3%) had a genetic or metabolic diagnosis prior to testing. Among them were 10 who had already been diagnosed with one of the syndromes studied. After the screening was conducted, there were 14 individuals with one of the “microdeletions/duplication syndromes” studied among the 620 adults with intellectual disability, resulting in a prevalence of at least 2.3% (95% CI 1.1-3.4%). Because of the presence of a marker chromosome the Prader Willi duplication is not taken into account in this prevalence figure.

**Table 5:** Prevalence of “microdeletion / duplication syndromes” before and after screening compared to prevalence in general population in literature

Syndromes	Centralized location (n= 401)	Community-based home (n=219)	Number before screening (n=620)	Total after screening (n=620)	Estimated prevalence (/1000 in residential care)	Prevalence in literature (/1000 in general population)
Williams	2	2	4	4	6.5 (95% CI 0.1 to 12.8)	0.13 <sup>1</sup>
22q11	1+ 1 newly diagnosed	1 newly diagnosed	1	3	4.8 (95% CI 0.0 to 10.3)	0.25-0.5 <sup>2,3</sup>
Prader Willi	1	1	2	2	3.2 (95% CI 0.0 to 7.7)	0.04-0.1 <sup>4</sup>
Prader Willi “ duplication” due to marker chromosome	0	1 newly diagnosed	0	1	1.6 (95% CI 0.0 to 4.8)	not reported
Angelman	2	0	2	2	3.2 (95% CI 0.0 to 7.7)	0.08 <sup>5</sup>
1p-deletion	0	1 newly diagnosed	0	1	1.6 (95% CI 0.0 to 4.8)	0.1-0.2 <sup>6</sup>
Sotos	0	1 newly diagnosed	0	1	1.6 (95% CI 0.0 to 4.8)	0.05-0.07 <sup>7</sup>
Saethre Chotzen	0	1	1	1	1.6 (95% CI 0.0 to 4.8)	0.02-0.04 <sup>8,9,10</sup>
Miller-Dieker	0	0	0	0	0	not reported
Smith-Magenis	0	0	0	0	0	0.02-0.03 <sup>11</sup>
Alagille	0	0	0	0	0	0.01 <sup>12</sup>
Total (nine micro-deletions)	7	7+1 marker chrom	10	14 +1 marker chrom	22.5* (95% CI 10.9-34.3)	

\*Because of the presence of a marker chromosome the Prader Willi duplication is not taken into account in this prevalence figure.

<sup>1</sup>(Stromme et al., 2002), <sup>2</sup>(Cassidy SB and Allanson, 2005), page 615 <sup>3</sup>(Arimami, 2006) <sup>4</sup>(Lee, 2002) <sup>5</sup>(Steffenburg et al., 1996) <sup>6</sup>(Slavotinek et al., 1999) <sup>7</sup><http://www.sssac.com/genchar.asp> accessed 12-10-2004 <sup>8</sup>(Paznekas et al., 1998) <sup>9</sup>(Krebs et al., 1997) <sup>10</sup>(Lee et al., 2002) <sup>11</sup>(Struthers et al., 2002) <sup>12</sup>(Brooks and Dooijes, 2003)

## Discussion

This is the first screening among adults with intellectual disabilities of unknown aetiology, living in central and community-based residential facilities, applying a non-invasive technique: Multiplex Ligation Dependent Probe Amplification on DNA obtained with buccal smears, resulting in new diagnoses in 5 out of 300 participants. The outcomes are representative for the study population without an aetiological diagnosis.

### Feasibility

The MLPA technique has already been established as a fast and reliable screening method, potentially suitable for use in routine diagnostics (Kirchhoff et al., 2006; Rooms et al., 2004). We conclude that the MLPA technique on buccal smears has a high feasibility for persons with an intellectual disability: undesirable 2.1% (95% CI 0.8-3.4), resistance 0.7% (95% CI 0.0-1.6), successful sample 83.9% (95% CI 79.7-88.1). In case of resistance combining the buccal smear with a visit to the dentist might be advisable. The buccal smear test is a painless and fast method, yet for some (2.1%, 95% CI 0.8-3.4) individuals with mild intellectual disability it was considered too stressful in advance by their psychologist or physician. The reason was mostly their suspicious attitude. We heard some of the participating individuals with a mild intellectual disability compare the buccal smear test with a police investigation, seen in a television series. For a small subgroup of individuals with paranoid tendencies this might form an objection. A majority (83.9%, 95% CI 79.7-88.1) of first swabs yielded enough DNA to successfully apply the MLPA technique; this success rate was equal in the second attempt. Using more than one swab in one session will probably diminish the necessity of repeated buccal smears, but also raise the laboratory workload and costs, which is not preferable in a screening setting. For the population with intellectual disabilities the disadvantage of a potential repeated attempt seems outweighed by the painlessness and simplicity of the buccal smear procedure, as compared to venapuncture. A current limitation of the technique of MLPA on buccal smears is the amount of DNA derived from one swab, which is sufficient for the detection of 9 microdeletions and duplications, but not for a larger number of tests. With advances in genetic techniques such limitations may be overcome in the near future.

### Prevalence estimate

Among 275 test results the following diagnoses were made: 22q11 deletion syndrome (n=2), 5q35 deletion (Sotos syndrome, n=1), 1p deletion syndrome (n=1) and a duplication of the Prader Willi-region (15q11-13) (n=1). A reliable result in 58% (275/471) of eligible participants increased the prevalence of the syndromes associated with the tested deletions/duplications in the total study sample from 1.6 to 2.3% (95% CI 1.1-3.4%), showing underdiagnosis in this sample. Combined with other (undiagnosed) genetic syndromes the high prevalence of syndromes with considerable co-morbidity can explain part of the higher morbidity and mortality among individuals with intellectual disability (van Schrojenstein Lantman-de Valk HM et al., 1997). However, epidemiologically, this study has some methodological limitations. The identification of (microdeletion) syndromes and brain injury as diagnosed prior to the screening, may have been less accurate for those living in the community, since for this group we used psychological instead of medical files. Detailed information on the molecular genetic basis of the syndromes diagnosed before screening was mostly unavailable in the patient files of care organisations. As a result, we do not know whether these are genetically based on a microdeletion, a mutation or e.g. uniparental disomy. This limits a direct comparison between syndrome prevalence before and after screening, since the MLPA method only diagnoses microdeletions and duplications. Due to the fact that we did not screen all 471 eligible participants and because of limitations of the MLPA technique, the prevalence described above is probably a minimum prevalence. Because of a lower inclusion of persons living in the community, the prevalence is not representative for the Dutch adult population with intellectual disabilities.

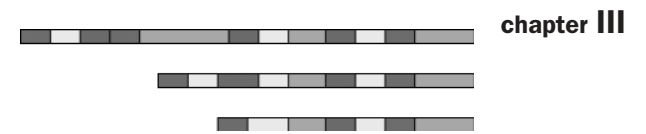
Our narrow confidence interval shows that sample size was sufficient to establish the prevalence of the syndromes associated with the 9 microdeletions/duplications among adults with intellectual disability with accuracy. However, our confidence intervals for separate syndromes are very broad (table 5). Higher accuracy for separate prevalence figures would therefore require a considerably larger screening sample. Non-participants with a history of perinatal asphyxia may have caused a bias in our study. Representatives who did not consent to buccal smear often mentioned a history of perinatal asphyxia as (sufficient) explanation for the intellectual disability of the eligible participant. However, there are suggestions that a genetic diagnosis might predispose for perinatal asphyxia. For example, 23% of neonates with Prader-Willi syndrome had a history of perinatal asphyxia vs. 1% in the general population (Wharton and Bresnan, 1989). The disability in these individuals can be wrongly blamed on the birth process, when it is in fact caused by the underlying genetic disorder and secondary complications. We analysed that the outcome of 5 new diagnoses and the feasibility measures are representative for the study population without aetiological diagnosis, but we emphasize that the prevalence figures in this study are rough estimates.

### *Outcome*

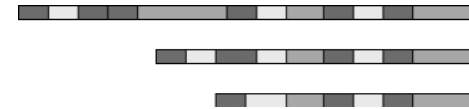
In 5 of 275 MLPA results (1,8%) a diagnosis could be made, compared to 15 of 258 individuals (5,8%) in the Kirchhoff study (Kirchhoff et al., 2006). However, as mentioned above, comparison is limited as the Kirchhoff study is a retrospective analysis of a selected population with dysmorphism, already suspected of a genetic aetiology, whereas we screened all individuals without an aetiological diagnosis for their intellectual disability. Remarkably only one out of five participants with a newly detected diagnosis was living in a centralized location - constituting the majority of our study population. A lower prevalence of undiagnosed syndromes in centralized locations compared to community-based homes is probably due to the fact that these individuals are treated by intellectual disability physicians, who are more aware of the necessity of genetic consultation than general practitioners.

The diagnosis in a centralized location concerned a woman with a severe intellectual disability with 22q11 deletion syndrome (table 4). We consider this diagnosis remarkable as the severity of intellectual disability in 22q11-deletion syndrome is described in literature as mild to moderate (IQ range 50-100) (Oskarsdottir et al., 2005). In individuals with severe intellectual disability, this syndrome might be overlooked.

We conclude that the MLPA technique on buccal smears is promising for the detection of microdeletions as well as duplications because of its high feasibility among individuals with intellectual disability. The prevalence of the syndromes associated with the 9 deletions and duplications tested increased as a result of our screening, which suggests an underdiagnosis of genetic syndromes. Diagnosing the aetiology of intellectual disability is very important, as it can be beneficial for the affected individual by implementing specific health care for co-morbidity. The burden of screening can be diminished by using this non-invasive test, enabling large-scale studies in the future.



### Dysfunctional motor behaviour



## Chapter IIIa

### Saccade dysmetria in Williams syndrome

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## Abstract

Numerous studies have described the poor visuo-spatial processing capacities of subjects with Williams-Beuren Syndrome (WBS), a genetically based developmental disorder. Since visual perception and eye movements are closely related we hypothesized that the poor visuo-spatial processing capacities of subjects with WBS might be related to a poor saccadic control. Thereto we recorded horizontal and vertical saccadic eye movements to targets using infrared video-oculography in 27 subjects with WBS and 8 healthy controls. In the WBS group saccadic gains were highly variable, both between and within individual subjects, and they often needed more than one correction saccade to reach the target. Ten (out of a subgroup of 22) WBS subjects showed a large number of hypometric and/or hypermetric saccades, and, also a left-right asymmetry in saccadic gains was observed in WBS. We conclude that the observed impairments in saccadic control are likely to affect the proper processing of visuo-spatial information.

## Introduction

The poor visuo-spatial abilities are one of the often described cognitive traits of subjects with Williams-Beuren Syndrome (WBS), a rare developmental disorder that occurs in about one in 20,000 births. WBS (Beuren et al., 1962; Williams et al., 1961) is clinically characterized by cardiovascular abnormalities (a.o. supravulvular aortic stenosis), occasionally infantile hypercalcemia, short stature, dysmorphic facial features and mental retardation (Donnai and Karmiloff-Smith, 2000; Bellugi et al., 1999; Lashkari et al., 1999). In more than 95% of the cases, the disorder is caused by a hemizygous deletion of approximately 1.5 Mb in chromosome band 7q11.23, which contains about 15 genes and is called the Williams Syndrome critical region (Korenberg et al., 2000; Ewart et al., 1993). WBS subjects exhibit a very specific cognitive profile (Mervis et al., 2000; Bellugi et al., 2000) which in combination with the relative low number of deleted genes provides the unique opportunity to study the relationship between genes and cognition (Bellugi et al., 1999). The outcomes of many studies using a large variety of tasks like pattern recognition, block-copying, drawing, visuo-spatial memory and visual search, suggested that the visuo-spatial processing deficits of WBS subjects are particularly expressed in tests of construction and do not seem to affect tests of perception (Farran and Jarrold, 2003). The deficits in visuo-spatial processing are in contrast to their relatively spared verbal capacities (Bellugi et al., 2000), although impairments have also been described in the latter domain (Karmiloff-Smith et al., 1997).

With respect to oculomotor behavior, toddlers with WBS (approximately of 29 months of age) were impaired in making double-step saccades, in which task they fail to plan a second saccade in a body-centered frame of reference in contrast to age-matched toddlers with Down syndrome and healthy controls, which suggested problems in using spatial representations to guide actions (Brown et al., 2003). In a recent block copying experiment subjects with WBS showed abnormal patterns of fixations, which was interpreted as a failure to represent spatial information properly, leading to significant changes in behavior (Hoffman et al., 2003). However, it is unclear why subjects with WBS should fail in building up adequate spatial representations. We hypothesized that the deficits in visuo-spatial performance of subjects with WBS might be related to impairments in the control of the accuracy of saccadic eye movements, as saccades play a very important role in visuo-spatial processing. Saccades are very fast goal-directed movements of the eyeball that serve to project objects onto the fovea. Since the fovea is dedicated to detailed inspection of objects, proper use of saccadic eye movements allows the detailed but also rapid inspection of the whole visual environment by orienting the eyes in sequence to the objects of interest. It is imperative that these saccadic eye movements are accurate. Inaccurate saccades call for secondary corrective eye movements that may severely slow down the processing of the visual environment. Therefore, poor control of saccadic accuracy in WBS may hamper a fast inspection of their visual world and, as a consequence, interfere with their processing of visuo-spatial

information. To investigate our hypothesis we studied the accuracy of goal-directed saccadic eye movements in subjects with Williams-Beuren syndrome.

## Methods

### Subjects

Informed consent was obtained from 27 subjects with Williams-Beuren Syndrome (WBS; 11-35 years of age, with (estimated) low to moderate IQ levels, i.e., below 80) and eight healthy controls (CS; 18-34 years of age) for this study, which was approved by the Medical Committee of the Erasmus MC, according to 1994 Declaration of Helsinki. All WBS subjects were contacted through the Dutch Williams Patients Association, and were genetically and phenotypically screened for Williams-Beuren syndrome in our laboratory. In all WBS subjects the submicroscopic deletion of genes (among which *ELN* and *CYLN2*) in chromosome band 7q11.23 was confirmed using fluorescence in situ hybridization (FISH). Furthermore, all WBS subjects had the phenotypical characteristics commonly associated with Williams-Beuren Syndrome (van Hagen et al., 2001; Franceschini et al., 1996). The control subjects were recruited from the university. All subjects were without spectacles and could easily see the targets on the computer screen.

### Stimuli and apparatus

Subjects were seated 70 cm in front of a 21-inch computer screen. Monocular eye position was recorded using infrared video-oculography (EyeLink 2.04, SensoMotoric Instruments, Germany (Van Der Geest and Frens, 2002) at a sample rate of 250 Hz. Head movements were restrained by means of a chinrest and good head stability was continually checked by the experimenter. Eye position was calibrated using the built-in automatic routine. Subjects looked at the stimuli with their preferred eye as estimated by classical preference test (Barbeito, 1981), while the other eye was patched. Subjects were encouraged to look to-and-fro between two black dots on a gray background. In each of the four trials the two dots were centered about the center of the screen and were either horizontally or vertically aligned. The distance between the dots was either 11 or 22 degrees of visual angle. Each trial in total was displayed for 20 seconds. All subjects grasped the task and did make saccades between the dots. This shows that the task was simple enough to be independent of the WBS-related visuo-spatial deficits that might play a role in more complex tasks.

### Analysis

Saccadic eye movements were extracted automatically from the raw data using a velocity criterion of 30 degrees per second. Each saccade and each raw data trace was checked manually to ensure proper detection. We wanted to analyze only saccades that were intended to bring the point of gaze toward one of the targets. Therefore, we included only those saccades that met all of the following criteria: the saccade started on one of the two dots (< 1 degree away) and was directed to the other dot; the amplitude of the saccade was more than 50 percent but less than 200 percent of the distance between saccade starting position and the position of the target; the saccade either landed directly on target or was followed by one or more correction saccades which brought the point of gaze onto the target; the vertical saccadic amplitude for horizontally aligned targets or the horizontal saccadic amplitude for vertically aligned targets was less than 10% of the distance between the two dots. Using these stringent criteria only goal directed, or primary saccades were included for further analysis, yielding about 30-50 saccades per direction per subject. Saccadic amplitude was defined as the distance traveled by the saccade, i.e., the difference between the positions 4 ms before and 4 ms after the saccade. The gain (G) of each saccade was defined as  $G = A/D$ , in which A is the saccadic amplitude and D is the distance between saccadic start position and target position. Note that D can vary (slightly) between saccades because not all saccades started exactly on a target position. So, a gain of 1.0 reflects a saccade that lands exactly on

target. For each primary saccade the number of subsequent correction saccades needed for foveation of a target was counted. Also the number of primary saccades was counted that were either hypo- or hypermetric (i.e., being too small or too large, respectively). Horizontal and vertical primary saccades were treated separately. Subjects were excluded if they made less than 20 saccades to allow for a proper quantitative analysis. The WBS group and the CS group were statistically compared using Students t-tests.

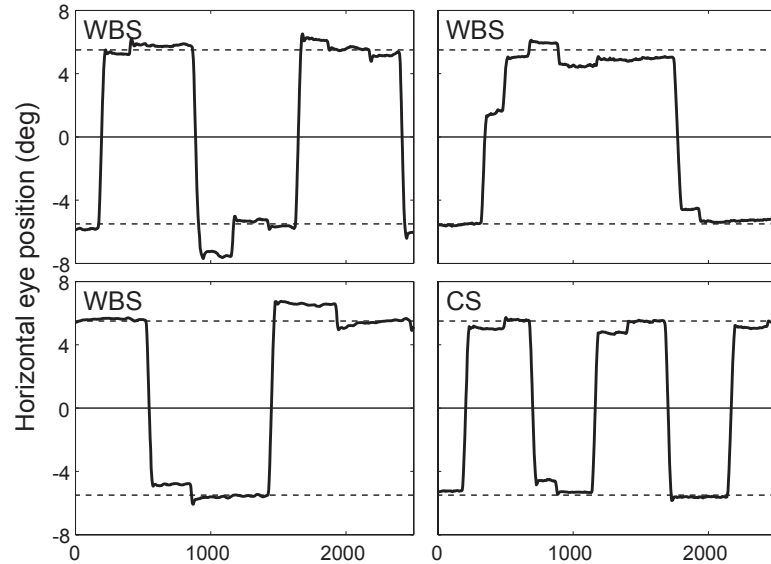
## Results

Preliminary analyses showed that the gains and the number of corrective saccades did not differ between the 11 degree and 22 degree stimuli and therefore the results are pooled over the two stimulus conditions. As the WBS group contained some children and adolescents as opposed to the control group, we performed all analyses excluding the six WBS patients younger than 18 years of age. The outcomes of these analyses did not have any consequences for the conclusions drawn on basis of the analyses presented below in which all WBS subjects are included. Furthermore, we did not observe any correlation between saccade performance and chronological age

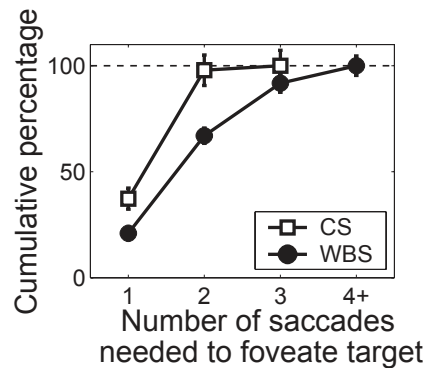
### Correction saccades

Figure 1 shows parts of typically observed horizontal eye movement traces of three subjects with WBS and one control subject. One can see that most of the primary saccades of the control subject undershoot the target position, and that often a correction saccade is needed for foveation. This is in line with the literature (Becker, 1991; Collewyn et al., 1988). However, WBS subjects show some irregularities: saccades are less accurate and have a larger scatter in amplitudes. Moreover, often more than one correction saccade is needed to foveate the target. This is quantified in figure 2. Here we show the number of saccades that is needed to foveate a target. Control subjects (CS) virtually always reach the target within two saccades, i.e. the primary saccade and perhaps one correction saccade in 98 % of their gaze shifts. WBS subjects need more than one correction saccade to foveate the target in 33% of their gaze shifts.

**Figure 1:** Horizontal eye position traces between 11 degree stimuli made by 3 subjects with Williams Syndrome (WBS, aged 19.1, 20.9, and 21.3 years of age) and 1 control subject (CS, aged 28.0 years of age). The dotted lines at 5.5 degrees excentricity represent the positions of the two targets.



**Figure 2:** The cumulative occurrence frequency of number of saccades needed to reach the target. Data from horizontal saccade trials, averaged across subjects. Error bars represent standard error of the mean across subjects



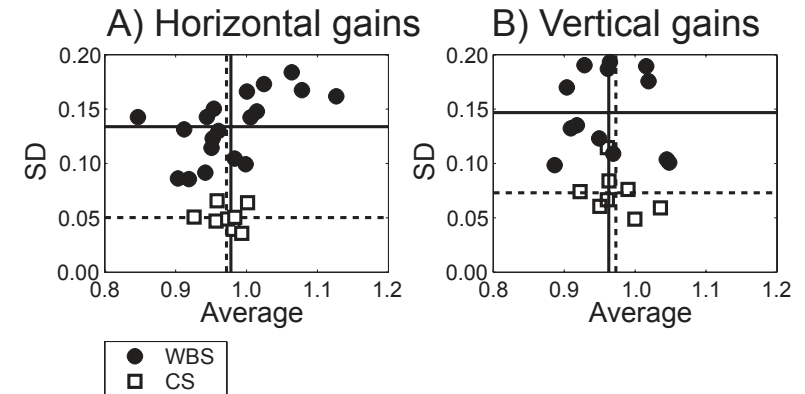
### Saccade Gain

We included 19 (of the 27 in total) WBS subjects in the quantitative analysis of horizontal gains, and 13 in the analysis of vertical gains. The other WBS subjects were excluded because they made too few (i.e., less than 20) primary saccades that met the stringent criteria to allow for a proper quantitative analysis.

