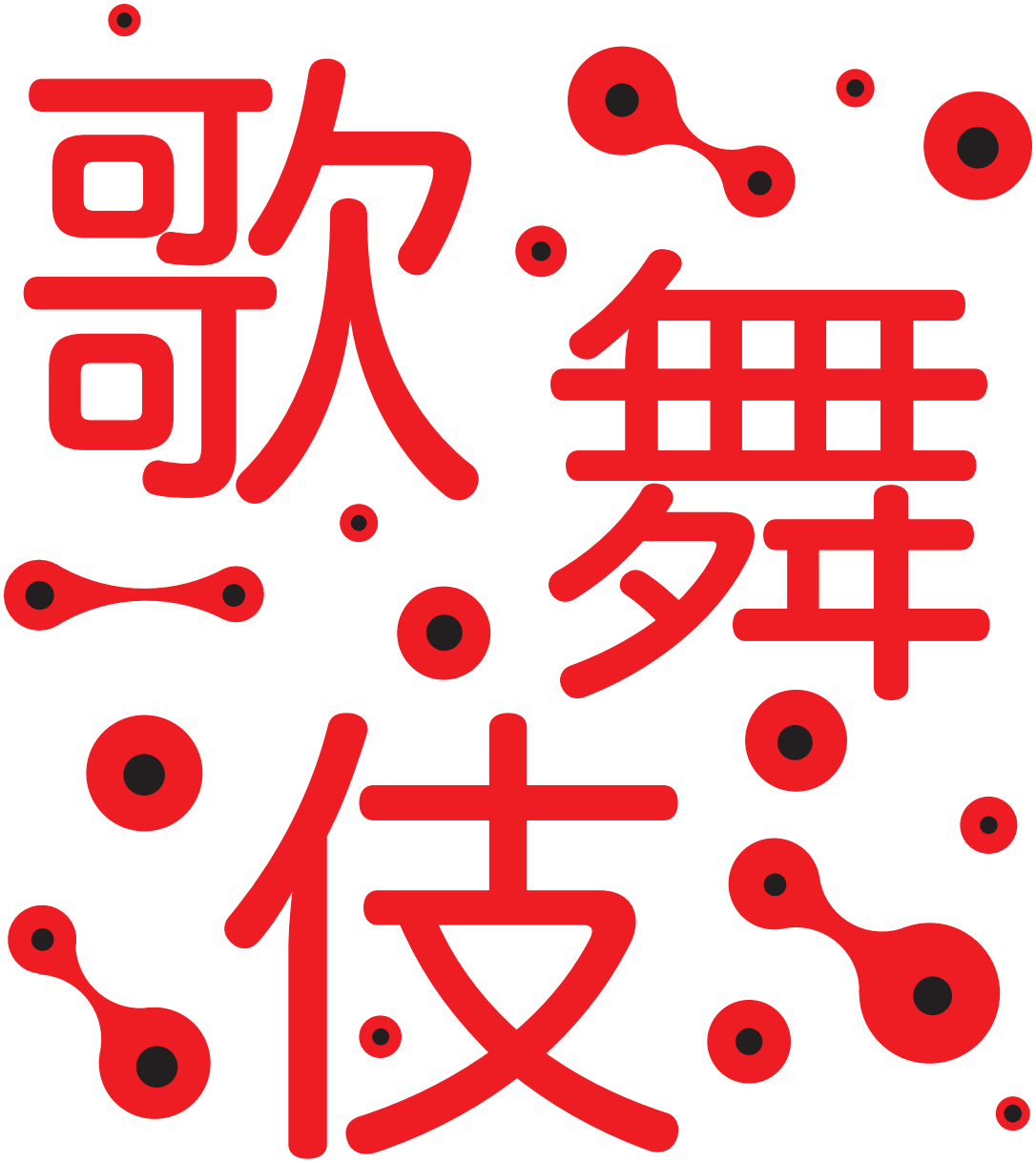
D.A. Schott



GROWTH HORMONE THERAPY IN KABUKI SYNDROME

prospective study on the metabolic and longitudinal growth effects of rhGH treatment in children with kabuki syndrome.

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GROWTH HORMONE THERAPY IN KABUKI SYNDROME

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GROWTH HORMONE THERAPY IN KABUKI SYNDROME

In memoriam
Meike van der Snee

Dedicated to my parents

CONTENTS

CHAPTER 1
INTRODUCTION

GENERAL INTRODUCTION AND AIMS OF THE THESIS

Dina A. Schott

INTRODUCTION

Kabuki syndrome (KS) was first described independently by Niikawa et al. and Kuroki et al. in 1981 (1,2). One of the cardinal manifestations in KS is short stature, as reported in many reviews and case reports with an incidence of approximately 80%. The cause of this growth retardation is still unknown. This study has been conducted to achieve more insight into growth, body composition, energy expenditure, cardiovascular markers, and hypermobility in children with Kabuki syndrome before and during human recombinant growth hormone treatment.

1.1 Kabuki Syndrome

KS is a multiple congenital anomalies syndrome (OMIM 147920). The prevalence of KS is estimated to be 1:32.000 live births based on data from 'Monitoring for Congenital Anomalies' in Kanagawa, Japan. KS is characterized by typical facial features, of which the eyebrows figure is prominent (100%). KS derives its name from Kabuki, a traditional form of Japanese theater (Figure 1). The resemblance between the characteristic faces seen in these patients and the make-up of the actors in the Japanese Kabuki theater gave this syndrome its name: Kabuki make-up syndrome. Nowadays, we simply speak of 'Kabuki syndrome' (1,3). Besides the arched and broad eyebrows, there are more classic facial features (Figure 2), which involve elongated palpebral fissures with eversion of the lateral third of the lower eyelid, short columellae with depressed nasal tip, and large and prominent or cupped ears. Further cardinal manifestations are persistence of fetal fingertip pads (93%), mild to moderate disability (92%), and postnatal growth deficiency (83%) (4,5).
Other findings may include: congenital heart defects, genitourinary anomalies, cleft lip and/or palate, gastrointestinal anomalies including anal atresia, widely spaced teeth, and hypodontia. Functional differences can include: increased susceptibility to infections and autoimmune disorders, endocrine abnormalities including isolated premature thelarche, feeding problems, and hearing loss. More recently, joint laxity (74%) figures as a frequently observed feature that was overlooked in previous descriptions (6). Although many additional, unique features have been described, these cases are of more limited prevalence.

Figure 1 and 2. Kabuki Make-up Theater actor and a child with Kabuki syndrome.

