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MULIBREY NANISM

Clinical characteristics and pathophysiologic features of growth restriction, insulin resistance and tumour development

Niklas Karlberg

ACADEMIC DISSERTATION

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To the treasures of my life
Susann,
Nicole and Axel

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to by the Roman numbers in the following text. In addition some unpublished new data is presented.

I. Karlberg N, Jalanko H, Perheentupa J, Lipsanen-Nyman M: Mulibrey nanism: Clinical features and diagnostic criteria. *J Med Genet* 2004; 41:92-98.

II. Karlberg N, Jalanko H, Kallijärvi J, Lehesjoki A-E, Lipsanen-Nyman M. Insulin resistance syndrome in subjects with mutated RING finger protein TRIM37. *Diabetes* 2005;54:3577-81.

III. Karlberg N, Jalanko H, Lipsanen-Nyman M. Growth and growth hormone therapy in subjects with Mulibrey nanism. *Pediatrics* 2007;120:e102-11.

IV. Karlberg N, Karlberg S, Karikoski R, Mikkola S, Lipsanen-Nyman M, Jalanko H. High frequency of tumours in Mulibrey nanism. *J Pathol* 2009;218:163-71.

ABBREVIATIONS

ACTH	adrenocorticotropic hormone
AGA	appropriate for gestational age
ALT	alanine aminotransferase
ALS	Acid-labile subunit
APECED	autoimmune polyendocrinopathy-candidasis-ectodermal dystrophy
ASD	atrial septal defect
AST	aspartate aminotransferase
BA	bone age
bp	base-pair
BMI	body mass index
BRCA1	tumour suppressor gene breast cancer 1
BWS	Beckwith-Wiedemann syndrome
CA	chronological age
CdLS	Cornelia de Lange syndrome
CFC	cardio-facio-cutaneous syndrome
CHF	congestive heart failure
CHH	cartilage-hair hypoplasia
CMV	cytomegalo virus
CNC	Carney complex
CNS	central nervous system
CT	computer tomography
DNA	deoxyribonucleic acid
DTD	diastrophic dysplasia
E1	ubiquitin activating enzyme
E2	ubiquitin conjugating enzyme
E3	ubiquitin ligase
ENaC	epithelial Na^+ channel
EPO	erythropoietin
EUGR	extrauterine growth restriction
FAS	fetal alcohol syndrome
GH	growth hormone
GHR	growth hormone receptor
GHRH	growth hormone releasing hormone
HDL	high-density lipoprotein
HECT	homologous to E6-AP carboxy terminus
HIF	hypoxia-inducible factor
HIV	human immunodeficiency virus
HPAA	hypothalamic-pituitary-adrenal axis
ICP	infancy-childhood-puberty model of growth
ICR	imprinting control region

IFG	impaired fasting glucose
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IGT	impaired glucose tolerance
IR	insulin resistance
IRS	insulin receptor substrate
IUGR	intrauterine growth retardation
LDL	low-density lipoprotein
LFS	Li-Fraumeni syndrome
LGA	large for gestational age
LTD	Laron-type dwarfism
MAS	McCune-Albright syndrome
MCV	mean cellular volume
MEN1	multiple endocrine neoplasia type 1
MRI	magnetic resonance imaging
MUL	Mulibrey nanism
NCEP	National Cholesterol Education Program
NF1	neurofibromatosis type 1
NS	Noonan syndrome
OFC	occipitofrontal circumference
OGTT	oral glucose tolerance test
OMIM	Online Mendelian Inheritance in Man
PBD	peroxisomal biogenes disorder
PHD	Plant Homeo-Domain
PEC	perivascular epitheloid cell
PPAR	peroxisome-proliferator-activated-receptor
PTC	premature termination codon
PWS	Prader-Willi syndrome
RBCC	RING-B-Box-Coiled-coil
RIA	radioimmunoassay
SDS	standard deviation score
SGA	small for gestational age
SHOX	Short Stature Homeobox-containing gene
SLE	systemic lupus erythematosus
SMA	smooth muscle actin
SRS	Silver-Russell syndrome
TS	Turner syndrome
TNF	tumour necrosis factor
TRAF	tumour necrosis factor receptor associated factor
TRH	thyreotropin releasing hormone
TRIM	tripartite motif
TSH	thyroid stimulating hormone
UPD	uniparental disomy
UPS	ubiquitin-proteosome system
US	ultrasonography

VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau disease
VLCFA	very-long-chain fatty acids
VSD	ventricular septal defect
WHR	waist-hip ratio
WFH	weight-for-height
WHO	World Health Organization
WPW	Wolf-Parkinson-White syndrome
WT	Wilms' tumour
X-ALD	X-linked adrenoleucodystrophy
ZS	Zellweger syndrome

1. SUMMARY

Background: Mulibrey nanism (MUL; Muscle-liver-brain-eye nanism; OMIM 253250) is an autosomal recessive growth disorder more prevalent in Finland than elsewhere in the world. Clinical characteristics include severe prenatal-onset growth restriction, cardiopathy, multiple organ manifestations but no major neurological handicap. MUL is caused by mutations in the *TRIM37* gene on chromosome 17q22-23, encoding a peroxisomal protein TRIM37 with ubiquitin E3-ligase activity. Nineteen different mutations have been detected, four of them present in the Finnish patients.

Objective: This study aimed to characterize clinical and histopathological features of MUL in the national cohort of Finnish patients.

Patients and methods: A total of 92 Finnish patients (age 0.7 to 77 years) participated in the clinical follow-up study. Patients' hospital records and growth charts were reviewed. Physical, radiographic and laboratory examinations were performed according to a clinical protocol. Thirty patients (18 females) were treated with recombinant human GH for a median period of 5.7 years. Biopsies and autopsy samples were used for the histopathological and immunohistochemical analyses.

Results: MUL patients were born small for gestational age (SGA) with immature craniofacial features after prenatal-onset growth restriction. They experienced a continuous deceleration in both height SDS and weight-for-height postnatally. In infancy feeding difficulties and frequent pneumonias were common problems. At the time of diagnosis (median age 2.1 years) characteristic craniofacial, radiological and ocular features were the most constant findings. MUL patients showed a dramatic change in glucose metabolism with increasing age. While the children had low fasting glucose and insulin levels, 90% of the adults were insulin resistant, half had type 2 diabetes and an additional 42% showed impaired glucose tolerance (IGT). Seventy percent fulfilled the National Cholesterol Education Program (NCEP) Adult Treatment Panel III criteria for metabolic syndrome as adults. GH therapy improved prepubertal growth but had only minor impact on adult height (+5 cm). Interestingly, treated subjects were slimmer and had less frequent metabolic concerns as young adults. MUL patients displayed histologically a disturbed architecture with ectopic tissues and a high frequency of both benign and malignant tumours present in several internal organs. A total of 232 tumorous lesions were detected in our patient cohort. The majority of the tumours showed strong expression of endothelial cell marker CD34 as well as α-smooth muscle actin (α-SMA). Fifteen of the tumours were malignant and seven of them (five Wilms' tumours) occurred in the kidney.

Conclusions: MUL patients present a distinct postnatal growth pattern. Short-term response of GH treatment is substantial but the long-term impact remains modest. Although MUL patients form a distinct clinical and diagnostic entity, their clinical findings vary considerably from infancy to adulthood. While failure to thrive dominates early life, MUL adults develop metabolic syndrome and have a tendency for malignancies and vascular lesions in several organs. This speaks for a central role of TRIM37 in regulation of key cellular functions, such as proliferation, migration, angiogenesis and insulin signalling.

2. INTRODUCTION

Finland was sparsely populated 10 000 years ago by few settlers of mostly unknown origin and remained so for thousands of years, mostly due to geographic reasons but also due to our language. Also, population-isolates distantly located from each other, often separated by large lakes or deep forests, were a common feature. This gradually resulted in loss of genetic variation due to marriages within the population. The occurrence of one mutation within this small founding population was followed by enrichment giving rise to a phenomenon known as the founder effect, where one single or only few mutations are present in the majority of the affected individuals (Provine 2004).

Today, 36 rare monogenic and mostly autosomal recessive disorders are overrepresented in the Finnish population. In many of these, the number of identified cases is greater in Finland than reported worldwide. Together these disorders are known as the Finnish disease heritage, with a disease spectrum extending to somewhat all branches of clinical medicine (de la Chapelle 1993, Norio 2003). Almost one third of them cause mental retardation, and many result in visual impairment or deafness. Also, congenital malformations, bone disorders and metabolic, neurological, or hematological diseases as well as multi-systemic syndromes are represented. A substantial part causes handicap and heavy burden to the patient and his or her family (Norio 2003). Over the years, several of these monogenic disorders have been characterized at the molecular level which has helped physicians and scientists to understand the pathophysiology behind common diseases. The heterogeneous nature of many common conditions, such as diabetes mellitus or cancer suggests that more research attention should be focused on phenotypically homogenous subgroups of patients (O'Rahilly et al 2005), precisely as the case is with disorders of the Finnish disease heritage.

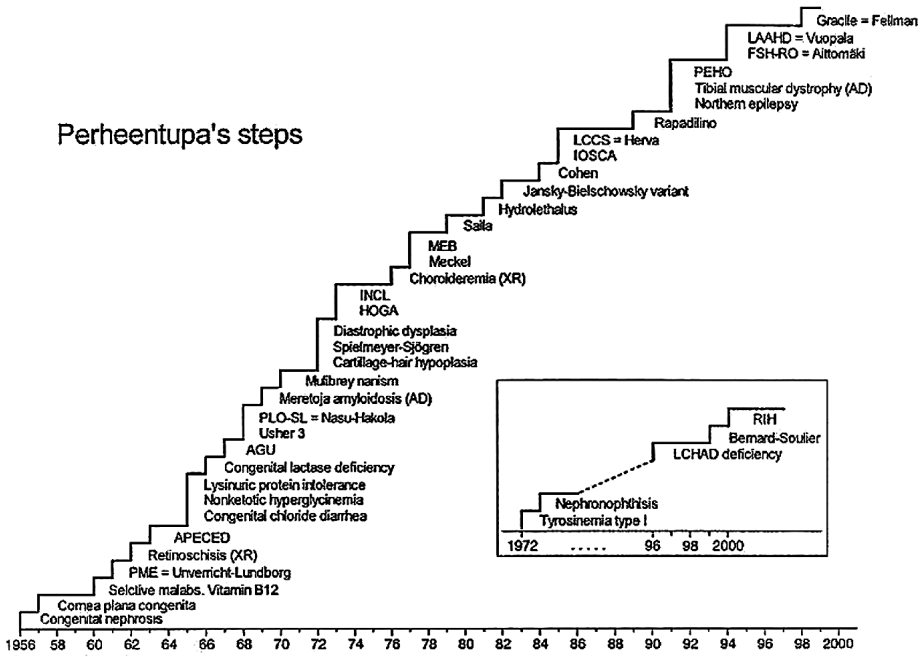

Figure 1. Perheentupa's steps showing the 36 rare disorders of the Finnish disease heritage. Thirty-two of them are autosomal recessive, two autosomal dominant, and two X-linked. Adapted from Norio et al 1973.

In the early 1970s doctors Perheentupa, Autio, Leisti, Raitta, and Tuuteri described a new Finnish dysmorphic growth disorder (Perheentupa et al 1970, 1973). The disorder was first entitled Perheentupa syndrome but later renamed Mulibrey nanism (MUL; OMIM 253250); an acronym comprising of muscle hypotonicity (**MU**scle), hepatomegaly (**LI**ver), enlarged ventricles and cisternae of the central nervous system (**BR**ain) and yellowish dots in the midperipheral area of the ocular fundi (**EY**e). The term nanism was adapted from Latin; meaning dwarfism or short stature. For decades, clinical care and follow-up was centred to the University of Helsinki Children's Hospital. This gave rise to unique clinical data collected for over more than thirty years, part of which has been analyzed in this study.

3. REVIEW OF THE LITERATURE

3.1 Normal growth

Growth can be defined as gain in size by increase in tissue mass. Growth is a combination of cellular hyperplasia (increase in cell number), hypertrophy (increase in cellular size) and apoptosis (controlled cellular death). Successful growth is a well suited combination of these three tightly controlled cellular events (Clayton and Gill 2001). Optimal environment and interaction will allow a fertilized egg to develop into a mature human being, whereas disruptions may interfere with the developmental process and result in variable degrees of whole-body or regional growth restriction (Clayton and Gill 2001).

Normal growth consists of four different stages; intrauterine, infant, childhood and pubertal growth, eventually resulting in adult stature (Figure 2) (Karlberg et al 1987a, Karlberg et al 1987b, Karlberg 1989, Clayton and Gill 2001). To achieve normal stature all these stages need to be successful. However, the control of growth is related to several complex interacting factors within the cell (intrinsic factors; i.e. genotype, epigenetic factors), or extrinsic factors (i.e. nutrition, environment, hormones and growth factors). Also, tissue growth will not end in all organ systems when the adult height is achieved and some cells maintain their capacity to proliferate, as is the case in the liver and endocrine tissues (Clayton and Gill 2001).

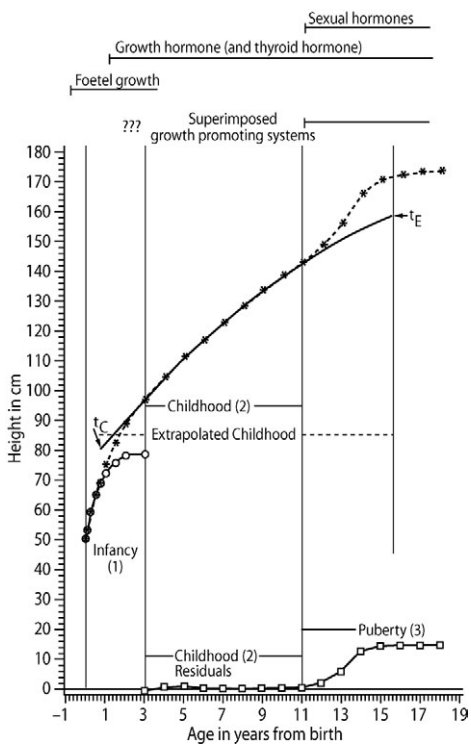

Figure 2. The ICP model of growth. Postnatal growth is modelled as a combination of three components; 1) rapid infant growth sharing features with the intrauterine growth, 2) steady childhood growth mainly regulated by GH and 3) the pubertal growth spurt driven by sex steroid hormones eventually resulting in adult stature. Adapted from Karlberg 1989.

3.1.1 Prenatal growth

Intrauterine growth is a complex, dynamic process where cell proliferation, differentiation, and migration together form the developing organ systems of the fetus (Clayton and Gill 2001, Bryan and Hindmarsh 2006, Maulik et al 2006a). During the first trimester of the pregnancy, tissue patterns and organ systems are forming from the three germ layers (ecto-, meso- and endoderm). Particularly rapid growth is seen between the gestational weeks 4 and 8 when all the major organ systems in the body are established. In the second trimester, the fetus undergoes cellular hyperplasia and rapidly increases in size. In the third trimester, the organ systems mature and the fetus is being prepared for extrauterine life (Clayton and Gill 2001).

Human growth hormone (GH) appears to have very little impact on prenatal growth, since individuals with complete GH deficiency or insensitivity display a nearly normal body size at birth (Rosenfeld et al 1994). Insulin, however, is the major growth mediator *in utero* affecting both intrauterine growth and size at birth, as seen in macrosomic infants born to diabetic mothers and in babies with congenital hyperinsulinism (Fournet et al 2004, Hadden 2008). Further, the importance of insulin-like growth factors (IGF-1 and IGF-2) in normal intrauterine growth has been established in some extraordinary case reports; one patient with a homozygous deletion of the IGF-1 gene (Woods et al 1996), one patient with mutated IGF-1 gene resulting in ineffective IGF-1 (Walenkamp et al 2005), and two patients with mutated IGF-1 receptor (Abuzzahab et al 2003), all displaying severe intrauterine growth restriction. Also mouse models with IGF-1 and IGF-2 deficiency have displayed restricted intrauterine growth (Accili et al 1999).

IGF-1 influences the growth both pre- and postnatally, whereas IGF-2 only appears to regulate growth *in utero*. During fetal growth the IGF-2 effect is mediated through the insulin receptor (Nakae et al 2001). Interestingly, imprinted genes at chromosome 11p15.5 have been associated with fetal growth (DeChiara et al 1990, Rossignol et al 2006). Genes in this cluster include the *IGF2* gene, and recent observations show hypomethylation of the imprinting control region (ICR) in some individuals with Silver-Russell syndrome (SRS; OMIM 180860) (Bruce et al 2009), a dysmorphic growth disorder with prenatal-onset growth restriction. Interestingly, opposite epigenetic mutations of the same locus result in the overgrowth syndrome Beckwith-Wiedemann (BWS; OMIM 130650), characterized by macrosomia, macroglossia, omphalocele, hemihyperplasia, peculiar external ears and a tendency for tumours in childhood (Gaston et al 2001, Eggermann et al 2008). As expected from these epigenetic findings, SRS and BWS subjects also display opposite tissue expression of *IGF-2* (Girquel et al 2005, 2008, Eggermann et al 2008). Analysis of tissue samples from patients with BWS revealed overexpression of *IGF-2* (Weksberg et al 2003), while lower IGF-2 expression was evident in SRS subjects (Girquel et al 2005).

3.1.2 Postnatal growth

During the first year the human baby grows rapidly in length and weight, although the growth rate is sharply decelerated in comparison with the intrauterine growth rate (Karlberg et al 1987a, Clayton and Gill 2001). This early postnatal growth is strongly influenced by nutritional intake and the hormones primarily regulating growth are similar to those involved in the intrauterine growth (Euser 2008, Maulik et al 2008a). At this stage the correlation between height and weight and mean parental size is poor (Rosenthal and Wilson 1994). When the intrauterine effect gradually fades and vanishes normally by the age of 2.0 years, the infant establishes his or her own growth pattern (Clayton and Gill 2001). Somewhere between 6-12 months of age the secretion of the 191 amino acid 22-kD human GH from the anterior pituitary gland (Lewis et al 1980) starts to increase and as the infant grows into childhood, GH becomes the dominant moderator of growth (Albertsson-Wikland and Rosenberg 1988, Clayton and Gill 2001).

The GH release is controlled by a balance between stimulatory growth hormone releasing hormone (GHRH) and inhibitory somatostatin both from the hypothalamus (Rosenbloom and Connor 2007). This balance is regulated by neurological, metabolic (i.e. glucose, amino acids, free fatty acids), and hormonal (estrogen and testosterone) influences, as well as sleep (Strobl and Thomas 1994). After release, GH exerts its effects on target tissues by binding to the GH-receptor (GHR), which activates an intracellular cascade resulting in enhanced gene transcription (de Vos et al 1992). The biological actions of GH can only be reached in the presence of a normal function of the GHR and an optimal intracellular signalling pathway (Laron 2004). Deletions and mutations in the extracellular domain of the *GHR* gene result in GH insensitivity known as Laron syndrome (LTD; OMIM 262500) (Laron et al 1968). The typical features of Laron syndrome are short stature, characteristic face, obesity, acromicria, hypoglycemia, high basal serum GH, and low-serum IGF-1 unresponsive to the administration of exogenous GH (Laron 2004). Most of the growth effect of GH is a result of increased expression of IGF-1 in peripheral tissues, particularly in the liver and epiphyseal cartilage of long bones (Jones et al 1995, Walenkamp and Wit 2006).

IGF-1 and IGF-2 are single chain polypeptide hormones with considerable structural homology to insulin and share common structures of receptors and post-receptor cascades (Rinderknech and Humble 1978, Nakae et al 2001). Indeed, IGFs can bind with a weaker affinity directly to the insulin receptor. IGFs are expressed in several tissues both under endocrine and paracrine regulation. Their actions on postnatal growth are mediated primarily by IGF-1 through its IGF-1 receptor (Jones et al 1995, Klammt et al 2008) and only to a lesser extent through the IGF-2 receptor (Nakae et al 2001). The expression of IGF-1 is controlled by many factors other than GH. For instance, nutrient deficiency and

hypoxia both decrease the IGF-1 gene expression and synthesis (Rosenbloom and Connor 2007). Also, insulin has growth promoting effects and it stimulates the IGF-1 synthesis. IGF-1 possesses insulin-like metabolic actions, such as increased uptake of glucose and fatty acids (Boulware et al 1992). Unlike insulin, the IGFs form complexes together with insulin-like growth factor binding proteins (IGFBPs). The most important of these binding proteins is IGFBP-3, which is increased both by GH and also directly by IGF-1 (Jones et al 1995, Klammt et al 2008). IGFBP-3 and IGF-1 bind together with a third glycoprotein, the acid labile subunit (ALS), to form a stable 150 kDa ternary complex that serves as an intravascular store for IGFs and prolongs their life-span in the bloodstream (Donaghy et al 1996, Limal et al 2006). Reduced levels of ALS has been implicated in growth impairment for instance in some patients with Noonan syndrome (Limal et al 2006).

GH is the major determinant of growth during childhood. At this particular time dysfunctions in pituitary GH release, GH-receptor signalling and GH-IGF-1 axis are usually diagnosed (Albertsson-Wikland and Rosenberg 1988, Rosenbloom and Connor 2007). GH deficiency can be congenital, as in genetic defects and syndromes affecting the hypothalamic-pituitary axis or acquired (i.e. tumour, trauma and asphyxia) (Bunin et al 1998). The thyroid hormones also play an important role in the control of childhood growth, and are like GH strictly under hypothalamic regulation. Thyreotropin releasing hormone (TRH) is secreted into the hypophyseal portal system, resulting in release of thyroid stimulating hormone (TSH) from the anterior pituitary gland (Morley 1981). Downstream TSH stimulates the thyroid gland to synthesize and secrete thyroid hormones (T_3 and T_4). A close interaction between GH and thyroid hormones also exists. In the presence of hypothyroidism the pituitary GH secretion is subsequently diminished and the growth usually decelerates dramatically (Rosenbloom and Connor 2007).