Because of the poor performance of the latter group, the outcome of this quantification may somewhat overestimate the quality of the saccadic performance of WBS subjects. In our view the data loss is most likely due to concentration problems of these patients resulting in, e.g., a lower rate of saccades in some WBS patients (see also figure 1) and in saccades made toward other objects in their environment. All eight control subjects were included in both analyses.

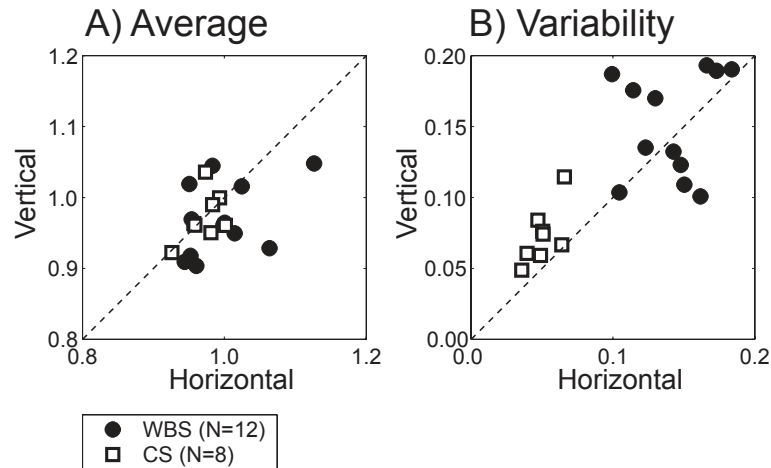
Figure 3 shows the standard deviation (reflecting the within-subject variability of the saccades) as a function of the within-subject average of the primary saccade gains.

**Figure 3:** The standard deviation of saccadic gain (within-subject variability) versus the within-subject average of saccadic gain. Each point represents an individual subject (WBS: Williams-Beuren syndrome subjects; CS: Control subjects). The left panel shows the horizontal saccadic gains and the right panel shows the vertical saccadic gains. The thinner lines show the average values for the control group, the thicker lines the average values for the WBS group.



For horizontal saccades, the individual average gains did not differ between the WBS group and the control group ( $0.98 \pm 0.06$  vs.  $0.97 \pm 0.02$ , respectively,  $p=0.73$ ), although the between-subject variability of the individual gains was higher between WBS subjects (gains between 0.85 and 1.13 in the WBS group versus 0.93 and 1.00 in the CS group). Note that for all control subjects the average gains were smaller than one. The standard deviation of the individual gains (within-subject variability) was significantly higher in the WBS group than in the control group ( $0.13 \pm 0.03$  deg vs.  $0.05 \pm 0.01$  deg, respectively,  $p<0.001$ ). Also for the vertical saccades, the individual average gains did not differ between the WBS group and the control group ( $0.96 \pm 0.05$  vs.  $0.97 \pm 0.03$ , respectively,  $p=0.61$ ), but again the within-subject variability was significantly higher in the WBS group than in control group ( $0.15 \pm 0.09$  vs.  $0.07 \pm 0.02$ , respectively,  $p<0.001$ ).

**Figure 4:** The vertical saccadic gain versus the horizontal saccadic gain values. The left panel shows the vertical versus horizontal average saccadic gain. The right panel shows the vertical versus horizontal within-subject variability of saccadic gain (the standard deviation in gain). Each dot represents one subject.

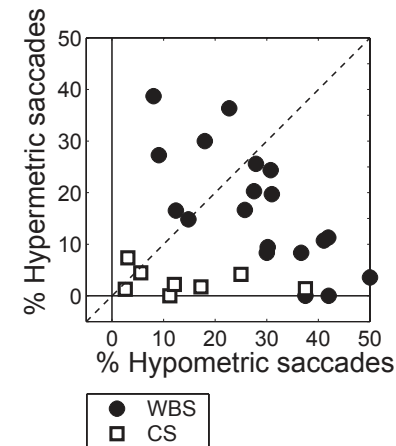


Although the within-subject variability is higher in the WBS group than in the control group for both horizontal and vertical saccades, there was no correlation between the gains for the two directions. This is shown in figure 4, where we plot the average gains (left panel) and the within-subject variability (right panel) of the horizontal and vertical saccades for those subjects who made more than 20 saccades in both directions (12 WBS and 8 CS). Neither within the CS group, nor within the WBS group, the correlations between the horizontal and vertical directions were significant.

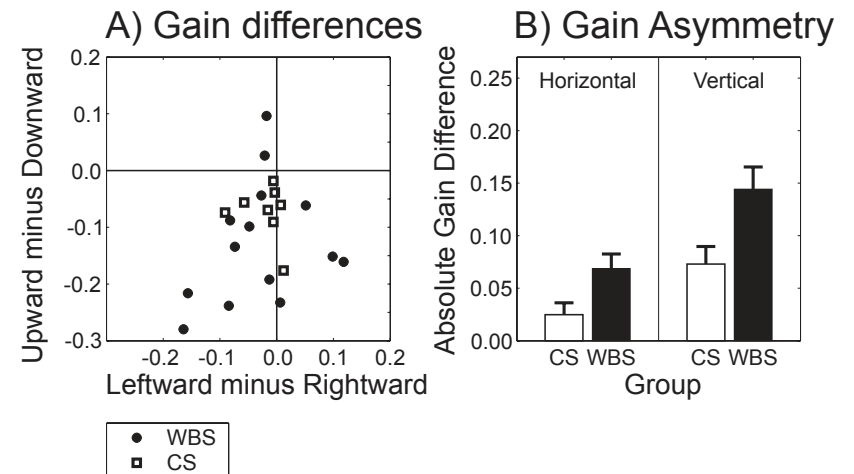
The variability in saccadic gains, also observed in normal subjects and in line with the literature (Becker, 1991), relates to the tolerance of the saccadic control system for small errors in saccadic gains. The values for the within-subject variability in saccade gain in the normal control group are close to those previously reported (Kowler and Blaser, 1995). However, larger errors, i.e., gains that deviate too much from one, will decrease the efficiency of saccadic eye movements. Moreover, saccadic undershoots minimize the time needed to foveate the target (Harris, 1995), and, indeed, saccadic gains are on average smaller than 1.0 in normal subjects (see, e.g., figure 2) and it has been argued that the number of saccades that are too large ('hypermetric') have to be minimized. Therefore we calculated the percentages of dysmetric horizontal saccades for each subject individually. Hypometric saccades were defined as having a gain smaller than 0.9, saccades with gains between 0.9 and 1.1 were classified as normometric and hypermetric saccades were defined as having a gain larger than 1.1.

Figure 5 shows that more than 95% of the saccades of normal controls are either normometric or hypometric. However, in almost half of the WBS subjects (10 of the 22 subjects who made more than 20 saccades) more than 15% of the saccades were hypermetric. Moreover, only 52% of all saccades made by the WBS group were normometric versus 85% of all saccades made by the control group.

**Figure 5:** The percentage of hypermetric saccades plotted versus the percentage of hypometric saccades. Each dot represents an individual subject.



**Figure 6:** The difference in saccadic gains between the two vertical directions (upward minus downward) versus the difference in saccadic gains between the two horizontal directions (left minus right) are plotted on the left. Each dot represents one individual subject. The averages of the absolute gain differences are plotted on the right for the two directions and the two groups. Error bars represent standard deviations.



## Discussion

### Summary of results

Saccadic eye movement performance was compared between a group of subjects with Williams-Beuren syndrome (WBS) and a control group. Most of the WBS subjects did perform well enough in this task to allow for quantitative analyses of saccadic control. The most prominent outcome of the present study were the high within-subject variability and the between-subject variability of saccadic amplitudes in the WBS group. For control subjects, figure 3 shows the expected narrow distribution of both horizontal and vertical saccadic gains around an average gain of slightly less than 1.0 (Becker, 1991; Collewijn et al., 1988). Only a small fraction of saccades made by the control subjects were hypermetric (figure 5). Furthermore, targets were reached within two saccades in 98% of their gaze shifts (figure 2). In contrast, the WBS group as a whole showed a broader distribution of saccadic gains. Dysmetric saccades occurred in a majority of the WBS subjects, and in 33 percent of their gaze shifts more than one correction saccade was needed for target foveation. Furthermore, saccadic accuracy was significantly decreased in a large number of WBS subjects. Although the average gains, for both horizontal and vertical saccades, did not differ between WBS and control subjects, the within-subject variability of saccadic gains was much higher for each WBS subject than for the control subjects. Furthermore, we observed a high between-subject variability in the WBS group with respect to their average gains. Additionally, a left/right asymmetry was observed in the WBS group for horizontal saccadic gains. In both groups an upward/downward asymmetry was observed, which is in line with the literature on vertical saccades (Zhou and King, 2002; Collewijn et al., 1988), but this asymmetry was larger in the WBS-group.

These outcomes clearly suggest abnormalities in the control system for saccade accuracy of subjects with Williams-Beuren Syndrome. Since eye movements and spatial perception are closely related, a poor saccadic accuracy control is likely to play a role in the poor visuo-spatial capacities of WBS subjects, although the question remains whether these poor visual capacities are indeed a result of their poor saccadic gain control or vice versa. For instance, too many secondary corrective saccades in between fixations to local elements may interfere with memorizing the positions of previously fixated elements. In turn, this may hamper the adequate processing of the global spatial information created by the spatial arrangement of these local elements. It can also be imagined that the observed asymmetries in saccadic gain control predict asymmetries in visual spatial performance employing tasks like pattern recognition in the different visual fields or in an experiment involving block-copying from left to right or vice versa.

### Age and IQ

In this study the ages of the Williams-Beuren syndrome subjects ranged between 11 and 35 years of age, and all subjects with WBS had (estimated) low to moderate IQ levels (<80). Therefore, a developmental delay in saccade control related to the chronological and/or mental age might account for the observed dysmetria. We believe strongly that this is not the case, however. In our study, we did not observe any correlation between saccade performance and chronological age. Furthermore, in our view, an explanation in terms of developmental delays seems less likely, since the oculomotor system is one of the first motor systems to mature with respect to saccadic gain control, already at a very early age (Hainline et al., 1984). Moreover, IQ level is unlikely to play an important role in the observed effects, since cognition is not involved in maintaining an optimal level of saccade accuracy. This is illustrated by the fact that accurate saccades are also observed in species like monkeys, cats and chameleons (Carpenter, 1988). We agree that the developmental level might be important for saccadic control on a higher, more cognitive level, for instance in planning saccade scan paths during visual search tasks and other saccade tasks involving instructions, intentions, memory or target selection. However, our study was carefully designed to exclude a possible interference with such cognitive processes.

### Neurophysiological origin

Of course we can only speculate about the neurophysiological background of our findings. However, based on the nature of the disturbance, and existing literature on Williams-Beuren syndrome, we propose that two possible neuro-anatomical structures might play a role in the poor saccade control of WBS subjects: the dorsal (occipito-parietal) stream of cortical processing, and the oculomotor vermis of the cerebellum (lobules VI and VII).

The dorsal pathway is involved in the processing of spatial information and visually guided actions, as opposed to the ventral (occipito-temporal) stream which is involved in processing object properties (Goodale and Milner, 1992). The results of many studies on object and spatial processing in WBS have implicated a relative sparing of information processing within the ventral stream and clear deficits in processing within the dorsal stream (Paul et al., 2002; Atkinson et al., 1997). On the genetic level, one of the deleted genes in WBS (*GTF2I*) has been linked to the dorsal pathway (Galaburda et al., 2002). Several areas in the dorsal stream are involved in the control of saccadic eye movements. For instance, activation in the parietal area (LIP) occurs prior to the execution of a saccade to one of multiple saccade targets (Goldberg et al., 2002). This area is allegedly related to selective attentional processes. However, a direct association between the dorsal stream and the control of saccade metrics has not been described yet.

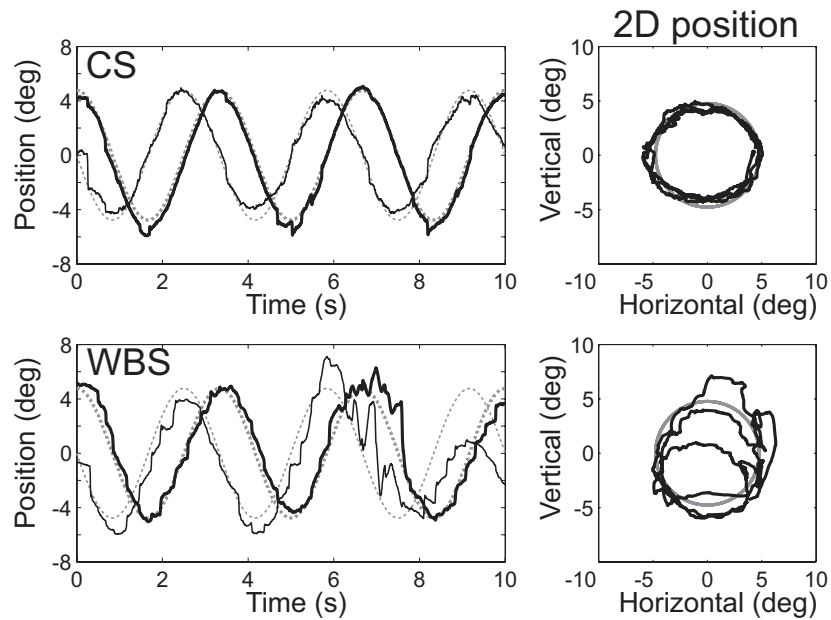
The cerebellum is more directly related to the control of saccade accuracy. The cerebellar oculomotor vermis is critically involved in fine-tuning saccade metrics in order to minimize post-saccadic errors and to ensure that the very fast saccades will indeed change the point of gaze as intended (Barash et al., 1999; Takagi et al., 1998; Optican and Robinson, 1980). Saccadic gain impairments, i.e. saccade dysmetria (especially hypermetria, but also hypometria) in the absence of kinematic deviations are generally associated with cerebellar disturbances (Leigh and Zee, 1999; Botzel et al., 1993). Main sequence analyses of the saccades made by our subjects, which will be presented elsewhere in detail, showed normal relationships between amplitudes and durations and between amplitudes and peak velocities of saccades in both the WBS group and the normal control group (e.g., the saturations level of the amplitude/peak velocity relation for horizontal saccades were not significantly different between the two groups:  $412 \pm 71$  deg/s for the WBS versus  $463 \pm 59$  deg/s for the NC group,  $p > 0.1$ ). Our observations are in good agreement with findings of saccade dysmetria in monkeys with cerebellar lesions, in which a large scatter of saccadic amplitudes around a normal average value was observed (Barash et al., 1999). The corresponding average gains between the control and the WBS group (see figure 3 and the left panel of figure 4) suggests that the assumed cerebellar role in saccade adaptation is relatively spared in subjects with WBS.

Furthermore, a cerebellar disturbance, whether unilateral or bilateral, is unlikely to yield similar effects for leftward and rightward saccades, and a left-right asymmetry in saccadic gains is likely to be observed, as in the present study. Rough motor commands for leftward and rightward saccade components are encoded at contralateral cortical and midbrain structures that are corrected by the ipsilateral cerebellar oculomotor vermis. If the correction is incomplete, different errors may remain for saccades with different horizontal components. The suggestion of a cerebellar origin is also in harmony with the observed morphological cerebellar abnormalities in Williams-Beuren syndrome, and more specifically, the alleged relative volumetric increase of the cerebellar oculomotor vermis (Schmitt et al., 2001b; Reiss et al., 2000) although the relationship between cerebellar size and functioning is, at present, unclear. On the genetic level, at least two commonly deleted genes are expressed in the cerebellar cortex (*CYLN2* in the Bergmann glia fibers in the cerebellar cortex (Korenberg et al., 2000; Hoogenraad et al., 1998), and *STX1A* in the molecular layer (Botta et al., 1999b). The absence of these genes (as in all of our WBS subjects) might therefore lead to cerebellar malfunction. Finally, on a neurological level, most subjects with WBS show cerebellar-related problems in motor coordination, tone and gait, for instance when walking stairs or uneven surfaces (Mervis et al., 2001; Chapman et al., 1996;

Withers, 1996;Bellugi et al., 1990;Trauner et al., 1989).

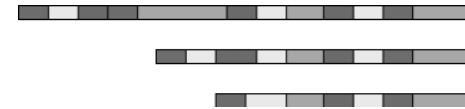
We did not systematically quantify other eye movement signs of cerebellar dysfunction, like gaze-evoked nystagmus or smooth pursuit disturbances in our WBS group. However, in the few WBS subjects tested we did observe abnormalities in smooth pursuit, e.g. showing a lot of catch-up saccades (see figure 7). Nonetheless, more research on other types of eye movements in WBS will be necessary to fully appreciate the results of the present study in relation to the alleged cerebellar dysfunctioning.

**Figure 7:** Smooth pursuit eye movements of a control subject and a WBS subject, following a target moving in a circular fashion. The left panels show the horizontal positions (thick lines) and the vertical positions (thinner lines) of the left eye (dark lines) and the target (grey lines); the right panels show the eye and target positions in 2D. The circular movement frequency was 0.3 Hz and the amplitude was 4.8 degrees, yielding a constant path velocity of 9 deg/s.



## Acknowledgements

We are grateful to the subjects who took part in this study, as well as to their families and the Dutch Williams Patient Association. During this research JNvdG was sponsored by NWO-MW (grant 903-68-394) and by the Revolving Fund of the Erasmus MC (00-157).



## Chapter IIIb

### Visual depth processing in Williams syndrome

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## Abstract

Patients with Williams-Beuren Syndrome (WBS, also known as Williams Syndrome) show many problems in motor activities requiring visuo-motor integration, such as walking stairs. We tested to what extent these problems might be related to a deficit in the perception of visual depth or to problems in using this information in guiding movements. Monocular and binocular visual depth perception was tested in 33 patients with WBS. Furthermore, hand movements to a target were recorded in conditions with and without visual feedback of the position of the hand. The WBS group was compared to a group of control subjects. WBS patients were able to perceive monocular depth cues that require global processing, but about 49% failed to show stereopsis. On average, patients with WBS moved their hand too far when no visual feedback of hand position was given. This was not so when they could see their hand. Patients with WBS are able to derive depth from complex spatial relationships between objects. However, they seem to be impaired in using depth information for guiding their movements when deprived of visual feedback. We conclude that the problems that WBS patients have with tasks such as descending stairs are not due to an inability to judge distance.

## Introduction

Williams-Beuren syndrome (WBS, also known as Williams Syndrome; OMIM database #194050, see <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=194050>), is a genetically based neurodevelopmental disorder, caused by a microdeletion on chromosome region 7q11.23 (Korenberg et al., 2000; Francke, 1999; Osborne et al., 1996). WBS patients have several marked features, such as mental retardation, dysmorphic facial features, supraaortic stenosis and transient infantile hypercalcemia (Bellugi et al., 1999; Lashkari et al., 1999). Concentration and attentional difficulties and high distractibility are common behavioral problems in WBS (Morris and Mervis, 2000; Davies et al., 1998). Furthermore, a specific cognitive profile is often observed in WBS (Mervis et al., 2000; Bellugi et al., 2000), with relatively preserved verbal and visual recall skills (Udwin and Yule, 1991), but moderate to severe impairments in visuo-spatial tasks, such as block copying (Atkinson et al., 2001; Farran et al., 2001; Nakamura et al., 2001) and drawing (Stiles et al., 2000). The worse performance on these tasks specifically suggest deficits in processing the global configuration of objects (Kovacs et al., 2001; Bihle et al., 1989). For instance, when patients with WBS copy drawings, local elements and details are often correctly reproduced whilst the global configuration, i.e., spatial relationships between the local elements is altered or left out (Bellugi et al., 1999). The development of visual functioning is also found to be abnormal in many WBS patients, and is marked by a high incidence of, e.g., strabismus, low visual acuity, and amblyopia (Atkinson et al., 2001).

Many individuals with WBS, especially children, show difficulties in motor activities involving visuo-motor integration (Chapman et al., 1996; Withers, 1996; Trauner et al., 1989; MacDonald and Roy, 1988), such as walking on non-uniform surfaces and descending stairs. These difficulties might be related to deficits in visual processing that hamper the proper visual guidance of one's movements. Indeed, reduced stereopsis, i.e., sub-optimal perception of binocular depth, is likely to be found in WBS (Atkinson et al., 2001; Olitsky et al., 1997; Sadler et al., 1996). However, typically developed individuals with no or sub-optimal binocularity can still function well using only non-stereoscopic (monocular) depth cues (von Noorden, 1996b). Binocular depth cues arise from the slightly different images projected on our two eyes. Monocular depth cues, such as occlusion, perspective and motion parallax, normally also provide abundant information about the relative distances between different objects and the distance between objects and oneself. In order to extract and use such information one has to integrate several features, e.g., the height of your eyes and the height of an object if you intend to step onto it.

In this paper we investigate the processing of visual depth in patients with WBS. The noted problems in



global processing in WBS might suggest that the use of relationships between visible structures to judge depth is disturbed. On the other hand, inadequate use of visual depth information for movements might also account for the difficulties in motor activities requiring the integration of visual and proprioceptive information. Here we will show that patients with WBS are able to extract depth information from their visual environment, but that the use of this information to guide their movements seems to be impaired.