1.2 Genetic cause

1.2.1. History

Until 2010, the diagnosis of KS was based on the "facial gestalt" in combination with mild to moderate developmental delay, hand anomalies, short stature, and a variety of other physical indicators. In 2010, mutations in the *KMT2D* (formerly called *MLL2*) gene were described by Ng et al. (7). Before that time, numerous case reports described chromosomal abnormalities. In individuals with Kabuki-(like) syndrome, features overlap the phenotype of the 22q11 deletion syndrome (e.g., cleft palate, congenital heart defects, and urinary tract anomalies) (8,9). Also, sex chromosome inheritance has often been implicated in KS. Overlap with Turner syndrome has been reported since both conditions share clinical manifestations. Ring X chromosome, 45,X cell lines and abnormal breakpoints of Xp11 or Yp11 were reported (10-14). However, an X-linked gene for KS was not identified until 2012 by Lederer et al. (15). Milunsky and Huang described for the first time an association between 8p22-p23.1 duplications and KS in 6 unrelated

patients (16). However, these results were not replicated by other research groups (17,18). The implication of the 2q37 region in KS was first reported by Cuscó et al (19). The clinical overlap between KS and the 2q37 microdeletion syndrome supports screening for 2q37 deletions particularly when evaluating young children, because typical KS eye features are not so evident before 3 years of age. Clinical overlap between Kabuki syndrome and CHARGE syndrome has been reported as well (20). CHARGE syndrome (OMIM 214800) is characterized by coloboma, heart defects, choanal atresia, ear anomalies and various other features and is caused by mutations in CDH7 (21). A molecular link between Kabuki and CHARGE syndrome was demonstrated by Schulz et al. (22). Both the *KMT2D* and the CHD7 gene are part of the same chromatin remodeling and chromatin modification 'machinery' via the WAR complex.
There is also a phenotypical similarity between KS and KBG syndrome (OMIM ID: 148050) which is characterized by macrodontia of the upper central incisors, distinctive craniofacial findings, short stature, skeletal anomalies, and neurologic involvement that includes global developmental delay, and intellectual disability. KBG syndrome is linked with mutations in the ANKRD11 gene encoding for ankyrin repeating domain-containing protein 11 involved in transcription repression (23-25).

1.2.2. Mutations

KS is an autosomal dominant condition that appears de novo in the majority of cases. In 2010, whole exome sequencing led to the identification of mutations in *KMT2D* (previously *MLL2*, OMIM ID: 602113) as the basis of KS subtype 1 (OMIM ID: 147920) (7) (Figure 3). Still, the underlying cause could not be identified in 20 to 45% of patients with presumed diagnosis of KS. *KMT2D* maps to 12q13.12 and encodes a histone H3 lysine 4 (H3K4)-specific methyl transferase that belongs to the human SET-domain methyltransferase superfamily (26). *KMT2D* functions as a part of multiprotein complex, ASCOM, which binds to the regulatory sites of target genes. It is important for epigenetic transcriptional activation, it interacts with estrogen receptor-α and is important for embryonic development (27). Mutations in this gene are found in 55 – 80% of cases (7,28,29). Most mutations concern a truncating mutation (ca. 70%).
In 2012, complete or partial de novo deletions of *KDM6A* (previously *UTX*, OMIM ID: 300128) gene were identified in three patients with KS (15) (Fig. 3). *KDM6A* is located at Xp11.3 and encodes the lysine demethylase 6A (*KDM6A*)

demethylating di- and trimethyl-lysine 27 on histone H3 (H3K27) (30). H3K4 methylation by *MLL2/3* is linked to the demethylation of H3K27 by *KDM6A*. Like *KMT2D*, *KDM6A* plays a role in embryogenesis and development. The mutation detection rate for *KDM6A* in KS is about 9-13% plus *KMT2D* (55-80%), suggest that additional gene(s) may be found. De novo or rarely inherited point mutations located in the coding region and splice junctions of the *KDM6A* gene have also been recently identified in approximately 3-8% KS individuals. Genetic variants in this second gene define X-linked KS subtype 2 (OMIM ID: 300867) (31).

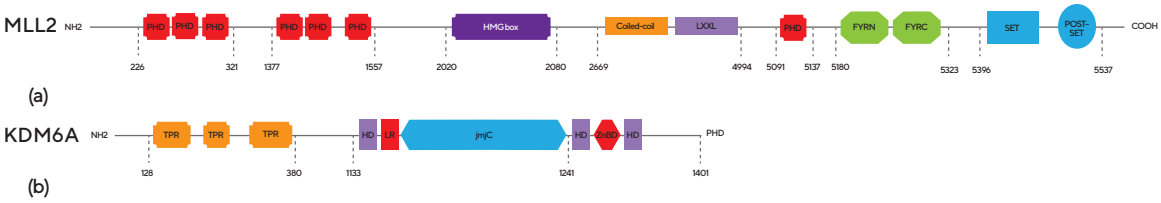

Figure 3. The genetic structure of the *KMT2D (MLL2)* and *KDM6A*.

1.2.3 Genotype-phenotype correlation

A different facial gestalt along with variations in some clinical features presents in *KMT2D* mutation-positive and –negative patients. Banka et al. reported that the majority of typical KS patients (based on facial KS morphology scores) have *KMT2D* mutations, implying that the genetic heterogeneity of KS may be minimal (29). The conclusions by Paulussen et al. were similar, even as the growth retardation (threshold -3 SD) was found more pronounced in *KMT2D* positive patients (28). Furthermore, more renal abnormalities were observed in the *KMT2D* mutation positive patients (32).

Two years later, the *KDM6A* was identified as a second gene in KS patients. The *KDM6A* deletions in KS patients have the same clinical features as seen in the *KMT2D* patients, such as facial dysmorphias, short stature, joint laxity etc. However, *KDM6A* patients have a broader phenotypic spectrum ranging from typical KS to milder clinical presentations in affected family members (15). *KDM6A* escapes X inactivation (33).

Both KS genes play comparable and critical roles in early vertebrate

development, and their reduced expression results in craniofacial, cardiac and brain abnormalities. Morpholino-based knockdown of *KMT2D* and *KDM6A* in zebrafish showed reduced posterior body length and abnormal curvature of the spine, as well as many other manifestations in KS (34).

1.2.4 Additional genes associated with Kabuki syndrome

Twenty to thirty percent of patients with a Kabuki (like) phenotype have no identified *KMT2D* or *KDM6A* gene mutation, so other genes must play a role. Whole exome sequencing has the power to find these genes, and indeed a growing number of articles shows this.

Mutations in *RAP1A* and *RAP1B* genes have also been reported in 2 cases with a clinical diagnosis of KS (35). In one patient with KS a mutation in *RAP1A* was converted to homozygosity as the result of uniparental disomy. In another patient with Kabuki-like syndrome a de novo dominant mutation was found in *RAP1B* gene.

Lintas and Persico provide a literature review unraveling molecular pathways shared by Kabuki and Kabuki-like syndromes. They list the cytogenetic abnormalities and candidate genes reported in Kabuki-like phenotypes and conclude that many of the genes identified so far have a functional role similar to *KMT2D* and/or *KDM6A* or share the same biological processes e.g. abnormal chromatin remodeling and transcriptional dysregulation (23). Two of those genes, for CHARGE - and KBG syndrome are describe in the above section 1.2.2.