Childhood growth is highly sensitive to environmental factors and dependent on normal hormonal and metabolic functions (Albertsson-Wikland and Rosenberg 1988). Glucocorticoid excess or deficiency and diabetes mellitus all influence negatively on growth (Clayton and Gill 2001). Moreover, chronic diseases such as gastroenterological, renal, cardiopulmonary, hematological disorders and cancer may decelerate growth. Also infections and use of certain drugs, such as corticosteroids for asthma, may deprivate growth. Lack of vitamin D, or minerals (e.g. phosphate, zinc and iron) and general malnutrition are still leading causes of growth deceleration or restriction in the third world countries. Psychosocial factors and stress can also be sources or worsening factors of growth deprivation (Clayton and Gill 2001).

In late childhood, additional hormonal changes occur (Cianfarani 2001). The adrenal glands begin to produce androgens from cholesterol and the gonads start to secret male or female sex hormones (testosterone and estrogen) under

hypothalamic control (Cutler 1997). Of the sex steroids, estrogen is the main determinant of pubertal growth in both sexes (Cutler 1997). This accelerates linear growth at the cartilage growth plates at the end of the diaphysis of long bones and the secondary sexual characteristics start to develop (Tanner and Whitehouse 1979, Cutler 1997). The pubertal growth displays an increased tempo (pubertal spurt) in growth, which usually reaches its peak velocity at Tanner stage 3 and 4 in girls and boys, respectively (Tanner and Whitehouse 1979). The pubertal spurt is determined both by GH and sex steroids (Aynsley-Green et al 1976, Karlberg et al 1987b, Kerrigan and Rogol 1992, Cutler 1997, Clayton and Gill 2001). The elevated levels of sex steroid hormones over time convert the cartilage cells into bone cells terminating the linear growth eventually resulting in adult stature (Cutler 1997, Clayton and Gill 2001).

There is a large variation in human height and even fairly unusual growth patterns can be regarded as normal. However, the stature is considered abnormally short when the height standard deviation score (SDS) is 2.5 below the mean height for age and gender or mid-parental height (Rosenthal and Wilson 1994). An important cause resulting in short adult stature is early onset of puberty, in which maturation of bone is accelerated resulting in earlier growth arrest and short adult stature. In familial short stature the children are short primarily because their parents are short. Temporarily slow growth with catch-up growth compensating for final height is typical for late-onset puberty (Bhangoo et al 2007). Despite the abundance of different etiologies such as nutritional deprivation, hypothyroidism, pharmacological administration of glucocorticoids or mutations in the GH receptor, up to one fifth of subjects with short adult stature remain without explanation for their restricted growth (Bhangoo et al 2007, Wit et al 2008a, 2008b).

3.2 Intrauterine growth impairment

Intrauterine growth restriction (IUGR) is defined as growth impairment *in utero*, in which infants do not reach their genetic growth potential due to various genetic and/or environmental influences during gestation (Ergaz et al 2005, Maulik et al 2006b). IUGR is especially important because of the higher incidence of morbidity and mortality and the potential for long-term complications in childhood. Newborns with IUGR face challenges like hypothermia, pulmonary hemorrhage, and life-threatening hypoglycemia more frequently compared to normal sized newborns (Cunningham et al 1997, Lee et al 2003a, Maulik et al 2006b). It has been estimated that an abnormal fetal karyotype would be responsible for about 20% of all intrauterine growth restricted fetuses (Snijders et al 1993, Kinzler et al 2008).

The term IUGR has been proposed to be limited to the process of compromised intrauterine growth rate detected by several ultrasonographic measurements.

If prolonged or severe enough it will lead to the delivery of a small for gestational age (SGA) infant. Early onset growth restriction is defined as clinically evident growth retardation before the completion of the 28th week of gestation (Wit et al 2006, Kinzler et al 2008).

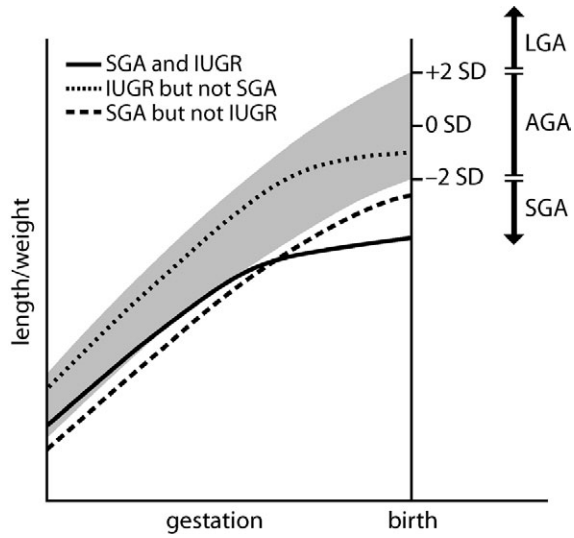

Figure 3. Schematic presentation of normal intrauterine growth, intrauterine growth restriction (IUGR), small (SGA), appropriate (AGA) as well as large for gestational age (LGA).

3.2.1 Small for gestational age

At birth, the newborn can have an appropriate weight and/or length for gestational age (AGA), be large for gestational age (LGA) or small for gestational age in weight and/or length (Battaglia and Lubchenco 1967). Of all newborns, 2.5% are born SGA. Traditionally, SGA refers to an infant with a birth weight below the 10th percentile for the gestational age. Among pediatric endocrinologists SGA is defined as birth weight and/or length more than 2.0 SD below the mean for gestational age (Figure 3) (Lee et al 2003b). Among SGA infants three subgroups can be distinguished; those with low weight but normal length (SWGA), those with short length but normal weight (SLGA) and infants with a combination of both low weight and short length for gestational age (SLWGA) (Albertsson-Wikland and Karlberg 1994, Karlberg and Albertsson-Wikland 1995).

3.2.2 Low birth weight

Small size at birth can also be determined according only to the weight at birth. WHO (World Health Organization) defines "low birth weight" as below 2500 g. About 15 % of all infants are born with low birth weight. The level in developing countries is twice as high compared to developed regions (WHO statistics 2005). The term "low birth weight" is used irrespective of the gestational age and includes babies born prematurely and subjects with monogenic disorders, as well as familial or sporadic syndromes (Saenger et al 2007).

3.2.3 Factors influencing intrauterine growth

The intrauterine growth is controlled and influenced by a large quantity of both genetic and environmental factors of maternal, placental or fetal origin (Table 1) (Bryan and Hindmarsh 2006, Maulik et al 2006a, Saenger et al 2007).

A significant portion (30-40%) of maternal factors is caused by hypertensive disorders of various etiologies. (Maulik et al 2006a). For instance, pre-eclampsia is associated with a 4-5 fold risk of delivering a SGA baby (Maulik et al 2006a). Other maternal factors leading to prenatal growth retardation include exposure to toxic substances, chronic diseases, malnutrition, birth order, multiple births and maternal genetic factors, size and age (Table 1) (Wollmann 1998, Bryan and Hindmarsh 2006, Maulik et al 2006a, Saenger et al 2007). Sometimes several factors overlap, such as chronic malnutrition, tobacco smoking, alcohol abuse and low socioeconomic status which can result in, for instance, the fetal alcohol syndrome (FAS) (Gluckman and Hanson 2004, Kleijer et al 2005). FAS is characterized by IUGR, microcephaly and immature facial features, as well as a high frequency of gross organ anomalies, most commonly heart malformations (Kleijer et al 2005). Congenital heart disease is seen in approximately 20% of Finnish FAS children (Autti-Rämö et al 2006).

The placenta is the life-line between the mother and the fetus and has an important role also in normal fetal growth. The growing fetus is vulnerable to nutrient deprivation and even mild placental dysfunction may restrict the transfer of nutrients and oxygen to the fetus so that growth deprivation gradually occurs (Miller et al 2008). Placental dysfunction can be a consequence of metabolic disturbances or vascular damage (i.e. placental infarction), partial placental separation, or simply a small placenta (Table 1) (Wollmann 1998, Bryan and Hindmarsh 2006, Maulik et al 2006a, Saenger et al 2007).

Chromosomal aberrations are a well-established cause behind restricted fetal growth and are estimated to account for approximately 20% of cases (Snijders et al 1993, Kinzler et al 2008). The presence of polyhydramnion, nuchal-fold, structural malformations, elevated α-fetoprotein and small fetal size may be

Table 1. Factors influencing intrauterine growth

Maternal factors
Maternal hypertension
Pre-eclampsia
Secondary hypertension
Renal disease
Gestational diabetes mellitus
Autoimmune disorders
Antiphospholipid syndrome, SLE
Thrombophilia
Cyanotic heart disease
Severe asthma
Anemia
Malignancy
Antepartum hemorrhage
Phenylketouria
Uterine anomalies
Large sub-mucous myomas
Uterus septae
Uterine fibroids
Life style
Smoking
Substance abuse; alcohol and drugs
Therapeutic agents and medications
Malnutrition
Low maternal weight gain and pregnancy BMI
Delivery at age < 16 or > 35 years
Environmental problems
Pollutions and toxic substances
High altitude
Low socioeconomic status
Placental factors
Placenta praevia
Placenta accreta
Abruptio placentae
Circumvallate placentae
Placental infarction
Placental hematoma
Placental hemangioma
Placental vascular malformation
Confined placental mosaicism
Fetal factors
Chromosomal abnormalities
Trisomy 13, 18 and 21
Genetic imprinting and uniparenteral disomy
Inborn errors of metabolism
Malformations
Heart; VSD, tetralogy of Fallot
Abdominal wall; gastroschisis, omphalocele, diaphragmatic hernia
Preterm birth
Multiple gestation
Infection; rubella, CMV, HIV, varicella zoster, herpes simplex, toxoplasmosis
Unexplained elevation of α-fetoprotein

Adapted from Maulik et al 2006a, Bryan and Hindmarsh 2006, Saenger et al 2007

indicative of chromosomal abnormality (Kinzler et al 2008). Uniparenteral disomy, inheritance of both homologes of a chromosome from a single parent, has also been associated with growth restriction *in utero* (Hannula et al 2001).

Multiple fetal organ malformations are also associated with an increased risk of growth restriction *in utero*. The heart and abdominal wall are the organs most frequently affected (Table 1). Endocrine dysfunction as well as intrauterine infections particularly during critical times of organ development, are fetal factors behind restricted intrauterine growth and account for approximately 5-10% of cases (Rosenthal and Wilson 1994, Ergaz et al 2005). Bacterial infections only rarely compromise the intrauterine growth (Maulik et al 2006a). Premature birth has been associated with intrauterine growth retardation for decades (Tamura et al 1984). Also, gestations of multiple fetuses challenge the maternal system to provide an optimal environment for growth. The incidence of SGA in twins is 15-30% and multiple gestations account for nearly 5% of all cases with intrauterine growth restriction (Maulik et al 2006a, Kinzler et al 2008).

3.2.4 Symmetric or asymmetric IUGR

Prenatal growth restriction can be classified according to its severity or by the effects on body proportions (i.e. symmetric or asymmetric) (Dasche et al 2000, Maulik et al 2006b). Symmetrical restriction is usually due to fetal causes such as viral infections, developmental factors or early onset placental defi-

ciency. Asymmetrical restriction on the other hand, is mainly caused by extrinsic factors, for example, placental insufficiency or teratogenic substances. Also genetic syndromes and malnutrition might result in asymmetry of different organ systems, when the fetus tries to prioritize nutrition to certain organs, namely the brain but also heart and kidney, to maintain normal growth or development (Cunningham et al 1997, Maulik et al 2006b). For instance, small head circumference for gestational age can be indicative of severe or early intrauterine growth restriction (Euser et al 2008), chromosomal aberrations, environmental factors (i.e. toxic substances and intrauterine infections) or a consequence of IGF-1 dysfunction (Walenkamp and Wit 2006), while in a part of the growth restricted conditions spared cranial growth with normal head circumference can be observed (Hannula et al 2001, Huber et al 2005, Bruce et al 2009). Microcephaly, defined as occipitofrontal head circumference (OFC) below 2.0 SDS for age and sex, is associated with a poorer mental capacity and frequently neurological handicap (Abuelo 2007).

3.3 Catch-up growth

Most (>70%) healthy SGA children show sufficient catch-up growth, usually beginning 2-3 months after birth, normalizing their stature commonly by the age of 2.0 years (Albertsson-Wikland et al 1998). This occurs especially in cases when the intrauterine growth has been restricted due to environmental factors. When these factors are removed after birth, the linear growth rapidly starts to normalize. Growth in very preterm infants, however, typically decelerates after exposure to extrauterine life, when their energy expenditure shifts to promote survival rather then growth (Gibson 2003, 2007, Euser et al 2008). However, approximately 10-15% of SGA children do not exhibit sufficient catch-up growth and remain persistently short throughout life (Albertsson-Wikland et al 1998).

3.4 Consequences of being small at birth

Understanding intrauterine and early childhood growth has assumed great importance since epidemiological observations firmly show a link between intrauterine and early extrauterine growth restriction and risk of chronic adult disease, such as cardiovascular disease and type 2 diabetes (Barker 1995). It is estimated that one-third of SGA children will eventually develop insulin resistance (Levy-Marchal et al 2004) and despite normal postnatal catch-up growth, they are still as adults prone to developing coronary heart disease, hypertension, type 2 diabetes (Barker et al 1993, Barker 1995, 1996) and metabolic syndrome (Veening et al 2003).

The entity metabolic syndrome was first described by Gerald Reaven as "syndrome X" in 1988. In the original description obesity was not included in the criteria (Reaven 1998). Today metabolic syndrome is defined as a constellation of glucose dysregulation, abdominal obesity, atherogenic dyslipidemia and hypertension. Various health organizations (World Health Organization (WHO), International Diabetes Federation (IDF) and National Cholesterol Education Program (NCEP) have all established their own guideline definitions. All of them include this cluster of risk factors and are quite similar in their cut-off levels for triglycerides, high-density lipoprotein and blood pressure. However, their definitions of obesity and glucose intolerance differs (Table 2) (Alberti and Zimmet 1998, NCEP 2001, Alberti et al 2005). In 2001, the NCEP Adult Treatment Panel III (ATP III) devised a definition for the metabolic syndrome (NCEP 2001, Fedder et al 2002), which was updated in 2005 by the American Heart Association and the National Heart Lung and Blood Institute (Grundy et al 2005). The NCEP ATP III definition form one of the most widely used criteria of metabolic syndrome (Table 2).

Table 2. Definitions for metabolic syndrome

NCEP	*IDF*	*WHO*
Three of the following:	**Waist circumference** men ≥94cm, women ≥80cm	**Insulin resistance* (IGT, IFG, T2DM or other evidence of IR)**
Fasting plasma glucose ≥ 5.6mmol/l	**and at least two of the following**	**and at least two of the following**
Waist circumference men ≥102cm, women ≥88cm		
Triglycerides ≥1.7mmol/l	Fasting plasma glucose ≥5.6mmol/l	WRH** men >0.90, women >0.85 or BMI*** >30kg/m^2
HDL-cholesterol, men ≤1.0mmol/l, women ≤1.3mmol/l	Triglycerides ≥ 1.7mmol/l	Triglycerides ≥ 1.7mmol/l or HDL-cholesterol ≤0.9mmol/l
Blood pressure ≥130/85mmHg or medication	HDL-cholesterol, men ≤1.0mmol/l, women ≤1.3mmol/l	Blood pressure ≥140/90mmHg or medication
	Blood pressure ≥130/85mmHg or medication	Microalbuminuruia

*IGT, impaired glucose tolerance; IFG impaired fasting glucose; T2DM, type 2 diabetes; IR, insulin resistance. **WHR; waist-hip ratio, BMI***; body mass index. Urinary albuminexcretion of ≥20ug/min or albumin-to-creatinine ratio of ≥ 30mg/g. Adapted from Alberti and Zimmet 1998, NCEP 2001, Fedder et al 2002, Grundy et al 2005, Alberti et al 2005.

3.4.1 "The thrifty phenotype"

In the early 1980s, epidemiologist David Barker and colleagues investigated mortality rates for coronary heart disease and other vascular diseases in England and Wales. They noted that the highest rates occurred in areas associated with relatively high unemployment and low socioeconomic status and that the rates of heart disease were closely associated with the distribution of infant mortality. David Barker suggested that coronary heart disease might have its origin in early or intrauterine life and that poor nutritional conditions in a pregnant mother might modify the organ development of her unborn child so that the fetus will be programmed for survival in an environment short of resources (Figure 4) (Barker 1995, Hales and Barker 1992, 2001).

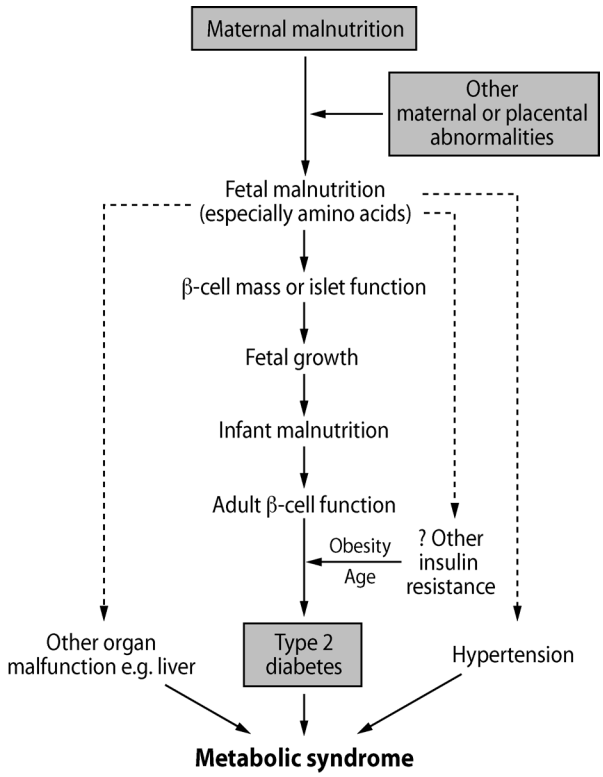

Figure 4. The original presentation of the "thrifty phenotype hypothesis" by Hales and Barker 1992

The etiologies of type 2 diabetes and cardiovascular disease are multifactorial and believed to arise from complex interactions between environmental and polygenic factors (Barker 1995, Jaquet et al 2000, Hales and Barker 2001, Cianfarani et al 2001). Today, many human diseases in adulthood are associated with poor growth in early life, suggesting early-life nutrition as the key mechanism. The "thrifty phenotype hypothesis" implicates that some metabolic adaptations occur early in life to promote survival by selecting an appropriate growth pattern in response to the given environment. Individuals with the thrifty phenotype are proposed to have a lowered metabolic rate and a smaller body size and be especially prone to develop obesity or type 2 diabetes in an affluent environment (Hales and Barker 1992, 2001), whereas those who have experienced a positive fetal environment will more likely adapt to good conditions and therefore are better able to cope with the rich diets of today (Barker 1995, Hales and Barker 1992, 2001).

The "thrifty phenotype" hypothesis has been challenged, but increasing amounts of evidence from different populations predict the same association (Eriksson 2001). However, alternative explanations to this association have also been suggested. For instance, low birth weight, impaired glucose tolerance, type 2 diabetes, and hypertension have all been suggested to be phenotypes of the same insulin-resistant or "thrifty" genotype (Hattersley and Tooke 1999, Frayling and Hattersley 2001). Further, insulin secreted by the fetal pancreas in response to maternal glucose concentrations has been shown as a key growth factor *in utero* (Fournet et al 2004). Monogenic diseases with impaired glucose sensing, lower insulin secretion or increased peripheral insulin resistance have been shown to manifest with impaired fetal growth (Hattersley and Tooke 1999, Frayling and Hattersley 2001).

Also, the IGF-system has been suggested to play a part in the early programming of metabolic consequences. Growth deceleration *in utero* and deprivated catch-up in early postnatal growth have been proposed to induce peripheral insulin resistance by increasing the amounts of peripheral IGF-1 receptors. This is proposed to promote faster growth but later predispose individuals to insulin resistance or type 2 diabetes (Cianfarani et al 1999, 2001).

3.5 Dysmorphic growth disorders

Failure to thrive is a condition where an infant is growing slower than expected with a concurrent poor weight progression. These patients are important to be recognized early since the deceleration in growth can be a sign of an underlying chronic disease such as an endocrine dysfunction (i.e. growth hormone deficiency or hypothyroidism) or problems like inadequate nutrition, recurrent infections or abnormal behaviour affecting food intake. However, growth retardation or failure to thrive can also be an early sign of an enduring congenital disorder (Clayton and Gill, Ergaz et al 2005).

A strong association between chromosome aberrations, IUGR and congenital malformations exists (Monk and Moore 2004). For example, fetuses with trisomies are frequently growth restricted. Children with Down's syndrome (trisomy 21; OMIM 190685) display postnatal growth retardation and later short adult stature (Cronk et al 1988). Also, many chromosome abnormalities include suboptimal growth of the fetus as one of the major hallmarks. In Turner syndrome (TS) the girls have sex chromosome abnormalities, either pure 45X or 46XX with aberrant X chromosome or mosaicism. The most frequent clinical finding in Turner syndrome is short stature, which has strongly been associated with dysfunction of the Short Stature Homeobox-containing gene (SHOX) on chromosome Xp22.33 (Sybert and McCauley 2004).

Several congenital disorders also present growth failure, typically with lack of catch-up in growth. A significant portion of them occur due to abnormal growth and remodelling of bone or cartilage (Baitner et al 2000). Over 250 different types of skeletal dysplasias are known (Alanay et al 2007). These disorders, generally referred to as chondro- or osteochondrodysplasias, are hereditary conditions with mutations for example in fibroblast growth factor receptors, collagens, different hormone receptors or sulphate transporters underlying them (Baitner et al 2000, Cohen 2002a). These disorders manifest with short stature and disproportionately short extremities as major clinical signs and often include other features that will require medical attention (Baitner et al 2000). Diastrophic dysplasia (DTD; OMIM 222600) and cartilage-hair hypoplasia (CHH; OMIM 250250) are congenital bone and cartilage disorders belonging to the Finnish disease heritage (Norio 2003).