## Methods

### Experimental procedures

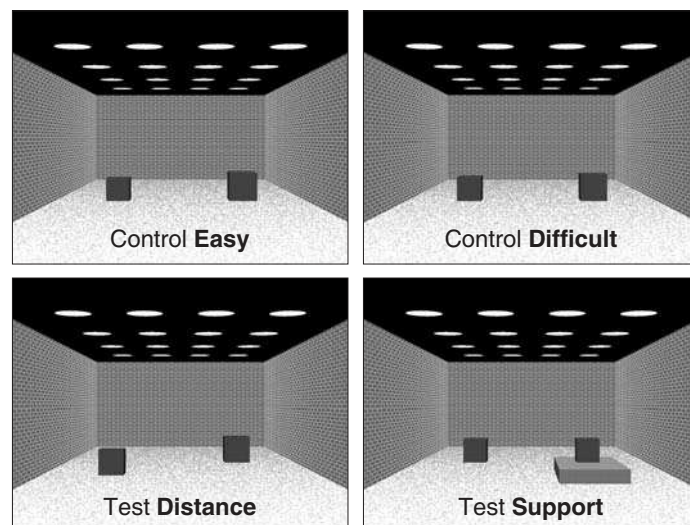
Subjects participated in four experiments. The first three experiments were aimed at the perception of depth using perspective, parallax and stereoscopic cues, respectively. The fourth experiment was aimed at the use of depth information to guide the movement of the hand. The procedures were approved by the medical ethical committee of the Erasmus MC, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

### Perspective cues

The perception of perspective cues was tested indirectly by asking for judgments of size. If two objects have retinal images that are the same size, the object that is judged to be further away will be judged to be larger. The perceived distance to an object is determined by its spatial relationship with other objects. For instance, an object seems to be further away when it is higher in the field of view.

In each of the 60 trials of this paradigm, a colored image was presented on a 21-inch computer screen 70 cm in front of the subject. Each image contained two blue cubes placed on the left and the right side of a textured room that was rendered with appropriate perspective for the subject's viewpoint. In each trial, the subjects had to decide by forced-choice which one of the two blue cubes in the room was the larger one. The order of 60 trials and the side on which the "larger" cube was presented was chosen randomly.

**Figure 1:** The four conditions in the monocular depth experiment. See text for explanation



There were four different conditions (see Figure 1). In the two control conditions, one of the two cubes was actually larger than the other (in pixels on the screen). The actual size difference could be large (18%; 'control-easy', 24 trials) or small (9%; 'control-difficult', 12 trials). These control conditions were included to verify that the subjects understood the task requirements.

In the two test conditions the two cubes had identical actual sizes (in pixels), but their sizes seemed different due to the configurations in the scene. Hence, if this influences the subjects' responses, we will have evidence that they were able to perceive the monocular depth cues. In the first test condition ('test-distance', 12 trials) one of two identical sized cubes was put higher on the screen than the other cube, and therefore seemed to be further away and, hence, larger than the other one. In the second test condition ('test-support', 12 trials) one of the cubes was placed on a gray block, and therefore seemed to be closer by and, hence, smaller than the other one (Meng and Sedgwick, 2001). The configurations in the both test conditions were construed in such manner that a full appreciation of the depth cues would yield an illusory size difference of 18% between the two blue cubes, as in the "easy" control condition.

Before the beginning of this paradigm subjects were shown several pairs of blue paper squares and were asked to indicate the larger of the two. All subjects were able to do this practice task correctly, showing that they understood at least the nature of the task. Note that a consistent response in this task can only be given when a subject is sensitive to the depth cues provided. The statistical chance of having less than 3 errors when a subject is purely guessing is less than 0.002% in the control-easy condition (24 trials), and less than 2% in the other three conditions (12 trials).

### Parallax: structure-from-motion

People can use motion cues alone to judge an objects' shape, which requires the perceptual combination of moving elements (Ullman, 1979). On a computer monitor, we presented two "circles" of dots with the same average velocity of dot motion. In one circle all dots moved back-and-forth in the same direction but the velocities were lower for dots nearer to the edges, as if they were placed on a rotating sphere. In the other circle all dots moved back-and-forth at the same velocity (simulating a moving plane behind a disk-shaped aperture). There were 500 dots in each image. The two images were presented simultaneously on the left and right side on a computer screen. In each of the 20 trials presented, subjects had to report which image looked more like a rotating sphere. The chance of making more than 15 correct choices in 20 trials by guessing is less than 1%.

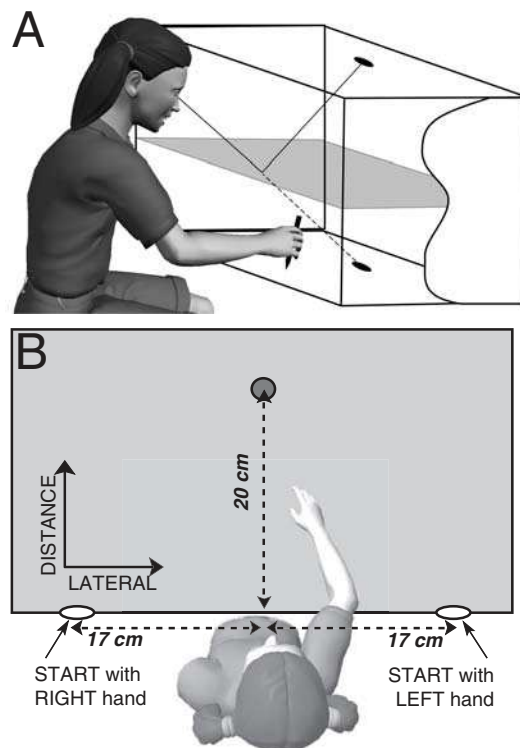
### Stereopsis

The perception of stereoscopic cues was tested using the commonly used Titmus Stereo Test (Stereo Optical Co., Chicago, Illinois, USA). In this test a patient is provided with properly oriented Polaroid spectacles so that each eye sees only one of a set of the two images that are polarized at 90° with respect to the other. This method induces a retinal disparity that can lead to a perception of depth at thresholds ranging between 3000 and 40 seconds of arc at a viewing distance of 40 cm (see (von Noorden, 1996a) for a description). The Titmus Stereo Test is especially suited for measuring stereopsis qualitatively in children. However, it should be noted that a failure to pass the test does not imply simply that the observer has no stereopsis and therefore a failure should not be regarded as entirely conclusive, that is (Ohlsson et al., 2001; von Noorden, 1996b). In the present study, performance was scored into one of four categories: 'failed' when no stereo-acuity could be measured; 'good' when stereo-acuity was better than 100"; 'medium' when stereo-acuity was between 400" and 100"; or 'coarse' when stereo-acuity was above 400". Statistics on the performance scores were performed using a Chi-square test with a significance level set at 0.05.

### Movements

The use of depth information for guiding movements was tested using a task in which subjects had to point to a target with visual feedback ('closed-loop') and without visual feedback ('open-loop') about the position of their hand. The target was projected on a plane seen via a see-through mirror. The target was at a fixed position straight ahead of the subject. The subject had to move a pen with the dominant hand on a digitizing tablet (Ultrapad A2, WACOM Technologies Corporation, Vancouver WA, USA) to the position at which the target was seen to lie on the tablet (see Figure 2 for a pictorial description of the setup).

**Figure 2:** Schematic drawings of the experimental setup in the hand movement paradigm from aside (panel A) and from the top (panel B). Subjects were seated at the center in front of the tablet, with the target at about 20 cm straight ahead. A movement started from 17 cm left or right, depending on the handedness of the subject. Lateral and Distance directions as used in the text are indicated.



Right-handed subjects (23 of the 30 WBS, 21 of the 22 CS, and 3 of the 5 MC subjects) started their pointing movements from the left (17 cm to the left and 20 cm below the target). Left-handed subjects (7 WBS, 1 CS and 2 MC subjects) started their pointing movements from the right (17 cm to the right and 20 cm below the target). In this way the required movement of the whole arm was similar for left- and right-handed subjects. The task consisted of two blocks of 10 trials each. In the first block the hand was visible through the mirror (closed-loop condition). In this condition, subjects could use both the visual information of the position of the target and the visual and proprioceptive information of the position of the hand to guide their hand to the target. In the second block, visual information about the position of the hand was removed by putting a sheet of paper beneath the mirror, so that only the target remained visible (open-loop condition). In this condition subjects could use the visual information of the target position, but only proprioceptive information of the hand to guide their invisible hand to the target. In other words, they had to integrate visual target information and proprioceptive hand information in order to point to the target.

The average and standard deviations of the lateral and distance positions (see figure 2) to which the subject pointed in each of the two conditions were calculated. The differences in the lateral and distance directions between the open-loop and closed-loop conditions were calculated individually. For the left-handed subjects the sign of the lateral difference was reversed. A negative lateral difference is a setting that is in the direction of the starting position. A positive distance difference is a setting that is above the target position. Statistics on these differences were performed using Student's t-tests with a significance level set at 0.01.

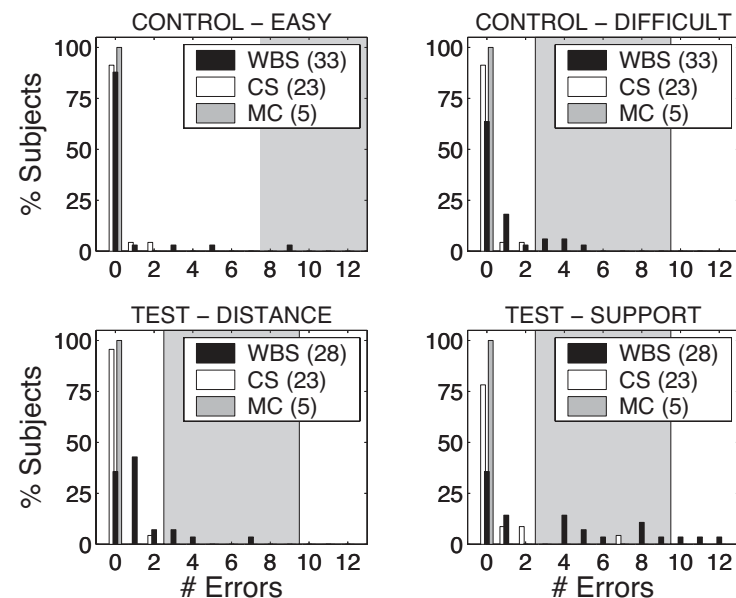
### Subjects

Informed consent to participate in this study was obtained from (parents of) 33 patients with WBS (age range of 10-39 years; mean  $18.9 \pm 7.5$  standard deviations). All patients showed the deletion of all genes (*ELN*, *CYLN2*, etc.) on the Williams Syndrome critical region on chromosome band 7q11.23 when tested genetically using fluorescent in situ hybridization (FISH) with four probes that cover the whole critical region. Furthermore, all patients showed the phenotypic characteristics of WBS. Based on parent and school reports all WBS subjects were low-functioning with estimated total IQs below 80. All WBS subjects participated in a large number of behavioral experiments, among which the experiments presented here. Thirty of the 33 subjects with WBS exhibited severe problems in descending stairs. For instance, they put two feet on each step taken and made their movements slowly and carefully. All 33 subjects participated in the two experiments on perspective and stereoscopic cues. 17 subjects also participated in the structure-from-motion task, which was added later to the protocol. 30 subjects (including the three subjects without difficulties in descending stairs) participated in the hand movement task. 23 control subjects (CS; age range of 6-30 years; mean  $15.9 \pm 9.0$ , matched as group on chronological age) were recruited from the clinics and departments of the Erasmus MC. None of the CS had any problems in walking stairs. All subjects participated in the experiment on perspective cues; nine CS participated in the structure-from-motion task and one CS did not participate in the hand movement task. Three control subjects who lack stereopsis were especially recruited to participate in order to control for the absence of stereopsis on the hand movement task. They were excluded from the analysis of the stereopsis test. A second group of atypical developing subjects of an unknown aetiology but without WBS consisted of 5 subjects (MC: age range of 16-19 years; mean  $17.7 \pm 1.2$ ) with a low level of cognitive functioning (on mean total IQ:  $75.8 \pm 7.8$ ). This group served as grossly matched control group on age and IQ for the WBS group. None of these five MC subjects had problems in walking stairs, and all participated in the experiments described here.

## Results

### Depth Perception

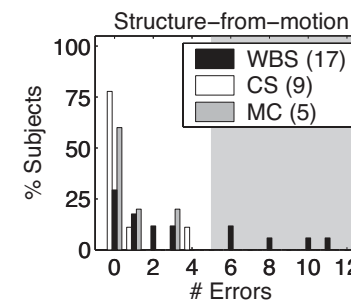
**Figure 3:** The percentage of subjects making a given number of errors in the four conditions of the monocular depth perception task. The light gray rectangles indicate the range within the 95% confidence interval for a subject performing at chance. This interval is different in the 'control-easy' condition because more trials were presented in this condition (24 trials) than in the other three (12 trials). The five WBS subjects performing below chance in the two control conditions were removed from the analysis of the two test conditions. Note that three WBS subjects systematically indicated the wrong cube above chance in the test-support condition.



The results of the four conditions in the first monocular depth task ('perspective cues') are presented in Figure 3. Choices were considered to be "errors" if they were inconsistent with the monocular depth cue that was provided. In the control conditions one of the cubes was actually larger than the other. The statistical chance of having less than 3 errors when a subject is purely guessing is less than 0.002% in the control-easy condition (24 trials) and less than 2% in the control-difficult condition (12 trials). All the 23 CS and all five MC subjects made fewer than 3 errors in these two control conditions, but five of the 33 WBS subjects made 3 errors or more in at least one of the control conditions. Therefore, we removed these five WBS subjects from further analysis of this task, since inability to respond correctly in the control condition confounds the results in the test conditions. In the test conditions the two cubes had identical sizes, but their sizes seemed different due to the configuration of the scene. The statistical chance of having less than 3 errors is less than 2% in these two conditions. In the test-distance conditions, all 23 CS, all 5 MC subjects and 24 of the 28 included WBS patients made fewer than 3 errors. In the test-support condition, 22 of the 23 CS, all 5 MC subjects and 14 of the 28 included WBS patients made fewer than 3 errors. Three of the 14

failing WBS patients systematically chose the "wrong" cube, perhaps indicating that they were considering the support as part of the object. The other 11 failing WBS subjects did not perform above chance. No correlation with age was observed ( $R^2=0.03$ ).

**Figure 4:** The percentage of subjects making a given number of errors in the structure-from-motion task. The light gray rectangles indicate the range within the 95% confidence interval for a subject performing at chance.



In the structure-from-motion task twelve of the 17 WBS subjects were able to indicate the simulated sphere on more than 15 of the 20 trials (see Figure 4), indicating that these subjects could judge shape from the coherent motion of the dots. Seven of the 9 tested CS and 3 of the 5 MC subjects made no errors at all and the other 4 control subjects made fewer than 5 errors. Note that the chance of making less than 5 errors in 20 trials when a subject is guessing is less than 1%. No correlation was found with the scores on the other monocular depth task, nor with age ( $R^2=0.08$ ).

### Stereopsis

**Table 1:** Stereopsis

The number of subjects (and percentages) in the group with Williams-Beuren syndrome (WBS) and the control groups (CS and MC taken together) who failed the stereopsis test, or coarse ( $>400''$ ), medium ( $100''-400''$ ) or good ( $<100''$ ) stereo-acuity. The three control subjects who were selected to participate because they were known to lack stereopsis are not included in this table.

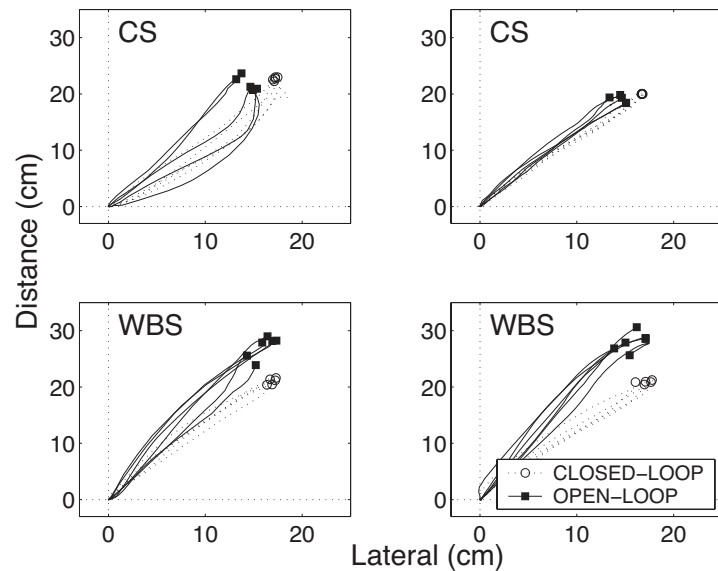
Group	N	Failed	Coarse	Medium	Good
WBS	33	16 (49%)	1 (3%)	13 (39%)	3 (9%)
CS+MC	25	3 (12%)	1 (4%)	3 (12%)	18 (72%)

Table 1 shows the distribution of the scores in the stereopsis test in the WBS group and the control groups. One subject in the MC group failed to pass this test. Both groups contained subjects in all four nominal categories of stereo-acuity. Group analysis showed that, although stereo-acuity in WBS was lower than in control subjects ( $X^2=25.3$ ), almost half of the patients with WBS showed fair stereovision, i.e., better than 400 seconds of arc. The three control subjects who were especially selected for lacking stereopsis (and indeed did not pass the stereopsis test) were excluded from this analysis. In the WBS group we did not observe any significant correlations with the results on both monocular depth tasks, nor with age ( $R^2=0.01$ ).

### Hand movements

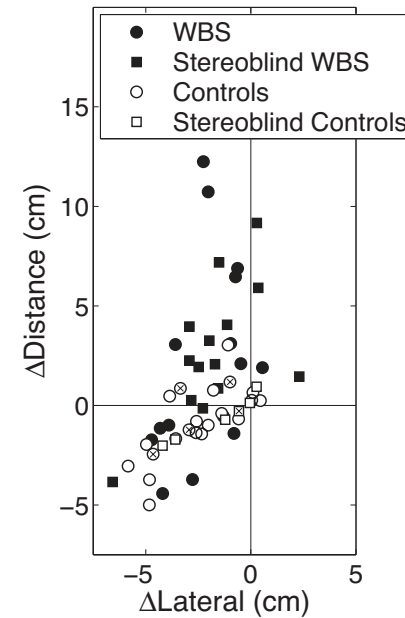
Figure 5 shows examples of hand movement traces of two WBS patients and two control subjects in the control condition and in the test condition. All subjects could accurately move to the target position when their hand was visible ('closed-loop condition'), but they often made systematic errors when the hand was invisible ('open-loop condition').

**Figure 5:** Example of trajectories and the endpoints of hand movements toward the target with visual feedback (dotted lines, open circles) and without visual feedback (solid lines, filled squares). The two top panels show control subjects. The two bottom panels show patients with WBS. Note that the target coincided with the endpoints in the closed-loop condition



The differences in mean lateral and distance positions between the closed-loop condition and the open-loop condition of all subjects are plotted in Figure 6. When analyzed individually, a significant overshoot (i.e., moving further than the target in the distance direction) was observed in 16 of the 30 WBS patients, participating in this experiment (vs. 6 of the 22 CS, and none of the 5 MC subjects). Furthermore, the radial differences between control and test condition (i.e., the absolute distance between target and pointing position) was significantly larger in the WBS group ( $4.7 \pm 2.7$  cm) than in the CS group ( $2.9 \pm 2.1$  cm) or the MC group ( $2.8 \pm 1.8$  cm).

**Figure 6:** The differences in pointing to the target between the settings in the closed-loop condition and the open-loop condition. For each subject the average distance difference is plotted against the average lateral difference. For left-handed subjects the sign of the lateral difference was reversed. Filled symbols represent WBS patients and open symbols represent control subjects (the combined CS and MC groups). Squares represent subjects without demonstrable stereopsis (stereoblind). The five control subjects of the MC group are marked with a cross.



Statistical group analyses across all 30 WBS subjects and 27 control subjects showed that, for the lateral direction, the WBS group did not differ significantly from two control groups ( $2.0 \pm 1.8$  cm versus  $2.0 \pm 2.2$  cm for the CS group,  $p=0.57$ ; and versus  $-0.9 \pm 3.1$  cm for the MC group,  $p=0.49$ ). Both the CS and the WBS group differed significantly from zero. This indicates that both WBS and the CS group showed a lateral difference in end point position in the direction of the starting positions (e.g., right-handed subjects moved their hand relatively too much to the left).

For the distance direction, however, the WBS group ( $2.3 \pm 4.2$  cm) differed significantly from the CS group ( $0.8 \pm 1.7$  cm) and the MC group ( $0.4 \pm 1.5$  cm). On average the WBS group moved their hand too far with respect to the target position. The CS and MC groups did not differ significantly from zero ( $p=0.03$  and  $p=0.60$ , respectively). In none of the groups a correlation with age was observed (all  $R^2 < 0.2$ ).

**Table 2:** Hand Movements

The mean (and standard deviations) of the lateral and distance differences (in cm) in the hand movement task for the WBS group and the control group (CS + MC combined) and for the two groups when split in several subgroups based on their problems in walking stairs and their performance on the stereopsis task [N: number of subjects in the group; SL<0: percentage of subjects showing a significant individual lateral shift in the direction of the starting point; SD>0: percentage of subjects showing a individual significant ( $p<0.01$ ) distance difference farther then the target position]. Note that the number of controls who failed the stereopsis test now includes the three subjects who were especially recruited for their lack of stereopsis.