Sobreira et al. performed targeted sequencing in a cohort of 27 probands with a clinical diagnosis of KS. Of these, 12 had causative variants in the two known KS genes. Ten of the 12 had a *KMT2D* variant, which does not have a known protein domain and has not been previously associated with KS. They also found two individuals with a novel de novo heterozygous missense or donor splice site variants in *KMT2A*, a histone methyltransferase gene previously associated with Wiedemann-Steiner syndrome (36). Wiedemann-Steiner syndrome has many overlapping features with KS, such as vertically narrow palpebral fissures, strabismus, broad nasal bridge/tip, external ear deformity, short stature, hypotonia, small hands, hip abnormalities, developmental delay, intellectual disabilities, feeding difficulties, heart anomalies, urological anomalies, and recurrent infections. These results indicate phenotypic overlap between KS and Wiedemann-Steiner syndrome.

The online tool GeneMatcher enabled the match of 2 probands with a de novo

missense variant in *HNRNPK*. The authors mention KS in their differential diagnosis, which is clear looking at the clinical pictures. Now a total of four patients with facial dysmorphisms clinically overlapping KS were found to have inactivating mutations in *HNRNPK*. *HNRNPK* has an important role in chromatin remodeling and all genes known to cause KS are chromatin regulators. This might explain the phenotypic overlap seen between KS and *HNRNPK* mutations. Particularly the facial gestalt resembles KS, although the distinctive KS-arched eyebrows are not evident (37-39).

1.3 Endocrinopathies in Kabuki syndrome

1.3.1 Hypothalamic pituitary axis abnormalities

In the literature, ten patients with KS were described with disturbances in the hypothalamic pituitary axis. Among them, GH deficiency was the most frequent finding (see section 1.4.2). Precocious puberty and central diabetes insipidus (DI) were identified in two cases. One patient had a combination of GH deficiency and central DI. Another was noted with an ACTH deficiency. Three out of the ten patients showed abnormalities in the pituitary region on the MRI scan. Two of the six patients with GH deficiency also manifested premature thelarche (40,41).

1.3.2 Premature thelarche

In KS, premature thelarche is a frequent finding. Premature breast development is reported in 40 of 94 (43%) female patients in the literature (42). Noticeably, girls with KS have increased sensitivity to estrogens during infancy (43). Endocrine studies have been reported in four patients. Three of them appear to present isolated premature thelarche, whereas one was documented to have true central precocious puberty (44-46).

1.3.3 Obesity

KS patients may become obese (BMI > 2.5 SDS) towards adolescence and a weight gain of 10 kg in 6 months would not be unusual (42). To date, no endocrine explanation has been given for this phenomenon. The fat distribution is mainly truncal (45).

1.3.4 Other endocrinopathies

There have been several reports concerning hypoglycemia after overnight fasting. Insulinoma was never documented, but treatment with a small dose of diazoxide was mentioned by Niikawa (1).
Several case reports mentioned cryptorchidism and micropenis (47).
One case reports an association between KS and type 1 diabetes mellitus (48).
Some KS patients showed hypothyroidism; one based on an autoimmune thyroiditis (49). Certain autoimmune diseases such as vitiligo and ITP have been reported to be co-exist with KS, thought the frequency is not particularly high (50-52).

1.4 Growth and growth hormone

1.4.1 General

Growth is an anabolic process. That is to say, a dynamic process with somatic changes in stature, body composition and body proportions, which involves cell hyperplasia, hypertrophy and apoptosis. Besides genetic factors, human growth is influenced by environmental and hormonal factors. Studies have revealed that non-pathological factors such as nutrition, psychological influences, physical activity and climate also play important roles in growth.
GH, the principal hormone involved in somatic growth and body composition, expresses its action directly or through its effects by IGF-I (53). The secretion and action of GH can be disrupted by mutations in genes affecting the synthesis of GH itself, its binding proteins and receptors or the production of pituitary transcription factors. Additionally, other hormones (eg. thyroid and adrenal hormones, glucocorticoids, sex steroids, ghrelin, insulin and leptin), interact with growth (54-56).
The growth hormone axis is very complex (Figure 4). The secretion of GH by the anterior pituitary is pulsatile and controlled by two neuro-hormones. GH secretion is stimulated by the GH releasing hormone (GHRH) and inhibited by somatostatin, also called GH inhibiting hormone (GHIH) (57). Pulses of GH secretion occur at times of maximum GHRH and minimum GHIH from the hypothalamus.
GH utilizes its effects through induction of somatomedins (inter alia IGF-I), a family of hormones secreted into the blood by the liver (and possibly other tissues) (58). IGF-I is structurally related to insulin, and is the agent that acts on

target tissues to promote mitosis and protein synthesis by increasing the rate of uptake of amino acids into muscle (insulin-like effect) (59). It is produced in the liver by stimulation of GH, but suppressed by malnutrition, GH insensitivity, GH receptor deficiency, or failures of the downstream signaling pathway of the GH receptor, including SHP2 and STAT5b. IGF-I is bound in ca. 98% to one of the six binding proteins (IGFBPs) of which IGFBP-3 is the most important, accounting for 80% of all IGF binding (60).
Furthermore, GH opposes fat deposition by decreasing glucose uptake and utilization in adipose tissue. Conversely, GH increases synthesis of the capillary lipases needed for dietary triglyceride uptake into adipose tissue and promotes the breakdown of the triglycerides, allowing fatty acids to be released. In this way, GH decreases storage of fat, and promotes the use of fatty acids for energy. This effect of GH on fat metabolism is seen only if the plasma insulin levels are low because insulin normally inhibits the catabolism of fat through its inhibition of intracellular hormones-sensitive lipases.

Final adult height is strongly corelated to genetic background. It has been estimated that 70-90% of a child's final height is genetically determined. Mutations in genes that influence the hypothalamo-pituitary axis may negatively affect the production of GH or its action. These include the GH gene, GH receptor gene, IGF-I gene, IGF-I receptor gene, and genes involved in the production of pituitary transcription factors (e.g. *POUF1, PROP1, HESX1, LHX3, LHX4*) which are associated with severe short stature. Also, non-hormonal abnormalities influence growth, such as defects in the fibroblast growth factor receptors (FGFR) or the short stature homeobox gene (SHOX). In conclusion, human growth follows a narrow, genetically defined path. Any deviation towards final adult height must be regarded with concern as to the child's general health.