Another category is genetic or monogenic syndromes characterized by growth restriction, short adult stature and dysmorphic craniofacial and constitutional features (Cohen 2002b) (Table 2). Several of them also manifest with a various degree of mental retardation. For instance, Prader-Willi syndrome (PWS; OMIM 176270), which is characterized by growth failure, mental deficiency, hyperphagia, obesity and hypogonodotropic hypogonadism, is caused by loss of expression of paternally inherited imprinted genes on chromosome 15q11-q13 (Goldstone and Beales 2008). Cornelia de Lange syndrome (CdLS; OMIM 122470, 300590, and 610759) typically displays growth failure, mental retardation, typical craniofacial features, bushy eyebrows, hirsutism, hypoplastic external genitalia, hypospadia and undescended testes. Mutations in the *NIPBL* gene have been found to underlie the condition (Krantz et al 2004). Seckels syndrome (OMIM 210600) manifests with growth retardation, mental deficiency, microcephaly, central nervous system abnormalities, clinodactyly of the fifth-finger and joint dislocations. These patients display a disproportionally prominent nose in relation to the face. The affected gene loci in Seckels syndrome are 3q22.1-q24 (Goodship et al 2000) and 18p11.31-q11.2 (Børglum et al 2001). William's syndrome (OMIM 194050) is characterized by growth failure, mental and developmental retardation, hypercalcemia, cardiovascular anomalies and typical facial features. William's syndrome is caused by a deletion in chromosome 7q11.23 (Morris and Mervis 2000).

Noonan syndrome (NS; OMIM 163950) is one of the most common syndromes transmitted by a Mendelian model. The incidence of affected individuals is estimated to be between 1:1000 and 1:2500. NS is a clinically heterogeneous disorder predominantly characterized by short stature, dysmorphic facial features, neck abnormalities, congenital heart disease and occasionally mild mental retardation (Allanson 2007). The head size in relation to height is mostly normal (Sharland et al 1992, Allanson 2007). Germline mutations affecting the RAS-MAPK (mitogen-activated protein kinase) pathway have been shown to be involved in the pathogenesis of Noonan Syndrome (Kratz et al 2007)

as well as in four other rare syndromes with similar clinical features; Leopard syndrome, cardio-facio-cutaneous syndrome (CFC; OMIM 115150), Costello syndrome (OMIM 218040) and neurofibromatosis type 1 (NF1; OMIM 162200) (Schubbert et al 2007). Patients with the 3-M syndrome (OMIM 273750) also have normal head circumference in combination with facial dysmorphism and short stature. Other features are midface hypoplasia, low set rotated ears, slender long bones, tall vertebral bodies, normal intelligence and a tendency to intracerebral and vascular aneurysms (Miller et al 1975, Huber et al 2005).

Table 3. Some important dysmorphic growth disorders and their clinical characteristics

Disorder	*Growth and head*	*Craniofacies*	*Other features*
Mulibrey nanism	Pre- and postnatal growth failure, normal head circumference and normal intelligence	Scaphocephaly, occipitofrontal bossing, triangular face, low nasal bridge and telecanthus, low-set rotated ears, small triangular tongue, J-shaped sella turcica	Short stature, general gracility, slender long bones, accentuated lumbar lordosis, fibrous dysplasia of long bone, high pitched voice, pericardial constriction, hepatomegaly, naevi flammei, yellowish dots in ocular fundi
Silver-Russell syndrome	Pre- and postnatal growth failure, normal head circumference and normal intelligence	Facial triangularity, frontal bossing, micrognatia with down-curved mouth corners and dental crowding, low-set rotated ears	Short stature, extreme constitutional gracility, asymmetry of limbs and body, clinodactyly of 5th finger, syndactyly of 2-3 toes, cryptorchidism
3-M syndrome	Pre- and postnatal growth failure, normal head circumference and intelligence	Gloomy face, frontal bossing, midface hypoplasia, low-set rotated ears	Short stature, constitutional gracility, slender long bones, tall vertebral bodies, normal endocrine function, intracerebral aneurysms
Noonan syndrome	Pre- and postnatal growth failure, mostly normal head circumference and mild mental retardation	Epicanthal folds, hypertelorism, low nasal bridge, down-slanting palpebrae, increased mouth width, retrognathia	Short stature, short neck with low nuchal hair line, spine deformity, pectus excavatum, cardiac anomaly, micropenis, cryptorchidism, coagulapathy, myopia, ptosis, strabismus
De Lange syndrome	Severe pre- and postnatal growth failure, microcephaly and mental retardation	Brachycephaly, low nasal bridge, bushy eyebrows, long and curly eyelashes, long philtrum and thin upper lip, micrognathia with down-curved mouth corners and high arched palate	Very small stature, clinodactyly of 5th finger, syndactyly of 2-3 toes, simian crease, proximal implantation of thumb, hirsutism, low nuchal hair line, testicular hypoplasia, hypospadia, weak voice and cry, myopia, ptosis
Prader-Willi syndrome	Normal birth length with postnatal growth failure, microcephaly and mild mental retardation	Almond-shaped face, up-slanting palpebrae, narrow bifrontal diameter, thin upper lip	Short stature, obesity, small hands and feet, fair hair and skin with tendency for pickles, micropenis, cryptorchidism, hypoplastic labiae and clitoris, scoliosis, kyphosis, osteoporosis, strabismus
Williams syndrome	Pre- and postnatal growth failure, mild microcephaly and mild mental retardation	Epicanthal folds, medial eyebrow flarem, blue eyes, low nasal bridge, long philtrum, prominent lips	Short stature, hypoplastic nails, hallux valgus, lordosis, spine deformity, cardiovascular and renal anomaly
Bloom syndrome	Pre- and postnatal growth failure, mild microcephaly and mild mental retardation	Dolicocephaly, malar hypoplasia, prominent ears, facial telangiectasic erythema excerbated by sunlight	Short stature, syndactyly and clinodactyly of 5th finger, hypo- and hyperpigmentation of skin, high pitched voice, propensity to develop malignancies and type 2 diabetes
Seckel syndrome	Severe pre- and postnatal growth failure, microcephaly and mental retardation	Receding forehead, prominent nose, large eyes, down-slanting palpebral fissures, low-set ears, micrognathia	Very small stature, clinodactyly of 5th finger, dislocation of hips, slender long bones, 11 pairs of ribs, cryptorchidism
Dubowitz syndrome	Pre- and postnatal growth failure, microcephaly and mental retardation	Small faces, low nasal bridge and telecanthus, epicanthal folds, small eyes, short palpebral fissures, broad nasal tip, micrognathia, prominent dysplastic ears, facial eczema	Short stature, brachyoclinodactyly of 5th finger, syndactyly of 2-3 toes, high pitched cry, propensity to develop malignancies, cryptorchidism and hypospadia, cardiac defects, iris hypoplasia, strabismus, and coloboma

Adapted from Lyons Jones K (Ed.), Smith's Recognizable Patterns of Human Malformation, 5th edition 1997.

SRS is a well known dysmorphic growth disorder, where subjects are born SGA after severe intrauterine growth restriction. The growth failure typically progresses postnatally and affected individuals are proportionately short throughout life. SRS subjects have mostly normal intelligence and their head circumference is close to normal standards. Patients display facial triangularity with a tiny jaw and a mouth that tends to curve down, lean constitutional appearance, skeletal asymmetry and fifth-finger clinodactyly (Silver et al 1953, Russell 1954). The average adult height of males is 151 cm and that of females is 140 cm. Many patients have feeding difficulties and hypoglycemia as infants (Hitchins et al 2001). SRS is a genetically heterogeneous condition and represents a phenotype rather than a specific disorder. The diagnosis is therefore primarily based upon identification of consistent clinical features, especially prenatal and postnatal growth retardation with a close to normal head circumference. Most SRS cases are sporadic, but some familial cases have been reported (Duncan et al 1990, Ounap et al 2004). Further, molecular studies have shown that 5-15% of SRS patients have maternal uniparental disomy (UPD) of chromosome 7 (Hannula et al 2001) and approximately 60% show hypomethylation of the *H19* ICR (Bruce et al 2009).

3.5.1 Mulibrey nanism

Mulibrey nanism (MUL) is a rare autosomal recessive disorder with severe growth failure of prenatal onset and characteristic dysmorphic features, first described from Finland in the early 1970s by Perheentupa and co-workers (Perheentupa et al 1970, 1973). By today, some 130 patients from different ethnic groups have been diagnosed with MUL worldwide (Thorén 1973, Cumming et al 1976, Voorhess 1976, Similä 1980, Finni and Herva 1981, Sánchez-Corona et al 1983, Cotton et al 1988, Haraldsson et al 1993, Lapunzina et al 1995, Seemanová and Bartsch 1999, Avela et al 2000, Jagiello et al 2003, Hämäläinen et al 2004, 2006, Doğanc et al 2007), a majority of them from Finland (92). Mulibrey nanism is a well-suited prototype of a disease belonging to the Finnish disease heritage (Figure 6). It is clearly more common in Finland than elsewhere, with an incidence of approximately 1:40 000 (Lipsanen-Nyman 1986). Most of the Finnish patients origin from clustered regions in Savo and North Carelia (Lipsanen-Nyman 1986). One mutation (Fin-major) is found in nearly all of the Finnish patients (Avela et al 2000).

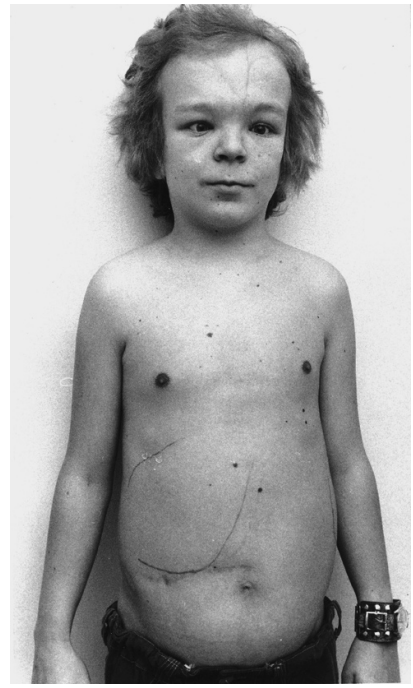

Figure 5. A picture of the first diagnosed Finnish MUL patient. He was referred to University of Helsinki Children's Hospital at the age of 5.0 years with the diagnosis dystrofia gravis. In 1962 his case was published by Pitkänen and Perheentupa as a glycogen storage disease. The diagnosis was later changed to MUL, when patients with similar problems and dysmorphic features were observed among growth restricted children.

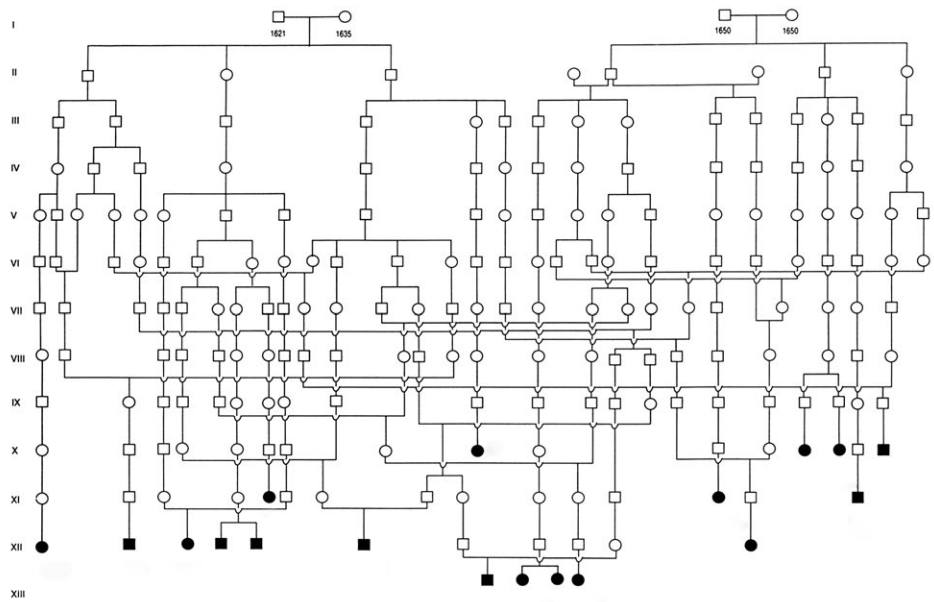

Figure 6. Pedigree of 18 MUL patients dating back to the shores of Lake Pielinen in North Carelia in the early 1600s, displaying a typical founder effect. (Avela and Lipsanen-Nyman, unpublished data)

The originally described patients displayed hypoplastic facial features with a prominent forehead and a J-shaped sella turcica (Perheentupa et al 1970, 1973). The tongue was small and their voice high pitched. Dental crowding was also commonly seen (Myllärniemi et al 1978). The patients also displayed typical yellowish dots in the midperipheral area of the retina and hypopigmentation and pigment dispersion with clusters of pigment in the ocular fundi (Figure 7) (Raitta et al 1974, Tarkkanen et al 1982). Furthermore, fibrous dysplasia of long bones, cutaneous naevi flammei and liver enlargement were encountered (Figure 7) (Perheentupa et al 1973). The most striking feature was the heart manifestation and patients were frequently compromised by constrictive pericarditis and congestive heart failure (CHF) (Tuuteri et al 1974). Hypoglycemia was observed in young children in the original cohort. However, no evaluation concerning the glucose metabolism has been conducted previously.

The long time course of the Mulibrey heart disease including the results of pericardiectomy was described in 2003 by Lipsanen-Nyman and colleagues. Constrictive pericarditis, myocardial hypertrophy and fibrosis constituted the main elements of the heart disease and more than half of the patients ultimately developed CHF. A considerable death rate, due to CHF, in early childhood was followed by no mortality between 10 and 20 years of age. Thereafter, cardiac deaths from CHF due to the heart disease again started to occur. The degree of myocardial involvement is the major determinant of prognosis (Figure 8) (Lipsanen-Nyman et al 2003).

Wilms´ tumour has been reported in one Finnish MUL patient (Similä 1980) and in two cases outside of Finland (Seemanová and Bartsch 1999, Hämäläinen et al 2006). The natural growth in MUL has been analyzed in a Finnish monography (Lipsanen-Nyman 1986). The results of GH treatment have not been evaluated before.

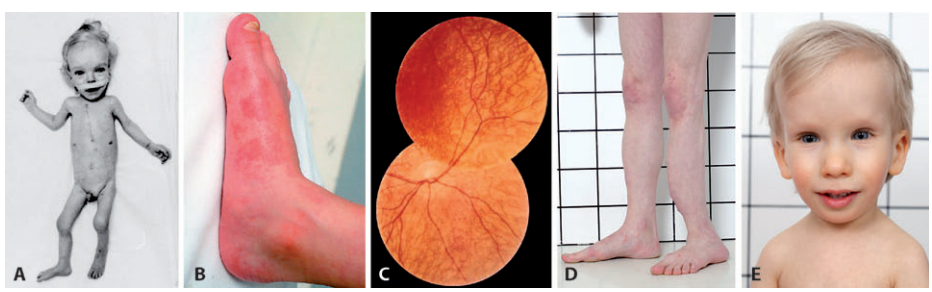

Figure 7. Typical clinical characteristics of MUL illustrated. A. General constitutional gracility. The boy has been pericardiectomized and has a feeding tube due to severe feeding difficulties. B. Cutaneous naevi flammei. C. Yellowish dots in ocular fundi. D. Deformed lower limb due to fibrous dysplasia of long bones. E. Typical craniofacial dysmorphism with frontal bossing, high hair-line, low nasal bridge and low-set ears.

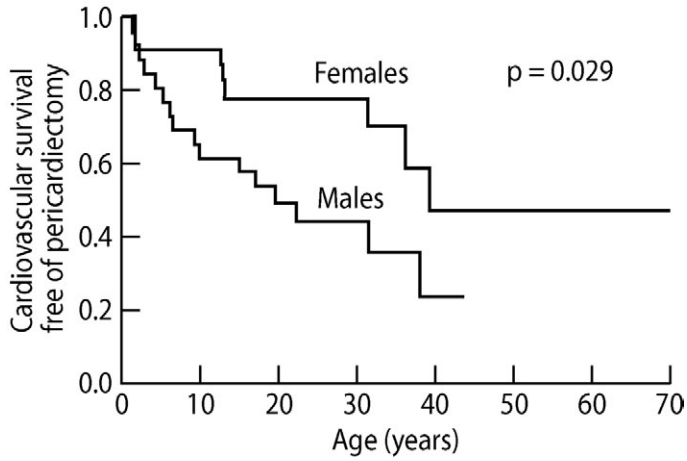

Figure 8. Kaplan-Mayer graphs for the cardiovascular survival free of pericardiectomy in MUL. Although the average survival is shortened (mean 52 years), a normal life span is not excluded: two elderly ladies with MUL are doing quite well at the age of 60 and 77 years. Adapted from Lipsanen-Nyman et al 2003.

3.5.2 *TRIM37* mutations underlie MUL

A positional cloning strategy was initiated in the early 1990s to identify the pleiotropic gene underlying MUL. In year 2000 four disease-associated mutations (two Finnish, one American and one Czech) were successfully identified (Avela K et al 1997, 2000) in a candidate gene corresponding to a previously uncharacterized 4111 base-pair (bp) KIAA0898 cDNA clone (Nagase et al 1998), coding for a novel 964-amino acid 130kD protein, a member of the RING-B-Box-Coiled-coil (RBCC) protein family. The identified gene (KIAA0898) located on chromosome 17q22-23 was named *MUL,* referring to Mulibrey nanism, but shortly renamed *TRIM37* as the causative protein TRIM37 was included in the newly characterized TRIM protein family (Raymond et al 2001).

Today, fifteen disease-associated mutations in the *TRIM37* gene have been published (Table 3) (Avela et al 2000, Jagiello P et al 2003, Hämäläinen et al 2004, 2006, Kallijärvi et al 2005, Doganc et al 2007). The most dominant mutation found (Fin-major) is present in 96% of all Finnish patients (Table 4) (Avela et al 2000). The Fin-major mutation is a c.493-2A>G transition in the 3´splice site of exon 7 resulting in an aberrant splicing at the next AG site which occurs after 3 base pairs producing a 5-bp deletion at c.493-497. The Finnish minor mutation (Fin-minor, c.2212delG), is a 1-bp deletion of a G at nucleotide c.2212 (Avela et al 2000). The Fin-minor mutation has only been detected in two Finnish patients, who both are compound heterozygotes for this and the Fin-major mutation (Avela et al 2000).

Table 4. Disease associated mutations in Mulibrey nanism

Mutation	*Predicted protein consequence*	*Origin of patient*	*Reference*
c.1346dupA	p.Ser450fs	American	Avela et al 2000
c.326C>G	p.Cys109Ser	Australian	H□m□□inen et al 2006
c.860G>A	p.Glu271_Ser287del	Australian	H□m□□inen et al 2006
c745C>T	p.Gln249X	Canadian	H□m□□inen et al 2004
c.965G>T	p.Gly322Val	Canadian	H□m□□inen et al 2004
c.1037_1040dupAGAT	p.Met347fs	Canadian	H□m□□inen et al 2004
c.1411C>T	p.Arg471X	Canadian, Tunisian	H□m□□inen et al 2004
c.838_842delACTTT	p.Thr280fs	Czech	Avela et al 2000
c.493-2A>G	p.Arg166fs	Finnish; *Fin-major*	Avela et al 2000
c.2212delG	p.Glu738fs	Finnish; *Fin-minor*	Avela et al 2000
c.227T>C	p.Leu76Pro	Finnish	Kallij□rvi et al 2005
c.1166A>G	p.Tyr389X	Finnish	* unpublished data
c.81delG	p.Cys28fs	French	* unpublished data
c.1233delA	p.Lys411fs	German	* unpublished data
c.1313+507_1668-207del	p.Arg439fs	Italian	H□m□□inen et al 2004
c.2056C>T	p.Arg686X	Saudi-Arabian	H□m□□inen et al 2004
1910_1911dupTA	p.Gln638fs	Swiss	* unpublished data
c.810-1G>A	p.Glu271fs	Turkish	Jagiello et al 2003
c.1894_1885delGA	p.Glu632fs	Turkish	Doganc et al 2007

* H□m□□inen et al unpublished data.

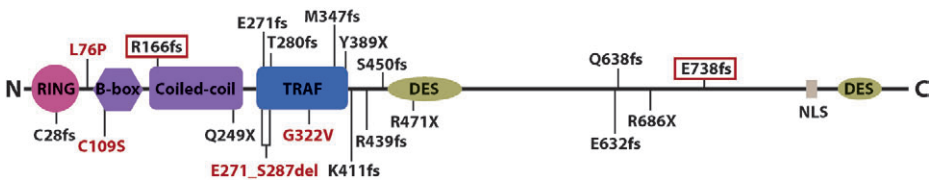

Figure 9. A schematic structure of the TRIM37 protein. Altogether 19 mutations underlying MUL are known. Fifteen of them produce premature termination codons (PTC) and are likely to trigger nonsense mediated mRNA decay. Of the mutations in red, three are missense mutations; L76P, G322V, C109S and one a 17 amino-acid deletion of the TRAF domain; E271_S287del. All 19 *TRIM37* mutations identified to this date seem to produce loss of function alleles and no genotype-phenotype correlation has been seen in MUL patients (Hämäläinen et al 2004, 2006). The Fin-major (R166fs) and Fin-minor (E738fs) mutations are visible in the red boxes above.

3.6 TRIM protein family

More than 60 TRIM proteins are known to this date (Ozato et al 2008). The TRIM domain is located in the N-terminus, while various other domains may be present in the C-terminus of the protein. The RING domain is defined by ordered arrangement of cystein (C) and histidine (H) residues that bind two zinc atoms (Freemont et al 1993). The TRIM, RING and B-Box domains are, for example, needed for protein-protein interactions and for promoting formation of larger protein complexes. (Raymond et al 2001).