Group	Stairs	Stereopsis	N	$\Delta$ Lateral (cm)	$S_L<0$ (%)	$\Delta$ Distance (cm)	$S_D>0$ (%)
WBS	Problems		30	-2.0 (1.8)	57	2.3 (4.2)	53
		Failed	27	-1.9 (1.9)	52	2.6 (4.3)	59
		Low Good	13	-1.7 (2.1)	54	3.0 (3.3)	69
	Normal		14	-2.1 (1.7)	50	2.3 (5.2)	50
		Failed	3	-2.5 (1.3)	100	-0.6 (0.4)	0
		Low Good	1	-2.3	100	-0.1	0
			2	-2.6 (1.8)	100	-0.8 (0.3)	0
Controls	All Normal		27	-2.0 (2.2)	57	-0.8 (1.7)	22
		Failed	6	-1.4 (2.1)	50	-0.6 (1.1)	17
		Low Good	21	-2.2 (2.2)	62	-0.8 (1.8)	24

As can be seen in Figure 6 and table 2, subjects without stereopsis did not differ from subjects with stereopsis, neither within the same group nor across the groups, and neither in the lateral nor the distance direction (all  $p > 0.3$ ). Furthermore, we did not observe any correlation between the performance on the perspective and structure-from-motion tasks and the results of the hand movement paradigm (maximum R2 obtained was 0.02, all  $p > 0.5$ ).

Finally, table 2 shows that the three WBS patients without difficulties in walking stairs were comparable to the CS group. These three patients showed a negative lateral shift (i.e., in the direction of the starting point) and a tendency to undershoot the target in the distance direction. Their behavior contrasts to the overshooting behavior of some (but not all) of the other WBS patients who did have difficulties in walking stairs.

## Discussion

In the present study the perception of monocular and binocular depth cues and the use of visual depth information were tested in a group of patients with Williams-Beuren syndrome. We were inspired by their notable problems in descending stairs, which are often regarded as deficits in the perception of depth. As for the visual perception of depth, our results show that patients with WBS were able to judge size and shape from the (spatial) relationships between structures using perspective and parallax cues. Half of the patients with WBS did make more mistakes in the monocular depth perception task when depth was induced by a rather complex configuration of the elements in the room (the 'test-support' condition). One might argue that the noted problems in global processing of patients with WBS might hamper the performance in such a complex situation, which represents one of the more extreme cues available in monocular depth perception. On the other hand, this result could also be attributed to more general deficits because the WBS group also made more errors in the other conditions of this task. We think that the poor performance of the five subjects, who were excluded from the analysis of the monocular depth perception task, is most likely due to a loss of interest, attention and/or concentration, since they did not differ

obviously from the other patients with respect to the characteristics of Williams-Beuren syndrome.

Nonetheless, 24 subjects of the 28 included subjects showed a performance in this task that can only be reached when they can process monocular depth cues properly.

About 49% of our WBS patients were unable to perceive stereoscopic depth information properly, which is congruent to the incidence of 44% reported previously (Atkinson et al., 2001). Such a high incidence of reduced stereopsis in WBS is not surprising since these patients show a much higher incidence of common visual problems in childhood, such as strabismus (Kapp et al., 1995) and reduced visual acuity (Atkinson et al., 2001), that are known to restrict the proper development of binocular processing of visual information. With respect to the use of depth in guiding movements, we observed that patients with WBS could move accurately to a target in depth when they could see their hand (see figure 4), indicating that patients with WBS do not show motor problems in performing this task. However, their hand movements were impaired when they had no visual feedback about the position of their hand. On average they tended to move their hand too far with respect to the target position. Although the monocular depth perception task and the pointing experiment were not matched, it is striking to see that in the pointing experiment in which subjects could use all possible cues (and not only texture and perspective), the performance of WBS subjects was the worst.

We can conclude that the majority of patients with WBS are able to perceive and report depth, although the scores of the WBS group are on average lower than those of the control group. This suggests that their difficulties in visuo-motor activities, such as walking stairs, are not related to problems in the perception of visual depth. Rather, the use of visual depth information in guiding movements seems to be impaired when the movement is executed without continuous visual feedback (Atkinson et al., 2003; Atkinson et al., 1997). However, we cannot rule out the possibility that problems in proprioception in WBS play a role in the outcomes of the present study, although one might argue that such proprioceptive problems are also likely to show up in the control condition of the hand movement task.

### Relevance of stereoblindness to motor control

As mentioned in the introduction, depth information can be extracted by both binocular and monocular cues in the visual environment. We did not observe any effect of the absence or presence of stereopsis on the accuracy of hand movements in either WBS or control subjects. Moreover, the five normal control subjects without stereopsis did not report any problems with walking stairs at all. Therefore, we conclude that the presence or absence of stereopsis is unlikely to have played an important role in the outcomes of our hand movement task. This is congruent with a previous report that stereo deficits were uncorrelated with performances on tests of spatial cognition (Atkinson et al., 2001). So, poor stereopsis cannot be the most prominent cause of the problems in walking stairs in Williams Beuren Syndrome. It seems likely that other factors, like motor problems, play a more significant role.

### Relation to walking stairs

Problems in motor behavior have often been described in the diagnostic literature concerning WBS (Chapman et al., 1996; Withers, 1996; Trauner et al., 1989; MacDonald and Roy, 1988). Severe problems are commonly noted in walking on stairs in a majority of patients with WBS (e.g., 30 of the 33 patients seen by our group). Also the parents of patients prominently reported the problems in walking stairs when they were asked for any motor abnormalities of their child.

Most marked is their inability to descend steps smoothly. They put two feet on the same step before continuing to the next step, they often use two hands for holding on to the banisters, and they tend to look down constantly to the steps ahead. Ascending stairs seems to pose fewer problems, although most of our subjects still climbed carefully. This observation might be related to the fact that a misstep during climbing has less serious consequences than a misstep during descending. Moving a foot too far during descending



leads to a miss of the next step of the stairs, and yields the unacceptable risk of falling down. Such an overshooting foot movement would correspond to the overshoot observed in our hand movement task when the hand was not visible. During ascending a misstep is most likely to lead to a (harmless) touch of the next step with your foot.

Walking stairs adequately involves both the proper perception of visual depth and subsequently the proper use of the visual depth information in guiding ones movements. Normal subjects are quite able to descend stairs using one foot per step and walk without much direct visual feedback about the position of feet and stairs. In contrast, patients with WBS seem to prefer constant visual feedback about the relative positions of their feet and the steps ahead. Hence, they are likely to move more slowly and carefully. In this way they ensure that the stairs are walked safely without risk of getting hurt. This is consistent with our finding that WBS patients perform their movements only correctly when visual feedback is present, although we acknowledge that this analogy is somewhat speculative as the depth cues provided in our experiments and those available in normal stair walking are quite different. In our group three patients with WBS did not show any problems in walking stairs, and, interestingly, their results in the hand movement task were similar to the control group. This suggests that, in contrast to the other patients, these three patients were able to use depth information in guiding their movements adequately, although all patients shared the common genotype and clinical symptoms of Williams-Beuren Syndrome.

### Limitations

In order to investigate depth processing in WBS we employed four experimental procedures. This inevitably puts limitations on the ecological validity of the experiments. For instance, in real life observers perceive and act on the basis of a real 3-dimensional world with all depth cues provided simultaneously, instead of being forced to use each one separately. On the other hand, the employed procedures allowed for a quantitative and controlled comparison between subjects.

The procedures themselves might have introduced some limitations. In the monocular tasks, for instance, the sensitivity might be too low, since we were only asking for ordinal relationships (rather than metric depth). Therefore, it could be argued that monocular depth vision in WBS might be subnormal, but still above the threshold set in our task. Presenting a more elaborated task is likely to reduce these limitations, and may yield subtle deficits in WBS subjects. However, this has the serious drawback of inducing effects of loss of interest, attention and concentration, which are common problems in WBS. These problems are probably already at the basis of the errors made by the WBS patients in the present experiments. This might be illustrated by the significant correlation between the total number of errors made in the two control conditions and the total number of errors made in the two test-conditions of the monocular depth perception task using the illusory room ( $R^2=0.55$ ,  $p < 0.001$ ). Finally, we should remark that no detailed matching or scoring of IQ was done for the present experiments, so we cannot relate the present results with the alleged visuo-spatial deficits often observed in WBS.

One has to realize that matching a control group to subjects is burdened with difficulty given the atypical cognitive profile of with Williams-Beuren syndrome. Nonetheless, in our opinion this possible shortcoming is unlikely to have had a significant effect on the outcome of the experiments. For instance, in none of the experiments an effect of age was observed at all (all  $R^2 < 0.2$ ). Also, the group of low-functioning subjects without WS (the MC group) performed similar to the other subjects of the control group (the CS group). Furthermore, it has to be noted that in that control group 10 children were present as well. We like to remark that the perception tasks were designed such that a successful performance is proof of the appreciation of depth cues. The control conditions included in the tasks ensured that subjects understood the task requirement, and by comparing the results within each subjects in the hand movement task, each subject served as his or her own control.

### Neurophysiological basis

Although speculative, the observed problems in visuo-motor coordination (hand movements and descending stairs) may be related to cortical deficits in the dorsal stream, since this occipito-parietal pathway is associated with the visuo-spatial processing and the visual control of action (Goodale and Milner, 1992). Other studies have already implicated a substantial problems of dorsal stream functioning in WBS (as well as in a number of other disorders (see, e.g., (Braddick et al., 2003)), whereas the ventral stream (occipito-temporal lobes) that is mainly involved in processing object properties seems to be relatively spared in WBS (Atkinson et al., 2003; Paul et al., 2002; Nakamura et al., 2002; Atkinson et al., 2001). Moreover, it has been suggested that WBS subjects seem to process visual information effectively but show problems in translating it into actions which would be indicative of dorsal stream deficits (Atkinson et al., 1997). Our observations may also reflect deficits in cerebellar functioning in WBS, since the cerebellum is strongly involved in motor activities. Structural MRI studies (Schmitt et al., 2001b; Reiss et al., 2000) and neurological observations (Chapman et al., 1996; Bellugi et al., 1990; Trauner et al., 1989) do indeed suggest cerebellar disturbances in WBS, which are recently related to the deletion of the gene *CYLN2* in WBS (Hoogenraad et al., 2002). However, we did not observe signs of gross cerebellar deficits, such as the presence of ataxia, nystagmus or vestibular dysfunction in our patients, although the saccadic eye movements of subjects with WBS seems to suggest some cerebellar disturbances (Van Der Geest et al., 2004).

### Conclusion

We conclude that most patients with Williams-Beuren syndrome are able to derive depth from spatial relationships between visual objects, despite their alleged problems in global visual information processing. Our results rather suggest impairment in using visual depth information for guiding movements adequately, especially in conditions without visual feedback of the movement.

### Acknowledgements

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## Linking genes to cognitive and motor coordination functions

### Contribution of *CYLN2* and *GTF2IRD1* to Neurological and Cognitive Symptoms in Williams syndrome

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Neurobiol Dis 2007;26:112-124

## Abstract

Williams Syndrome (WS, [MIM 194050]) is a disorder caused by a hemizygous deletion of 25-30 genes on chromosome 7q11.23. Several of these genes including those encoding cytoplasmic linker protein-115 (*CYLN2*) and general transcription factors (*GTF2I* and *GTF2IRD1*) are expressed in the brain and may contribute to the distinct neurological and cognitive deficits in WS-patients. Recent studies of patients with partial deletions indicate that hemizygosity of *GTF2I* probably contributes to mental retardation in WS. Here we investigate whether *CYLN2* and *GTF2IRD1* contribute to the motoric and cognitive deficits in WS. Behavioral assessment of a new patient in which *STX1A* and *LIMK1*, but not *CYLN2* and *GTF2IRD1*, are deleted showed that his cognitive and motor coordination functions were significantly better than in typical WS patients. Comparative analyses of gene specific *CYLN2* and *GTF2IRD1* knock-out mice showed that a reduced size of the corpus callosum as well as deficits in motor coordination and hippocampal memory formation may be attributed to a deletion of *CYLN2*, while increased ventricle volume can be attributed to both *CYLN2* and *GTF2IRD1*. We conclude that the motor and cognitive deficits in Williams Syndrome are caused by a variety of genes and that heterozygous deletion of *CYLN2* is one of the major causes responsible for such dysfunctions.

## Introduction

Williams Syndrome (WS, also known as Williams-Beuren syndrome, MIM 194050) is a genetically determined neurodevelopmental disorder, which is characterized by a rather unique combination of distinct facial features and medical complications on the one hand and characteristic behavioral patterns and cognitive disabilities on the other hand (for reviews see, e.g., (Tassabehji, 2003; Mervis, 2003; Morris and Mervis, 2000; Bellugi et al., 1999)). The facial features include a broad nasal tip, a long philtrum and full cheeks and lips, while the medical complications are dominated by supravalvular aortic stenosis (SVAS) and pulmonary arterial stenosis (PAS), growth retardation, intermittent hypercalcaemia, hyperacusis and dental abnormalities. The behavioral and cognitive abnormalities are also reflected in a wide variety of symptoms including abnormal gait, impaired stair and surface stepping, dysmetria of saccadic eye movements, a low mean IQ varying from 40 to 79, and poor visual-motor integration and attention deficits (Van Der Geest et al., 2005; Van Der Geest et al., 2004; Mervis et al., 2001; Chapman et al., 1996; Withers, 1996; Gosch and Pankau, 1996; Bellugi et al., 1990; Trauner et al., 1989). In contrast to the neuropsychological features listed above, language and musical skills of WS patients are relatively spared, and their personalities are usually friendly, social and engaging (Bellugi et al., 1999). Because of their outgoing character the mental retardation of WS patients is often underestimated.

The peculiar sets of symptoms and features of WS patients generally result from a 1.55 Mb submicroscopic deletion of 25-30 genes in chromosome band 7q11.23, which is called the WS critical region (Korenberg et al., 2000; Francke, 1999; Osborne, 1999; Ewart et al., 1993). However, some patients suffer from a smaller deletion comprising only a subset of the general spectrum of WS genes. Genotype-phenotype correlation studies of these patients have revealed important new insights in the genetic causes of some of the typical WS symptoms. So far, the strongest correlations have been found for some of the facial symptoms and medical complications. For example, heterozygous deletions of *GTF2IRD1* and Elastin have been found to cause the craniofacial abnormalities and vasculopathology, respectively (Tassabehji et al., 2005; Ewart et al., 1994; Ewart et al., 1993). Studies on the genotypical and phenotypical relations have also provided new insights in the genes that may contribute to the neuropsychological characteristics of WS patients, but due to the complexities of the brain and behavior these correlations often are harder to pinpoint to single genes and single symptoms. As it stands hemizygosity of *GTF2I* probably contributes to the cognitive, behavioral or neurological symptoms of WS (Morris et al., 2003; Hirota et al., 2003), while hemizygous deletions of



*STX1A* or *FZD9* (formerly known as *FZD3*) do not (Tassabehji et al., 1999; Botta et al., 1999a). The possible contribution of several other genes that are prominently expressed in the brain awaits further confirmation; these include the roles of *LIMK1* (Morris et al., 2003; Tassabehji et al., 1999; Frangiskakis et al., 1996), *GTF2IRD1* (Hirota et al., 2003; Bayarsaihan et al., 2002) and cytoplasmic linker protein II (*CYLN2*) encoding CLIP-115 (Hoogenraad et al., 2002; De Zeeuw et al., 1997). The potential contribution of these genes to the WS phenotype remains to be elucidated, because either conflicting data have been reported and/or we are still missing detailed behavioral examinations of WS patients with particular restricted lesions in the telomeric region. Here we address the question as to what extent the genes *CYLN2* and *GTF2IRD1* may contribute to the cognitive and motoric deficits in WS. We describe a new patient in which *STX1A* and *LIMK1* are hemizygotously deleted, while *CYLN2* and *GTF2IRD1* are preserved. Furthermore, we investigated the brain morphology and behavior of two strains of mutant mice in which the expression of *CYLN2* (Hoogenraad et al., 2002) and *GTF2IRD1* (Durkin et al., 2001) are specifically affected. Since hippocampal and cerebellar structures are likely to be affected in WS patients (Van Der Geest et al., 2004; Jones et al., 2002; Hoogenraad et al., 2002), we focused our behavioral assessments on potential deficits associated with these brain regions.

## Methods & Materials

### Patient

Our patient (ws75) is a boy of 16.8 years of age. His parents gave their written informed consent for the genetic analysis and the behavioural tests. The results of these tests were compared to a group of typical WS patients and healthy controls, who participated in a large study on Williams Syndrome in the Netherlands. The study was approved by the Medical Committee of the Erasmus MC and the VU University Medical Center.

### FISH analysis

Chromosome analysis was performed on the blood using standard resolution techniques. Fluorescent in situ hybridisation (FISH) with the probes RP11-451M8, B315H11, CITB51J22, B270D13 and B39H04 was performed on the proband's metaphase spreads according to standard protocols. These probes were selected from a set of 25 probes "PKZD Berlin" and used within the diagnostic cytogenetic department for routine WS diagnostics. In brief the probes were alternated labelled with Bio-16-dUTP/Dig-11-dUTP. The chromosome slides and probes were denatured simultaneously and incubated overnight at 37 °C. The detection was performed in one single layer containing streptavidine-Alexa 594 (Invitrogen) and anti-digoxigenin-FITC (Roche). Hybridisations were analysed with an epifluorescence microscope (Zeiss Imaging II), images were captured with an image analysis system (MetaSystems). To determine whether a given probe was deleted more than 50 metaphase cells were evaluated and scored for the presence or absence of a signal from the test probes hybridized simultaneously with a 7qter located control probe (RP1-3K23).

### DNA analysis

Screening for deletions of genes in the Williams syndrome critical region was performed using MLPA kit SALSA P029 (MRC Holland, The Netherlands). This kit contains probes for 8 genes in this region: *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*. For *ELN* and *CYLN2* probes for various exons are present in the kit. Polymorphic repeat markers D7S2476, *ELN* and *LIMK1GT* were used to determine the parental origin of the boy's deletion. The locations of these markers are shown in figure 2c.

### Motor control tests for WS75

Eye movements were recorded with an infrared eye-tracking device (EyeLink, SMI, Germany, see van der Geest & Frens, 2002) at a sample rate of 250 Hz whilst head movements were restrained by means of a chinrest. In one task, the subject was encouraged to make saccadic eye movements back-and-forth between two stationary targets on a computer screen. In a second task, the subject was instructed to follow a moving dot on a computer screen, which normally evokes a smooth pursuit eye movement response (see (Van Der Geest et al., 2004) for details). The adaptation of hand movements were examined using a prism-adaptation task. Here the patients were instructed to make pointing movements toward a target without being able to see his hand (see (Van Der Geest et al., 2005) for details of the experimental setup). This was done before and after a 10 minute adaptation period in which the subject was wearing prism glasses that effectively induced a mismatch between the visual and proprioceptive sensory systems (Deuschl et al., 1996).

### Cognitive tests for WS75

We used a variety of psychometric tests to assess the cognitive abilities on both verbal and visual-spatial domains. Five of these tests were taken from a Dutch version of the WISC with Dutch norms (Wechsler Intelligence Scale for Children-Revised (WISC-RN), (van Haasen et al., 1986)), because neither the WISC III nor the DAS (Differential Abilities Scales) are validated for the Dutch population. These tests were *Digit recall*, *Vocabulary*, and *Similarities*, the *Block Design* and *Object Assembly* test. Passive and active verbal performance level was measured by the *Peabody Picture Vocabulary Test, 3rd edition, adapted for Dutch persons* (Dunn and Dunn, 1981), the *Boston Naming Test* (Kaplan et al., 1983) and *Verbal Fluency* (Lezak, 2007). Non-verbal visual-spatial tests were the *Beery - Visual Motor Integration* test (Beery, 1989), the *Raven Coloured Progressive Matrices* (Raven, 1960), and the *Line Orientation Test* (Benton, 1983) and the *Trailmaking Test* (Reitan and Wolfson, 1985). Table 1 provides a brief description of the cognitive domain each test aims to assess. All tests were administered in a quiet room at home.

All raw scores were expressed in mental age levels. The performances of subject WS75 were compared to those of a group of typical WS patients and a age-matched subgroup of these patients who were aged between 14-21 years (7 females, 5 males). It should be noted that some tests were too difficult and that not all subjects participated in all tests (see table 1). In particular, the tests taken from the WISC-RN turned out to be too difficult for most typical WS subjects, and only one typical WS subject was able to perform well in the Trailmaking Test. The data were analyzed using non-parametric tests, and percentile scores are presented.

In our lab we also developed two computerized tasks to investigate visual-spatial and visual memory functioning in children with Williams Syndrome. In the *Visual-Spatial Memory task*, the display on a monitor showed a 5 by 5 grid of squares. In each trial a specific number of these squares were marked one after another. After the presentation, the subject had to indicate by mouse click the positions of these squares in the same order as they were presented. Furthermore, there were two types of trials: structured trials (VS S) and random trials (VS R). In the structured trials, the marked squares formed a distinctive pattern, for instance a straight line consisting of five squares in order. In the random trials, the marked squares did not form such a coherent pattern at all. A trial was counted as correct when all squares were correctly indicated in place as well as in sequence. During the experiment the number of marked squares increased gradually. The highest number of correctly memorized positions made up the score. In the *Visual Recognition Memory* task, the subject had to memorize a series of pictures that was presented for one second sequentially on the center of the computer screen. After presentation of each trial the subject had to indicate whether a target picture, which was shown after the series, had been either absent or present. During the experiment the number of pictures increased gradually. The test was ended when the subject made three mistakes in a row. The pictures were simple colored drawings of for instance a house, a car, etc. On this test feedback was given on the correctness of the response. The number of pictures of the last correct answered series

made up the score. The results of WS75 were compared to the results of a group of WS subjects with the common deletion (age range 6-40 years, on average 17.3 years) and a normal developing group of subjects (age range 9-30 years, on average 14.1 years). Stereoscopic Depth vision was measured using the standard Titmus Fly Stereo Test (Ohlsson et al., 2001), while visual acuity was assessed using the standard Snellen card.