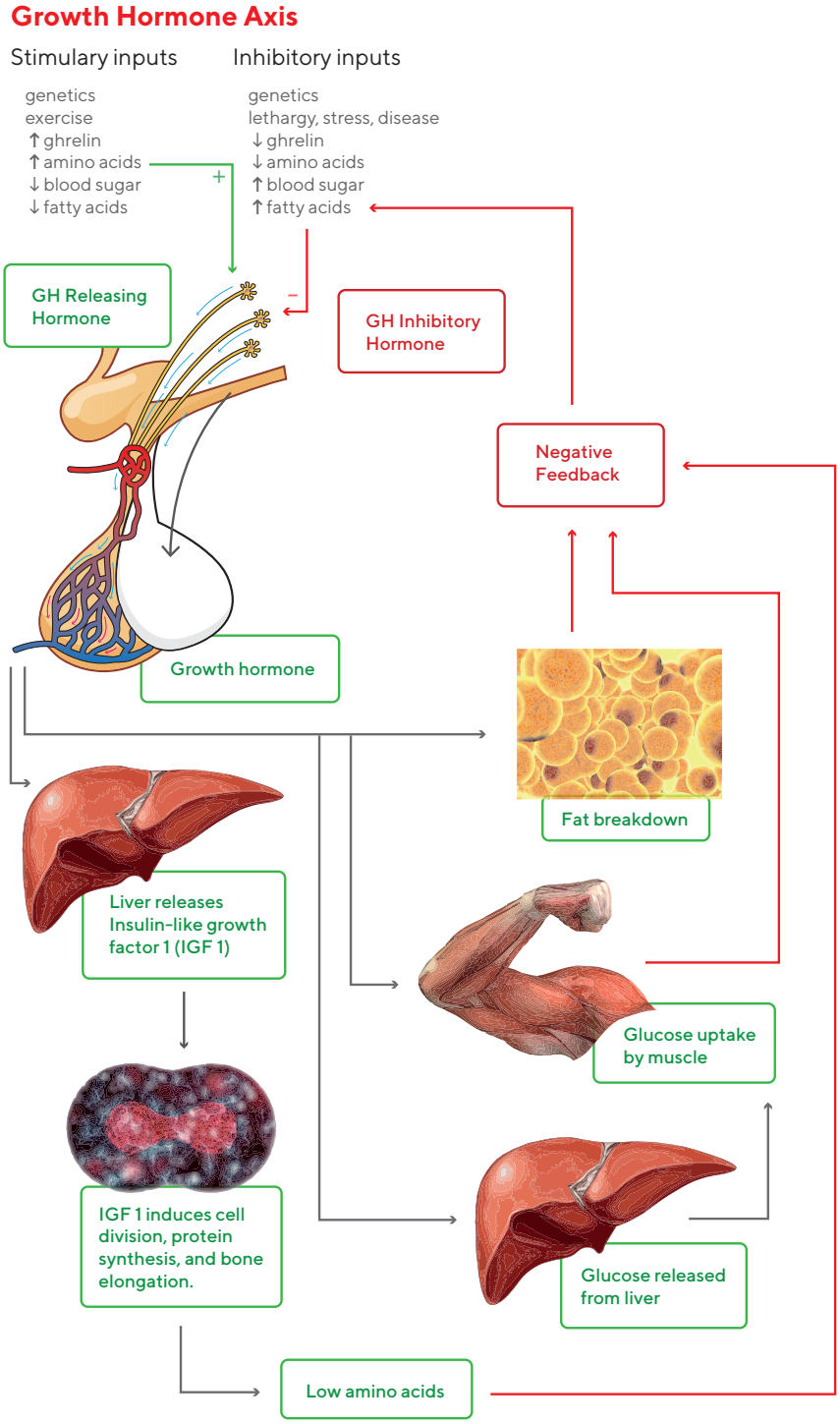

Figure 4. The growth hormone – IGFI axis. Adapted from Lanzillo et al. (61)

1.4.2 Growth in genetic syndromes

In genetic conditions, such as Turner syndrome (TS), Noonan syndrome (NS), Prader-Willi syndrome (PWS), Silver-Russell syndrome, Leri-Weill syndrome, and Kabuki syndrome, short stature is a common feature. Recently, the increasing availability of recombinant human growth hormone (rhGH) has expanded interest in GH secretion and therapy related to these developmental syndromes. Children with some of these syndromes (eg. TS and PWS), but without a real GH deficiency, have benefited from rhGH therapy at supra-physiological doses, obtaining a greater final height than expected according to the natural history. PWS as well as KS children are characterized by several signs and symptoms, including feeding problems, muscular hypotonia, psychomotor delay, trunk obesity and short stature.

Since KS children share many of the same features of PWS children, we hypothesize that KS children would experience the same positive growth and metabolic effects of rhGH treatment as these children do.

1.4.3 Growth and growth hormone in Kabuki syndrome

In the first publication on KS, Niikawa described a normal birth weight and length for Japanese KS patients (10). Many patients develop a postnatal growth retardation several months after birth (mostly -2 SD level). Final height is expected to be about 152 cm for Japanese patients (10). Schrander-Stumpel et al. reported that 70% of the patients had a height below the 3rd percentile for age (42). Consequently, growth retardation is a major criterion of KS. The causative factors for the short stature in KS have not been clarified.

Growth hormone deficiency (GHD) has been reported in KS at least eight times: Twice by Schrander-Stumpel et al. along with seven more reference in the literature (10,44,45,62-65). Gabrielle et al. reported on a 9-year-old boy with KS. GH stimulation test showed a partial GH deficiency (CLO 5.2 ng/ml and ARG 5 ng/ml) with a low normal IGF-I level. The bone age was equal compared to the chronical age, also during treatment after four years. Height velocity before start was 3.5 cm/yr, and accelerated to 9.5 cm during the first year and thereafter to 6 cm/yr (63). Devriendt et al. describe a girl of 23 months with an insufficient glucagon stimulation test (9.8 ng/ml), and undetectable IGF-I levels. rhGH treatment showed a catch-up growth. Tawa et al. mentioned a boy of almost 5 years with a short stature (height -4.1 SDS), retarded bone age, and a low height velocity. GH stimulation test did not confirm GH deficiency (INS 21.2

ng/ml and GLU 14.3 ng/ml), despite a low level of IGF-I. However, the pulsatile secretion of GH during deep sleep was deteriorated, perhaps compatible with GH neurosecretory dysfunction. After 6 months of rhGH treatment both serum IGF-I level and the growth velocity (8.2 cm/yr) were increased (44). Kadawa et al. studied a boy aged ten-years old with a short stature (-2.9 SD) and partial GH deficiency (INS 8.5 ng/ml and L-dopa 9.5 ng/ml). rhGH treatment was started, and the height velocity increased from 3.5 to 5.3 cm/yr. Handa et al. portrayed a girl with a cleft lip and palate. Because of growth retardation and pseudo-precocious puberty, an Arginine and LHRH test was done. The maximum GH level was 8.6 ng/ml, but the report did not mention anything about rhGH treatment (66). Satoh also reported a four-year-old boy with GHD and cleft palate. On MRI imaging the pituitary stalk was not identifiable and the anterior lobe looked slightly atrophic. GH provocation tests were abnormal (GLU 0.84 ng/ml and CLO 0.74 ng/ml) but no other endocrine abnormality was noticed. After start of rhGH treatment, catch-up growth was perceived (62).
The KIGS[1] database has indicated that rhGH treatment increases growth rate in KS children. Twenty-one KS children treated with rhGH therapy achieved an increase in height SDS from -3.41 to -2.58 SDS after one year of treatment (67). Long-term reports of controlled studies are not available.