The RING domain is thought to be the crucial site for some TRIM proteins in mediating their effects on signalling pathways (Ozato et al 2008). RING fingers can act solely or as a multi-subunit RING E3-ligase (Pickart 2001a, 2001b) and have been shown to mediate the transfer of ubiquitin from the ubiquitin conjugating enzyme to its substrate and act as an ubiquitin E3-ligase (Joazeiro and Weissman 2000). The RING domains of many TRIM family members, including TRIM5, TRIM8, TRIM11, TRIM22, TRIM25, and TRIM37, have now been shown to confer E3-ubiquitin ligase activity, that allows them to mediate ubiquitin events (Toniato et al 2002, Kallijärvi et al 2005, Diaz-Griffero et al 2006, Ishikawa et al 2006, Sabile et al 2006, Gack et al 2007, Barr et al 2008, Yamauchi et al 2008). Recent studies have shown that many members of the TRIM protein family are involved in a broad range of biological processes, including innate immunity and some that underlie genetic disorders, neurological diseases and cancers (Table 4) (Raymond et al 2001, Meroni and Diez-Roux 2005, Ozato et al 2008).

Table 5. TRIM protein family in human disease

Protein	Disease	Ubiquitin E3-ligase
TRIM1	X-linked FG syndrome	-
TRIM5	HIV	yes
TRIM8	Unknown	yes
TRIM11	Alzheimer□s disease-like neuronal insults	yes
TRIM18	X-linked Opitz syndrome	-
TRIM19	Promelocytic leukemia	-
TRIM20	Familial Mediterranean Fever	-
TRIM21	SLE* and Sj□gren syndrome	yes
TRIM22	Unknown	yes
TRIM24	Murine leucemia	-
TRIM25	Antiviral innate immunity	yes
TRIM27	Thyroid carcinoma	-
TRIM32	Limb-girdle dystrophy	-
TRIM33	Thyroid carcinoma	-
TRIM37	Mulibrey nanism	yes
TRIM68	SLE* and Sj□gren syndrome	-

Monogenic disorder, * SLE; systemic lupus erythematosus. Adapted from Raymond et al 2001, Ozato et al 2008

3.6.1 TRIM37

The defective protein in MUL, TRIM37, possesses a typical N-terminus tripartite motif (TRIM) comprising a RING domain, a single B-Box domain, and a Coiled-coil region (Figure 9) (Borden 1998, 2000, Raymond et al 2001). The TRIM unit is followed by a Tumour necrosis factor (TNF)-Receptor-Associated Factor TRAF domain (Zapata et al 2001). TRAF proteins are in general involved in TNF receptor signalling, they serve as adaptor proteins for a wide variety of cell surface receptors or act as ubiquitin E3-ligases regulating a wide range of important cellular functions (Paul 2008). The C-terminus half of TRIM37 has no known functional domains (Avela et al 2000).

TRIM37 localizes in immunofluorescence studies to peroxisomes in cell cultures. However, no known peroxisomal targeting signals have been shown in TRIM37 and the localization can be dependent on interaction partners (Kallijärvi et al 2002). Peroxisomes are small single membrane-bound organelles that participate in a variety of metabolic functions in the eukaryote cell. The peroxisomes play a central role in lipid metabolism and hydrogen peroxide detoxification. However, they are also needed for normal intracellular signalling and in regulating normal development (Titorenko and Rachubinski 2004). Defective assembly of peroxisomes results in metabolic or developmental disorders or are lethal (Wanders et al 2004).

Peroxisomal disorders are divided into two categories; peroxisomal enzyme deficiencies and disorders of the peroxisomal biogenesis (PBD). PBDs result from mutations in the *PEX* genes, encoding for peroxins that are required for appropriate assembly of peroxisomes (Wanders 2004). Typical clinical features of PBDs include dysmorphic craniofacial features, neurological disabilities and skeletal, hepatological and ocular abnormalities (Wanders 2004). One of the most severe phenotypes of PBDs is seen in Zellweger syndrome (ZS; OMIM 214100), in which patients have multiple congenital anomalies and rarely survive the first year of life (Brosius and Gärtner 2002). Refsum's disease (OMIM 266510, hereditary motor sensory neuropathy type IV, heredopathia atactica polyneuritiformis) is an autosomal recessive disorder including features like retinitis pigmentosa, blindness, anosmia, deafness, sensory neuropathy and ataxia. Patients also display cardiomyopathy and cardiac arrhythmias as a consequence of phytanic acid tissue accumulation (Leys et al 1998).

Single peroxisomal enzyme deficiencies generally result in a much milder phenotype than seen in the PBDs. The most common is the X-linked adrenoleukodystrophy (X-ALD; OMIM 300100) caused by impaired peroxisomal oxidation of very-long-chain fatty acids (VLCFA) and subsequent accumulation of VLCFA in peripheral tissues. The clinical phenotype can vary from manifestations occurring only in the adrenal cortex or the Leydig cells of the testes to more severe

symptoms arising from the central nervous system (CNS) (Gärtner et al 1998, Moser et al 2000).

MUL shares some features with known peroxisomal disorders, particularly with the PBDs. These include growth restriction, facial dysmorphism, retinal pigmentary changes, muscular hypotonicity, hepatomegaly and in Refsum´s disease cardiomyopathy (Leys et al 1989, Sacksteder et al 2000). However, peroxisomal disorders, particularly PBDs typically display mental retardation and neurological abnormalities, which MUL-patients mostly lack. Due to overlapping clinical findings in MUL and PBD the peroxisomal function has previously been assessed in a small cohort of MUL-patients (Schutgens et al 1994). The plasma concentrations of phytanic acid, pristanic acid, tri- and dihydroxycholestanic acid, and cerotic acid, however, were found to be normal in MUL. Also, the profile of very-long-chain fatty acids and *de novo* plasmalogen biosynthesis in fibroblasts were within normal limits. Although no biochemical evidence of gross peroxisomal dysfunction has been found (Schutgens et al 1994), MUL has since 2002 been regarded as a peroxisomal disorder (Kallijärvi et al 2002).

3.7 Ubiquitin proteasome system

The exact biological function of TRIM37 is unknown. It is expressed in several tissues during different stages of development in mice (Lehesjoki 2002, Kallijärvi 2006) and has been shown to possess TRIM-domain dependent ubiquitin E3-ligase activity (Kallijärvi et al 2005). Ubiquitination is a post-translational modification of proteins in the eukaryotic cell discovered in the 1980s by Hershko and colleagues (Hershko 1983, Hershko and Ciechanover 1998). The process of ubiquitination is highly conserved and the ubiquitin-proteasome system (UPS) plays a pivotal role in upholding protein homeostasis in the cell by regulating many central cellular functions, including cell proliferation, adaptation to stress, cell death and apoptosis (Hershko and Ciechanover 1998, Pickart 2001a, 2001b). UPS eliminates misfolded, unassembled, oxidized or otherwise damaged proteins that could form potentially toxic aggregates in the cell. These abnormal proteins are rapidly removed from the endoplasmic reticulum, transported to the cytosol and targeted for destruction by the UPS (Mukhopadhyay and Riezman 2007, Paul et al 2008).

Ubiquitination is an ATP-dependent covalent attachment of an ubiquitin molecule to its target protein via a cascade of three separate biochemical reactions (Figure 10). The ubiquitin process is then usually repeated resulting in a polyubiquitin chain (Pickart 2001a). Substrates marked with a Lys48-linked polyubiquitin chain are then targeted to the 26S proteasome in the cell for destruction (Hicke and Dunn 2003, Mukhopadhyay and Riezman 2007). On the other hand, monoubiquitination or Lys63-linked polyubiquitin chains mainly regulate cellular func-

tions, such as, endocytosis, transcription and most importantly also DNA repair (Weissman 2001, Hicke and Dunn 2003, Mukhopadhyay and Riezman 2007).

E3 ubiquitin-ligases can be divided into three groups: 1) E3s containing a HECT-domain (homologous to E6-AP carboxy terminus) (HECT), 2) E3s containing a RING finger and 3) E3s containing a U-Box or a PHD (Plant Homeo-Domain). The RING finger ubiquitin ligases constitute the largest subgroup of E3s. The ubiquitin E3-ligases regulate the final and most crucial step in the ubiquitination process and act as specific substrate-recognition elements or "quality controllers" of the UPS. The specificity of the ubiquitin-mediated regulation is conferred by the low number of E3-ligases, probably about 1000 in the human genome. Each of them acts alone with a single or a few E2s to recognize specific amino acid sequences (Borden 2000, Pickart 2001a, 2001b).

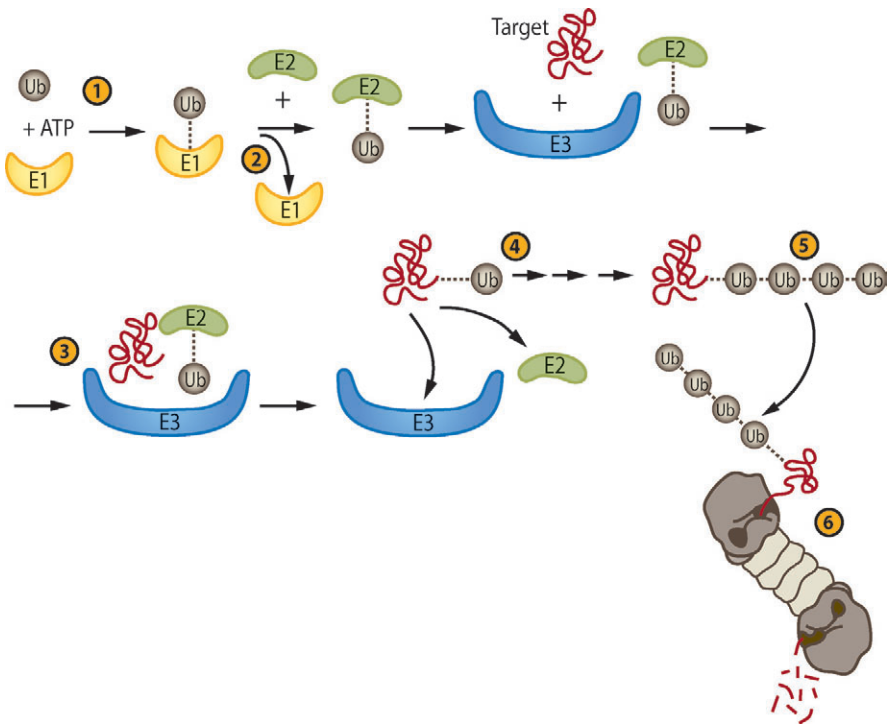

Figure 10. In step one, the ubiquitin-activating enzymes (E1) form a bond between the ubiquitin molecule and a cysteine residue on the activated site of the enzyme. In step two, the activated ubiquitin is transferred and bound to the ubiquitin-conjugating enzyme (E2). In step three, the ubiquitin E3-ligase recognizes the specific E2. In step four, E3 tags the target protein with ubiquitin. Then, in step five, the ubiquitin process is usually repeated resulting in a polyubiquitin chain. Finally in step six, the polyubiquitined protein is targeted to the 26S proteasome for proteolysis. Adapted from Hershko and Ciechanover 1998.

3.7.1 Disorders of UPS

UPS controls the degradation of numerous proteins and regulates signalling pathways either by activating or inhibiting them. Dysfunction of UPS could alter normal cellular homeostasis and cause a wide range of pathologies in different organ systems. In fact, UPS dysfunction has been associated with diabetes mellitus, obesity, growth failure and a wide range of cardiac diseases (hypertrophy and failure, myocardial ischemia, atherosclerosis and cardiomyopathy) (Wang et al 2008). Also genetic or acquired syndromes (i.e. cystic fibrosis, Sjögren syndrome), metabolic disorders (Fanconi anemia and α_1-antitrypsin deficiency) and neurodegenerative disorders (i.e. Alzheimer's disease, Parkinson's disease, Huntington disease, prion-like lethal disorders and amyotrophic lateral sclerosis) (Hegde et al 2007) have been linked to UPS. Also, several cancers (i.e. colon, breast, ovarian, renal, Kaposi's sarcoma and multiple myeloma) have recently been connected to UPS (Chen et al 2002, Corn 2007, Bénichou et al 2003), most likely by affecting the stabilization of oncoproteins or destabilization of tumour suppressor genes (Sun 2006, Paul 2008).

Defects in all three ubiquitin ligase groups have been associated with human diseases (Jiang and Beaudet 2004). Mutations in the *UBE3A* gene, encoding for a HECT family ubiquitin ligase UBE3A, have been found to result in Angelmans' syndrome with severe motor and mental retardation (Kishino et al 1997). Liddle syndrome, a hereditary form of early onset hypertension, is caused by mutations in the epithelial Na^+ channel (ENaC). The mutated region serves as a binding site for a HECT family E3-ligase. As a consequence, the endocytosis is impaired which leads to an increased Na^+ and fluid reabsorption in the distal nephron, resulting in elevated blood volume and high blood pressure (Rotin 2008).

APECED (autoimmune polyendocrinopathy-candidasis-ectodermal dystrophy), a disorder of the Finnish disease heritage, has been found to be caused by a defective protein AIRE, which is a PHD type zinc finger ubiquitin ligase (Nagamine et al 1997). In APECED the main findings are recurrent candidal infections, chronic ectodermal alternations and several endocrinological dysfunctions (most importantly hypoparathyroidism and adrenocortical insufficiency) appearing in childhood and after that during subsequent decades (Perheentupa 2002).

Recent evidence links intrauterine growth restriction with impaired ubiquitin E3-ligase activity (Provot and Shipani 2007, Sarikas et al 2008). Dysregulation of Cullin7 (CUL7), a protein which under normal circumstances assembles a RING finger ubiquitin E3-ligase, has been shown to result in the autosomal recessive 3-M syndrome, characterized by low birth weight, profound pre- and postnatal growth retardation, facial dysmorphism and radiological abnormalities (Miller et al 1975, Huber et al 2005). In addition, *CUL7* $^{-/-}$ mice often die immediately

after birth due to respiratory distress, and show progressive growth retardation already during embryonic period. The mice further present abnormal vascular morphogenesis which is seen also in the placenta. Dermal and hypodermal hemorrhage is detected in mutant embryos, suggesting disturbed vascular morphogenesis as one of the underlying causes for the growth problem in 3-M syndrome (Arai et al 2003). Recent identification of insulin receptor substrate-1 (IRS1), a critical mediator of insulin and insulin-like growth factor-1 signalling, as the proteolytic target of the CUL7 E3-ligase, provide a molecular link between CUL7 and a well-established growth and metabolic regulatory pathway in humans (Xu et al 2008). CUL7 has also been demonstrated to interact with the p53 tumour suppressor protein, further associating CUL7 and RING finger E3-ligases to the control of tissue growth (Sarikas et al 2008).

Tumours have strongly been associated with the ubiquitin pathway. The prototypic example of this is the tumour suppressor protein p53, which plays an essential role in the cell cycle control, DNA repair, and apoptosis, and may enhance cancer through its inadequate actions. P53 is regulated by E3-ligases, mainly by the RING-type Mdm2. In the vast majority of cancers Mdm2 regulates p53 both by inhibiting its transcription and by increasing its turnover by targeting p53 for ubiquitination and proteosomal degradation (Sun 2006, Paul 2008).

A number of functions have been ascribed to the tumour suppressor protein BRCA1, including transcriptional regulation, DNA repair, and induction of apoptosis. BRCA1 possesses N-terminal RING finger and has been shown to be an E3-ligase (Fang et al 2002). Tumour-associated mutations in the RING finger have been shown to abolish its E3 activity. Indeed, inactivating mutations of BRCA1 have frequently been reported in breast cancer (Hashizume et al 2001) and in ovarian carcinoma (Paul 2008). Also, down-regulation of tumour-suppressing sub-chromosomal transferable fragment cDNA (TSSC5) has been observed in certain tumours, particularly Wilms' tumour. TSSC5 encodes for a transporter-like RING-finger protein, RING105, acting as an ubiqutin E3-ligase (Sun 2006, Scott et al 2008).

The von Hippel-Lindau tumour-suppressor gene (*VHL*) is inactivated in the von Hippel-Lindau disease (VHL; OMIM 193300), which is an autosomal dominant cancer syndrome that results from germline mutations in the *VHL* gene on chromosome 3p25-26 (Sun 2006, Jung et al 2006). *VHL* is also inactivated in sporadic cases of clear-cell renal cell carcinoma (Paul 2008, Shehata et al 2008). The VHL protein (pVHL) functions as part of an ubiquitin E3-ligase complex that targets proteins for 26S proteasomal degradation. Its best-characterized substrate is the hypoxia-inducible factor-alpha (HIF-α). Loss of pVHL and subsequent up-regulation of HIF target genes has been shown to cause tumours of highly vascular nature (Jung et al 2006). Additional functions of pVHL may also be important in preventing the development of clear-cell renal cell carcinoma by maintaining the stabilization of p53 (Paul 2008).

4. AIMS OF THE STUDY

The aims of this study were to analyze the clinical and histopathological characteristics of MUL in the national cohort of Finnish patients. MUL is a dysmorphic growth disorder caused by recessive mutations in the *TRIM37* gene on chromosome 17q22-23. The specific aims were:

1. To characterize the clinical features of MUL from infancy to the time of diagnosis, and to propose new clinical diagnostic criteria for MUL.

2. To analyze and describe the key elements of the glucose and lipid metabolism in subjects with MUL.

3. To evaluate and describe the natural growth pattern of MUL patients and to analyze the outcome of long-term GH treatment.

4. To study the specific histopathological and immunohistochemical characteristics of MUL in different organ systems.

5. PATIENTS AND METHODS

Since the early 1970s, the clinical care of the Finnish MUL patients has mostly been centred to the University of Helsinki Children's Hospital. This has generated a large amount of clinical unpublished data. A significant part of this retrospective data together with the new data from a clinical follow-up study initiated in year 2000 make up the material of this thesis.

5.1 Subjects

The series included all known 92 Finnish MUL patients diagnosed before 2007, 88 of them homozygous for the Fin-major mutation (c.493–2A>G) of the *TRIM37* gene (Avela et al 2000). Four patients were compound heterozygotes; two for the Fin-major and Fin-minor (c.2212delG) mutations (Avela et al 2000), and the remaining two patients had a c.227T>C/Fin-major (Kallijärvi et al 2005) and c.1166A>G/Fin-major (Hämäläinen et al unpublished data) genotype, respectively. The diagnosis has been genetically confirmed directly in all but five of the patients. In four of them, DNA was obtained from family member(s); one parent couple, two single parents, and one sibling, all carriers of the Fin-major mutation (c.493–2A>G). The phenotype of the remaining patient left no doubt about the diagnosis. In seven of the families, there was an affected sibling. In five of these siblings, the clinical findings were constant, but in two families the phenotype showed minor variation.

5.2 Clinical data collection

The hospital records of 89 patients (49 females) were retrospectively analyzed (I, II, IV). Data on clinical events, appearance and dysmorphic features were recorded and the patients were photographed. Obstetric and growth data were collected from local hospitals, child welfare centres and schools. The patients' parents, relatives, and physicians were interviewed for missing details.

All patients underwent a physical examination at 6-12 month intervals during childhood and puberty with assessment of height, weight, and pubertal stage according to the criteria of Tanner (Tanner and Whitehouse 1979). Height and weight measurements were made by the clinician or a trained nurse. Standing heights were measured with a stadiometer to the nearest millimetre and the mean of three measurements was used. The weight was measured with an ordinary scale to the nearest 100 g. The blood pressure was measured three times while the subjects were seated, and the last two measurements were averaged for analysis. GH treated patients were reviewed at baseline and at 3, 6, 9 and 12 months after commencement and subsequently every 6 months.

Growth data from birth to final height on 72 (40 females) patients were collected (I, III). A standard arginine- or insulin-arginine tolerance test was used to evaluate the GH secretion. A peak serum GH response >10 µg/l was considered normal and a value between 5 and 10 µg/l indicated partial GH deficiency. The patient was considered GH deficient if the peak serum level was <5 µg/l in two separate tests. Thirty of the patients (18 females) were treated with recombinant human GH since early 1990´s for a median period of 5.7 years. The efficiency of GH treatment was analyzed based on data from these patients, while the natural growth was evaluated from the untreated patients and data on the treated patients prior to commencement of GH therapy (III).

Finnish growth standards were used for height and weight analysis (Sorva et al 1984). Height standard deviation score (hSDS) was calculated for calendar age. Final hSDS was calculated from the standards of the Finns at the age of 18 years (I, III). The weight of the patients was defined as the percent deviation from the age- and sex-specific median weight-for-height (WFH) (Pere 2000) (I, II, III). Adult weights were expressed also as body mass index (BMI) (II, III). The birth length and weight of patients born preterm (before 38 weeks of gestation) were extrapolated to term by using Finnish standards of prenatal growth (Pihkala et al 1989) (I, III). The onset of puberty was defined as stage P2G2 in boys and a stable breast stage of M2 in girls (Tanner and Whitehouse 1979) (II).

Also radiological [x-rays, ultrasonography (US), computer tomography (CT), and magnetic resonance imaging (MRI)] (I, II, III, IV), and histological data (biopsy and autopsy samples) (III, IV), and data on the clinically followed patients were retrospectively analyzed. Fifteen patients in the survey were evaluated only from hospital and autopsy records and samples obtained at autopsy (I, II, III, IV). Two of the patients died during follow-up and an autopsy was performed.

5.3 Laboratory examinations

Blood glucose (venous whole-blood glucose), glycohemoglobin A1C and serum insulin were measured after an overnight fast with concomitant measurement of serum leptin and plasma uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase and triglycerides as well as plasma total cholesterol, high-desity lipoprotein (HDL), and low-density lipoprotein (LDL) (I, II, III). In patients receiving GH serum IGF-1 was measured every 6 months. Blood counts, blood glucose and serum concentrations for thyroid and liver function were followed as safety parameters annually (III).