#### *GTF2IRD1- and CYLN2-knockout mice*

A high degree of synteny and linkage conservation exists between human chromosome band 7q11.23 and mouse chromosome 5G1-G2. The orthologous region in the mouse lacks the characteristic duplicated blocks but has the full complement of genes, which is inverted with respect to the human map. The heterozygous *GTF2IRD1*-mutants (line 166.8) we used harbor a hypomorphic allele of *GTF2IRD1* (Durkin et al., 2001), while the heterozygous *CYLN2*-mutants we used were derived from the null-mutant of CLIP-115 (Hoogenraad et al., 2002). Both types of mutants mimic (part of) the genetic deletion in Williams Syndrome in that a suboptimal dosage of the respective gene is present. The researchers who did the manual work were blind to the type of mouse being analyzed.

#### *Magnetic resonance imaging (MRI)*

The mice were anaesthetized with 5% isoflurane induction and 1% isoflurane maintenance, administrated in a mixture of 30% oxygen and 70% N<sub>2</sub>O. The head of the mouse was firmly fixed in a stereotactic device, positioned in the center of the RF coil. During MRI, the temperature of the mouse was kept constant at 37.0 ± 0.5 °C, using a rectal probe (PT100) and electrical heating pad (Uty Nelson). MRI imaging was performed at 300 MHz on a 7T MR microscope (SMIS, Guildford, UK). High resolution coronal slices of the mouse brain were obtained using a 3D Fast Spin Echo sequence (256 x 128 x 64 matrix, TR 2500 ms, TE: 35, 60, 85, 110 ms); the echo train length reduced the imaging time by a factor of 4 (Kooy et al., 1999; Fransen et al., 1998; Yuan et al., 1993). The imaging parameters were chosen to obtain 3D images of the brain with optimal contrast between the ventricles and the surrounding brain tissue: cerebrospinal fluid in the ventricular system was white on the T2-weighted scans. The imaging procedure took about 85 minutes. The MR data was reconstructed to an 256x256x256 image matrix (78x78x78 \_m3 voxel size). Brain and ventricular structures were defined on coronal MR images according to the mouse brain atlas (Paxinos and Franklin, 2003). Their positions were defined as antero-posterior distance to the interaural line (IA). A semi-automatic 3D segmentation technique was applied on the 3D MR images data set to extract quantitative volumes (Sijbers et al., 1997). The volumes of the hippocampus, cerebellum, amygdala and the ventricular system were segmented using Surfdriver software. The ventricular system was divided into the fourth ventricle, the aqueduct of Sylvius, the third ventricle and the lateral ventricles according to the mouse brain atlas (Paxinos and Franklin, 2003). Values are indicated as means ± s.e.m., and statistical analyses were performed with a two-tailed Student's t-test.

#### *Histology*

The mice were anaesthetized (Nembutal; 50 mg/kg) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed, cryoprotected in sucrose, embedded in gelatin, and cut on a cryotome in sagittal 40 µm thick sections. The sections were reacted for hematoxylin/eosin, mounted on glass slides, coverslipped, and investigated under the light microscope. In addition, sections were processed for immunocytochemistry using various primary antisera such as against calbindin, choline-acetyltransferase, etc. After treatment with 10% normal goat serum in 0.1 M Tris-buffered saline (TBS), the sections were incubated with rabbit primary antibody diluted 1:1000 in 0.1 M TBS for 72 hours, rinsed with Goat anti-rabbit-biotin for 90 minutes, and ultimately reacted with avidine-biotin-peroxidase and diaminobenzidine for visualization of the antigen. Subsequently, the sections were mounted, coverslipped, and investigated under the light microscope (see (De Zeeuw et al., 1997; De Zeeuw et al., 1989) for details).

#### *Fear conditioning of mouse mutants*

Computer-assisted 24 hours contextual fear conditioning was performed as described by Anagnostaras and colleagues (Anagnostaras et al., 2000). Mice were placed into the conditioning chamber and after a 2 minute baseline period, they received a foot shock (2 second, 1.0 mA). Twenty four hours later, the mice were placed back into the same conditioning chamber for a 2 minute contextual freezing test. We scored freezing time digitally on a frame by frame basis using a webcam at 5Hz and customary written software. Values were means ± standard error of the mean of percentage of freezing; statistical analyses were performed with a two-tailed Student's t-test.

#### *Motor coordination of mouse mutants*

Wild type and mutant mice were analyzed for motor coordination (see (De Zeeuw et al., 1998) for details). All behavioral experiments were carried out double blind. First, open field behavior tests (50x50 cm<sup>2</sup>) were performed to assess spontaneous motor activity. Mice always started from the same corner of the arena and were recorded for 5 minutes using a computerized video tracking system to measure walking distance, and moving episodes and moving time at the horizontal and vertical planes. Subsequently, motor coordination and balance were assessed by measuring the ability of the mice to stay on the accelerating rotarod. The rotarod consists of a smooth plastic roller (8 cm diameter; 14 cm long) flanked by two large round plates (30 cm) to prevent animals from escaping. Mice were initially placed on the roller for 10 seconds at a constant velocity (2 rpm). The rotational speed was gradually increased in 2 minutes to 12 rpm. Mice were trained at day one (on a stationary rotarod) and tested over the next five days. Three trials were carried out for each mouse every day. The latency (i.e. the time the mouse could remain on the rotarod) was measured. Finally, the mice were subjected to the circadian running wheel test, which measures the 24 hour cage activity. Mice were placed in cages with running wheels for one week. After this acclimatization period, the running wheel activity of the mice was measured during 24 hours (van der Horst et al., 1999). In all behavioral experiments described above, statistics were performed with a two-tailed unpaired Student's t-test.

## Results

### *Subject WS75*

#### *Clinical description*

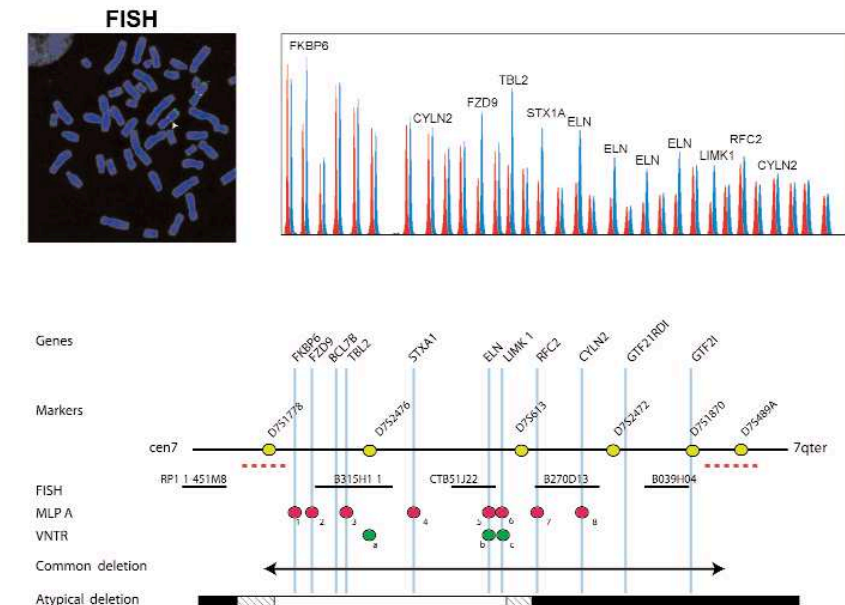
Subject WS75 is the first child of non-consanguineous parents of Turkish origin. Pregnancy and delivery were uncomplicated. Psychomotor development appeared normal. The boy started to walk without support and spoke his first words at one year of age. After receiving extra Dutch lessons the boy is now attending regular schooling. His intelligence is slightly below normal (full scale IQ 85). Because of the detection of a supravalvular aortic stenosis and multiple peripheral pulmonary arterial stenoses (at the age of one year and 5 months), he was suspected of having a deletion in the WS region. Otherwise, the boy has no documented history of hypercalcaemia, hyperacusis, inguinal or umbilical hernia or joint problems, and did not present any of the other typical features of WS in the past. At the time of examination WS75 was 16.8 years old. His length was 162.5 cm (< -1 Standard Deviation), his weight 50 kg (< 0 SD) and his head circumference 54 cm (-1.5 SD). No dysmorphic features were noticed (Figure 1) and the neurological examination did not show abnormalities. He had no problems descending stairs or stepping over surface changes. Stereoscopic depth vision and visual acuity were normal. In contrast to typical WS patients he had a shy appearance.

**Figure 1:** Photograph of WS75, aged 16.8 years*FISH and DNA analyses*

Chromosome analysis on peripheral blood lymphocyte culture revealed a normal male karyotype (46, XY). The FISH analysis performed utilized five probes spanning the common deleted region. For B315H11 and CITB51J22 signals were observed on only one of the chromosomes 7, while for RP11-451M8, B270D13 and B39H04 signals were detected on both chromosomes 7 (figure 2a). Using multiplex ligation-dependent probe amplification (MLPA), hemizygosity for *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN* and *LIMK1* was detected, but not for *RFC2* and *CYLN2* (Figure 2b). Polymorphic repeat-marker analysis on peripheral blood DNA from the boy and his parents confirmed hemizygosity for the markers D7S2476, LIMK1GT and ELN and the paternal origin of the deletion. These analyses indicate that WS75 has a partial deletion of the WS critical region excluding the genes *CYLN2* and *GTF2IRD1* (Figure 2).

**Figure 2.** Results of genetic analysis of WS75.

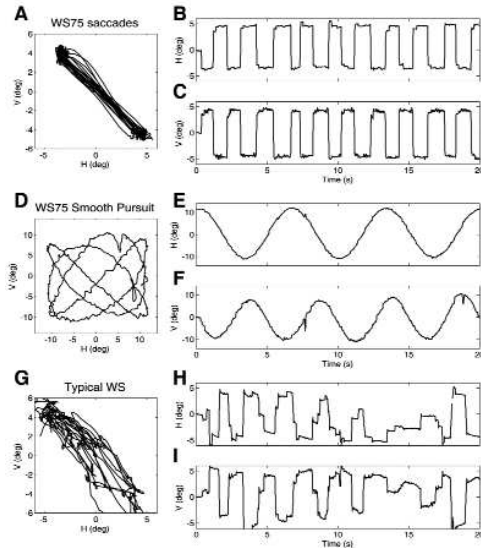
(A) FISH result showing a deletion, indicated by the arrow, for CITB51J22 (green) and the normal signals for B270D13 (red) and the control probe (RP13K23; in green) on 7qter. (B) Multiplex ligation-dependent probe amplification. Normalised MLPA peak pattern from the index patient (red) and from control DNA (blue) plotted in one figure for easy comparison. Peaks from genes in the Williams syndrome critical region are labelled with their name; unlabelled peaks represent control genes. For ELN and CYLN2 probes for various exons are present in the MLPA kit. (C) A physical map of the deleted region in the common WS population and subject WS75. The five FISH probes are represented by black lines, the MLPA probes by red dots (1: FKBP6; 2: FZD9; 3: TBL2; 4: STX1A; 5: ELN; 6: LIMK1; 7: RFC2 and 8: CYLN2) and the VNTR markers studied are represented by green dots (a: D7S2476; b: ELN and c: LIMK1GT). The common deleted region is presented in a black dual arrowhead line, while the atypical deletion of WS75 is depicted in a white box flanked by the not deleted area in black.

*Motor control tests*

As described above, WS75 did not show any obvious sign of ataxia or disturbed locomotion. To quantify the level of cerebellar motor performance and motor learning we also investigated his eye movements. The boy was able to make accurate saccadic eye movements between small targets (Figure 3a-c). For instance, his amplitude gains of horizontal saccades (mean of 0.96) had a normal low variability (0.077), which is in clear contrast to the high variabilities observed in a group of typical WS patients ( $0.13 \pm 0.03$ ) (Van Der Geest et al., 2004). Furthermore, he showed a normal smooth pursuit response to sinusoidal target motions. Gains for horizontal and vertical smooth pursuit were close to 1 with a negligible phase lag and a low number of saccadic intrusions (Figure 3d-f). In contrast to WS patients with complete deletions, he showed no signs of cerebellar dysfunction, such as an increase in the number of correction saccades or saccade dysmetria (Figure 3g-i), or an asymmetry between leftward and rightward saccades (Van Der Geest

et al., 2004; Botzel et al., 1993). The performances of typical WS patients do not correlate with age or gender (Van Der Geest et al., 2004). Furthermore, spontaneous nystagmus was absent and the velocity profiles of his saccades were normal. Finally, the prism adaptation task also showed a normal modification of pointing movements indicating a normal adaptive control of hand movements.

**Figure 3.** WS75 showed normal saccadic eye movement (panels A-C; 10 degree oblique targets) and a normal smooth pursuit response (panels D-F). The saccadic eye movements of an age-matched typical WS patient for the same 10 degree oblique targets are shown in panels G-I.



### Cognitive tests

WS75 was subjected to two major categories of psychometric investigations including verbal tests and (non-verbal) visual-spatial tests (see tests 1 to 6 and 7 to 12 in Table 1, respectively). On most tests he performed significantly better than a group of typical WS patients.

Table 1: Outcome of standard psychometric tests: Note that the performances of WS75 (16.8 years old at the moment of the tests), the group of all typical WS patients and the subgroup of age-matched typical WS patients (aged between 14-21 years), are expressed in both mental age levels, as well as raw test scores. The median (50th percentile) as well as the 25th and 75th percentile scores of the typical WS group and subgroup are given for a number of subjects (N). Test marked with a (W) are part of the WISC-RN, which was too difficult for most WS patients. The first six tests (Digit Span - Verbal fluency) concern verbal tests, while the latter six non-verbal test concern visual-spatial cognition (Block Design- Trailmaking). The scores of WS75 marked with a # are significantly ( $P < 0.01$ ) better than those of the group of typical WS patients; scores marked with a \$ are significantly better than those of his age-matches peers.

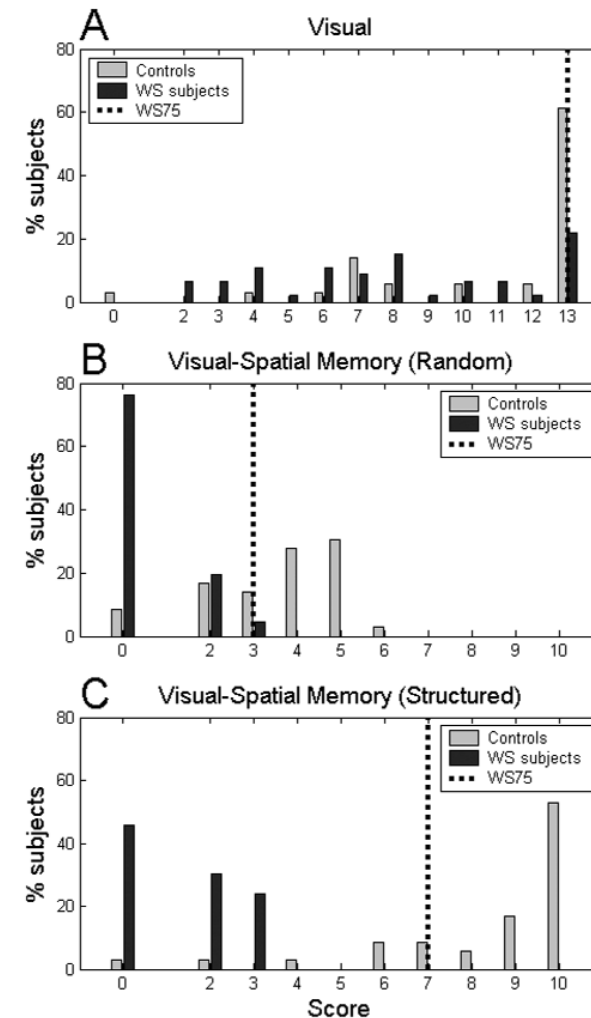
**Table 1**  
Outcome of standard psychometric tests

Psychometric test	Cognitive domain	Name	Score	WS75			Typical WS			Age-matched WS subgroup		
				n	MA	Raw	n	Median	25th/75th percentile	n	Median	25th/75th percentile
1	Digit Recall <sup>(W)</sup>	Short-term auditory memory	MA	13.5 #, \$	11	6.3	11	6.3	5.7/6.9	2	6.2	5.9/6.6
2	Boston Naming Test	Picture-naming vocabulary	Raw	12 #, \$	50	5.3	50	5.3	3/6	10	3	4/5
3	Vocabulary <sup>(W)</sup>	Knowledge of word meanings	Raw	37 #, \$	11	7.5	11	7.5	5.0/7.3	2	5.5	5.0/7.1
4	Peabody Picture Vocabulary Test	Receptive (hearing) vocabulary attainment	Raw	6.7	14	16	14	16	20/34	2	28	25/34
5	Similarities <sup>(W)</sup>	Verbal abstract reasoning	Raw	11.4 #, \$	55	8.3	55	8.3	6.2/8.0	2	6.1	6.1/6.2
6	Verbal Fluency	Speed and flexibility of verbal thought processes	Raw	140 #, \$	10	7.5	10	7.5	10/19	12	10	9/11
7	Block Design <sup>(W)</sup>	Visual abstract ability	Raw	10.7 #, \$	54	6.0	54	6.0	5.4/10.9	12	8.6	7.4/10.8
8	Object Assembly <sup>(W)</sup>	Visual analysis and construction of objects	Raw	20 #, \$	10	109	10	109	71/136	1	113	99/135
9	Beery-VMI	Visual perception and fine motor coordination	Raw	10.2 #, \$	53	4.8	53	4.8	6.8/8.0	1	5.6	5.4/7.9
10	Raven Coloured Progressive Matrices	Ability to form perceptual relations	Raw	18 #, \$	11	6.3	11	6.3	10/15	12	6.0	5.4/7.9
11	Line Orientation Test	Visual-spatial processing without motor skills	Raw	7.5 #, \$	10	6.3	10	6.3	8/15	11	11	9/14
12	Trailmaking	Scholar skills and visual information processing: (A) digits (B) Digits and letters	Raw	26	11	7.0	11	7.0	6.2/6.5	1	6.2	5.9/6.6
			MA	7.5 \$	52	5.8	52	5.8	14/22	14	14	6.5/6.8
			Raw	34	30	20/38	30	20/38	6.0/8.5	2	6.3	6.5/6.8
			MA	12.8 #, \$	53	4.8	53	4.8	4.4/5.7	12	4.8	4.8/5.5
			Raw	37	7	5.9	7	5.9	25	25	22/28	4.8/5.5
			MA	> 10 #, \$	52	5.8	52	5.8	4.8/6.9	12	5.7	5.0/6.4
			Raw	31	17	13/21	17	13/21	7	7	7/8	5.0/6.4
			Raw	7.4	0	0	0	0	14/20	12	18	14/20
			Raw	12.6	1	9.3	1	9.3	0	0	0	0
			Raw	10.4	8.5	8.5	8.5	8.5	0	0	0	0

For the verbal tests, he scored relatively well on Digit Span, Boston Naming Test, Peabody Picture Vocabulary Test, Similarities and Verbal Fluency, performing better than the average WS patient, but somewhat less than the normal controls. However, Vocabulary formed an exception in that WS75 performed below the median of the typical WS group. With regard to the non-verbal tests addressing visual-spatial cognition, WS75 performed superior to the group of typical WS subjects, except for the Object Assembly test. These outcomes were also observed when he was compared to a smaller group of typical WS patients, who were matched for chronological age. Moreover, the tests taken from the WISC-RN were too difficult for the vast majority of age-matched typical WS subjects. Only 2 of the 7 females, and none of the 5 males aged between 14 and 21 years, were able to perform in the tests taken from the WISC.

Furthermore, on the Line Orientation Test he reached a performance of 7.4 years, while this test is usually too difficult for people with typical WS (Farran and Jarrold, 2003; Bellugi et al., 1999). In addition, he scored better on the Trailmaking test. In general WS75 showed fear of failure during the examinations, so he worked quite concentrated and motivated to the expense of being slow. This strategy probably influenced his scores on those tests that had to be performed within stringent time limits such as the Object Assembly and Verbal Fluency. In the two non-spatial memory tasks WS75 performed similar to a control group. In the *Visual Recognition Memory* task WS75 reached the maximum score of 13 items (versus  $8.0 \pm 3.7$  items in 47 WS subjects, and  $10.9 \pm 3.2$  items in 36 control subjects; figure 4A). In the Digit Recall test he reached a correct response level of 12 (11 WS subjects scored  $4.6 \pm 2.0$ ; 36 normal controls reached  $13.2 \pm 2.7$ ; table 1). The *Visual-Spatial Memory* test showed that the easily recognizable pattern in the structured trials aided his memory for positions considerably, whereas such a pattern did not influence the performance of a typical WS patient (figure 4BC). WS75 reached a score of 3 positions for the random condition and 7 positions for the structured condition (versus  $0.5 \pm 1.0$  and  $1.3 \pm 1.3$  positions, respectively of 47 typical WS subjects, and  $3.6 \pm 1.6$  and  $8.5 \pm 2.4$  positions, respectively, for 36 normal control subjects). Thus taken together we can conclude that, although WS75 scored below his age level on the psychometric tests, in general he performed significantly better than would be expected from a typical WS patient (Mervis et al., 2000; Bellugi et al., 2000).