1 KIGS: Pfizer Growth Study Program. This is a multicenter observational database and study to gather information about children's growth response and long-term effects from growth hormone replacement therapy using Genotropin®. This study provides information to doctors prescribing Genotropin to treat children and adolescents, ultimately helping physicians to use it more effectively.

1.5 Energy expenditure, body composition and growth hormone

1.5.1 Energy expenditure

Energy expenditure is the amount of energy (or calories) a person requires to carry out physical functions such as breathing, circulating blood, digesting food, or physical movement. Total energy expenditure (TEE) is the total number of calories a person burns each day. TEE can vary from person to person depending on body size, gender, body composition, genetics and activity level. To prevent weight gain, energy intake or calorie intake must be balanced with energy expenditure. TEE can be divided into three components: basal metabolic rate (BMR), diet induced thermogenesis (DIT) and activity related energy expenditure (AEE) (Figure 5).

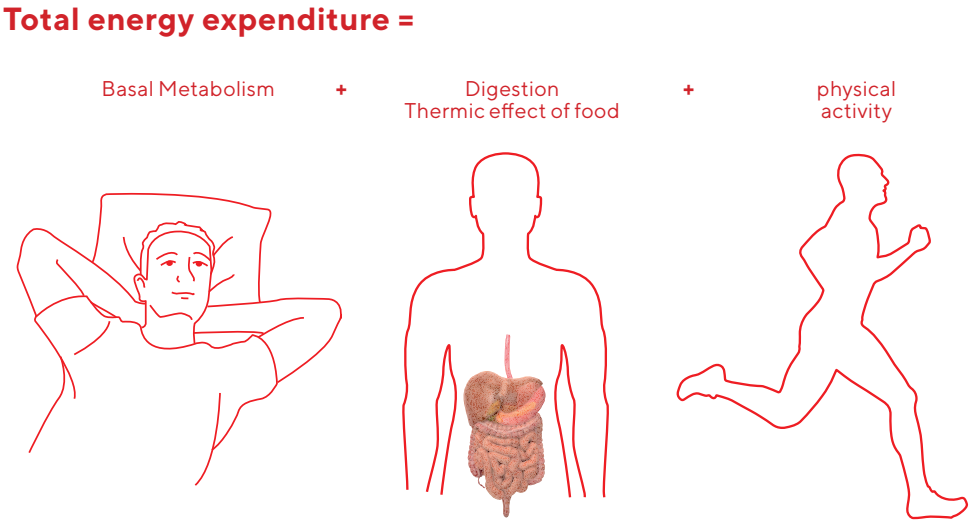

Figure 5. Total energy expenditure is the sum of energy expended at rest, during digestion, and during physical activity.

TEE can be measured with the doubly labeled water (DLW) technique. The DLW method makes use of two isotopes, ^{2}H and ^{18}O, and has become the gold standard for measuring TEE under free-living conditions (68,69). It was introduced for human use approximately 30 years ago (70). The principle of the method is as follows. Subjects receive a loading dose of water labeled with the stable ^{2}H and ^{18}O isotopes, and these isotopes mix with the hydrogen and oxygen in body water within a few hours. As energy is expended, CO_2 and water are excreted. The CO_2 is lost from the body only through the breath, while the water (including both ^{2}H and ^{18}O) is lost not only by the breath but also in urine, sweat, and through other means such as evaporation. During a period of 4-20 days, the difference between the rate of loss of ^{18}O and ^{2}H from the body reflects the rate at which CO_2 is produced (Figure 6.), which in turn can be used to estimate EE by using a modified Weir's formula based on the CO_2 production rate (rCO_2) and respiratory quotient (RQ) (71).

Weir's formula: $TEE\ (kcal/day) = 22.4\ \{3.9(rCO_2/FQ) + 1.1(rCO_2)\} \times 4.184/1000$

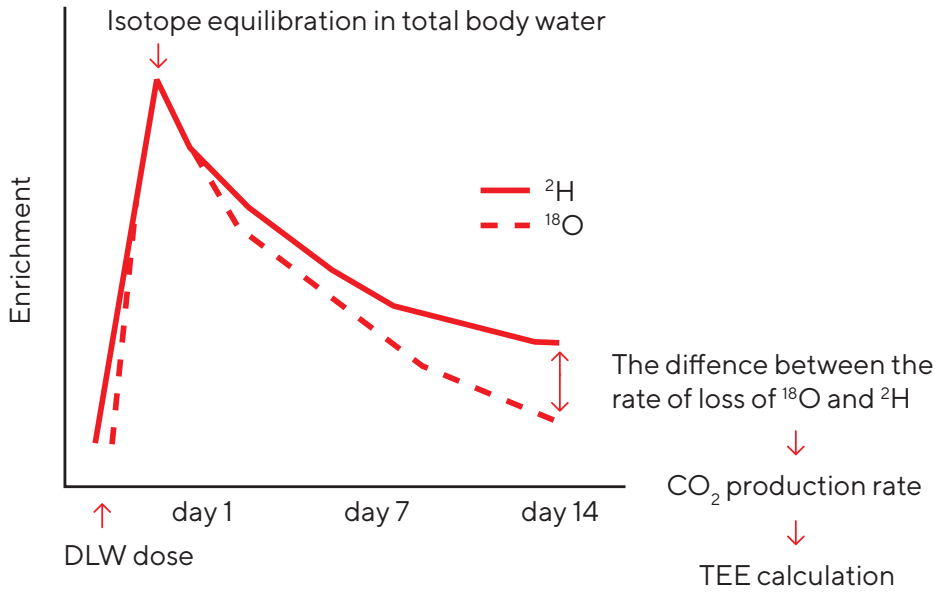

Figure 6. Decline of ^{2}H and ^{18}O levels in total body water during a doubly labeled water experiment. The difference between disappearance rates of ^{2}H and ^{18}O approximates the CO_2 output, which can be used to calculate the TEE (Adapted from Heijman and Roberts (72)).