Serum IGF-1 levels were measured by radioimmunoassay (RIA) (Incstar, Stillwater, Minnesota USA) and serum leptin levels were assessed by specific RIA (Linco Research Inc, St. Louis, Missouri USA) (II, III). GH concentrations were measured

by monoclonal immunoradiometric assay (Gif-sur-Yvette, Cedex, France) prior to the year 1995 and thereafter by AutoDelfia time-resolved immunoflurometric assay (PerkinElmer, Wallac, Turku, Finland).

A comprehensive evaluation of the glucose and lipid metabolism was performed in 65 (37 females) patients (II). An oral glucose tolerance test (OGTT; glucose load 1.75g/kg, maximum 75 g) with concomitant measurements of blood glucose and serum insulin was used to classify the subjects as having normal glucose tolerance, impaired glucose tolerance (IGT; the 2-h venous whole blood glucose value from 6.7-9.9 mmol/l), or type 2 diabetes (fasting venous whole blood glucose ≥6.1 mmol/l or the 2-h value ≥10.0 mmol/l) on the basis of WHO criteria (Alberti and Zimmet 1998). The degree of insulin sensitivity was determined from the ratio of fasting glucose and insulin levels (Vuguin P et al 2002) and the whole-body insulin sensitivity index was calculated from the OGTT values according to Stumvoll (Stumvoll et al 2000, 2001). Metabolic syndrome was determined according to the NCEP Adult Treatment Panel III (ATP III 2001, Fedder et al 2002) and hypertension as blood pressure >95th percentile for age and sex.

5.4 Radiology

A radiological survey with X-rays of the skull, thorax, and long bones was conducted (I, III, IV). US of the abdomen (children and adults) (II, IV) and thyroid gland (adults) (IV) were performed and in cases of tumour suspicion, MRI was arranged without delay (IV). The bone age (BA) was determined from an x-ray of the palm and wrist according to the method of Greulich and Pyle (Greulich 1950, Greulich and Pyle 1950) (III).

5.5 Histology and immunohistochemistry

The tissue specimens were obtained on clinical grounds at surgery or as percutaneous core needle biopsy samples or at autopsies of 17 MUL patients. Formalin-fixed paraffin-embedded samples were used for the histological and immunohistochemical studies. Immunoperoxidase staining was performed in a traditional way, using sections of formalin-fixed, paraffin-embedded tissue samples. Microwave treatment in 10 mM/l citric acid for 10 min was performed, or Dako Target Retrieval Solution (S1699) (DakoCytomation, Glostrup, Denmark) was used to improve the antibody penetration. The slides were then incubated with antibodies against endothelial cell markers CD34 (Dako-Cytomation, Glostrup, Denmark) and CD31 (PECAM-1; Dako), α-smooth muscle actin (α-SMA; Dako), perivascular epithelioid cell (PEC) marker HMB-45 (Dako) and cellular proliferation marker MIB-1 (KI-67; Dako). Amplification of the primary antibody reaction was achieved by incubating the sections with biotinylated secondary an-

tibody (Vector Elite ABC Kit; Vector Laboratories Inc., Burlingame, CA, USA) or TSA Indirect (Pyramide Signal Amplification Kit, Perkin-Elmer LAS Inc., NEL700). Histological specimens were reviewed by the experienced pathologist Dr. Riitta Karikoski.

5.6 Statistics

Clinical, laboratory and metabolic parameters were correlated with age, weight for height (II, III), and with the hSDS increment during the preceding year (III). The explanation rate was expressed as an R^2-value. The Wilcoxon test was used to compare parameters between the GH treated and untreated patient groups. P-value less than 0.05 were defined as statistically significant.

5.7 Ethics

The study was approved by the Institutional Ethical Review Board at the University of Helsinki. The use of clinical and autopsy material for research purposes was approved by the National Authority for Medico-Legal Affairs in Finland (TEO). Patients and/or their guardians gave informed consent. Also, approval for the use of patient photographs for scientific purposes was obtained.

6. RESULTS

The clinical and histopathological characteristics of MUL were analyzed in the national cohort of Finnish patients mostly homozygous for the Fin-major mutation in the *TRIM37* gene. The results indicate that MUL forms a distinct clinical entity, although organ manifestations vary considerably from early childhood to adulthood. The most consistent findings in our cohort were the characteristic dysmorphic features, pre- and postnatal progressive growth failure, severe insulin resistance with tendency to abbreviations in glucose and lipid metabolism with increasing age, and high frequency of malignant tumours and benign adenomatous and vascular lesions, as well as disturbed organogenesis.

6.1 Clinical characteristics of MUL

Clinical features were evaluated in 85 (46 females) subjects with MUL. Most of the pregnancies preceded without major problems. Nineteen of the pregnancies (23%) had been monitored due to poor fetal growth. Other complications included pre-eclampsia (three cases), anti-Rhesus immunization (a pair of siblings), gestational diabetes (one), and varicella zoster infection (one). Fifteen mothers (18%) had experienced miscarriages priorly. Length of gestation varied from 32 to 42 weeks (median 39 weeks); 90% of the children were born at full term. Labour was normal in all but three cases, in which an urgent cesarean section was performed because of threatening asphyxia. Cardiac involvement was evident in only one newborn with Wolf-Parkinson-White syndrome (WPW) causing paroxysmal supraventricular tachycardial attacks already *in utero* (Tikanoja and Taipale 2004). Fourteen babies needed supplementary oxygen after birth. Respiratory distress syndrome was diagnosed in three; two of them were born prematurely. Apgar score at 5 minutes was on average 8 (range 2-10).

After a relatively normal newborn period half of the infants failed to thrive. Feeding difficulties and upper respiratory infections, including frequent pneumonias were the most common clinical problems (Table 5). Episodes of respiratory failure induced by an infection occurred in 25% and nine of the 85 infants had to be resuscitated (Table 6). Four and nine patients had congestive heart failure by the age of 1.0 and 2.0 years, respectively; four of them died. Psychomotor development was normal or slightly delayed in a great majority of the infants. Mild hypotonicity was evident in nearly half of the infants, and some delay in both motor development and speech was noted in nearly one third. Age at the first steps and the first words ranged from 0.8-2.6 years (mean 1.2 years) and 0.9-3.1 years (mean 1.3 years), respectively. A mildly hydrocephalic skull with frontal bossing and abnormally wide fontanelles evoked suspicion of hydrocephalus in 15% of the infants (Table 6).

Table 6. Problems in infancy in 85 patients with MUL

Feature	Frequency (%)
Neonatal problems	
Respiratory problems	31
Ventilatory assistance needed	20
Cyanosis while crying	15
Feeding difficulties	30
Nasogastric feeding tube needed	17
Suspicion of hydrocephalus	6
Suspicion of sepsis	6
Cardiac arrhythmia	1
Growth failure	96
Infections	
Upper respiratory infections >4/year	51
Pneumonia	47
Necessitating hospital care	30
Three or more pneumonias	27
Ventilatory assistance needed	25
Intubation needed	14
Middle ear infections >4/year	38
Feeding difficulties	50
Vomiting	39
Difficulties in sucking	31
Nasogastric feeding tube needed	31
Delay in switching to solid foods	24
Fatigue during eating	21
Percutaneous gastrostomy needed	6
Muscular hypotonicity	46
Suspicion of hydrocephalus	16
Hypoglycemia	15
Congestive heart failure	11
Resuscitation during first year of life	11
Due to infection	9
Due to aspiration	1
Due to arrhythmia	1
Death during infancy	5
Due to infection	2.5
Due to congestive heart failure	2.5

Adapted from the Journal of Medical Genetics 2004 (I)

The diagnosis was commonly made in early childhood (median age 2.1; range 0.02 to 52 years), so that one third were younger than 1 year and two thirds were younger than 5 years. At the time of diagnosis, the characteristic craniofacial features combined with poor growth were the dominant characteristics in the great majority (Table 7, Figure 11), and in all but four of the patients (95%) the hSDS was below -2.5. Also, characteristic radiological findings including slender long bones with thick cortex and narrow medullary channel and a low and shallow (J-shaped) sella turcica were present in nearly all patients (Table 7, Figure 11). Fifteen patients (18%) had signs of heart disease; ten of them had congestive heart failure (Table 6), and four had pericardial constriction necessitating pericardiectomy. Thirty-eight patients (45%) had hepatomegaly and six of them tumour-like lesions in the liver. Wilms' tumour was detected in two patients before MUL was diagnosed. Both had non-symptomatic hematuria with a palpable abdominal mass as the only signs.

Gross organ anomalies of the heart and urogenital system were also observed (Table 7). A peculiar high pitch and slight coarseness of the voice was noted in nearly all of the prepubertal patients. Characteristic ocular findings were yellow dots in the midperiphery of ocular fundi, seen in 80% of the patients. Additional ocular findings were retinal hypopigmentation and pigment dispersion with clusters of pigment in the retina. Two thirds of the patients had cutaneous naevi flammei and 15% had fibrous dysplasia, most commonly in the lower limbs (Figure 11). A slight asymmetry of the extremities was noted in 17 patients (20%), which in two thirds was due to shortening by fibrous dysplasia. At diagnosis, the only abnormal laboratory parameters were the elevated serum levels of the aminotransferases seen in nearly half of the patients; AST up to 110 U/l (mean 61 U/l) and ALT up to 193 U/l (mean 41 U/l).

Table 7. Clinical features at the time of diagnosis in 85 MUL patients

Feature	Frequency (%)
Head	
Characteristic face: triangular face, high and broad forehead; low nasal bridge and telecanthus	90
Scaphocepaly with occiptofrontal bossing	90
Low and shallow (J-shaped) sella turcica	89
Laterally rotated orbital fossae	80
Hypoplastic tongue	80
Small hypoplastic face	71
Orbital hypertelorism	64
Low set located posteriorly rotated ears	54
Dental crowding	50
High hairline	45
Wide fontanelles and sutures in infancy	37
Abnormally wide metopic suture	31
Cleft palate	2
Body	
Thin extremities	99
Accentuated lumbar lordosis	96
General gracility	95
Narrow shoulders	94
Small bell shaped thoracic cage	94
Barrell-like trunk	92
Cutaneus naevi flammei	65
Hypoplastic buttocks	52
Constitution	
Slender long bones	93
Relative proximal shortness of limbs	90
Large head relative to stature	90
Large hands and feet relative to stature	67
Fibrous dysplasia of long bone	15
Skeletal asymmetry	15
Heart	
Signs of heart disease	18
Cardiomegaly	14
Dyspnoea	12
Prominent veins on upper body	11
Cardiac anomaly	15
ASD	8
VSD	5
Open ductus arteriosus	3
Congestive heart failure	12
Pericardial constriction	6
Liver	
Hepatomegaly	45
Ascites	11
Liver tumour-like lesions	9
Urogenital	
Renal anomaly	8
Hydronephrosis	6
Abnormally located kidney	5
Hypoplastic kidneys	5
Horseshoe kidney	12
Male genital anomaly*	6
Cryptorchidism	6
Female genital anomaly	2
Hyperplastic labiae	1
Prominent clitoris	2
Wilms tumour	3
Nervous system	
Yellowish dots in the ocular fundi	79
Muscular hypotonicity	68
Large cerebral ventricles and basal cisternae	44
Slow speech development	30
Slow motor development	29
Skin	
Cutaneous naevi flammei	65

*Males (n = 39); females (n = 46)

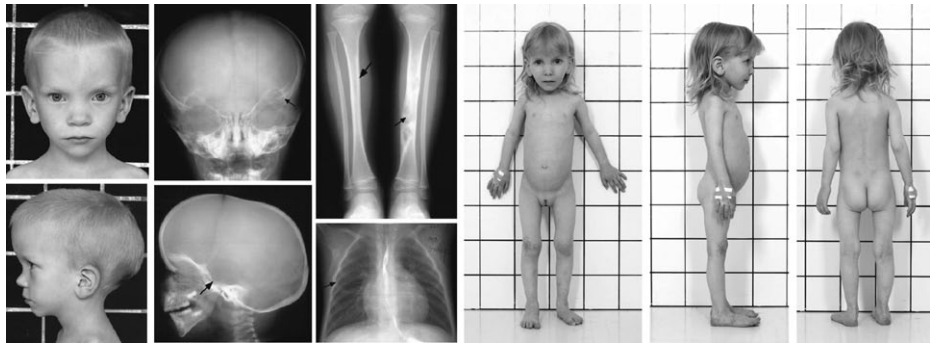

Figure 11. Typical craniofacial, radiological and constitutional features of MUL. The face is triangular and the skull scaphocephalic with a J-shaped sella turcica (arrow). The orbital fossae are slightly upward-slanting (arrow) and fibrous dysplasia of the slender long bones was frequently seen (arrows). The heart appears large in relation to the small and bell-shaped thoracic cage. The ribs are also thin (arrow). Patients display general gracility and hands and feet apperar large in relation to the height. Figures partly adapted from the Journal of Medical Genetics 2004 (I).

6.2 Natural growth in MUL

Growth data were analyzed in 72 (40 females) MUL patients. At birth, MUL infants were both short and light and 95% were born SGA. The median birth length and weight adjusted to 40 weeks of gestation were 44.8 cm (median hSDS -3.0) and 2300 g (median SDS -3.0) for the girls and 45.0 cm (median hSDS -2.8) and 2350 g (median SDS -2.9) for the boys, respectively. The median SDS for OFC was -0.5 (range -0.9-0.8) indicating spared cranial growth with macrocephaly relative to birth length. The growth failure progressed in early infancy with a median hSDS decrement of 1.1 from birth to 2.0 years of age (Figure 12). Growth in children with severe feeding difficulties (five children; median hSDS -5.1, range -3.8 to -5.9) and congestive heart failure (nine children; median hSDS -5.4, range -4.3 to -7.4) was more compromised than in subjects without these problems (median hSDS -4.4). Two children (3%) were born severely premature at gestation week 32, with a birth length SDS of -6.4 and -4.0, respectively. Their postnatal growth was most severely affected so that their hSDS at 2.0 years of age was -7.8 and -7.3, respectively.

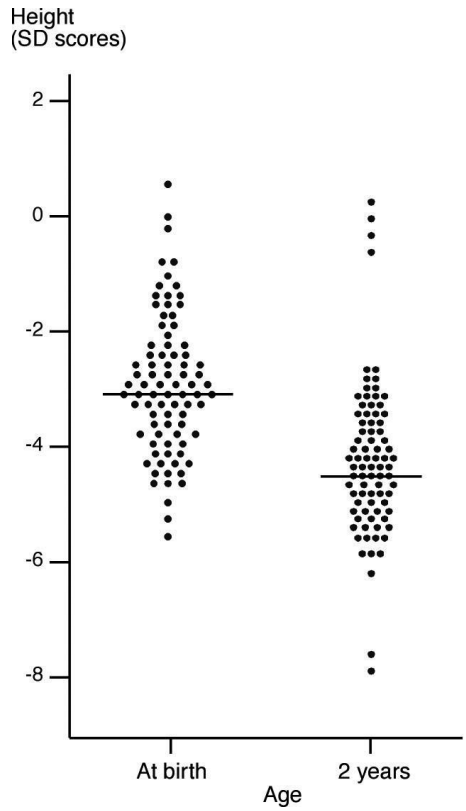

Figure 12. Ninety-five percent of MUL babies were born SGA and their growth failure progressed postnatally. Figure with permission from the Journal of Medical Genetics 2004 (I).

Catch-up in growth with a gain in hSDS of more than 1.0 occurred only in five (6%) of the infants. At the age of 2.0 years, the hSDS was on average -4.4 (range 0.1 to -7.8) (Figure 12). WFH decelerated continuously during the two first years (Table 8, Figure 14). Infant growth deceleration was followed by a spontaneous but incomplete catch-up growth, which lasted up to school age (Figure 14). The children remained slim until puberty (Table 5, Figure 14). MUL subjects showed a wide individual variation in the onset of puberty with a median age of 12.5 and 13.0.years for boys and girls, respectively (Table 5). The puberty was spontaneous but the development of the secondary sexual characteristics was slow and incomplete in both sexes. At the onset of puberty the median hSDS was -3.6 (range -6.3 to -1.0, Table 8, Figure 15). Postpubertally, WFH started to increase particularly in the females (Table 8, Figure 14, 15). The final adult height averaged 136 cm (range 130-155 cm; median hSDS -5.1) in females and 150 cm (range 147 to 162 cm; median hSDS -4.1) in males.

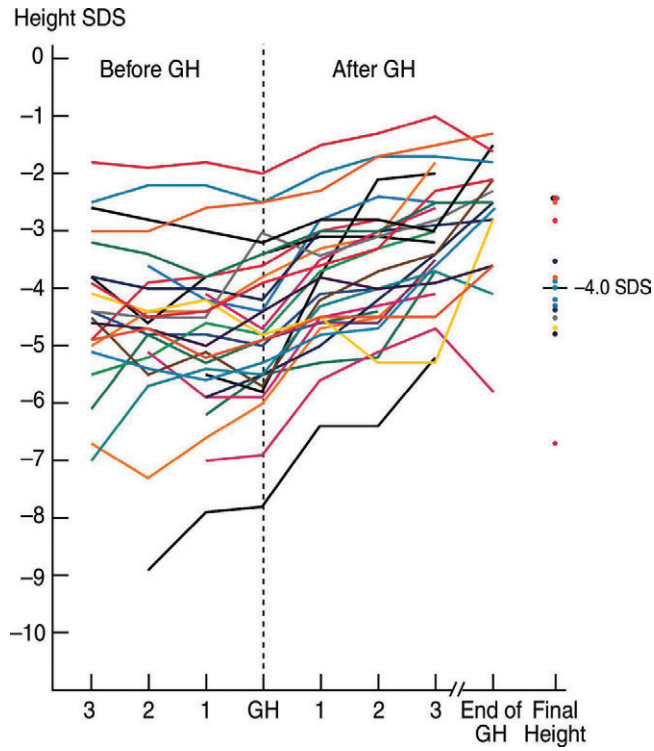

Figure 13. GH treatment improved growth temporarily so that the growth velocity reached its peak 12 to 18 months after the start. The long-term effect however remained modest. A median gain of 5 cm in height was achieved. Figure with permission from Pediatrics 2007 (III).

6.3 Growth hormone treatment in MUL

GH production was assessed in 38 MUL children at a median age of 5.1 years (2.0-15 years). The serum peak GH level averaged 8.2 µg/l (range 1.3-23.2). The response was interpreted as normal in 26%, subnormal in 58%, and low in 16%. The distribution was similar in those individuals who were started on GH treatment (normal 27%, subnormal 62%, low 11%). Serum IGF-1 levels were normal, but half of the prepubertal children had an IGF-1 value in the lowest quartile of the normal range.

Thirty (18 females) patients were treated with GH for a median period of 5.7 (range 1.8-13.4) years (Figure 13). At commencement (median age 4.4) their median hSDS was -4.7 (range -7.8 to -2.0). During the first year the hSDS increment was on average 1.0 SDS, and by the onset of puberty it was 1.8 (range 0.2-2.7). Also, WFH increased after commencement of GH therapy (Table 5). At the onset of puberty the median height was 0.7 SD greater in the GH treated patients ($p<0.01$, Table 8, Figure 14). Bone maturation accelerated after 8.0

years of age in the GH treated patients, so that a significant difference (1.09 vs. 0.92) in the BA/CA ratio was evident at the age of 10.0 years ($p<0.01$) compared to the untreated subjects. The difference remained significant throughout puberty so that at the age of 14 years this ratio was 1.05 and 0.95 in the treated and untreated patients, respectively ($p<0.03$) (Figure 14). Also the duration from stage 2 breast development to menarche was significantly shorter and menarche occurred nearly 3.0 years earlier in the GH treated as compared to untreated girls (Table 8). Sixteen patients (8 females) receiving GH have reached their final height with a median of 142 cm (range 137-154 cm, median hSDS -4.2) and 155 cm (range 150-163 cm; median hSDS -3.6) in females and males, respectively (Figure 13, 14, Table 8). The GH treated females and males reached their final height 1.1 and 0.6 years earlier than their untreated counterparts ($p<0.002$ and $p<0.02$, respectively). At final height, the difference in hSDS between the treated and untreated patients was 0.6 (approximately 5 cm) (Table 8, Figure 13, 14). Females seemed to benefit slightly more (by 0.4 SDS) than the males ($p<0.05$).

Table 8. Growth from early infancy to final height in MUL.