**Figure 4:** The percentage of subjects in the control group and in the WS group who reached a particular score in the visual-recognition memory test (A) and the visual-spatial memory test (B & C). The scores of subject WS75 are indicated by a dotted line. Memory for visual-spatial positions is better for structured patterns than for random patterns in both the control group and in subject WS75, but not in typical WS subjects.



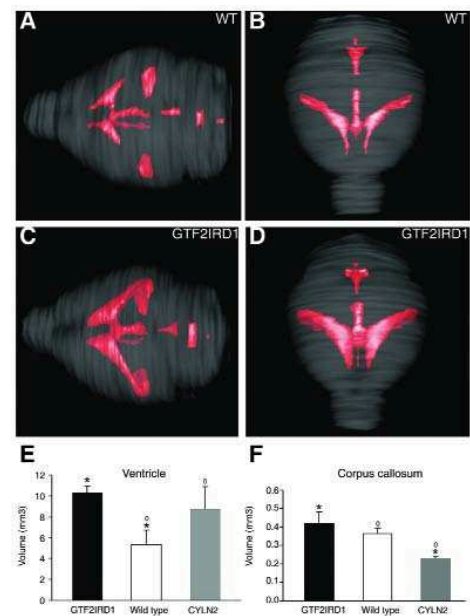


GTF2IRD1 and CYLN2 mouse mutants

Volumetric measurements by MRI

As no patients with specific deletions of either *GTF2IRD1* or *CYLN2* are available, we set out experiments to compare the phenotype of mouse mutants of *GTF2IRD1* to that of *CYLN2* mutants. Both types of mutants mimic the genetic deletion in Williams Syndrome in that a suboptimal dosage of the respective gene is present. Structural Magnetic Resonance Imaging showed that the volumes of the cerebellum, cerebrum, hippocampus and amygdala did not differ significantly among *GTF2IRD1*-mutants (n = 6) and wild types (n = 6; all p-values > 0.2; ANOVA). However, the total size of the brain ventricles of *GTF2IRD1*-mutants was significantly larger (p < 0.01; ANOVA) than that of wild types (Figure 5). This increase in volume was not paralleled by a decrease in size of the corpus callosum as we found previously for the *CYLN2*-mutants. Thus, the ventricles were enlarged in both the *GTF2IRD1*-mutants and *CYLN2*-mutants, while the corpus callosum was only significantly affected in the *CYLN2*-mutant (Hoogenraad et al., 2002).

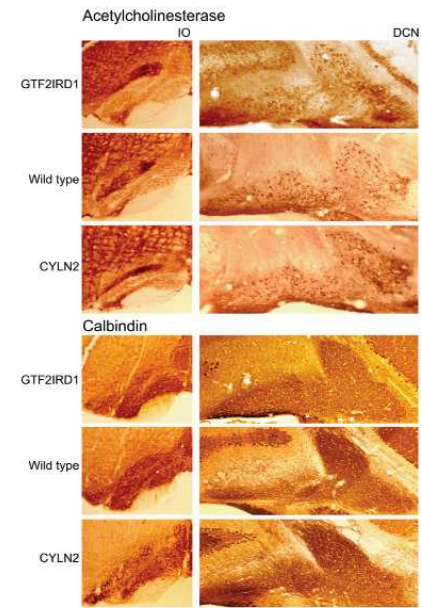
**Figure 5:** The results of the MRI in mice. (A–D) 3D surface rendering of the segmented ventricles in the brain of a wild type (WT) and *GTF2IRD1* mouse. The 3D reconstruction is viewed from above at two different caudal–rostral angles. All major ventricles in the *GTF2IRD1* mouse are enlarged. (E) Total ventricles volumes as determined by high-resolution MRI. Ventricular volumes (mean ± SEM) are enlarged in both *GTF2IRD1* and *CYLN2* mice, as compared to wild types (p < 0.05), but did not differ significantly from each other. (F) Corpus callosum volumes were significantly smaller in the *CYLN2* mice (p < 0.05) but not in the *GTF2IRD1* mice.



Histology

Staining of the brains of both the *GTF2IRD1*-mutants and *CYLN2*-mutants with hematoxylin/eosin did not reveal any gross abnormalities in the distribution of neurons, neuropil, glia or white matter. In addition, the cyto-architectonic layers in both the hippocampus and cerebellum appeared unaffected. The enlarged ventricles that were observed with the use of the volumetric MRI measurements were also apparent in the histological examination. Particularly, the enlargement of the lateral ventricles and the IVth ventricle could be readily observed under the microscope (data not shown). Additional stainings with acetylcholine esterase, silver staining or immunocytochemistry for dopaminergic, cholinergic, GABAergic or calbindin-positive neurons did not reveal any abnormality (for stainings of olivocerebellar system, see Figure 6).

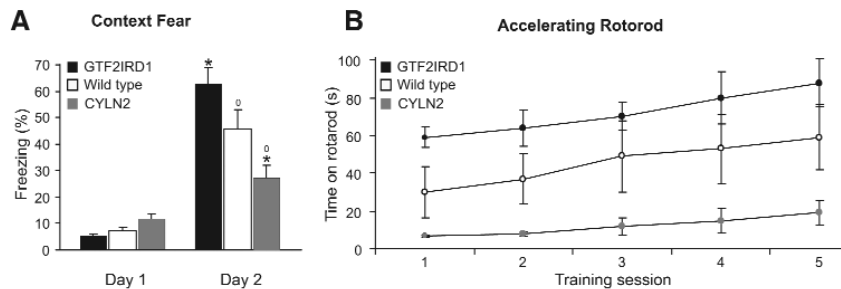
**Figure 6:** Histochemical markers show no abnormalities in mouse mutants with respect to the distribution cells in the inferior olive (IO) or the deep cerebellar nuclei (DCN). Top three panels show labeling in *GTF2IRD1*-mutants, wild types and *CYLN2*-mutants following acetylcholine esterase histochemistry. Bottom three panels show labeling in the same mice following immunocytochemistry for calbindin.



Fear conditioning

In the contextual fear conditioning paradigm the *GTF2IRD1*-mutants (n = 14) did not show any sign of hippocampal dysfunction, in contrast to *CYLN2*-mutants (Hoogenraad et al., 2002). The *GTF2IRD1*-mutants showed a significant increase in their average freezing time when they were put back into their context-specific test-cage 24 hours after a foot shock (p < 0.0001; t-test). While this increase was comparable to that observed in wild types (Figure 7A), the increase in *CYLN2*-mutants was significantly less than that observed in the *GTF2IRD1*-mutants or wild types (p < 0.001 and p < 0.002, respectively; t-test).

**Figure 7:** The behavior of the mouse mutants. (A) The increase freezing time in *CYLN2*-mutants was significantly less than that observed in the *GTF2IRD1*-mutants or wild types in the contextual freezing paradigm (B) In the rotarod paradigm the *GTF2IRD1* and wildtype (WT) mice showed both a higher level and better improvement of motor performance than *CYLN2* mutants.



### Rotarod

In the accelerating rotarod paradigm *GTF2IRD1*-mutants did not show any cerebellar motor deficits and performed comparable to wild types, whereas such deficits could be readily observed in *CYLN2*-mutants (Hoogenraad et al., 2002). After the *GTF2IRD1*-mutants were adjusted to the equipment, they were able to stay on average 59 seconds on the rotating rod at the first trial. This duration increased up to 88 seconds ( $\pm 12$ ; SEM) at the fifth trial. The wild types performed slightly less, but this difference was not significant ( $p = 0.23$ ; ANOVA) and the learning curve was similar to that observed in the *GTF2IRD1*-mutants (Figure 7B). In contrast, however, the *CYLN2*-mutants stayed significantly shorter on the accelerating rotarod than *GTF2IRD1*-mutants and control mice ( $p < 0.01$  and  $p < 0.05$ , respectively; ANOVA). In addition, *GTF2IRD1*-mutants did not show the highly erratic motions and ejections in the running wheel that were frequently shown by the *CYLN2*-mutants (data not shown).

### Discussion

In this paper we describe a subject with an atypical deletion in the Williams Syndrome critical region, but without any of the common features of Williams Syndrome except for the congenital heart malformation. Genetic analysis showed that subject WS75 had a deletion of genes *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN* and *LIMK* but not of the genes *RFC2*, *CYLN2*, *GTF2IRD1*, and *GTF2I*. The results of the tests performed in this patient and the results of the tests in the mutant mice strongly suggest a role for *CYLN2* or *GTF2I*, rather than *GTF2IRD1*, in the motoric and cognitive features commonly present in Williams Beuren syndrome. The normal motor performance of subject WS75 stands in remarkable contrast to the abnormal motor behaviour of typical Williams patients who show abnormal adaptation of motor control. For instance, saccade dysmetria, which would be consistent with a cerebellar functional deficit, was not observed (Van Der Geest et al., 2005; Van Der Geest et al., 2004). In addition, the prism-adaptation task showed normal adaptive control of hand movements suggesting normal cerebellar functioning with respect to motor learning (Baizer et al., 1999; Deuschl et al., 1996). He also had no problems in walking, descending stairs and stepping over surface changes as can often be observed in WS patients. In addition, the cognitive performance of subject WS75 did not resemble the profile commonly seen in Williams Syndrome with its grossly impaired visual-spatial capacities (Mervis et al., 2000; Bellugi et al., 2000). The boy performed much better in visual-spatial tasks than can be expected in a subject with the full deletion of the WS critical

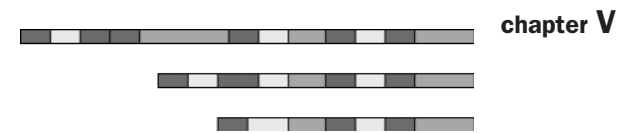
region, even when matched for age. For instance, his score on the Beery-VMI tests was much higher than any of the 53 typical WS subjects, and he even succeeded in performing well in the Line Orientation test and the Trailmaking test. The scores on his visual-spatial memory test were also much better than any of the typical WS subjects.

Our data largely fit in with the results of other partial deletion studies (listed in Table 2), although not all of these studies have described the motor or cognitive behavior in detail (Tassabehji et al., 2005; Doyle et al., 2004; Morris et al., 2003; Heller et al., 2003; Gagliardi et al., 2003; Hirota et al., 2003; Karmiloff-Smith et al., 2003; DelCampo et al., 2002; Korenberg et al., 2000; Tassabehji et al., 1999; Botta et al., 1999a). For example, Karmiloff-Smith and colleagues (2003) as well as Hirota and colleagues (2003) described patients in which the genes centromeric to *CYLN2* are deleted, who had no or mild visual-spatial impairments and a cognitive profile above average. Still, in many of the tests WS75 performed worse than would be expected (Table 1). Thus, our findings in WS75 strongly support the possibility that deletions of *CYLN2* and/or *GTF2IRD1* and/or *GTF2I* play a crucial role in the motoric and cognitive abnormalities of Williams Syndrome (Hoogenraad et al., 2004; Danoff et al., 2004; Morris et al., 2003; Hirota et al., 2003) (Table 2), but they do not rule out the possibility that other genes such *LIMK1* contribute equally as well (Frangiskakis et al., 1996) and they do not allow us to discriminate between the differential contributions of *CYLN2* and *GTF2IRD1*. While the role of *GTF2I* in the cognitive and neurological symptoms of WS is relatively well established (Morris et al., 2003; Hirota et al., 2003), as of yet, no patients are available in which *CYLN2* or *GTF2IRD1* are specifically deleted (Table 2). To investigate the extent in which either of these two genes may contribute specifically to the neuropsychological symptoms in WS, we performed experiments in mutant mice in which either *GTF2IRD1* or *CYLN2* are affected. No major morphological abnormalities of the brain were observed in *GTF2IRD1*-mutants, except for somewhat larger brain ventricles. Also no abnormalities were observed in adult *GTF2IRD1*-mutants with respect to hippocampal and cerebellar functioning. On the other hand, *CYLN2*-mutants showed both morphological brain abnormalities (ventriculomegaly and a smaller corpus callosum) and behavioral abnormalities that can be attributed to deficits in cerebellar and hippocampal functioning. Thus, our results obtained in the mouse mutants suggest that *CYLN2*, rather than *GTF2IRD1*, contributes significantly to the cerebellar and hippocampal dysfunctions in Williams Syndrome. Taken together, the findings in our atypical patient and the mutant mice suggest a prominent, but not a sole, role for *CYLN2* in the motoric and cognitive characteristics of Williams Syndrome.

Table 2: Schematic overview of patients with partial hemizygous deletions. For the full WS population typical milestones of motor development (sitting, walking, and talking, expressed in months) are the averages of 218 patients (Pankau et al., 2000). (Symbol meanings: + = present; - = absent/month; ± = borderline intellectual functioning; A = also described by Tassabehji et al. (1999); B = also described by Frangiskakis et al. (1996); e10 = deletion from exon 10 onward; part = partially deleted; tel = deletion of telomeric portion; pos = possibly deleted; valve = pulmonary valve stenosis; ba = balance adequate; N = normal; Oom = age of onset in months)

The authors are grateful to the boy and his parents for their cooperation to the study. We like to thank the participants (and their parents) in the “Rotterdam/Amsterdam Williams Syndrome Study” and the Dutch “Network Williams Syndroom” for their cooperation. This research was supported by grants from the Netherlands Organization for Scientific Research (NWO; Zon-MW), Neuro-Bsik (Senter), EU (SENSOPAC), Prinses Beatrix Fonds, and by the Revolving Fund of the Erasmus MC.





## General discussion - future perspectives

Williams syndrome (WS) is one of the most common multiple congenital anomalies/mental retardation syndromes with an estimated prevalence between 1: 7.500 and 1:10.000 (Stromme et al., 2002; Morris et al., 1988). Historically, diagnosis of malformation syndromes is based on the clinical identification of a specific pattern of phenotypic abnormalities. Unfortunately, the phenotypical expression of WS is variable and establishing a clinical diagnosis can be difficult. In chapter II we have presented studies that tackle these diagnostic difficulties. Using a smaller number of items than Selicorni and colleagues (Selicorni, 1996) and the Committee on Genetics of the American Academy of Pediatrics (Committee on Genetics, 2001), but including "hyperacusis" and "difficulty descending stairs", we were able to improve diagnosis with respect to older scoring systems. Furthermore, we showed that Multiplex Ligation-dependent Probe Amplification (MLPA) can be a reliable alternative for FISH to detect a deletion of the WS region on chromosome 7q11.23. Finally, we proved that MLPA on buccal smears is feasible as a screening method for (WS) deletions and duplications among adults with an intellectual disability of unknown aetiology (chapter IIc). These improvements are worthwhile because establishing a WS diagnosis enables an appropriate health watch program. One of phenotypic characteristics in WS that tend to be ignored are the commonly observed deficits in motor behaviour, such as an abnormal gait and problems descending stairs and stepping over surface changes. In chapter III we showed that patients with WS also show deficits in oculomotor behaviour and visual depth processing. These studies suggest that part of the motor deficits may have a cerebellar origin. In chapter IV we explored the alleged link between a cerebellar disturbance and the neurological deficits observed in WS. Based on the studies in *CYLN2* and *GTF2IRD1* mouse mutants and an individual with an atypical (smaller) deletion we suggest that the deletion of *CYLN2*, a gene expressed in the cerebellum, has a prominent role in the motoric deficits of people with WS. Furthermore, this study also suggests that the deletion of *CYLN2* is likely to contribute to the neurological and cognitive phenotype of WS. In the remainder of this chapter, we will discuss the diagnosis of WS and the correlation between genes and phenotype in greater detail.

## strategies for establishing a clinical and molecular WS diagnosis

### Clinical diagnosis

In table 1 of the introduction of this thesis the frequencies of medical problems in 59 WS patients have been presented and compared with the frequencies found by the Committee on Genetics of the American Academy of Pediatrics (Committee on Genetics, 2001). According to the Committee, impaired visuospatial constructive cognition can be found in 95% of WS patients. However, the committee does not present a percentage of WS patients that have problems descending stairs or show a reluctance in changing the surface. In our study those percentages were 92 and 46 respectively. Based on these numbers we developed a new scoring system: the Dutch WS Questionnaire. This questionnaire promise to be an easy and reliable diagnostic tool. It is probably also applicable for adults with an intellectual disability living in institutions or community-based homes. However, the oldest WS patient in our research group was a male of 39 years of age (figure 2 introduction). Therefore we have not been able to test the Dutch WS Questionnaire in older adults with WS syndrome. The items used in both steps of our scoring system are probably not influenced by advanced age (Howlin and Udwin, 2006; Cherniske et al., 2004) but knowledge about childhood features may not be available. Further studies to validate our scoring system in older WS patients are indicated.

### Mutational mechanisms of WS deletions and its impact on molecular diagnosis

Several mapping reports shed light on the complex genomic structure of the 7q11.23 region rearranged in patients with WS. Three large region-specific segmental duplications or low-copy repeat elements (centromeric, medial and telomeric LCRs), each composed of three differentiated blocks called "A", "B", and "C", flank the WS common deletion region (Peoples et al., 2000; Valero et al., 2000). Block B in the medial location (Bm) contains three genes: *GTF2I*, *NCF1* and *GTF2IRD2* (Bayes et al., 2003). Corresponding (putative pseudo-)genes are located at the centromeric B block (Bc); *GTF2IP1*, *NCF1P1* and *GTF2IRD2P1*, and telomeric B block (Bt); *GTF2IP2*, *NCF1P2*, and *GTF2IRD2P2*. Most WS patients exhibit a 1.55 Mb deletion caused by recombination between block B copies (Bc-Bm, or Bt-Bm in inversion mediated deletions). "1.55 Mb deletions" can vary in the number of functional copies of *NCF1* and *GTF2IRD2* because the deletion breakpoints are variable. Moreover, *NCF1* has been shown to be functional only from a single locus located in the medial block B (Bm) and *GTF2IRD2* appears to be functional in two of the three copies (Bm and Bt). Only a small number of WS patients displays a larger 1.84 Mb deletion, caused by recombination between centromeric and medial block A copies (Bayes et al., 2003). The *GTF2IRD2* (Bm) locus is always deleted in the 1.84 Mb deletion (figure 1) (DelCampo et al., 2006; Bayes et al., 2003). Because of the variability in deletion size and the occasional occurrence of partial deletions (Edelmann et al., 2006; Howald et al., 2006; Tassabehji et al., 2005; Doyle et al., 2004; Morris et al., 2003; Heller et al., 2003; Gagliardi et al., 2003; Hirota et al., 2003; Karmiloff-Smith et al., 2003; DelCampo et al., 2002; Korenberg et al., 2000; Tassabehji et al., 1999; Botta et al., 1999a) and duplications of the WS critical region (Kriek et al., 2006; Kirchhoff et al., 2006; Somerville et al., 2005) we suggest use of MLPA, instead of FISH with commercial probes, in a diagnostic setting. We also think that it might be worth while to add MLPA-probes for *GTF2IRD1*, *GTF2I*, *NCF1* and *GTF2IRD2* to the diagnostic MLPA kit Salsa P029 (containing probes for *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*) (Chapter IIb).

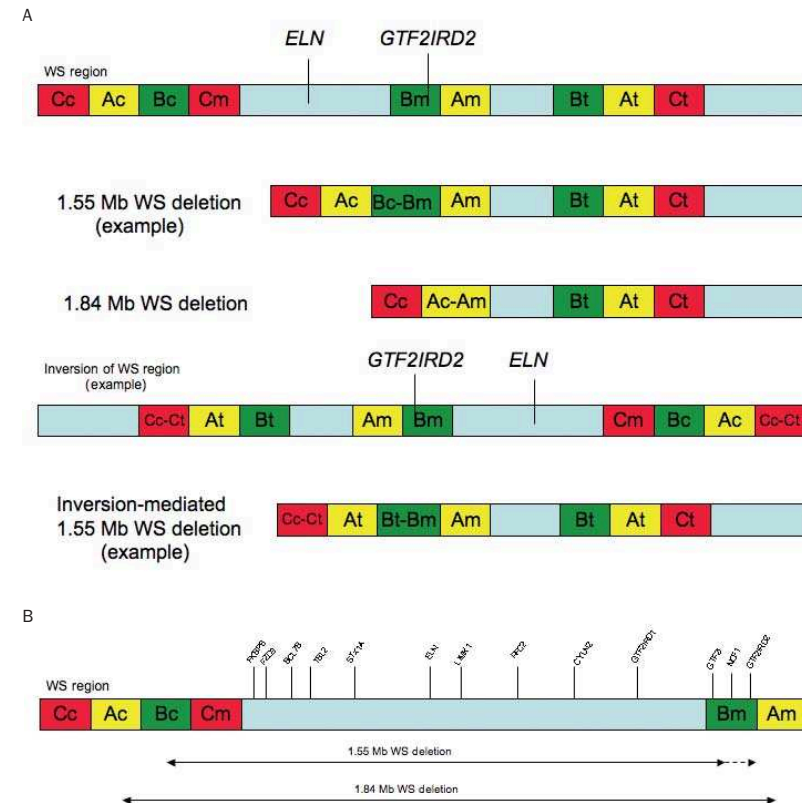
**Figure 1**

A. Mutational mechanisms of WS deletions.

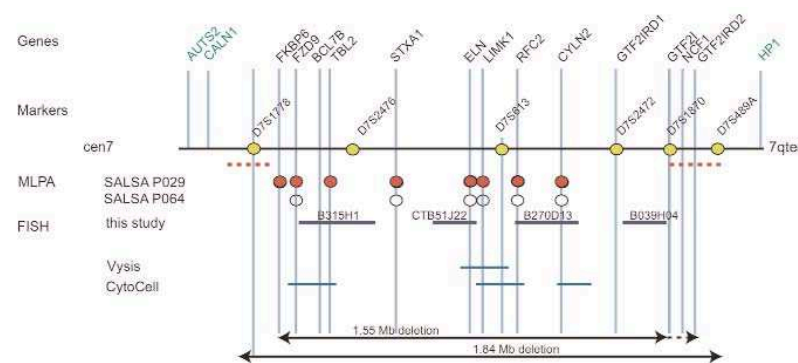
WS deletions can originate by three main mechanisms; 1: recombination between centromeric and medial block B copies (1.55 Mb deletion), 2: recombination between centromeric and medial block A copies (1.84 Mb deletion), 3: recombination between telomeric and medial block B copies in inversion-mediated deletions (1.55 Mb deletion).