BMR is the energy needed to maintain all vital body functions at rest. Some of those processes are breathing, blood circulation, controlling body temperature, cell growth, brain and nerve function, and contraction of muscles. The measurement of basal metabolic rate must meet four conditions: the measurement occurs in a thermo-neutral environment, the subject should be awake, has fasted long enough to eliminate DIT, and is at rest to eliminate AEE. BMR can be measured using an open-circuit, ventilated hood system (Figure 7). A transparent plastic hood is placed over the head and neck of the subject, who lies down on a bed and breathes normally. The outside air is led through the hood and the breathed air is analyzed. By determining the quantity of air flowing through the hood and measuring the oxygen uptake and CO_2-concentration, the energy expenditure at rest can be calculated.

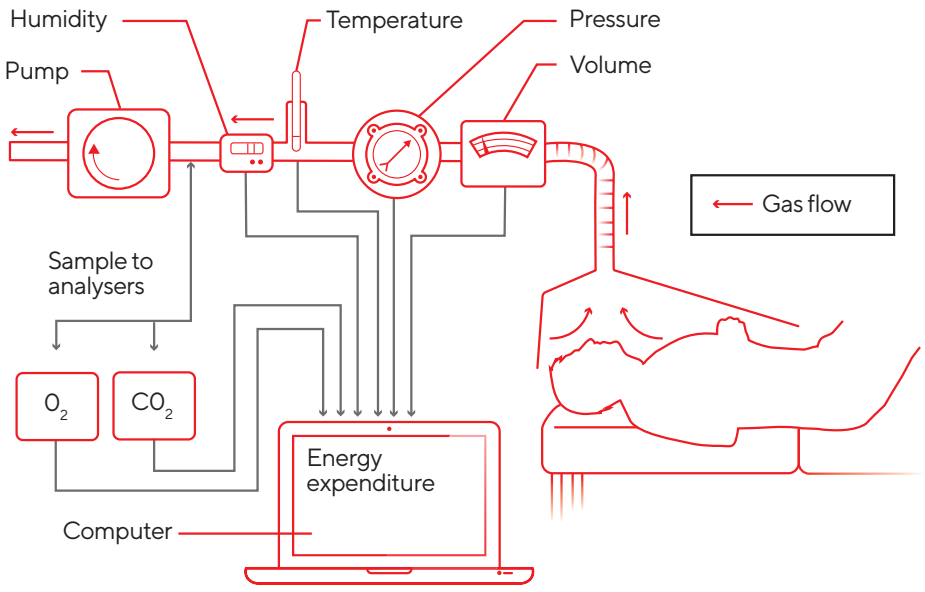

Figure 7. Indirect calorimetry measuring oxygen uptake and CO_2-production with the ventilated hood method.

Physical activity and physical fitness are important for health. Inactivity increases the risk of diseases, like coronary artery disease, hypertension and diabetes mellitus, all components of the metabolic syndrome (73-75). Physical inactivity is becoming more and more characteristic of our modern way of life. At the same time, obesity is becoming an increased problem among adults and children (76). As a consequence of these negative effects of physical inactivity, the interest to measure physical activity levels increases.

Physical activity can be monitored using various methods. Using the DLW method, physical activity can be measured over a period of one to two weeks under free-living conditions, without influencing natural activity behaviors (77). The physical activity level (PAL) can be calculated as TEE/BMR. However, since DLW is expensive and scarce, this method is not suitable for regular use. Accelerometers, on the other hand, can measure physical activity intensity and activity patterns. These have been proven reliable for the assessment of physical activity in adults as well as in children (78,79). The Tracmor$_D$, a triaxial accelerometer, is a lightweight, portable device that check the acceleration in the vertical, horizontal and mediolateral planes and appears to be well correlated with the DLW method (Figure 8) (80). The triaxial accelerometer calculates the sum of the rectified and integration acceleration curves from the anteroposterior, mediolateral and vertical directions of the trunk. Research demonstrates that the Tracmor$_D$ is a highly accurate instrument for predicting free-living energy expenditure (81).

Figure 8. DirectLife activity monitor or accelerometer Tracmor$_D$.

1.5.2 Body composition

Body composition is a sum of five compartments: water, fat, protein, minerals and glycogen. Understanding the fat content of the body has medical importance since the amount of fat may influence morbidity and mortality (82). Knowledge about, it is important, especially since the prevalence of overweight and obesity in children and adolescents is rapidly increasing (83). For those affected, the extra weight consists not only of fat but also of fat free mass. Since it is impossible to measure these compartments separately, Siri divided the body partitions into two compartments: fat mass (FM) and fat free mass (FFM) (82). There are many techniques to measure the FM and FFM, but the accuracy and reliability of these techniques are critical. Reference techniques such as the measurement of total body water by deuterium dilution is a good option and is considered by several investigators as the preferred means for the assessment of body composition in both adults and children (84,85).

1.5.3 Effects of growth hormone on body composition and energy expenditure

Besides its growth-promoting effect, GH also influences metabolism (86). Its increasing nitrogen retention within days of starting treatment reflects the increased protein synthesis and fat free mass, which in turn is associated with increased energy expenditure (87,88). Therefore, GH improves exercise capacity, body composition, and psychological well-being (89-91).
Treating GH deficient adults with rhGH has been found to cause a shift in body composition towards an increase in fat free mass and an increase in basal metabolic rate (92). After the introduction of rhGH therapy in GH deficient children, the same effect was visible (87). Gregory et al. showed a significant increase in BMR and TEE in children receiving rhGH treatment. The Maastricht study by Hoos et al. demonstrated that the increase in fat-free mass (measured by deuterium dilution) after six weeks of rhGH treatment was strongly correlated with growth after one year of treatment (93). Gerver et al. showed that discontinuation of growth hormone treatment had the opposite effect (94). Meta-analysis in Prader-Willi syndrome patients showed that rhGH treatment improved body composition, with an increase in lean body mass and a reduction in total body fat and subcutaneous and visceral fat (95). These changes in body composition have also been reported during rhGH treatment in other conditions such as Turner syndrome, short stature and Noonan syndrome (96-98).

1.6 Cardio-vascular risk in growth hormone deficiency

Growth hormone deficiency in adults is associated with an increase of adiposity, adverse serum lipid profiles, vascular endothelial dysfunction, and reduced exercise capacity (99-101). Lethargy, low mineral bone density, and excess mortality, partly the result of increased cardiovascular deaths, have also been reported (88,102,103). Investigations showed that GH induce metabolic effects: increased protein synthesis, mobilization of fatty acids and decreased peripheral glucose utilization. The results of placebo-controlled trials with rhGH treatment on cardiovascular risk factors were beneficial for lean and fat body mass, total and HDL cholesterol levels and diastolic blood pressure, but negative on plasma glucose and insulin levels (104). It is known that GH is an anabolic hormone and has lipolytic effects, increasing the mobilization of triacylglycerol's from adipose tissue (105,106). GH inhibits lipoprotein lipase in adipose tissue, reduces de novo lipogenesis in the liver, and increases overall fat oxidation rates, which may contribute to its positive effects on lipids (107).