		Natural growth			GH-treatment			
Stage of growth	**Unit**	**Median**	**Range**	**Number**	**Median**	**Range**	**Number**	**p-Value**
Early infancy								
Birth length	SDS	-3.3	-1- -5.4	43	-3.6	-1.5- -6.4	29	NS
Birth weight	SDS	-2.7	-1.8- -3.6	43	-3.0	-1.4- -4.0	29	NS
Infancy								
Height at 2.0 years	SDS	-4.4	-0.7- -6.2	41	-4.7	-1.3- -7.8	29	NS
Weight-for-height at 2.0 years	%	-22	-2- -28	32	-22	2- -35	27	NS
Childhood								
Height at 5.0 years	SDS	-3.8	-2.0- -6.9	26	-4.2	-2.0- -6.5	27	NS
Weight-for-height at 5.0 years	%	-20	-2- -32	26	-23	-2- -32	27	NS
Height at 8.0 years	SDS	-3.5	-0.3- -6.5	23	-3.5	-1.5- -5.9	24	NS
Weight-for-height at 8.0 years	%	-17	16- -27	23	-17	15- -29	24	NS
Puberty								
Pubertal stage P2G2/M2	years	12.5	9.2-15	26	11.6	8.8-15	15	NS
Menarche	years	15	10.5-17	13	12	10-15.5	8	0.05
Height at P2G2/M2	SDS	-3.6	-6.3- -1.4	22	-2.9	-4.2--0.9	12	0.01
Weight-for-height at P2G2/M2	%	-14	27-22	22	-14	21--24	12	NS
Height in mid-puberty	SDS	-3.5	-5.8- -1.3	19	-2.9	-5.3--1.8	14	0.02
Weight-for-height in mid-puberty	%	-4	-21-58	19	-8	-21-20	14	0.02
Adulthood								
Final height	SDS	-4.6	-6.7- -2.2	26	-4.0	-4.9- -2.1	14	0.05
Weight-for-height at final height	%	20	-2-75	26	3	-22-34	14	0.002

GH, recombinant human growth hormone at a dose of 0.035 mg/kg/day

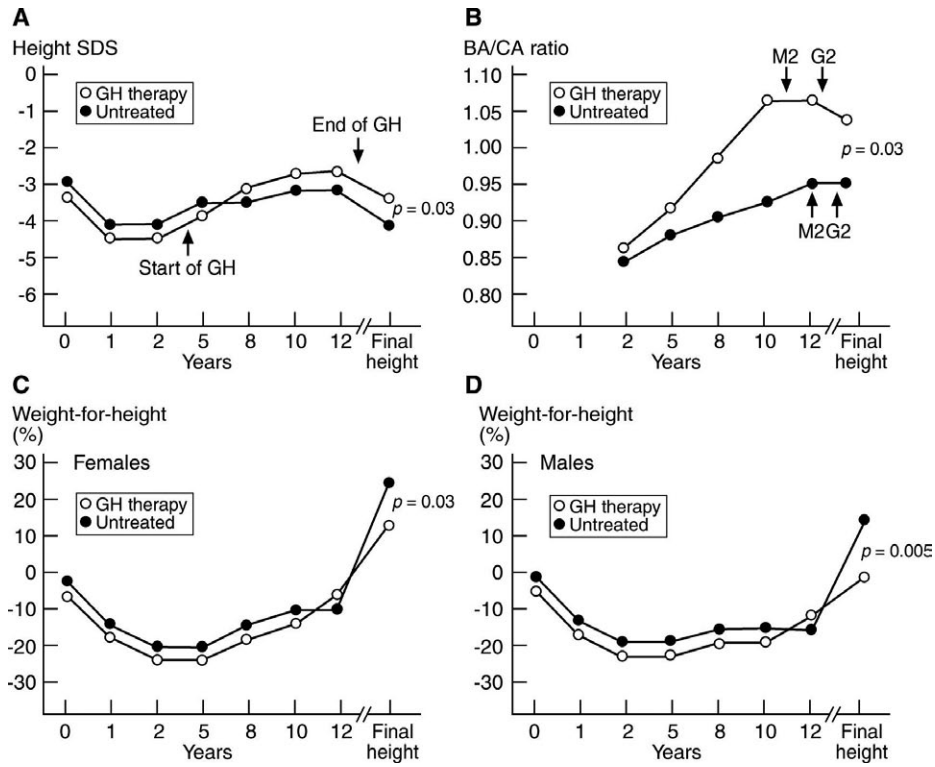

Figure 14. Median hSDS from birth to final height (A). GH therapy had only minor impact on final height (hSDS +0.6). Progression of bone age (BA) in relation to chronological age (CA) in GH-treated and untreated subjects (B). GH therapy accelerated bone maturation after 8 years. Median weight-for-height (WFH) for females (C) and males (D). At adult height, subjects treated with GH were slimmer in both genders. Figure with permission from Pediatrics 2007 (III).

6.4 Glucose and lipid metabolism in MUL

Glucose metabolism and lipid metabolism were studied in 65 MUL patients aged 1.1-55 years (Table 9). A dramatic change in the glucose metabolism was observed with increasing age. While children (<10 years) had low fasting insulin and frequently (50%) showed hypoglycemic values (blood glucose <3.4 mmol/l), 90% of adults showed high fasting and post-load peak insulin values ranging up to 1450 mU/l (median 430 mU/l). Serum basal and post load insulin levels correlated well with the C-peptide levels, and the explanation rate (R^2- value) was 0.6121 and 0.657, respectively. The fasting glucose and insulin ratio and Stumvoll indexes showed a 10- and 4-fold decrease with advancing age, indicating the development of severe insulin resistance. Half of the adults (aged >20 years) had type 2 diabetes and an additional 42% showed IGT. The median ages revealing IGT and type 2 diabetes were 18.3 (range 3.1- 42.3) and 26.0 (9.5-55.2) years, respectively.

GH treatment did not significantly worsen the pre-existing insulin resistance. Contrary, at final height, the subjects treated with GH remained significantly slimmer than the untreated patients ($p<0.002$) (Figure 14). Serum leptin, uric acid, total cholesterol, and triglycerides also increased with age (Table 9). Hypertension (>95th percentile) was observed in 81%, and 70% of the adults fulfilled the criteria for metabolic syndrome according to the NCEP Adult Treatment Panel III (NCEP 2001, Fedder et al 2002). As adults, the frequency of metabolic syndrome was twice as high (64% vs. 33%) in the untreated (N=11; median age 23.9 years) compared to the GH treated subjects (N=12; median age 24.2 years).

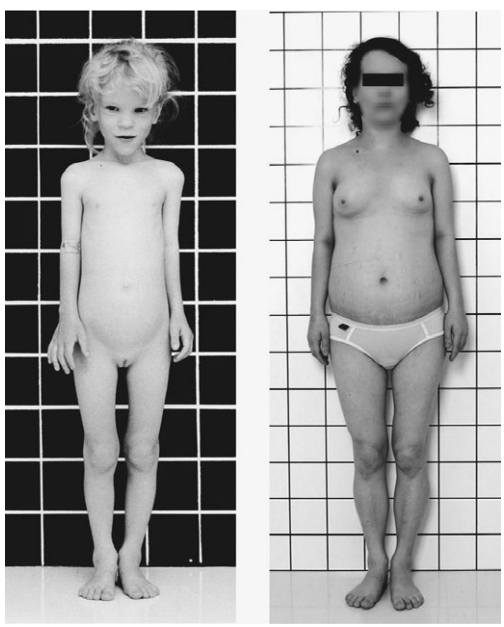

Figure 15. The development of insulin resistance was accompanied by changes in body composition and lipid metabolism. While prepubertal children were thin, clinically evident abdominal obesity started to develop after puberty. Of adults 42% were overweight (WFH >20%). Note the same female subject at 6.0 years and 20 years of age. The transformation from a thin child to a mildly overweight adult is evident. Figures partly adapted from Pediatrics 2007 (III).

Table 9. Glucose and lipid metabolism in 65 patients (37 females) aged 1.1 to 55 years with Mulibrey nanism

Feature	Normal range	*1-10 years of age*				*11-20 years of age*				*>20 years of age*			
		Median	Range	Abnormal value		Median	Range	Abnormal value		Median	Range	Abnormal value	
Anthropometrics													
Height	-2.0- +2.0 SDS	-4.0	-6.1- -1.0	30/33	(90)	-3.4	-5.5 - - 1.4	26/27	(94)	-4.2	-6.2- - 2.1	35/35	(100)
Weight-for-height	-20- +20 %	-17	-31- +16	0/33	(0)	-8	-22- +75	5/27	(19)	16	-24- +45	16/35	(46)
Body mass index	<91th percentile	14	11 - 18	0/33	(0)	16	12 - 32	2/27	(7)	21	14 - 26	2/35	(6)
Blood pressure (mmHg)	<95th percentile	102/65	85-140 / 50-65	10/28	(36)	116/70	97-140 / 55-85	2/11	(18)	139/85	105-174 / 70-118	21/26	(81)
Glucose metabolism													
Fasting B-glucose	4.0 - 6.1 mmol/l	3.5	2.2 - 5.6	0/33□	(0)	4.4	2.9 - 6.1	1/27□	(4)	4.6	3.8 - 10.9	6/35□	(17)
2-hour B-glucose	<6.7 mmol/l	6.1	3.8 - 10.9	8/23	(35)	7.0	4.6 - 11.2	14/27	(52)	10.1	4.2 - 21.9	33/35	(94)
B-HbA1c	4.9 - 6.0 %	4.4	4.0 - 5.6	0/33	(0)	4.4	4.0 - 5.7	0/27	(0)	5.4	4.6 - 6.7	5/35	(14)
Fasting S-insulin	2-15 mU/l	5	1 - 22	4/33	(12)	12	2 - 50	0/27	(0)	25	3 - 178	27/35	(77)
2-hour S-insulin	<75 mU/l	29	3 - 203	5/23	(22)	106	17 - 551	17/27	(63)	280	41 - 1100	22/35	(63)
Peak S-insulin	<150 mU/l	61	14 - 291	7/23	(30)	178	60 - 620	18/27	(67)	430	88 - 1450	31/35	(89)
Insulin sensitivity													
FGIR-index	-	0.80	8.2 - 0.18	-	-	0.34	1.95 - 0.09	-	-	0.21	1.53 - 0.04	-	-
Stumvoll-index	-	0.119	0.134 - 0.084	-	-	0.107	0.126 - 0.070	-	-	0.080	0.122 - 0.012	-	-
Lipid metabolism													
Total S-cholesterol	<5.0 mmol/l	3.2	2.3 -5.7	0/33	(0)	3.8	3.2 - 5.6	3/27	(11)	5.3	3.3 - 7.3	22/35	(63)
S-LDL cholesterol	<3.5 mmol/l	1.7	0.6 - 3.1	0/33	(0)	2.0	0.9 - 3.9	0/27	(0)	2.6	0.9 - 4.6	13/35	(37)
S-HDL cholesterol	>1.0 mmol/l	1.3	0.6 - 2.3	7/33	(21)	1.2	2.1 - 0.7	7/27	(26)	1.1	1.7 - 0.7	12/35	(34)
S-triglycerides	<1.7 mmol/l	0.9	0.4 - 2.4	2/33	(6)	1.2	0.5 - 2.3	6/27	(22)	2.4	0.7 - 4.7	25/35	(71)
S-leptin (µg/l)	-	3.8	1.7 - 10.0	-	-	6.8	1.9 - 34.4	-	-	16.1	2.0 - 49.9	-	-
Metabolic asseys													
S-AST*	<35 U/l	51	36 - 147	30/33	(91)	38	19 - 74	16/27	(60)	38	12 - 205	21/35	(60)
S-ALT**	<35 U/l	44	16 - 316	26/33	(79)	40	19 - 85	16/27	(60)	37	12 - 109	17/35	(49)
S-gamma GT***	<50 U/l	27	10 - 130	9/33	(27)	40	14 - 104	14/27	(52)	75	29 - 382	29/35	(83)
S-uric acid	150 - 350 µmol/l	372	200 - 560	21/33	(64)	340	208 - 490	17/27	(63)	380	233 - 505	16/35	(46)

Measurements out of the normal range presented in each group as abnormal value of the total amount and as percentage (%). □Abnormal fasting venous whole blood glucose >6.1mmol/l. *AST, aspartate aminotransferase. **ALT, alanine aminotransferase. ***gamma GT, gamma glutamyltransferase. Adapted from Diabetes 2005 (II).

6.5 Organogenesis and tumours in MUL

Findings in various organs were evaluated by radiology [US and MRI] in 76 patients, aged 0.7 to 77 years, and from autopsy samples in 17 deceased patients. Overall, 232 tumorous lesions were encountered (Table 10). Also, ectopic tissues and abnormal organ development were evident in the histological samples.

6.5.1 Histological findings

Disturbed organ development was seen in several internal organs. The general architecture of the adrenal gland was clearly disturbed. Cortical and medullar structures intermingled and the adrenal parenchyma commonly penetrated through the capsule into the surrounding fat or renal tissue (Figure 16). Also, fetal-type cortical architecture was present in several specimens. One female patient was found to have a focus of ovarian tissue in her kidney. The pancreatic gland was atypically folded in all histological specimens, with lipid deposits within and around the gland. The spleen was congestive in all patients examined post mortem. It contained lymphocytes but the white pulpa lacked lymph follicles. Similarly, a severe lymphatic depletion in the lymph nodes was evident.

A high frequency of benign tumours was noted in different organ systems. The majority of the lesions were cystic and benign adenomatous lesions. In radiology or histology 52% of MUL subjects had renal cortical cysts, 26% had pancreatic cysts, 20% had thyroid cysts, 15% had cysts in the central nervous system and 11% had cysts in the reproductive organs. Bilateral nodular cortical hyperplasia of the adrenal gland was present in 87% of the histological samples analyzed (13/15) and all histological samples of the pancreas (13/13) revealed hyperplastic islets, as in focal endocrine hyperplasia. Twenty-two (25%) patients had fibrous dysplasia of long bones, four had nodular thyroid goitre, two had renal angiomyoliopmas, one had a renal hamartoma containing ovarian tissue, one had a pancreatic serous cystadenoma and one infant had a small medullar pheochromocytoma. Moreover, six adrenal cortical adenomas and two thyroid adenomas were encountered (Table 8). More than half of the postpubertal females (12/22) had benign ovarian fibrothecomas (Karlberg et al 2004).

Fifteen malignant tumours were encountered in thirteen patients (15%), seven of them occurred in the kidney. Wilms' tumour was diagnosed in five (6%) patients (mean age, 2.5 years; Table 10). Two patients were diagnosed with renal papillary carcinoma and two with a thyroid papillary carcinoma. A medullar thyroid carcinoma combined with squamous-cell metaplasia was also encountered. Other malignancies were two gynecological cancers (ovarian carcinoma and endometrial adenocarcinoma), one gastrointestinal carcinoid tumour, one neuropituitary Langerhans cell histiocytosis and one case of acute lymphoblastic leukemia.

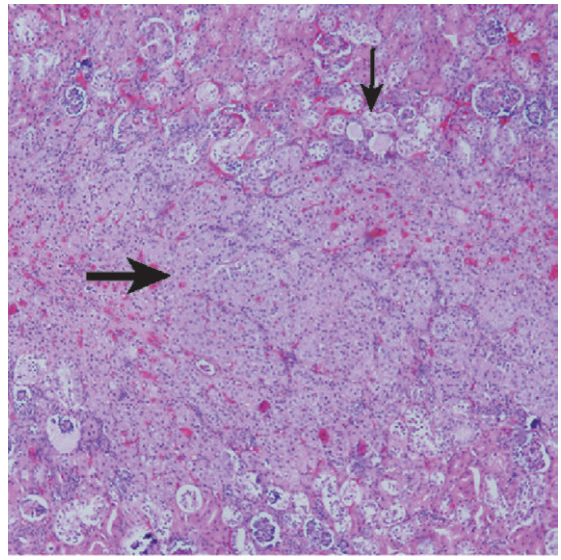

Figure 16. The renal and adrenal tissues mixed frequently, and in one case the adrenal gland (large arrow) was totally surrounded by renal tissue (small arrow).

6.5.2 Liver peliosis

The most consistent tumour lesion was found in the liver. The lesions (5-60 mm) were noted in almost half (35) of the 72 MUL patients studied by US (Table 10, Figure 17 A). Most lesions resembled hemangiomas, but a minority had a more cystic appearance. The lesions usually enlarged with time, but in approximately one-third of the patients the lesions fluctuated in size during the follow-up. Ten of the lesions clearly shrank and temporarily disappeared in five patients. Intense homogeneous tumour-suspect lesions were detected in 20 of the 22 patients studied by MRI (Figure 17 B). Some of the larger lesions appeared cystic with a more heterogeneous signalling.

The liver was enlarged and congestive in all 17 patients at autopsy (0.7-48 years). Sixteen (94%) had multiple macroscopic hemangiomatous lesions (5-70 mm). Histological specimens from the 17 autopsies and 20 liver biopsies (2.4-40 years) revealed marked sinusoidal dilatation (peliosis; Table 10, Figure 19) and disorganized sinusoidal pattern in relation to central veins and portal tracts. The basic lobular arrangement was focally disturbed and the central veins in these areas were dilated and had unusually thick walls. The portal regions and biliary tracts were mostly normal, but the degree of fibrosis increased with time. Hepatocytes were normal or enlarged, containing variable amounts of micro- and macrovesicular lipid droplets (20-90%). Fatty liver was also frequently noted in the liver biopsies (14/20) and in the autopsy specimens (13/17).

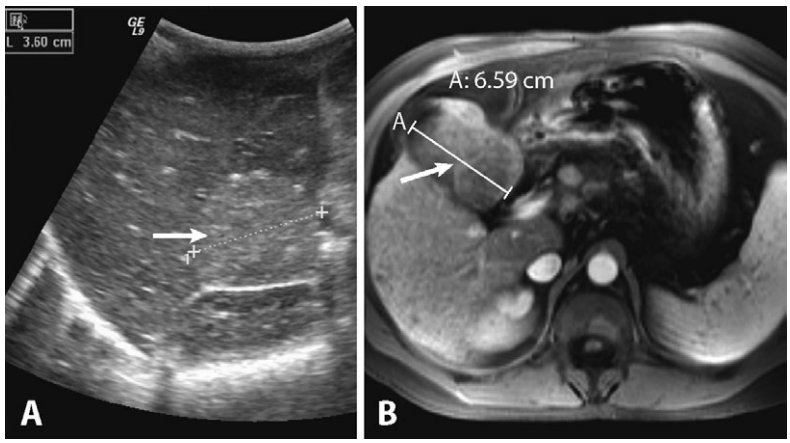

Figure 17. Tumour suspect lesion of the liver seen in US (A) and in MRI (B). The lesions consist of disorganized sinusoidal architecture and increased dilatation and rupture of the liver sinusoids (liver peliosis).

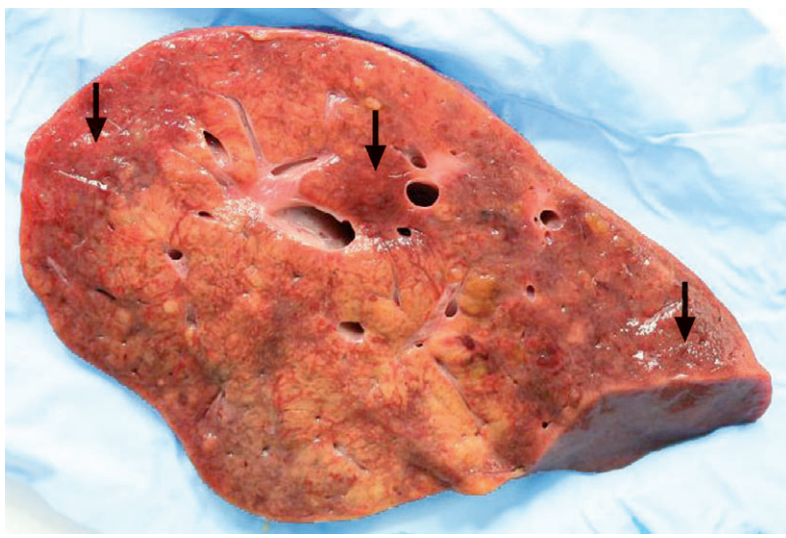

Figure 18. Macroscopic section of a liver taken at autopsy of a twelve year old boy. The liver appears nodular and the colour is heterogenous. The peliotic areas are more intensely red in colour (arrows).

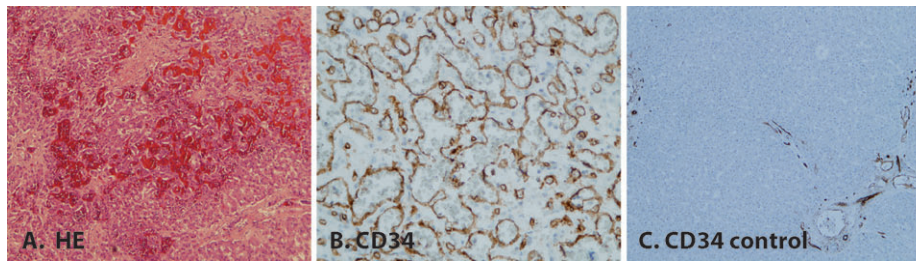

Figure 19. A. Strong sinusoidal dilatation and rich vascularity of a peliotic liver lesion. B. Strong staining with vascular endothelial marker CD34 in the peliotic liver in comparison with healthy controls (C).

Table 10. Radiological and histological findings in 89 MUL patients

	Radiology		Histology		Age of detection (years.)	
Organ	US	MRI	Autopsy	Biopsy	Median	Median
Liver						
Peliosis	35/72	20/22	16/17	20/20	14.7	0.8□37
Kidney						0.3-42
Cysts	30/72	18/22	16/17	6/6	13.6	
Renal hamartoma	0/72	0/22	1/17	0/6	40	
Angiomyolipoma	1/72	1/22	1/17	0/6	40	
Wilms□tumour	3/72	5/22	0/17	5/6	2.5	2.2-3.7
Renal papillary carcinoma	0/72	0/22	2/17	1/6	22.3	17-28
Pancreas						
Cysts	13/72	11/22	10/11	2/2	17.5	9.8-48
Serous cystadenoma	0/72	0/72	0/11	1/2	24	
Endocrine hyperplasia	0/72	0/22	11/11	2/2	16.7	0.7-41
Thyroid gland						
Cysts	12/30	3/3	6/12	3/3	24.5	1.7-42
Adenoma	0/30	1/3	0/12	2/3	25.5	14-36
Papillary carcinoma	1/30	0/3	1/12	1/3	32.1	28-40
Medullary carcinoma	0/30	0/3	0/12	1/1	40	
Parathyroideal adenoma	0/30	0/3	0/12	1/1	36	
Adrenal gland						
Nodular cortical hyperplasia	15/72	9/22	11/13	2/2	22.5	1.4-49
Adrenocortical adenoma	2/72	3/22	5/13	1/2	32.3	1.4-40
Pheocromocytoma	0/72	0/22	1/13	0/2	1.4	
Urogenitalia						
Ovarian fibrothecoma	12/22	0/0	1/9	12/12	29	16-52
Ovarian cysts	1/22	0/0	0/0	0/0	11	
Ovarian carcinoma	1/22	0/0	0/0	1/12	33	
Endometrial adenocarcinoma	1/22	0/0	0/0	1/12	45	
Epididymic cysts in males	6/15	0/0	3/9	0/0	24	2-42
Central nervous system						
Cysts	0/0	6/15	7/15	0/1	8.9	0.8-36
Neuropituitary histiocytosis	0/0	1/15	0/15	1/1	8.6	
Other						
Carcinoid tumour	0/1	1/1	0/0	1/1	21	
Acute lymphoblastic leukemia	0/0	0/0	0/0	1/1	3.6	
Fibrous dysplasia of long bone	22/89*	0/0	0/0	7/7	7.5	1.3-19

* Fibrous dysplasia detected with x-rays of long bones.