B. Physical map of the 1.55 Mb and 1.84 Mb deleted WS region

Centromeric (c), medial (m) and telomeric (t) low-copy repeat elements, each composed of three differentiated blocks "A" (red), "B" (green), and "C" (yellow) are depicted as coloured boxes.



However, for genotype/phenotype correlation analyses it may be worthwhile to add MLPA probes for Huntingtin-interacting protein 1-gene (HIP1), Calneuron 1-gene (CALN1) and Autism susceptibility candidate 2-gene (AUTS2), in order to determine the boundaries of the deletion more precisely (Kriek et al., 2006) (figure 2).

**Figure 2:** WS region and probe positions (not drawn to scale)

In the 1.55 and 1.84 Mb WS deletions these three genes will not be deleted. It is of interest to note that a deletion of *HP1* has been found in a patient with an atypical deletion (2.4-3.1 Mb starting from intron 1 of *GTF2IRD1*) showing some WS features and autism (Edelmann et al., 2006). Haploinsufficiency of *HP1* might be a cause of autism, a characteristic of some WS patients described in the literature. One could speculate about a second locus for WS in this region, especially because an *ELN*-containing deletion has not been found in all patients with a WS phenotype (Lowery et al., 1995).

A disadvantage of the MLPA kit Salsa P029 (even if extended with the probes mentioned above) is that no difference can be made between non-inversion and inversion-mediated deletions. For instance, differentiation between a 1.84 Mb deletion and an inversion mediated 1.55 Mb deletion will not be possible because in both cases *GTF2IRD2* may be deleted. Therefore, for research purposes another technique will be necessary to complement the MLPA findings, e.g., genotyping of paralogous sequence variants, also called "site-specific nucleotides" (SSNs). SSN 11 can be used to detect a gain of a Bt-sequence and a loss of a Bc-type in inversion-mediated deletions (Bayes et al., 2003). Additional information can also be provided by applying interphase FISH to tissue from the parents to detect a 7q11.23 inversion polymorphism.

#### Combining clinical and molecular diagnosis

Identifying the aetiology of MR in individual patients offers a means for health watch programs, parental support and recurrence risk counselling (table 1) (Lewis, 2004; Curry et al., 1997).

**table 1:** The benefits of genetic diagnosis based on Curry and colleagues (Curry et al., 1997)

#### The benefits of genetic diagnosis

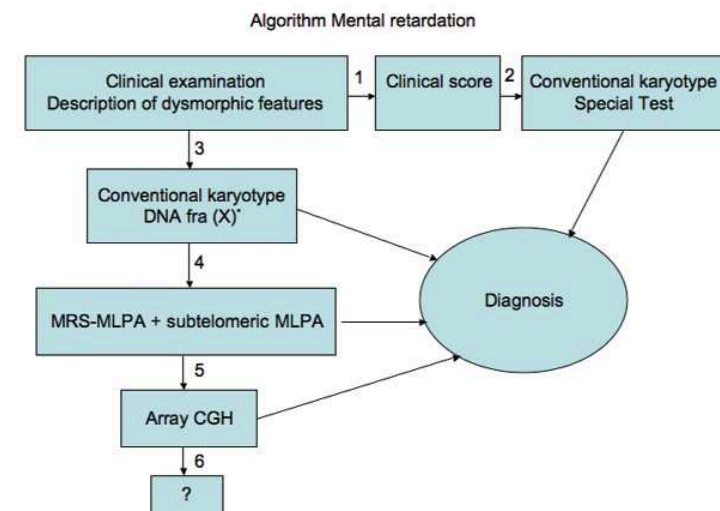
##### For the individual patient

- Identification of appropriate medical and non-medical therapies
- Pre-symptomatic screening for associated complications/functional disabilities
- Educational planning
- Elimination of unnecessary testing and evaluations

##### For the family and caregivers

- Referral to support groups
- Reproductive counselling, carrier testing, prenatal diagnosis
- Family networking

The first step in establishing a reliable diagnosis in a patient suspected of WS is the careful selection of questions followed by a description of dysmorphic features. The dysmorphic features will be revealed by a physical examination or by the use of photographs. The clinical score presented in chapter IIa of this thesis provides a useful tool. The clinical diagnosis may be substantiated using deletion detection, e.g., with an MLPA test, to confirm the diagnosis genetically (chapter IIb). This strategy will eliminate unnecessary tests and evaluations and will save expenses. However, not every WS patient will be suspected of having the disorder. To prevent WS patients from remaining unrecognized, we suggest performing a combination of subtelomeric MLPA and MRS-MLPA in a clinical algorithm for mental retardation (figure 3) (Kirchhoff et al., 2006). The MRS-MLPA kit (SALSA P064 probe set, MRC-Holland) contains probes for 1p36-deletion, Sotos syndrome, Saethre Chotzen syndrome, Williams syndrome, Prader-Willi syndrome, Angelman syndrome, Miller-Dieker syndrome, Smith Magenis syndrome, Alagille syndrome and 22q11 deletion syndrome (Chapter IIc). With this algorithm, a deletion of 7q11.23 and several other rather frequent deletions, e.g., 1p36 deletion (1: 5.000) and 22q11 deletion (1:4.000), will always be detected. The question remains whether patients with a WS phenotype and no deletion 7q11.23 exist. The Dutch WS Questionnaire identified 5 subjects without a deletion as having likely WS but after re-evaluation by 3 medical doctors who participated in the study the diagnosis WS was rejected in all 5 cases. Lowery and colleagues found a deletion in 95% of WS patients (Lowery et al., 1995). In the literature several patients with a WS phenotype and a chromosomal aberration are described (Menko and Stouthart, 1992; Colley et al., 1992). In our opinion the diagnosis WS should be restricted to patients with a 1.55-1.84 Mb deletion of chromosome 7q11.23. In accordance with the literature we think that phenocopies exist.

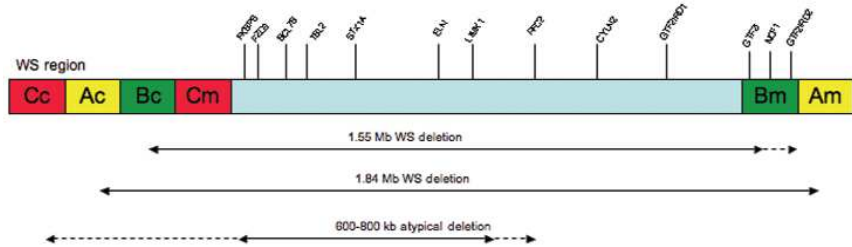
**Figure 3:** Algorithm for mental retardation (Adapted from Kirchhoff et al.). When a clinical diagnosis, e.g. WS, is suspected first a clinical score (if available) and a special test directed towards the diagnosis will be performed: step 1 and 2. If no diagnosis has been established after step 2 or no diagnosis is suspected at all, step 3 will follow. In case no diagnosis is established after step 3, step 4 will follow etc. (Fra(X) = fragile X syndrome, CGH = comparative genomic hybridisation, \* in men/boys *SLC6A8* mutation analysis (X-linked creatine transporter defect) could be considered (Salomons et al., 2003).)

The role of brain related genes from the WS region

Genotype-phenotype correlations

The excessive friendliness, the incapability of putting together puzzles, and the problems descending stairs and stepping over surface changes are all indications that people with WS may have deviant brain functions. It is unlikely that there is a simple one-to-one relationship between genes and brain functions. Moreover, not all genes within the WS critical region are expressed in brain tissue. However, it has been tempting to link specific genes to specific neurological functions (see table 3 of the introduction). Here we will review the current knowledge about the relationships between genes and brain function in WS. In this context, especially the studies we performed in a boy with an atypical deletion proved very valuable. The boy had a deletion of genes *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN* and *LIMK1* but not of the genes *RFC2*, *CYLN2*, *GTF2IRD1* and *GTF2I* (figure.4).

Figure 4: WS region showing the 1.55 Mb deletion, 1.84 Mb deletion and the atypical deletion of the boy described in this thesis



**FZD9** is selectively expressed in the hippocampus. Since *FZD9* knockout mice show severe deficits on tests of visuospatial learning and memory, *FZD9* is considered a contributing factor to the neurodevelopmental and behavioral phenotype of WS patients. The patient with the atypical deletion presented in chapter IV, however, had a deletion of *FZD9*, but did not have neurological problems or severe deficits on tests of visuospatial domains. Furthermore, studies in another patient with a partial deletion including *FZD9* also suggest that haploinsufficiency of this gene has no visuospatial effects (Karmiloff-Smith et al., 2003). Therefore, we think that the ideas about the function of *FZD9* are still inconclusive.

**STX1A** is prominently expressed in the brain. However, it has probably no role in the neurological symptoms found in WS patients. This is in accordance with the studies performed in atypical deletion patients. For instance, our patient of chapter IV has a deletion of *STX1A* but no neurological symptoms.

**LIMK1** regulates dynamic aspects of the cytoskeleton of the cell via the actin filament system. It has been suggested that *LIMK1* is linked to the striking impairment in visuospatial skills that is found in WS patients, but the *LIMK1* story is confusing: researchers have identified individuals missing one copy of the gene who show none of the WS cognitive defects (Gray et al., 2006). The patient with the atypical deletion we tested also had just one copy of *LIMK1* but performed much better in visual-spatial tasks than is expected in WS patients. Because of these results we think that *LIMK1* is no longer a candidate gene for the visuospatial problems found in WS patients.

**CYLN2** is expressed in the cerebellum and regulates dynamic aspects of the cytoskeleton of the cell through the microtubule network (Hoogenraad et al., 2004). The neurological abnormalities in WS include hypotonia, hyperreflexia, problems descending stairs and stepping over surface changes, and motor coordination

deficits with respect to eye and hand movements. These abnormalities suggest that cerebellar dysfunction may underlie (part of) the behavior of WS patients (Morris et al., 1990). As discussed in chapters III and IV of this thesis we think that diminished function of CLIP 115, the protein encoded by *CYLN2*, plays a major, but not an exclusive, role in the motor and cognitive characteristics of WS. Evidence for this hypothesis was found by analysis of the atypical deletion patient. This patient (no *CYLN2* deletion) had no motor problems, e.g. no problems walking stairs. The hypothesis was strengthened by studies of mice models in which single genes within the WS critical region (*CYLN2* or *GTF2IRD1*) had been deleted. The *CYLN2* knockout mice displayed an enlarged ventricle size and decreased corpus callosum size in the brain, and behavioural deficits including typical motor coordination defects. The *GTF2IRD1* mouse mutants had enlarged ventricles and a normal corpus callosum size. They did not show any motor deficits when walking on an accelerating rotarod. CLIP-115 is also prominently expressed in the hippocampus and amygdala (Hoogenraad et al., 2004; Hoogenraad et al., 2002; De Zeeuw et al., 1997). In WS patients reduced activation of the amygdala for threatening faces and increased activation of the amygdala for threatening scenes is noted (Meyer-Lindenberg et al., 2005). *CYLN2* knockout mice have altered fear responses (Hoogenraad et al., 2002). The boy with the atypical deletion (no *CYLN2* deletion) had a shy appearance. Combining all these findings one could speculate about a role for *CYLN2* in the diminished fear of strangers, and the increase of other fears and phobias in WS.

**GTF2IRD1 and/or GTF2I** are ubiquitously expressed and belong to a family of transcription factors. In accordance with the literature and the results we found in the atypical deletion patient, hemizygosity of these genes could contribute to impaired visuospatial construction, an overly friendly personality accompanied by excessive non-social anxiety, and language delay. However, *GTF2IRD1* is not prominently expressed in the hippocampus and amygdala and *GTF2IRD1* mouse mutants have no altered fear responses (this thesis). Therefore, we conclude that the diminished fear of strangers and fears and phobias in WS are probably not caused by haploinsufficiency of *GTF2IRD1*. It is puzzling that in WS patients increased expression of *GTF2IRD1* is found in transformed lymphocytes and decreased expression of *GTF2IRD1* is found in cultured skin fibroblast (Merla et al., 2006; Edelmann et al., 2006). In summary, our studies elucidate the possible role of some of the deleted genes that are expressed in the brain. Our ideas on the presumed effects of the deletion of specific genes are listed in table 2.

Table 2: Presumed neurological function of several brain related genes from the WS region

Gene	Neurological effect of haploinsufficiency
<i>FZD9</i>	None
<i>STX1A</i>	none
<i>LIMK1</i>	None
<i>CYLN2</i>	Role in the motor and cognitive characteristics of WS Diminished fear of strangers Fears and phobias
<i>GTF2IRD1</i>	Impairment in cognitive development Impaired visuospatial construction Language delay
<i>GTF2I</i>	Mental retardation Impaired visuospatial construction Diminished fear of strangers Fears and phobias

*Friendly faces, unusual minds, genes and environment*

In the previous part of this discussion we attempted to make a genotype-phenotype correlation for several brain-related genes from the WS region. However, due to the complexity of the brain it is not to be expected that single symptoms can be pinpointed to single genes. The genes in the WS region are probably interacting among themselves and with other genes in innumerable ways and it is also possible that normal-copy genes that map close to the deletion are candidate genes for the cognitive and motor features. Merla and colleagues proved that normal-copy neighboring genes can have decreased expression (Merla et al., 2006). They especially found this phenomenon for the genes HIP1, POR and KCTD7. Genetic variants in the nondeleted allele, subtle imprinting effects and the genetic background may also contribute to the complex neurocognitive profile of WS.

The environment is probably also a factor in the WS phenotype. Since the genes influence social behaviour very early on in WS individuals, their unusual social behaviour in turn is likely to construct an abnormal social environment - that is, other people will socially interact in a different way with a WS child than with a child without the syndrome (Bhattacharjee, 2005).

**A look at the (laboratory) future**

In a previous part of this discussion we presented an algorithm for mental retardation (fig 3). Genome-wide array CGH is a further step in this algorithm. This technique is still expensive and laborious, and validation is difficult and time-consuming. Moreover, a second technique is necessary to confirm the array CGH findings and parental investigations are required to determine the meaning of the results. For these reasons this new technique is not yet in standard practice. However, this will probably change in the near future. When array CGH will be less expensive and much easier to use, it probably will be "a routine-test" for patients with mental retardation. With this technique (atypical) WS deletions will be detected, even if this diagnosis had not been considered. However, linking molecular to clinical phenotype will remain essential.

**Recommendations for future genetic counselling**

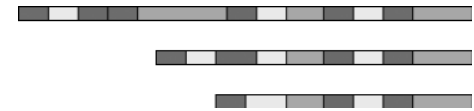
After establishing a WS diagnosis, genetic counselling is necessary. For the individual patient it will probably become more and more important to determine the deletion size as exactly as possible. There are indications that the loss of a functional copy of NCF1 protects a proportion of WS patients from hypertension, probably through a lifelong reduction of angiotensin II-mediated oxidative stress. Therefore, anti-NADPH agents, inhibitors of the angiotensin-converting enzyme and blockers of the angiotensin II receptor could all have a specific indication in WS patients (without a NCF1 deletion) who suffer from hypertension (DelCampo et al., 2006). In the future more knowledge about the function of other genes from the WS region will become available and it is reasonable to assume that this knowledge will also influence the treatment of the individual WS patient.

In addition, it will probably be worthwhile to determine the origin (maternal or paternal) and the mutational mechanism of the deletion. Genomic imprinting that affects genes in the WS region might play a role in determining head size (DelCampo et al., 2006; Perez Jurado et al., 1996).

In our research group of 59 WS patients all patients were sporadic. Until now it has not been clear whether parents with an inversion of the WS region have a higher recurrence risk than parents without the inversion. This issue can probably be resolved in the near future if additional investigations in parents are performed.

**Conclusions and prospects**

In this thesis we have studied various aspects of WS. People with WS are very special and their social behaviour may be an example to many of us. A correct diagnosis is important for correct medical guidance. We have presented some tools to make a clinical and molecular diagnosis. We have also looked at genotype-phenotype correlations especially regarding *CYLN2*, a gene from the WS region. Hopefully, in the near future WS patients will be recognized at a younger age and older, so far unrecognized patients will have a correct diagnosis at last.



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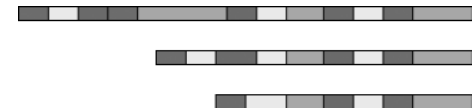
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**Summary**

**Samenvatting**



## Summary

Williams syndrome (WS, MIM 194050) is a neurodevelopmental disorder caused by a submicroscopic deletion that includes the elastin gene (*ELN*), on chromosome band 7q11.23. Our knowledge on WS is still limited, despite the growing body of research in the last years. In the first chapter of this thesis (General Introduction) an overview is presented on our current understandings of WS, with respect to the history, the clinical features, diagnosis, neurogenetics and medical management. Clinically, WS is associated with dysmorphic facial features, supravalvular aortic stenosis and other cardiovascular diseases, growth retardation, hyperacusis and intermittent hypercalcaemia. The mean IQ of individuals with WS is low, about 60 (between 40-100). Their cognitive profile, however, is unevenly distributed. It is characterized by relatively good verbal and face recognition abilities alongside a low level of spatial and constructive organisation. They tend to show overly friendly behaviour to strangers and display a general lack of social judgement. Furthermore, individuals with WS have problems descending stairs and stepping over surface changes. They also tend to show extreme anxiety in new situations when unexpected things might happen.

The three central questions of the studies described in this thesis are: 1. Can we improve the clinical and molecular diagnosis of WS?; 2. Can we quantify the deficits in motor behaviour in WS?; 3. Can we establish a link between the deleted genes and the phenotype in WS?

We have looked into both clinical as well as genetic aspects of the diagnosis of WS. The detection of a deletion of *ELN* can confirm the clinical WS diagnosis, but genetic deletion detection is invasive, time consuming and relatively expensive. Therefore the clinical diagnosis is important. However, existing scoring systems for clinical diagnosis are rather elaborative.

In **chapter IIa** we evaluated two existing scoring systems in a Dutch population of 69 individuals and aimed to develop a new questionnaire for the clinical diagnosis of WS. The Dutch WS questionnaire indicated deletion detection for all individuals with a WS deletion, whereas both other scoring systems missed some WS individuals. We conclude that the Dutch WS Questionnaire may provide a simple and easy tool in diagnosing Williams Syndrome using 10 easy questions.

In **chapter IIb** we investigated whether Multiplex Ligation-dependent Probe Amplification (MLPA) is a reliable alternative for Fluorescence In Situ Hybridisation (FISH), which is commonly used to detect the deletion in WS for diagnostic purposes. The MLPA kit (SALSA P029) contains probes for eight genes in the WS critical region: *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* and *CYLN2*. The experimental FISH assay that was used consist of four probes covering the WS critical region. A total number of 63 individuals was tested; in 53 individuals, a deletion was detected both with FISH and MLPA (P029), in ten individuals both techniques failed to demonstrate a deletion. In only one individual, a deletion was detected which was not previously detected by two commercial FISH probes. This individual appeared to carry a small, atypical deletion. We conclude that MLPA is a reliable technique to detect WS. Compared with FISH, MLPA is less time-consuming and has the possibility to detect also smaller, atypical deletions and duplications in the WS critical region.

Because the genetic cause of WS is known only since 1993, elderly individuals may be unrecognized or wrongfully diagnosed. The stress of a necessary venapuncture is often mentioned as a prevailing reason for individuals with mental retardation not to consent to genetic testing. In **chapter IIc** we investigated whether detection of deletions in DNA samples from an oral swab can be used as a non-invasive technique in adult institutionalised individuals. In a random sample of 300 adult clients of Dutch intellectual disability services without an aetiological diagnosis, DNA was collected on site using oral swabs. MLPA was applied to screen for nine microdeletions/duplications related to mental retardation syndromes (Williams, 22q11 deletion, 1p-deletion, Miller-Dieker, Smith-Magenis, Prader-Willi, Alagille, Saethre-Chotzen and Sotos syndrome). Prior to the consent procedure, for 2.1% (10/471 eligible participants) the method was considered undesirable.

In 0.7% (2/300 participants) oral swabs failed due to resistant behaviour, while in 16.1% (48/298 swabs) analysis was unsuccessful due to insufficient amounts of DNA. A repeated attempt yielded an equal success rate. Microdeletions were diagnosed in 4 participants: 22q11 deletion (n=2), 5q35 deletion (Sotos-syndrome) (n=1) and 1p deletion (n=1). One participant had a duplication of the Prader Willi-region (15q11-13) due to mosaicism of a supernumerary marker chromosome 15. We conclude that oral swabs are a feasible method for collecting DNA to be used in MLPA-based genetic tests in adults with mental retardation.