Adipose tissue is a key organ for the regulation of energy metabolism. It contains adipocytes, macrophages and endothelial cells, the three major cell types in adipose tissue, which all are immunologically active. Macrophages secrete many cytokines and chemokines and are promoted in obesity (108,109). Human adipocytes can produce pro-inflammatory (IL-1, IL-6, and TNF-alfa) and anti-inflammatory cytokines (IL-4, IL-10 and IL-13) as well as a broad panel of chemokines (IL-8, MIP-1, MCP-1). In addition, endothelial cells have been shown to produce a whole range of cytokines such as TNF-a and chemokines like IL-8. At early ages, rhGH therapy appears to exert beneficial effects on these cardio-vascular parameters (110-113).

1.7 Hypotonia and hypermobility in Kabuki syndrome

Children with Kabuki Syndrome have a delayed mental and motor development. Early hypotonia is very common in KS patients, which improves with time and physiotherapy. Many KS children have hypotonia in combination with feeding problems during neonatal period of which the severity is variable. A prevalence of 70-90% is reported in literature. Joint laxity has been defined in about 75% of the KS patients, although the prevalence in a review article by Bogershausen et al. was wide spread by 6-97% (47,114). Hypermobility is a commonly present problem in children with genetic syndromes. However, this literature reports

on generalized hypermobility. There is no information about the location and treatment of this joint laxity.
Hypermobility can cause joint dislocations. Congenital hip dislocation was already reported in 1981 in 33% of the KS patients (1,2). Though, they did not report about joint laxity. Since in KS patients' scoliosis, pectus excavatum and mitral valve prolapse has been identified, an underling connective tissue disorder has been proposed. But, biopsy performed in some cases failed to detect any significant abnormalities (6). Despite the high incidence of joint hypermobility, this has never formerly been assessed within a KS population.

1.8 Aims of the study and outline of the thesis

1.8.1 Aims of the study

The study was initiated to contribute to the knowledge of the physiology of growth patterns, growth hormone secretion, growth hormone treatment and the metabolic effects of rhGH treatment in children with Kabuki syndrome.

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

- Growth pattern in KS in general
- The characteristics of body proportions in children with KS:
  - How are the body proportions in Kabuki syndrome children?
  - Are the body proportions in children with Kabuki syndrome different compared to the normal population?
- Catch-up growth during rhGH treatment in KS
- Metabolic effects of rhGH treatment in children with KS:
  - Increase in total energy expenditure during 6 weeks of treatment with rhGH in children with Kabuki Syndrome
  - The relation between the short-term (6 weeks) change in TEE and the long-term change in height SDS during treatment with rhGH after one years
  - The effect of rhGH treatment on metabolic risk parameters typical for the metabolic syndrome in adults
  - To assess the long-term (at start and after one year of treatment) term safety of growth hormone therapy on metabolic risk parameters and body composition

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

- The characteristics of hypermobility in children and adults with Kabuki syndrome:
  - The prevalence of hypermobility
  - Which limbs / joints are affected by hypermobility, with or without (sub) luxation's
  - Does GH treatment lead to a diminished degree of hypermobility and, because of that, to less (sub)luxations?

1.8.2 Outlines of the thesis

After the introduction in chapter 1, the growth pattern of 39 individuals with Kabuki syndrome are described in detail in chapter 2. In chapter 3 the body proportions were investigated using photogrammetric anthropometry in 11 KS patients compared with 21 control subjects. Because of the unknown cause of short stature in KS patients, the GH secretion was explored by GH stimulation tests in 18 prepubertal KS children and described in chapter 4. Since short stature is an important feature in KS, results of one-year rhGH treatment in 18 KS children are presented in chapter 5, and the metabolic effects of the treatment are described in chapter 6 by means of energy expenditure and body composition. In chapter 7 the effects of GH treatment were evaluated on cardiovascular parameters in terms of safety and prevention of the metabolic syndrome. While, KS subjects are prone to joint laxity and luxation, we investigate the frequency, location and results of rhGH treatment on these hypermobility in chapter 8. In chapter 9 we discuss the significant findings presented in this thesis, make our conclusions and suggestions for future research. The social and clinical relevance is debated in chapter 10. In the last chapters 11-12 we summarize the findings of the studies in this thesis in English and Dutch.

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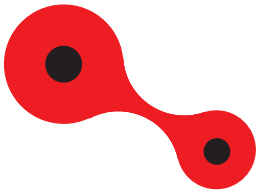

GROWTH PATTERN IN KABUKI SYNDROME WITH A KMT2D MUTATION

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ABSTRACT

Kabuki syndrome is a multiple congenital malformation syndrome with a spectrum of clinical features including short stature. Since there is no growth data on Kabuki syndrome patients with a proven *KMT2D* gene mutation, further research on growth and growth patterns is indicated. Data for this growth study on subjects with Kabuki syndrome were collected from referring clinicians. Subjects were eligible for inclusion in the study if the following criteria were met: a genetically confirmed diagnosis of Kabuki syndrome and no current treatment with growth hormones or other drugs that could influence growth. We present a report on growth data (n=39) in Kabuki syndrome patients. The data showed that postnatal growth retardation is a clinical feature in all cases. All Kabuki syndrome subjects showed a growth deflection during childhood and a diminution of the pubertal growth spurt. A genotype-phenotype correlation was not observed. Further research is required in order to determine whether a defect in the growth hormone/IGF-I axis and estrogen receptor plays a role in the growth retardation.

INTRODUCTION

Kabuki syndrome (KS) is a multiple congenital malformation syndrome that was independently described by Niikawa (1) and Kuroki (2) in 1981. The estimated incidence of KS is 1 in 32,000 in the Japanese population (1). Clinical features of KS include developmental delay or learning disability, hypotonia, postnatal growth retardation, abnormal dermatoglyphics, presence of fetal fingertip pads and facial dysmorphology characterized by long palpebral fissures with eversion of the lateral portion of the lower eyelid, broad, arched eyebrows with lateral sparseness, short columella with depressed nasal tip and large, prominent or cupped ears (3,4). In 2010, Ng et al. performed whole exome sequencing in ten cases clinically diagnosed with KS and discovered that it is caused by mutations in the *KMT2D* gene (also known as the *MLL2* gene) (5-7). *KMT2D* mutations have been identified in approximately 70% of the clinically diagnosed individuals (8). One of the key features in KS patients is growth deficiency. Niikawa et al. (9) were the first to describe the natural growth of male subjects and showed that birth weight and length are generally within the normal range, while in the first year of life many patients showed postnatal growth retardation. Furthermore, syndrome-specific growth charts are useful for a variety of reasons. They are needed to understand the pathogenesis of the underlying growth disorder, for monitoring health status and in the evaluation of growth-promoting therapies.
Lacking reference growth curves for the western population of KS individuals with a proven *KMT2D* mutation, we performed a retrospective data analysis among Dutch and Belgian KS subjects. As our second aim, we studied the growth pattern in order to provide insights into the mechanisms involved in this growth retardation.