6.5.3 Non-compaction cardiomyopathy

The heart was enlarged in all 17 patients examined post mortem (0.7-48 years). The endocardium appeared thick and the pericardium was adherent to the underlying myocardium, but no inflammatory cells were present as reported before (Lipsanen-Nyman M et al 2003). The thickness of the myocardium varied and in large areas closely resembled the papillary muscles, with highly trabeculated fibres suggestive of non-compaction cardiomyopathy (Figure 20). The myofibrils were mostly hypertrophic but were in some areas thin and angulated, as in acute ischemia. Histologically, the blood vessels had unusually thick walls and were folded, as in other internal organs. Atherogenic vascular changes were noted in more than half of the autopsied patients (N=10, median age 29 years). Five of them had died of CHF due to severe cardiomyopathy; two had died of cancer and three of other causes.

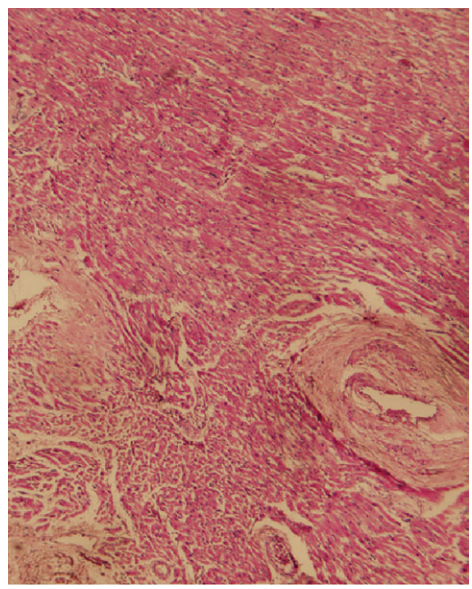

Figure 20. Non-compaction cardiomyopathy seen in histology. The myocardium is especially trabeculated and the myocytes abnormally short resulting in cardiac restriction. The blood vessels of the heart are unusually folded and thick walled.

6.5.4 Immunohistochemistry

The blood vessels had unusually thick walls and the capillaries and larger vessels were strongly dilated and folded not only in the liver and heart but also in the kidneys, lungs, pancreas, brain, adrenal and thyroid gland, as well as in the hilar region of the lymph nodes, were the blood vessels were exceptionally large and folded (Figure 21). In liver the endothelial cell markers CD31 and especially CD34 were strongly positive (Figure 19). Also, the staining for the myocyte marker α-SMA was intensely positive (Table 11). These staining patterns were clearly different from control livers, where the staining of all markers was restricted to portal areas (Figure 19). The liver lesions showed little mitotic activity and a weak expression of the proliferation marker MIB-1 (Table 11). Also, the marker for PEComa, HMB45, was not expressed in these lesions (Table 11).

In general, there was a strong positive staining for the endothelial cell markers CD31 and CD34, and for α-SMA in the internal organs (Table 11, Figure 19, 21), while the staining for MIB-1, p53 and HMB-45 were negative. Blood vessels in the lymph nodes showed very strong staining for CD34. The expression of α-SMA was intense around larger vessels, particularly in the hilar region. Control sections stained negative.

Table 11. Immunohistochemial analysis of paraffin-embedded tissue specimens in MUL

Organ	*Nr*	*CD34*	*CD31*	*α-SMA*	*HMB-45*	*MIB-1*	*p53*
Liver	5	+++	++	+++	-	-	-
Kidney	5	+++	++	+++	-	-	-
Adrenal gland	4	+++	+	++	-	-	-
Thyroid gland	4	++	+	++	-	-	-
Pancreas	4	++	+	++	-	-	-
Lung	4	+++	+	++	-	-	-
Lymph node	4	+++	-	+++	-	-	-

number of tissue specimens analyzed

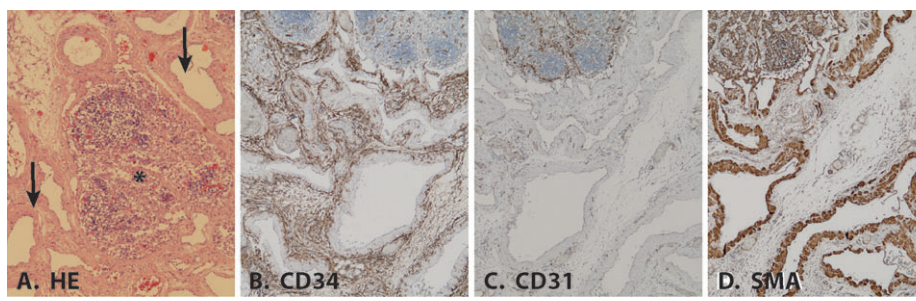

Figure 21. The staining pattern of lymph nodes. Large, abnormally folded blood vessels (arrows) in the hilar region seen in haematoxylin and eosin staining (A). The staining was strongly positive with CD34 (B) and α-SMA (D). However, the vessels were surprisingly negative for CD31 (C). This combined with the strong positivity of CD34 indicates a disturbed angiogenesis. *Lymph node.

7. DISCUSSION

Mulibery nanism (MUL) is an autosomal recessive growth disorder with multiple organ manifestations and no major neurological handicap enriched in the Finnish population. MUL is caused by mutations in the *TRIM37* gene on chromosome 17q22-23, encoding for TRIM37 with ubiquitin E3-ligase activity. The clinical and histopathological characteristics were analyzed in the Finnish cohort of MUL patients mostly homozygous for the Fin-major mutation. The results show that MUL patients form a distinct clinical and diagnostic entity, although clinical features vary from infancy to adulthood. While poor growth, feeding difficulties and respiratory infections dominates early life, young adults were especially prone to develop metabolic syndrome, as well as benign and malignant tumours in several organs.

7.1 Clinical features of MUL

Clinical features of MUL have previously been reported in the original Finnish cohort (Perheentupa et al 1970, 1973, Lipsanen-Nyman 1986) and as sporadic cases from different populations (Thorén 1973, Cumming et al 1976, Voorhess 1976, Sánchez-Corona et al 1983, Cotton et al 1988, Haraldsson et al 1993, Lapunzina et al 1995, Seemanová and Bartsch 1999, Avela et al 2000, Jagiello et al 2003, Hämäläinen et al 2004, 2006, Doğanc et al 2007). These reports are partly based on genetically unconfirmed patients. Our present series is the largest and most comprehensive describing the key clinical features of MUL from infants to adults, as well as at the time of diagnosis. Our results show that MUL patients are born SGA and are generally both abnormally short and light for length. Moreover, they fail to exhibit early or late catch-up in growth. The distinct early growth pattern in MUL resembles the postnatal growth of infants born very preterm at week 23-25th of gestation experiencing extrauterine growth restriction (EUGR) (Gibson et al 2000, 2003, Niklasson et al 2003, Finken 2006, Wit et al 2006, Pilling et al 2008). However, the very preterm children commonly catch up within 48 months after birth (Gibson et al 2003), while MUL infants display a continuous deceleration both in relative height and weight through infancy and early childhood. This wasting is followed by a spontaneous, but incomplete catch-up in growth until school-age.

MUL infants, although born at term, display immature features resembling those of premature infants, such as the early growth pattern and relative macrocephaly (Gibson 2007, Finken 2006). The craniofacial skeleton displays immaturity and the peculiar shape of sella turcica resembles the shape of the bony sphenoid before the cartilaginous dorsum sellae has ossified (Myllärniemi et al 1978). Also, the scaphocephalic shape of the skull with its wide sutures and prominent forehead and the size relationship between the skull and face are all supportive of delayed pre- and postnatal maturation in MUL (Myllärniemi

et al 1978). These features may also evoke false impression of hydrocephalus. Other major problems that MUL subjects share with preterm and SGA children are feeding difficulties. In general, swallowing is poorly coordinated in infants born prematurely or SGA and the degree of dysfunction is inversely related to the degree of immaturity and growth failure (Tuchman 1988, Selley et al 1990). The oral anatomy of mature infants is particularly well suited for eating by sucking, with the tongue being relatively large, filling the oral cavity (Stevenson and Allaire 1991, Derkay and Schechter 1998, Matsuo and Palmer 2008). Infants with MUL, however, have a hypoplastic and anteriorly triangular-shaped tongue. This is evident in nearly 70% of these infants. The craniofacial hypoplasia, maturational delay (Myllärniemi et al 1978) and muscular hypotonicity probably all contribute to the feeding difficulty. Early recognition and management are important because dysphagia may contribute to the failure of catch-up in growth and poses a threat to the child's nutrition and respiratory function (Kelly et al 2007a, 2007b).

Patients with MUL are also prone to respiratory tract infections and have an exceptionally high risk for pneumonia. No immunological defects have been demonstrated in Finnish MUL patients and the basis of this susceptibility is not known. However, in autopsy specimens a severe lymphatic depletion in lymph nodes was frequently evident and interestingly, recent studies have shown that TRIM proteins are expressed in response to interferons and involved in biological control of innate immunity (Raymond et al 2001, Zou and Zhang 2006, Ozato et al 2008). For instance, TRIM25, which is an ubiquitin E3-ligase, has been shown to be important in antiviral activity (Gack et al 2007). Interestingly, the gene encoding for TRIM25 is located close to *TRIM37* on chromosome 17q23.2. In our patient cohort, two patients who died of an infection early in infancy were post-mortem found to have adrenal cortical hypoplasia. In autopsy samples from deceased MUL-patients, patchy proliferation of myofibroblasts with thickening of the alveolar walls and hemosiderine-containing macrophages were frequent findings in the lungs, while hypoganglionosis or aganglionosis of the enteric plexus were frequently observed in the intestine. The frequent pneumonias have been suspected to be a combined consequence of dysphagia due to aspiration and a lower respiratory capacity. As infections quite easily tend to result in respiratory failure, a suboptimal immune and stress response could further complicate the situation.

7.2 Diagnosis of MUL

The clinical diagnosis of MUL is challenging during the first months of life. Infants with intrauterine growth failure constitute a heterogeneous group of patients and many of them have chromosome abnormalities, monogenic disorders or familial or sporadic syndromes (Albertsson-Wikland and Karlberg J 1994, Albertsson-Wikland et al 1998, Hannula et al 2001, Wit et al 2005). Diagnosis of

MUL should be considered in infants born SGA with progressive growth failure and poor weight gain, hepatomegaly and characteristic craniofacial features. The analysis of the clinical features in this large cohort of Finnish MUL-patients led to revised diagnostic criteria. Importantly, while none of the clinical features was constant, 99% of our patients presented with at least three of the major and one of the minor signs. One patient had only two major signs, but he had three of the minor signs (Table 12).

Table 12. Diagnostic signs and their prevalence in 85 MUL-patients (46 females and 39 males). For diagnosis three major signs with one minor sign are required or two major signs with three minor signs.

Signs	Frequency (%)
Major signs	
Growth failure (A or B or C)	
A) small for gestational age (SGA), lacking catch up growth	95
B) height in children 2.5 SDS below population mean for age	94
C) height in adults 3.0 SDS below population mean	90
Characteristic radiological findings (A or B)	
A) slender long bones with thick cortex and narrow medullar channels	93
B) low and shallow (J-shaped) sella turcica	89
Characteristic craniofacial features	90
Scaphocephaly, triangular face, high and broad forehead, low nasal bridge and telecanthus	
Characteristic ocular findings	
Yellowish dots in retinal mid peripheral region	79
Mulibrey nanism in a sibling	17
Minor signs	
Peculiar high pitched voice	96
Hepatomegaly	70
Cutaneous naevi flammei	65
Fibrous dysplasia of long bone	25

Adpted from J Med Genet 2004 (I).

Identification of the causative gene, *TRIM37,* has allowed genetic confirmation of the diagnosis. Since the Fin-major accounts for 96% of all Finnish MUL-associated mutations (Table 4) (Avela et al 2000), screening for this mutation seems sufficient enough in Finnish families. Genetic confirmation in non-Finnish patients requires sequencing of the whole *TRIM37* gene. Molecular genetics can also provide MUL diagnosis prenatally. However, since MUL is an extremely rare disorder, prenatal diagnosis is rational only in families known to be affected with this disorder.

Altogether 19 mutations underlying MUL are known (Table 4). Fifteen of them produce a premature termination codon (PTC) and are likely to trigger nonsense mediated mRNA decay; three are missense mutations; Leu76Pro, Gly322Val, Cys109Ser and one a 17 amino-acid deletion of the TRAF domain; Glu271_Ser287del. All 19 *TRIM37* mutations identified to this date seem to produce loss of function alleles since no genotype-phenotype correlation has been seen in MUL patients (Hämäläinen et al 2004).

7.2.1 Differential diagnosis

While prenatal onset growth failure and craniofacial features are important clinical characteristics of MUL, they are not unique and are seen in other dysmorphic conditions (Figure 22). For instance, subjects with SRS display a similar growth pattern with pre- and postnatal growth restriction, constitutional gracility, normal head circumference and craniofacial dysmorphism, precisely as MUL patients (Silver et al 1953, Russell 1954, Escobar et al 1978, Wollmann et al 1995, Price et al 1999, Hannula et al 2001). In early infancy, feeding difficulties and failure to thrive are commonly seen in both conditions (Price et al 1999, Hannula et al 2001, Hitchins et al 2001). The clinical overlapping makes SRS the most important differential diagnosis (Table 13, Figure 22). However, both these conditions have their own distinct characteristics. Clinodactyly, small face with marked triangularity, micrognathia with down-turned mouth corners, and skeletal asymmetry are characteristics of SRS (Price et al 1999, Hannula et al 2001, Hitchins et al 2001). Hepatomegaly, heart failure due to pericardial constriction or cardiomyopathy, yellow dots in ocular fundi, fibrous dysplasia and cortical thickening of long bones are key characteristics of MUL and do not occur in SRS. However, SRS is considerably heterogeneous both clinically and genetically (Hannula et al 2001, Hitchins et al 2001, Gicquel 2005, 2008, Abu-Amero et al 2008, Rossingnol et al 2008, Bruce et al 2009). The classical features of SRS (Silver et al 1953, Russell 1954, Escobar et al 1978, Price et al 1999), those that are shared with MUL, are associated with hypomethylation of the *H19* ICR locus on chromosome 11p15.5 (Bruce et al 2009). A milder SRS phenotype exists in patients with maternal uniparenteral disomy of chromosome 7 (Hannula et al 2001). Also, three atypically mild cases of MUL have been diagnosed in the Finnish patient cohort, all homozygous for the Fin-major mutation.

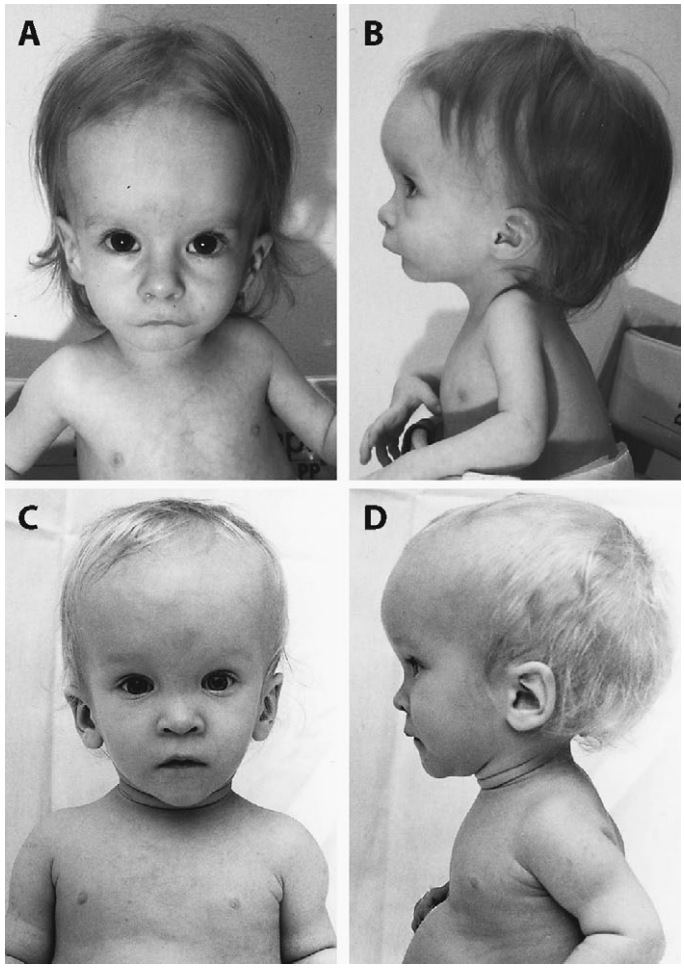

Figure 22. An infant with SRS (A and B) and an infant with MUL (C and D) at an age close to 2.0 years. The craniofacial features resemble each other considerably, making SRS the most important differential diagnosis of MUL.

Table 13. Differences in phenotype between SRS and MUL

Features	SRS	MUL
Craniofacial		
relative macrocephaly	100%	90%
facial triangularity	100%	89%
bossed forehead	96%	86%
micrognathia	100%	20%
dental crowding	65%	50%
down turned mouth corners	58%	-
low-set backwards rotated ears	77%	54%
J-shaped sella turcica	-	89%
Growth		
short stature	100%	95%
birth length (SDS)	-3.7	-3.0
birth weight (SDS)	-3.1	-2.9
2.0 year height (SDS)	-4.5	-4.4
2.0 year weight-for-height (%)	-26	-22
Musculosceletal		
clinodactyly of 5th finger	85%	-
skeletal asymmetry	88%	15%
syndactyly of 2-3 toes	50%	-
mucsle hypotonicity	46%	68%
thick cortex of long bone	-	93%
fibrous dysplasia of bone	-	25%
Heart and liver		
congestive heart failure	-	51%
hepatomegaly	-	70%
Metabolics		
*fasting blood glucose in prepubertal children	4.4 mmol/l	4.2 mmol/l
Urogenitalia		
cryptorchidism	62%	6%
Other features		
high pitched voice	38%	96%
yellowish dots in ocular fundi	-	80%
cutaneous naevi flammei	-	65%
feeding difficulties	38%	50%
slow speech development	27%	30%
slow motor development	27%	29%

* Fasting blood glucose from 24 prepubertal MUL subjects (median age 9.4 years) and 12 prepubertal SRS subjects (median age 9.2 years) (Karlberg et al unpublished data). Other findings in SRS patients constitute the features seen in 26 patients with *H19* ICR methylation <35%, Bruce et al 2009.

7.3 Long-term GH treatment in MUL

The importance of the GH-IGF-axis is indisputable in postnatal growth (Walenkamp MJ and Wit 2006). GH deficiency and resistance have previously been reported in short children born SGA (de Waal et al 1994, Boguszewski et al 1995, Saenger et al 2007) as well as reduced levels of IGF-1 and IGF-2 (de Waal et al 1994, Murphy et al 2006). Also, gene knockout studies have shown a major impact of IGF-1 on pre- and postnatal growth (Murphy et al 2006). Recent discoveries show variation in methylation patterns regulating imprinting and expression of IGF-2 in SRS (Girquel et al 2008, Bruce et al 2009). The role of GH-IGF-axis in the poor growth of MUL-patients is unclear, but some patients presented a partial GH deficiency and showed low normal levels of serum IGF-1.

The long term GH treatment had minor impact on the final height of MUL subjects (hSDS increment of 0.6 = 5 cm), which is in line with results reported previously in some dysmorphic growth disorders and skeletal dysplasias (Azcona et al 1998, Cianfarani 1999, Kelnar 2003, Hagenäs et al 2005). The gain in height was mainly achieved before the onset of puberty, as has been seen in previous studies on SGA (Dahlgren et al 2005). A substantial catch-up growth was observed especially during the first two years of treatment. At discontinuation of the GH therapy (BA 14 years, height velocity <2cm / year), a median hSDS increment of 1.9 compared to baseline data was observed. Bone maturation and growth arrest, however, occurred early in patients receiving GH, which explains why the final adult height in MUL patients still remained very poor as compared to the general population. Importantly, no adverse effects of long-term GH treatment, in regard to heart disease or tumour frequency, have emerged in the treated subjects. Nevertheless, the modest impact on the final height should be kept in mind when considering the grounds for this treatment.

Interestingly, the *GH1* gene encoding for human GH is located on chromosome 17q22-24, close to *TRIM37* (17q22-23). Mutations in the *GH1* have been reported to result in bioinactive GH and short adult stature (Moseley and Phillips 2000). After secretion, GH is bound to its receptor (de Vos et al 1992), which requires an active ubiquitin system to mediate biological actions of GH, such as promoting growth through increased expression of IGF-1 in peripheral tissues (Strous and van Kerkhof 2002). New evidence links normal UPS function even stronger to the regulation of human growth. Recently discovered mutations in the gene *cullin7 (CUL7)* on human chromosome 6p21 have been identified to result in the autosomal recessive 3-M syndrome, with features resembling those of MUL, including severe pre- and postnatal growth retardation, facial dysmorphism, normal head circumference and intelligence (Miller et al 1975, Huber et al 2005). Interestingly, the CUL7 protein is crucial in assembling an ubiquitin E3-ligase complex and thereby promotes ubiquitination (Huber et al 2005, Sarikas et al 2008). Mutations in the *CUL7* gene strongly link UPS dysfunc-

tion to the pathogenesis of prenatal-onset growth failure in humans (Huber et al 2005, Sarikas et al 2008). MUL seems to be the newest addition to this list.