An abnormal gait, problems descending stairs and stepping over surface changes, are commonly described deficits in the motor behaviour of individuals with WS. However, the background of these phenotypical characteristics in WS have so far not been adequately explained. Numerous studies have described the poor visuo-spatial processing capacities of individuals with WS. We hypothesized that the deficits in motor behaviour are related to the deficits in visuo-spatial performance.

Saccades (very fast goal-directed movements of the eyeball) play a very important role in visuo-spatial processing. Therefore the phenotypical characteristics in motor behaviour in WS might be related to impairments in the control of the accuracy of saccadic eye movements in WS. In **chapter IIIa** we looked into the saccadic eye movements of individuals with WS. We recorded horizontal and vertical saccadic eye movements to targets using infrared video-oculography in 27 individuals with WS and eight healthy controls. In the WS group saccadic gains were highly variable, both between and within individuals, and they often needed more than one correction saccade to reach the target. Ten (out of a subgroup of 22) WS individuals showed a large number of hypometric and/or hypermetric saccades, and, also a left-right asymmetry in saccadic gains was observed in WS. We conclude that the observed impairments in saccadic control are likely to affect the proper processing of visuo-spatial information and therefore contribute to the motor deficits in WS.

In **chapter IIIb** we tested to what extent the motor problems might be related to a deficit in the perception of visual depth. Monocular and binocular visual depth perception (stereopsis) was tested in 33 individuals with WS. Furthermore, hand movements to a target were recorded both with and without visual feedback of the position of the hand. The results of the WS group were compared to those of a group of control individuals. Although WS individuals were able to perceive monocular depth cues that require global processing, about 49% failed to show stereopsis. With visual feedback of the hand position both the control group and the WS group moved the hand straight to the target. In contrast to the control group, however, the average hand movement of individuals with WS went over the target when they could not see their hand. Although individuals with WS can derive depth from complex spatial relationships between objects, they seem to be impaired in using this depth information for guiding their movements when deprived of visual feedback. Therefore, we conclude that motor problems of WS individuals with tasks such as descending stairs are not due to the inability to judge distance.

Several of the 25-30 deleted genes in WS are expressed in the brain, including those encoding cytoplasmic linker protein-115 (*CYLN2*) and general transcription factors (*GTF2I* and *GTF2IRD1*). These specific genes may therefore contribute to the distinct neurological and cognitive deficits in WS individuals.

Recent publications about individuals with partial deletions indicate that hemizygosity of *GTF2I* probably contributes to mental retardation in WS. Our studies showed that the deficits in motor behaviour in WS might be related to impairments in the control of the accuracy of saccadic eye movements in WS.

The cerebellar oculomotor vermis is involved in the fine-tuning of saccades and *CYLN2* is expressed in the cerebellum. Therefore we suggested that haploinsufficiency of *CYLN2* might contribute to the saccade dysmetria.

In **chapter IV** we further investigated this hypothesis. In order to determine whether *CYLN2* or *GTF2IRD1* contributes to the motoric and cognitive deficits in WS, we compared the characteristic features of known WS individuals with the features of a male individual with an atypical deletion, including *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN* and *LIMK1* but not *RFC2*, *CYLN2*, *GTF2IRD1* and *GTF2I*. The male individual with the atypical deletion did not show any of the commonly described deficits in motor behaviour of individuals with WS. Furthermore, he was able to make accurate saccadic eye movements between small targets and performed significantly better on most used verbal and visual-spatial psychometric investigations than a group of WS individuals. These findings support the idea that haploinsufficiency of *CYLN2* and/or *GTF2IRD1* play a crucial role in the motoric and cognitive characteristics of WS. Because there are no known individuals with specific deletions of either *CYLN2* or *GTF2IRD1*, we compared the phenotype of mouse mutants of *CYLN2* to that of *GTF2IRD1*-mutants, to determine whether hemizygosity of *CYLN2* or *GTF2IRD1* contributes to the motor dysfunction of individuals with WS. In *GTF2IRD1*-mutants neither motor dysfunctioning or major morphological abnormalities of the brain (except somewhat larger brain ventricles) were observed. *CYLN2*-mutants, however, showed both morphological brain abnormalities (ventriculomegaly and a smaller corpus callosum) and motor coordination dysfunction. Therefore *CYLN2* (and not *GTF2IRD1*) seems to contribute significantly to the motor coordination dysfunction of individuals with WS.

## Conclusion

We have developed a simple and easy-to-use clinical scoring system for WS, and showed that MLPA and DNA samples from oral swab can be used to confirm this clinical diagnosis. The quantification of the motor deficits enabled us to elucidate the specific contributions of several of the deleted genes, such as *CYLN2*, to the behaviour of individuals with WS. These findings could aid in establishing correct diagnoses and improve genetic counselling of WS.

## Samenvatting

Het Williams syndroom (WS, MIM 194050) is een ontwikkelingsstoornis die veroorzaakt wordt door een deletie van verscheidene genen, waaronder het elastine-gen (*ELN*), gelegen in chromosoomband 7q11.23. Het aantonen van een deletie van *ELN* geldt momenteel als bevestiging van de diagnose WS. Hoewel de kennis over WS gedurende de afgelopen jaren is toegenomen, is nog steeds veel onbekend. In het eerst hoofdstuk van dit proefschrift hebben we een algemeen overzicht van de huidige inzichten gegeven met betrekking tot de geschiedenis, de kenmerken, de wijze van diagnosticeren, de genen die mogelijk de neurologische kenmerken veroorzaken en de richtlijnen omtrent de medische begeleiding. Klinisch wordt WS gekenmerkt door een bijzonder gelaat, supravulvulaire aortastenose en andere cardio-vasculaire afwijkingen, groei-achterstand, hyperacusis en intermitterende hypercalciëmie. Het gemiddelde IQ van mensen met WS is laag (ongeveer 60) en varieert tussen 40 en 100. Mensen met WS hebben een bijzonder ontwikkelingsprofiel: de taalvaardigheid en het vermogen gezichten te herkennen zijn relatief goed ontwikkeld, terwijl het ruimtelijk inzicht relatief slecht is ontwikkeld. Daarnaast zijn zij opvallende vriendelijk naar onbekenden. Zij vinden het moeilijk om de trap af te lopen en hebben moeite met een wisseling van de ondergrond. In onverwachte situaties kunnen mensen met WS extreem angstig zijn. Dit proefschrift beschrijft verschillende onderzoeken binnen een cohort van 74 mensen met (mogelijk) WS. Bij deze onderzoeken stond allereerst de vraag centraal of de klinische en moleculaire diagnose WS verbeterd kon worden. Vervolgens is bepaald of de motorische kenmerken van mensen met WS in een objectieve maat uitgedrukt konden worden. Tot slot is nagegaan of er een verband gelegd kon worden tussen de deletie van bepaalde genen en het WS-fenotype. De definitieve diagnose van WS kan alleen gesteld worden door (cyto-)genetische-analyse. De kosten hiervan zijn echter hoog, de procedure arbeids-intensief en het noodzakelijke bloedprikken wordt veelal als onaangenaam ervaren. Met klinische diagnostiek kan een goede inschatting worden gemaakt of dit alles geïndiceerd is. Als hulpmiddel bij het stellen van een diagnose zijn diverse score-lijsten ontwikkeld. Deze score-lijsten blijken in de praktijk echter te bewerkelijk te zijn.

In **hoofdstuk IIa** hebben we twee bestaande scoresystemen geëvalueerd binnen een Nederlandse populatie van mensen met (mogelijk) WS. Tevens hebben we een nieuwe score-lijst ontwikkeld: "The Dutch WS Questionnaire" (Nederlandse Vragenlijst voor Williams Syndroom). Bij alle mensen met een deletie bleek de indicatie voor het aanvullend onderzoek met behulp van deze Questionnaire goed te zijn ingeschat. Bij gebruik van de twee bestaande score-lijsten kwam bij een aantal mensen met een deletie de indicatie voor aanvullend onderzoek niet naar voren. We menen dan ook met de Dutch WS Questionnaire een eenvoudige 10 items bevattende score-lijst te hebben ontwikkeld die bruikbaar is voor de klinische WS diagnostiek.

In **hoofdstuk IIb** hebben we onderzocht of de genetische techniek Multiplex Ligation-dependent Probe Amplification (MLPA) een betrouwbaar alternatief is voor Fluorescentie In Situ Hybridisatie (FISH), de techniek die door de meeste diagnostische laboratoria wordt gebruikt. De MLPA kit (SALSA P029) bevat probes voor 8 genen uit de WS regio: *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN*, *LIMK1*, *RFC2* en *CYLN2*. De experimentele FISH assay die werd gebruikt bevat 4 probes die samen de gehele WS regio omvatten. In totaal werden 69 mensen getest. In 53 mensen werd zowel met FISH als met MLPA (P029) een deletie aangetoond. In 10 mensen werd met geen van beide technieken een deletie ontdekt. In één persoon werd een deletie aangetoond, die niet eerder ontdekt was met twee commerciële FISH probes. Deze persoon bleek uiteindelijk een kleine, atypische deletie te hebben. Onze conclusie is dan ook dat MLPA een betrouwbare techniek is om WS te diagnosticeren. Vergeleken met FISH is de MLPA-techniek minder tijdrovend. Daarnaast kan deze techniek zowel atypische deleties als duplicaties van de WS regio aantonen. De genetische oorzaak van WS is pas sinds 1993 bekend. Daardoor zijn er mogelijk oudere personen met

WS die niet als zodanig zijn gediagnosticeerd of bij wie ten onrechte de diagnose WS is gesteld. Genetisch onderzoek naar de aanwezigheid van een WS deletie gebeurt in de regel in bloed. Spanning van de patient rondom een bloedafname lijkt vaak een reden om van dit onderzoek af te zien. In **hoofdstuk IIc** hebben we onderzocht of deleties opgespoord kunnen worden met behulp van MLPA op DNA dat uit wangslimvlies wordt verkregen. In een a-selectie steekproef van 300 volwassenen met een verstandelijke handicap zonder oorzakelijke diagnose, wonend in voorzieningen voor mensen met een verstandelijke beperking, is wangslimvlies afgenomen. Vervolgens is DNA-onderzoek verricht met behulp van de “Mental Retardation 1 MLPA kit” die probes bevat voor 1p (1p- deletie syndroom), 5q35 (Sotos syndroom), 7p21 (Saethre-Chotzen syndroom), 7q11.23 (Williams syndroom), 15q11-13 (Prader Willi-/Angelman syndroom), 17p11 (Smith-Magenis syndroom), 17p13 (Miller-Dieker syndroom), 20p12 (Alagille syndroom) en 22q11 (22q11-deletie syndroom). Voorafgaand aan de consent-procedure werd voor 2.1% (10/471 mogelijke deelnemers) de methode als onwenselijk aangeduid. Bij 0.7% (2/300 deelnemers) was het door “verzet” niet mogelijk wangslimvlies af te nemen, terwijl bij 16.1% (48/298 wangslimvliessaftnames) DNA-diagnostiek mislukte omdat er een onvoldoende hoeveelheid DNA beschikbaar was. Bij een tweede afname was het succespercentage identiek. Alhoewel tijdens dit onderzoek geen mensen met WS opgespoord zijn, werden wel één persoon met 1p- deletie syndroom, één persoon met Sotos syndroom, één persoon met een duplicatie van de 15q11-13 regio veroorzaakt door een mozaïek marker-chromosoom 15 en twee personen met een 22q11 deletie syndroom ontdekt. Op basis van dit onderzoek kan geconcludeerd worden dat MLPA op DNA verkregen door middel van een wangslimvlies-afname een geschikte methode is voor het screenen op deleties (en duplicaties) bij volwassenen met een geestelijke handicap.

Motorische problemen die voor kunnen komen bij mensen met WS zijn een opvallende manier van lopen met naar voren gebogen schouders en problemen met traplopen en overgangen van ondergrond. De oorzaak van deze motorische problemen is nog niet bekend. Wel is uit een groot aantal onderzoeken gebleken dat mensen met WS een verminderd ruimtelijk inzicht hebben. Zij hebben bijvoorbeeld vaak moeite met het maken van puzzels of het vinden van de weg. Daarom veronderstelden we dat de motorische problemen van mensen met WS verband houden met het verminderde ruimtelijke inzicht. Saccades (zeer snelle bewegingen van de ogen naar een bepaald doel) spelen een belangrijke rol bij het verwerken van visuele informatie over de ruimte om ons heen. Problemen met de aansturing van saccades zou mogelijk kunnen bijdragen aan een slechtere verwerking van deze visuele informatie en leiden tot een verminderd ruimtelijk inzicht.

In **hoofdstuk IIIa** van dit proefschrift hebben we saccades van mensen met WS nauwkeurig onderzocht. Van 27 mensen met WS en 8 mensen zonder verstandelijke handicap zijn de saccadische oogbewegingen gericht op een doel (stip) met behulp van een infrarood videocamera geregistreerd. De deelnemers werden daarbij aangespoord om tussen twee zwarte stippen op een grijze achtergrond heen en weer te kijken. In de WS groep bleek dat de amplitudes (A) van de saccades ten opzichte van de afstand tussen het startpunt van de saccade en de positie van de stip (D) erg variabel waren. Deze grotere variabiliteit van de “gain (A/D)” werd gevonden zowel tussen WS personen onderling als tussen de saccades van elke individuele deelnemer afzonderlijk. Daarnaast viel op dat mensen met WS vaak meer dan een correctie-saccade nodig hadden om het doel te bereiken. In 10 personen met WS (voortkomend uit een subgroep van 22) werd een groot aantal te kleine of te grote saccades gemeten. Op grond van de gedane metingen kan de conclusie getrokken worden dat de motorische problemen van WS mogelijk deels veroorzaakt worden door afwijkende oogbewegingen. Deze afwijkingen zouden veroorzaakt kunnen worden door een stoornis in de functie van de kleine hersenen (cerebellum) die betrokken zijn bij de precieze controle van de nauwkeurigheid van saccadische oogbewegingen.

Onze conclusie aangaande de saccades is in overeenstemming met de bevindingen van het onderzoek dat in **hoofdstuk IIIB** van dit proefschrift wordt beschreven. In dit onderzoek is onderzocht of de opvallende

motoriek van mensen met WS mogelijk te maken heeft met het beperkt zien van visuele diepte. In 33 personen met WS hebben we gekeken naar hun vermogen om visuele diepte te kunnen waarnemen met één oog (monoculair) en twee ogen (binoculaire). Daarnaast werden handbewegingen naar een bepaald doel geregistreerd in twee verschillende test-situaties: met of zonder visuele informatie over de positie van de hand (wel of geen feedback). De bevindingen van de WS groep werden vergeleken met bevindingen in een eerste controle-groep bestaande uit 23 mensen zonder verstandelijke handicap en een tweede controle-groep bestaande uit 5 mensen met een verstandelijke handicap. De mensen met WS bleken normaal monoculaire diepte informatie te kunnen waarnemen. Het diepte zien met twee ogen bleek echter in 49% van de personen niet voldoende ontwikkeld te zijn. Daarnaast vonden wij dat mensen met WS hun handen voorbij het doel bewogen als zij geen visuele informatie kregen over de positie van hun hand, terwijl mensen uit de twee controle groepen hun hand voor het doel lieten stoppen. Wanneer er wel visuele feedback aanwezig was (zowel de hand als het doel was dus zichtbaar), bewoog iedereen precies naar het doel toe. Mensen met WS zijn dus in staat om diepte te zien door naar de onderlinge verhoudingen van objecten te kijken. Zonder visuele feedback blijken zij echter niet in staat om de informatie over de ruimte te gebruiken om een handbeweging aan te sturen. Uitgaande van een analogie tussen de handbewegingen en het traplopen kan geconcludeerd worden dat de motorische problemen van mensen met WS niet veroorzaakt worden door het feit dat zij helemaal geen diepte kunnen zien.

Een aantal van de 25-30 genen, die bij mensen met WS ontbreken, komen tot expressie in de hersenen. Hieronder zijn de genen *CYLN2*, *GTF2I* en *GTF2IRD1*. De deletie van deze genen (haploinsufficiëntie) kan dus mogelijk bijdragen aan de neurologische en cognitieve verschijnselen van mensen met WS. Recentere publicaties hebben aangetoond dat een deletie van *GTF2I* waarschijnlijk bijdraagt aan de verstandelijke handicap die aanwezig is in de meeste mensen met WS. Onze onderzoeken toonden aan dat de motorische problemen van mensen met WS mogelijk gerelateerd zijn aan een stoornis in het functioneren van de kleine hersenen (cerebellum), die onder andere tot uitdrukking komt door de afwijkingen in de aansturing van saccades. Daarnaast heeft de vermis van het cerebellum bij mensen met WS een relatief groot volume en komt *CYLN2*, een van de genen uit de WS regio, tot expressie in het cerebellum. Op basis van deze drie bevindingen is gesteld dat haploinsufficiëntie van *CYLN2* mogelijk een cerebellaire functiestoornis veroorzaakt. Deze zal zich op functioneel niveau uiten in een stoornis van de oogbewegingen en op klinisch niveau in problemen met traplopen, aarzeling bij wisseling van ondergrond en een weerzin tegen een zandige bodem. In **hoofdstuk IV** van dit proefschrift is deze hypothese nader onderzocht. Het gedrag en de symptomen van een jongen met een atypische, gedeeltelijk deletie (deletie van *FKBP6*, *FZD9*, *TBL2*, *STX1A*, *ELN* en *LIMK1* maar niet van *RFC2*, *CYLN2*, *GTF2IRD1* en *GTF2I*), werden vergeleken met die van mensen met WS, waarbij de deletie totaal is. Daarbij viel op dat de jongen met de atypische deletie geen problemen had met (trap-)lopen en het stappen over een wisseling van ondergrond. Ook maakte hij normale saccades en presteerde hij beter op de neuropsychologisch testen van verbale vaardigheden en ruimtelijke inzicht.

Al deze bevindingen ondersteunen de hypothese dat haploinsufficiëntie van *CYLN2* en/of *GTF2IRD1* een cruciale rol speelt in de motorische en cognitieve verschijnselen van WS. Omdat er geen mensen met een specifieke deletie van *CYLN2* of *GTF2IRD1* beschikbaar zijn voor onderzoek, besloten we om testen te ontwikkelen waarmee het fenotype van *CYLN2*- en *GTF2IRD1*-mutante muizen met elkaar vergeleken kon worden. Behoudens wat vergrote ventrikels werden geen anatomische hersenafwijkingen gevonden in *GTF2IRD1*-mutante muizen. Ook werden geen motorische problemen geobserveerd in volwassen mutante *GTF2IRD1*-muizen. In *CYLN2*-mutante muizen werden echter zowel vergrote ventrikels en een dun corpus callosum als problemen met de motoriek geconstateerd tijdens het lopen en een rad. De resultaten van alle bovengenoemde onderzoeken suggereren dat haploinsufficiëntie van *CYLN2* (en niet van *GTF2IRD1*) sterk bijdraagt aan de speciale motoriek die gevonden wordt in mensen met WS.

### **Conclusie:**

In dit proefschrift beschrijven we een eenvoudig en makkelijk te gebruiken score-lijst voor de klinische diagnose WS. Daarnaast hebben we aangetoond dat MLPA op DNA verkregen uit bloed of wangslijmvlies een goede techniek is om de klinische diagnose door middel van een detectie van de deletie te bevestigen. Door oogbewegingsonderzoek hebben we een objectieve maat gevonden voor de motorische problemen in WS. Hierdoor hebben we ook meer helderheid gekregen over de bijdrage van sommige gedeleteerde genen aan de neurologische verschijnselen van het WS. Mogelijk dat deze bevindingen kunnen bijdragen aan het correct diagnosticeren van het WS en de genetische counseling kunnen verbeteren.

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## Curriculum Vitae

Annet van Hagen was born on June 5th 1963 in Amsterdam, the Netherlands. After finishing secondary school (Gymnasium B) she went on to study medicine at the Medical Faculty of the Erasmus MC, Rotterdam. She graduated in February 1988 and started to work at the department of Internal Medicine at the Medical Center Alkmaar (MCA). Next she moved to Eindhoven, where she worked in the departments of Pediatrics and Neonatology of the St. Joseph Hospital. From 1990 until 1992 she continued working in Neonatology at the Leiden University Medical Center (LUMC ). In these years she obtained a great interest in congenital malformations and hereditary disorders. In October 1992 she started working at the department of Clinical Genetics of the VU University medical center in Amsterdam and in January 1994 she started her training as a clinical geneticist at this hospital. She was registered as a clinical geneticist in July 1997. From 1997 until present she works as a staff member at the department of Clinical Genetics of the VU University Medical Center, Amsterdam. Her main interest is dysmorphology and in 2000 she started her PhD-project "Williams syndrome: from genes to clinical features", in collaboration with the departments of Neuroscience and Clinical genetics of the Erasmus MC Rotterdam, which resulted in this thesis. Annet has been a member of the multidisciplinary team for patients with cleft lip and palate for many years. She also is involved in the training of medical students, residents in clinical genetics, pediatricians and dentists. Next to her clinical and research activities she is a member of the board of the Vereniging Klinische Genetica Nederland (VKGN). Since 1998 she participates in a genetic clinic at the Medical Center Alkmaar, the hospital in which she started her career.



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