7.4 Metabolic concerns of MUL

Hypoglycemia is a frequent concern in SGA and preterm babies (Gibson 2007, Saenger et al 2007, Miller et al 2008). Brain function is highly dependent on glucose as major fuel. When exposed to hypoglycemia neurophysiologic changes occur (Beardsall et al 2007). Stress effector systems like the hypothalamic-pituitary-adrenal axis (HPAA) are activated to increase peripheral blood glucose levels important for survival. Suggestions have been made that pre- and antenatal stress could permanently alter the function of developing organ systems and result in metabolic concerns, such as insulin resistance, type 2 diabetes and cardiovascular disease, later in life (Fall et al 2002, Kajantie et al 2002, 2006). For instance, insulin resistance has been associated with SGA caused by unfavourable mother-child environment followed by early postnatal weight gain to obesity (Eriksson et al 2003, Veening et al 2003). In MUL, however, the prenatal-onset growth failure is caused by a fetal gene defect and only a modest weight gain is observed before puberty. Moreover, the link between SGA and insulin resistance is not definitive as prepubertal subjects with SRS, who have a phenotype and growth pattern similar to MUL-patients, do not show corresponding abnormalities in their glucose metabolism (Karlberg et al unpublished data).

In most monogenic diseases leading to type 2 diabetes, the pathogenic process can be explained by dysfunction of the pancreatic β-cells or obesity (O'Rahilly et al 2005, Farooqi and O'Rahilly 2005, McCarthy and Hattersley 2008). Contrary to this, MUL-subjects presented exceptionally high post load serum insulin levels and fatty liver as well as acanthosis nigricans often detectable already in slim, prepubertal MUL children. Additionally, the weight gain did not correlate with development of insulin resistance. On the other hand, lipodystrophy with low leptin secretion (O'Rahilly et al 2005, Herranz et al 2008) was not involved in the development of insulin resistance. In MUL, serum leptin levels were unexpectedly high compared to the weight gain.

Under normal healthy conditions leptin plays a central part in regulation of body weight (Rector et al 2008). High leptin levels are associated with obesity and also with accumulation of fat in the liver (Fishman et al 2007). However, both lean and obese insulin resistant individuals have been shown to have excess fat in their liver. Lipid accumulation can occur either through increased uptake or synthesis of fatty acids or as a consequence of reduced or defective fatty acid oxidation (Shulman 2000). The reasons why some patients accumulate fat in the liver or in other organs are poorly known. Data from human and animal studies connect ectopic lipid accumulation in muscle and liver to the

development of insulin resistance. In animal models excess fat deposits have also been shown to cause organ dysfunction of the heart (Cheng et al 2004, Lee et al 2004) and pancreas (McGarry 2002). Ectopic fat deposits, in particular liver fat, correlates well with increased levels of serum liver enzymes (S-ALT and S-AST) (Bellentani et al 2000, Kotronen et al 2007) and all components of the metabolic syndrome (Kotronen et al 2007, Kotronen and Yki-Järvinen 2008). The accumulated fat possibly interferes with the normal intracellular insulin signalling leading to insulin resistance (van Herpen and Schrauwen-Hinderling 2007). The insulin resistance can initially be overcome by an increased insulin secretion, but when the secretory capacity of the beta-cells decline, type 2 diabetes develops (Poitout 2004). New evidence strongly suggests that lipid accumulation in the pancreatic islets is a central trigger of beta-cell dysfunction in diabetes (Sharma et al 2004, Maedler et al 2003). This lipotoxity could be one explanation why insulin resistant individuals cannot meet the increasing insulin demand and eventually develop type 2 diabetes (McGarry 1992, van Herpen and Schrauwen-Hinderling 2007). In MUL the highly elevated serum leptin levels together with the early development of fatty liver, suggest that accumulation of fat in the liver and also in the pancreas might be the crucial step in the development of type 2 diabetes in these subjects.

TRIM proteins regulate a broad range of biological processes in humans (Ozato et al 2008). Some are associated with genetic disorders, neurological diseases or even cancer (Meroni and Diez-Roux 2005). Many TRIM proteins have been shown to confer ubiquitin E3-ligase activity crucial for mediating their biological effects on signalling pathways within the cells (Raymond et al 2001, Ozato et al 2008). UPS has been shown to affect the regulation of insulin signalling cascades and insulin action (Rome et al 2004). Moreover, UPS is involved in the internalization of the insulin receptor, in the control of the amount of insulin receptor substrates (IRS1 and IRS2), and in cellular insulin degradation. UPS also regulates transcription factors and nuclear receptors mediating insulin-induced gene expression. For instance, the peroxisome-proliferator-activated-receptors (PPARs) are involved in the regulation of lipid and glucose metabolism and in the control of inflammatory response (Blanquart et al 2002, Floyd and Stephens 2002, Rome et al 2004). Interestingly, inherited variation in the *PPAR*-gamma gene has been implicated in the pathogenesis of type 2 diabetes (Altshuler et al 2000) and also in reduced fetal growth (Jaquet et al 2002). Recent reports indicate that altered UPS function might be one of the molecular mechanisms of insulin resistance in many pathological situations. Insulin resistance is suggested to develop from inappropriate degradation of important molecules in the insulin signalling pathway, such as IRS1 and IRS2 (Rui et al 2002, Wing 2008). In addition, defective insulin secretion has been proposed to occur due to UPS-mediated degradation of IRS2 in the β-cells of the pancreas (Casas et al 2007). The UPS also appears to be involved in regulating lipid synthesis in adipocytes and lipid production by the liver and could influence the development of obesity (Donaldson 1979, Qi et al 2006, Wing 2008). TRIM37 is

the first member of the TRIM superfamily to influence the insulin homeostasis. The effects of TRIM37 on the insulin signalling cascades are still unknown.

7.4.1 Metabolic aspects of GH therapy

Since GH is an insulin antagonist and may induce hyperinsulinemia, we were concerned about the glucose metabolism in the GH treated MUL patients. Importantly, the pre-existing insulin responsiveness did not change significantly during GH treatment in prepubertal or pubertal children. Moreover, the adult patients who had received GH therapy had lower WFH and BMI and the deterioration of their glucose metabolism was less severe as compared to untreated MUL subjects. Similar favorable changes in body composition, BMI and the lipid atherogenic index have been presented in large cohorts of SGA children on GH therapy (van Pareren et al 2003, Boonstra et al 2003). Thus, the GH therapy appears at least to some extent slow down the development of the metabolic syndrome in MUL.

In MUL children the greatest increment in height was observed in those with the highest serum fasting and post load peak insulin concentrations suggesting that insulin might improve the spontaneous childhood growth, and accelerate the short term catch-up growth after commencement of GH therapy. Indeed, insulin is a well known growth promoting factor both pre- and postnatally. It regulates the IGF-1 concentration by facilitating the binding of GH to its receptor, stimulates the production of IGF-1, and increases bioavailability of IGF-1 by suppressing the hepatic synthesis of IGFBP (Böni-Schnetzler et al 1991, O'Brien et al 1991). Moreover, in a recent study involving non-GH treated SGA girls a rapid progression in pubertal growth tempo and progression to menarche driven by insulin was observed (Ibáñez et al 1998, 2006). Our results are in line with this, suggesting that the aggravated levels of insulin might underlie the faster pubertal tempo to menarche seen in MUL females on GH.

7.5 Cardiac problems in MUL

The heart disease comprises of two components; constrictive pericarditis (Tuuteri et al 1974) and thickening of the myocardium resulting in cardiac restriction (Lipsanen-Nyman et al 2003). The degree of heart involvement dominates the clinical state as well as the prognosis (Lipsanen-Nyman et al 2003). However, only 12% of our patients had congestive heart failure at the time of diagnosis (median 2.1 years) and half of the adult patients have been reported to be free of major heart problems (Lipsanen-Nyman et al 2003).

In histological analysis we found that the normal development of the heart was disturbed, resulting in a focally trabeculated myocardium and non-compaction cardiomyopathy (Finsterer et al 2002, Wessels et al 2008). UPS has been shown

to be involved in pathophysiological processes in muscle tissues (Fagan et al 1987). Skeletal muscle degeneration that follows denervation, immobilization and catabolic states, such as starving, fasting, and cancer-induced cachexia, has been shown to activate the UPS (Sun 2006, Paul 2008). In muscle, specific ubiquitin E3-ligases (muscle-specific RING-finger proteins or MuRFs) exist. MuRFs are the key regulatory components of the UPS in heart and skeletal muscle. They are responsible for the degradation of skeletal muscle proteins in wasting (Fagan 1987, Fischer et al 2000), and also for maintaining the anabolic and catabolic balance in cardiomyocytes (Toth et al 2006, Wang et al 2008). Interestingly, the role of UPS appears to be essential for up-holding normal cardiac function and muscular architecture of the heart (Powell 2006, Mearini et al 2008). Studies indicate that down-regulation of ubiquitin E3-ligases or UPS dysfunction may result in ischemic injury, cell death and the development of certain cardiomyopathies (Stöllberger et al 2002, Date et al 2005, Zolk et al 2006, Mearini et al 2008). In mice, the absence of pVHL in heart muscle has been shown to cause lipid accumulation, myofibrillar disorganization, cardiac muscle degeneration and the development of severe CHF (Lei et al 2008). How UPS dysfunction affects the progressive heart phenotype seen in MUL is yet to be enlightened.

7.6 Vascular abnormalities in MUL

Liver enlargement was obvious in half of the patients at the time of their diagnosis. Tumorous lesions of the liver, detected by US and MRI, were often present already in young MUL children, and 10% had liver lesions at the time of diagnosis (median age 2.1 years). These lesions were the most frequent tumors encountered in MUL patients. They comprised of large blood-filled dilated hepatic sinusoids (hepatic peliosis) (Tsokos et al 2005, Iannaccone et al 2006), which in the US follow-up tended to fluctuate in size and shape. This is in agreement with the fact that peliosis is associated with an increased pressure in the hepatic circulation, caused by cardiac failure and pericardial constriction (Tsokos et al 2005). In MUL, liver enlargement is regarded as an early clinical sign of the heart disease. Interestingly, peliosis can also occur secondary to wasting illnesses, infections, organ transplantation, certain drugs, immunological deficiencies and diabetes mellitus or as a paraneoplastic phenomenon (Tsokos et al 2005, Iannaccone R et al 2006). Besides heart failure, diabetes and wasting are also typical for MUL.

Vascular anomalies in MUL were not restricted to the liver. Cystic blood vessels were seen in other organs as well, such as adrenal and thyroid glands, lymph nodes, kidneys, lungs and brain. These lesions stained extensively for the endothelial marker CD34, which is expressed in both progenitor and mature endothelial cells (Pusztaszeri et al 2006). The CD34 staining has been found to be negative in normal hepatic sinusoids but positive in liver malignancy (Haratake

et al 1990, Coston et al 2008), i.e. hepatocelluar carcinoma, highlighting an increased capillarization of the sinusoids (Dhillon et al 1992). Benign lesions may randomly show focal CD34 positivity, but the strong staining for CD34 seen throughout the liver, as well as in the lymph nodes, in MUL is clearly abnormal and suggestive of a strongly disturbed angiogenesis (de Boer et al 2000, Pusztaszeri et al 2006, Hes and Morreau 2009). The staining for α-SMA was also prominent, indicating that the lesions contained myocytes. HMB-45, the marker for PEComa was negative (Hornick et al 2006). The minimal staining for the proliferation marker MIB-1 was in agreement with the benign nature of the lesions.

7.7 Ectopic tissues in MUL

Ectopic tissues and disturbed architecture were also present in other organ systems as well, indicating a defective control of cell migration. Both ovarian and adrenal tissues were found in the MUL kidneys. Ectopic or accessory adrenal tissues are usually found along the path of descent of the gonads but very rarely in kidneys (Lack et al 1997). The structure of the adrenal gland was also distorted in several MUL patients, showing fetal-type architecture, and mixing of cortical and medullar tissue. These findings, together with frequent gross organ anomalies, indicate disturbed organ development in MUL patients. The problem does not seem to be restricted to prenatal life, since several organs show progressive changes postnatally, such as hyperplasia of pancreatic islets, nodular hyperplasia of the adrenal gland and pericardial constriction. The molecular basis for the disturbed organogenesis remains to be fully elucidated.

7.8 Tumours in MUL

The results indicate that MUL is associated with an increased frequency of benign and malignant tumours and shows disturbed control of organ development. Radiology and histopathological examinations revealed tumorous lesions in 76% of the subjects. The benign tumours included cysts, vascular abnormalities (peliosis), adrenal adenoma, parathyroid adenoma, thyroid goitre, pancreatic cystadenoma, renal angiomyolipoma, ovarian fibrothecoma and pheochromocytoma. Thirteen (15%) of the 89 MUL-patients had a malignant tumour, such as Wilms' tumour, renal papillary carcinoma, thyroid papillary and medullar carcinoma, ovarian and endometrial carcinoma, acute lymphoblastic leukemia, Langerhans cell histiocytosis and carcinoid tumour.

Adrenal tumours have been described in rare genetic syndromes (Barlaskar et al 2007, Horvath et al 2008), such as the Li-Fraumeni cancer syndrome (LFS; OMIM 151623), which is caused by germline mutations of the tumour suppressor gene *p53* on chromosome 17q13 (Tabori et al 2008). The actions of p53

protein are known to be regulated by the UPS (Sun 2006). Adrenocortical tumours have also been described in endocrine syndromes, such as multiple endocrine neoplasia type 1 (MEN1; OMIM 131100). Affected subjects with MEN1 frequently show tumours in the endocrine pancreas, parathyroid, pituitary, thymic and thyroid gland. Adrenocortical adenomas or nodular hyperplasia is seen in 25-40% of subjects with MEN1 (Falchetti et al 2008, Horvath et al 2008). McCune-Albright syndrome (MAS; OMIM 174800), characterized by polyostotic fibrous dysplasia, café au lait skin lesions, precocious puberty and endocrine overfunction, and the overgrowth syndrome BWS are two other syndromes associated with adrenocortical neoplasia (Horvath et al 2008). Interestingly, both loss of heterozygosity of the 17q22-24 region and somatic mutations of the *PRKAR1A* gene located on 17q22-24 have been shown to occur in sporadic adrenocortical tumours (Bourdeau et al 2006). Also, inactivating mutations of the *PRKAR1A*, have been found to cause nodular adrenocortical hyperplasia and the multiple endocrine neoplasia syndrome Carney complex (CNC; OMIM 160980), an autosomal dominantly inherited syndrome responsible mainly for spotty skin pigmentation, endocrine overactivity, cardiac myxomas and adrenocorticotropic hormone (ACTH) independent Cushing's syndrome due to nodular adrenocortical changes (Bourdeau et al 2006, Horvath et al 2008). *PRKAR1A* and *p53* are known genes on chromosome 17q participating in adrenocortical tumorgenesis (Bourdeau et al 2006, Sun 2006, Tabori et al 2008) and *TRIM37* could probably be added to this list.

A growing number of cancer-associated proteins have been linked to the ubiquitin proteasome pathway; for instance, in the overgrowth syndrome BWS down-regulation of TSSC5 is associated with Wilms' tumour (Scott et al 2006, 2008). TSSC5 encodes a RING-finger protein, RING105, acting as an ubiquitin E3-ligase (Sun 2006). The tumour suppressor gene *BRCA1*, located on chromosome 17q21, encodes for a RING finger protein BRCA1 with ubiquitin E3-ligase activity. Mutations in the RING finger have been shown to abolish its E3 activity. Interestingly, inactivating mutations have frequently been reported in ovarian carcinoma (Paul 2008) and breast cancer (Hashizume et al 2001). The familial cancer syndrome VHL results from germline mutations of tumour suppressor gene *VHL* located on chromosome 3p25-26 encoding for a tumour suppressor protein pVHL with E3-ligase activity (Jung et al 2006). In VHL, HIF-1α is up-regulated due to loss of function of pVHL, leading to overexpression of vascular endothelial growth factor (VEGF) and erythropoietin (*EPO*), and eventually enhanced vascular growth (Jung et al 2006, Shehata et al 2008). The elevation of VEGF also increases the vascular permeability, causing oedema which is seen as multiple cysts in different organ systems in VHL (Jung et al 2006, Shehata et al 2008).

VHL and MUL share some histological and radiological features and both predispose the patients to multiple cystic and vascular lesions, primarily in endocrine tissues (Tattersall et al 2002, Jung et al 2006, Shehata et al 2008). These two

conditions, however, have their own distinct characteristics. Hemangiomas of the cerebellum, spine and retina are pathognomonic lesions for VHL (Tattersall et al 2002, Shehata et al 2008), while vascular lesions in the liver rarely occur. Moreover, the most common malignant tumour in VHL is renal clear-cell carcinoma (Tattersall et al 2002, Jung et al 2006, Shehata et al 2008) and not papillary carcinoma or Wilms' tumour, as in MUL. Although the tumour spectrum of VHL and MUL clearly differs, loss of TRIM37 seems to result in cellular effects resembling those of VHL, including enhanced angiogenesis, development of multiple cysts and to some extent even tumour growth. Intriguingly, the pathogenetic mechanism in VHL could at least to some extent be applied to MUL as well; MUL patients have low oxygen pressure (pO_2) in blood-gas analysis (Karlberg et al unpublished data), have a restrictive cardiopathy, and frequently need supplementary oxygen during stress and infections. Moreover MUL subjects have an increased mean cellular volume of red blood cells (MCV) due to reticulocytosis probably as a reactive response to hypoxia (Karlberg et al unpublished data). Whether the loss of TRIM37 causes overexpression of VEGF or triggers some other vascular growth promoting pathway, will hopefully be answered in ongoing studies.

The malignant tumours found in MUL do not show a clear recognizable spectrum as in classic cancer syndromes, such as VHL (Jung et al 2006, Shehata et al 2008). Also, the malignancies in MUL do not occur at an especially early age (Hes and Morreau 2009). For instance, Wilms' tumours in MUL were detected at a median age of 2.5 years as is the case in sporadic tumours (Scott et al 2006, 2008). The age of Wilms' tumour diagnosis is typically closer to 1.0 years in syndromes caused by mutations in the *WT1* gene on chromosome 11p13 (Scott et al 2006, 2008). However, the incidence of Wilms' tumour was clearly elevated in MUL (6.5%, 6/92) and somewhat equal to the risk presented in BWS (4-10%) (Scott et al 2006). In MUL, the malignancies do not display multicentricity or bilateralism as in classical cancer syndromes (Hes and Morreau 2009), but the benign tumours in MUL do. In addition to the two bilateral adrenocortical adenomas, renal cysts were typically seen in both kidneys. Moreover, six females had a bilateral ovarian fibrothecoma and one female patient had an angiomyolipoma in one kidney and a hamartoma containing ovarian and adrenal tissues and a metanephric adenoma in her other kidney. Also, one MUL patient was diagnosed with a thyroid adenoma in both lobes.

Totally 232 tumorous lesions were detected in 70 of the 92 patients (76%). This shows that some MUL patients are not affected by tumour growth or have not simply been diagnosed with tumours, while some patients clearly present more than one tumour, which is typical for cancer syndromes (Hes and Morreau 2009). There are many examples of genes responsible for cancer syndromes being defective in sporadic tumors with similar morphology and location (Jung et al 2006, Hemminki et al 2008, Karlberg et al 2009) and dysregulation of TRIM37 has recently been implicated in the pathogenesis of sporadic fibrothecomas

(Karlberg et al 2009). If Mulibrey nanism claims a place among established cancer syndromes remains to be seen.

7.9 Limitations of the study

Although our MUL patient cohort is the largest in the world, the major limitation with this study is the small number of patients and their wide age range (0.7 to 77 years). Due to the small number of patients statistical analyses were a challenge and not always possible to carry out. Also, the possibility of statistical errors cannot totally be excluded due to small sample size. On the other hand, also clinically significant features failed to reach statistical significance. When assessing the glucose metabolism in MUL (II), age-, sex- and BMI-matched controls would have been valuable and further increased the scientific impact. Also, a control group in the analysis of GH treatment in MUL (III) would have been valuable.

The diagnostic criteria presented are based on the Finnish patients and different TRIM37 mutations may not result in the exact same phenotype. In particular, all patients reported outside of Finland have been notably compromised by the heart disease or had Wilms' tumour. Presumably, while MUL is relatively well known to the Finnish pediatricians, it is elsewhere mainly recognized by the characteristic heart disease or Wilms' tumour.

8. CONCLUSIONS

Our results show that Finnish MUL patients with recessive mutations in *TRIM37* gene present a distinct clinical phenotype but clinical variation exists. Also different *TRIM37* mutations may not lead to the precisely same phenotype. The diagnosis remains a challenge. However, in subjects born SGA, lacking postnatal catch up growth, and having poor weight gain, hepatomegaly, and characteristic craniofacial features, MUL should be considered. Although rare, this condition might also underlie CHF, failure to thrive or Wilms' tumour in absence of a clear characteristic dysmorphology.

MUL patients are born SGA with immature features and display a continuous deceleration in both height and weight throughout infancy. Feeding difficulties and pneumonias are frequent early life concerns. GH therapy improved prepubertal growth but the long-term impact on final height remained modest. In MUL, a dramatic change in glucose metabolism occurs with advancing age. While the children have low fasting glucose and insulin levels, the vast majority of the adults are insulin resistant and develop a full-blown metabolic syndrome. Notably, the GH treated subjects remained leaner and had less frequent metabolic concerns than the untreated individuals. Importantly, MUL patients display disturbed organogenesis and have a high frequency of benign adenomatous and vascular lesions, as well as malignant tumours detectable in several internal organs. Additionally, MUL patients displayed lipid accumulation in both liver and pancreas.

The extensive phenotype of MUL including common human pathologies like type 2 diabetes and tumours, speaks for a central role of the TRIM37 protein in cellular functions, such as insulin signalling, proliferation, migration and angiogenesis. Elucidation of the underlying molecular biology in Mulibrey nanism can provide new aspects for a better understanding of common human diseases.

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