MATERIALS AND METHODS

Data for this growth study in individuals with KS were collected in close collaboration with the Dutch Kabuki Syndrome Network and referring clinicians from The Netherlands and Belgium. Subjects were eligible for inclusion in the study if the following criteria were met: a genetically confirmed (*KMT2D* mutation), diagnosis of KS and no current treatment with growth hormones or other drugs that could influence growth. In the Netherlands, 56 subjects met the inclusion criteria for this study and were sent a consent form and questionnaire. Subjects from Belgium were asked to participate in the study by their referring specialist.

The project was approved by the Medical Ethics Committee of the Maastricht University Medical Centre. Written permission was obtained from the subjects or their parents/legal guardians to retrieve growth data from the infant welfare centre and paediatricians.

The parents/legal guardians of the subjects completed the questionnaire, documented information on pregnancy and labor, growth, feeding and nutrition during the first year of life. They also provided the growth data (height, weight and head circumference) from the "growth booklet", which were collected by the infant- and child-welfare centres (*Kind en Gezin*, i.e,Child and Family) or--if available–by the welfare centres themselves. These centres offer medical examinations throughout childhood and adolescence, including anthropometric measurements. Additionally, when possible, growth data from the treating paediatrician were retrieved. Only growth data collected by professionals were used. The study population comprised both full-term and infants born at a gestational age of 25-40 weeks.

The growth data from the research population were compared to reference values reported by Gerver and de Bruin. The measurements values were expressed as standard deviation scores (SDS) or z-scores described by Gerver and Bruin (10). The values were corrected for gestational age if the gestational age was different from the full term, 40 weeks. The last measured value for height in patients aged 18 years and older was considered the final height.

To assess a possible correlation between the type of *KMT2D* mutations (genotype) and the degree of growth retardation (phenotype), the protein-truncating *KMT2D* mutations were sorted according to the position of the premature protein translation termination codon within the gene together with

the corresponding height SDS values for each patient. The two patients with a missense mutation and six patients with an RNA-splice mutation were excluded from this analysis for consistency, as missense and RNA-splice mutations might have a different or more indirect effect, respectively, on protein function compared to nonsense and frame-shift mutations.
Data were expressed as mean ± standard deviation. The Pearson correlation was used to measure the strength of association between the variables. All statistical analyses were performed using the software SPSS version 22 for Mac (SPSS Inc, Chicago, IL, USA) and Excel version 14.6.2 for Mac (Microsoft, Redmond, WA, USA). A P-value of <0.05 was considered significant.

RESULTS

Table I lists all baseline characteristics of the 39 subjects (32 from the Netherlands and 7 from Belgium). There were 15 males and 24 females (median age 9.48 years, range 3.2 to 58.55 years) who returned the questionnaire. Data on pregnancy and labor revealed one incidence of polyhydramnios, mild vaginal bleedings in three cases and gestational diabetes in one case. Two infants had a 2-vessel umbilical cord. The mean gestational age was the same for boys and girls, namely $37^{5/7}$ (range $32^{3/7}$ - 41) weeks. Twenty-eight percent were born premature and one child met the definition of born small relative to gestational age for weight. Thirty-two percent of the subjects had tube feeding in the first year of life. Of all subjects, 23% had a cleft palate and 36% were born with a heart defect.

Table I. Data on pregnancy and labor, and other factors that could influence growth during first year of life.

	Male (*n=15*)	Female (*n=24*)	Total (*n=39*)
Pregnancy			
Premature birth	4 (27%)	7 (29%)	11 (28%)
2-vessel umbilicus	-	2 (8%)	2 (5%)
Vaginal bleeding	2 (13%)	1 (4%)	3 (8%)
Gestational diabetes	-	1 (4%)	1 (3%)
Polyhydramnion	1 (7%)	-	1 (3%)
Smoking	2 (13%)	3 (13%)	5 (13%)
Labor			
Caesarean	1 (7%)	1 (4%)	2 (5%)
Vacuum pump	1 (7%)	1 (4%)	3 (8%)
Initiating	1 (7%)	1 (4%)	2 (5%)
Meconium amniotic	1 (7%)	2 (8%)	3 (8%)
Small for Gestational Age			
for length	-	-	-
for weight	1 (7%)	-	1 (3%)
Tube feeding	10 (67%)	21 (88%)	32 (82%)
Congenital heart defect	6 (40%)	8 (33%)	14 (36%)
Ventricle septum defect	6 (40%)	7 (29%)	13 (33%)
Atrial septum defect	3 (20%)	2 (8%)	5 (13%)
Coarctatio aortae	3 (20%)	1 (4%)	4 (10%)
Aorta stenosis	1 (7%)	-	1 (3%)
Cleft palate	3 (20%)	6 (21%)	9 (23%)

Anthropometric measurements from the 39 subjects with KS were retrospectively collected. Table II shows the characteristics for weight, height and head circumference at different ages. Since the number of subjects varies by gender and age, the numbers are displayed by category in parentheses. Fifty-eight percent of the infant welfare centres and 72% of the paediatricians provided the growth data we requested. The missing data were obtained from the parents/legal guardians by means of the "growth booklet". Only data measured by experts were used. All data regarding gestational age, birth weight and birth length were compared to the Dutch reference data.

The mean birth weight SDS for the whole group in boys was 0.34 SDS (2941 g; n=14) and in girls 0.65 SDS (2931 g; n=19); and for length in boys -0.14 SDS (48.96 cm; n=12) and in girls 0.11 SDS (47.95 cm; n=19).

Weight and height at one year of age were available in 36 individuals. Mean weight SDS in boys was -1.96 SDS (8.2 kg; n=14) and -1.93 SDS (7.4 kg; n=22) in girls at one year of age. The overall mean height SDS in boys was -2.28 SDS (72.2 cm; n=15) and -1.86 SDS (70.4 cm; n=19) in girls.

The mean adult height (age 18 and above) in females was 147.7 cm (SDS -3.86; n=5) and in males 162.0 cm (SDS -2.16; n=5).

Table II. Anthropometric characteristics for weight, height and head circumference at different age groups.

Anthropometry at birth		
Birth Weight	**Boys (n=14)**	**Girls (n=19)**
Mean birth weight in grams	2941	2931
Mean birth weight in SDS (range)	0.34 (-2.29 – 3.86)	0.65 (-0.51 – 1.47)
Birth Length	**Boys (n=12)**	**Girls (n=19)**
Mean birth length in cm	48.96	47.95
Mean birth length in SDS (range)	-0.14 (-1.74 – 1.42)	0.11 (-1.86 – 1.50)
Birth Head Circumference	**Boys (n=4)**	**Girls (n=8)**
Mean birth HC in cm	33.70	33.20
Mean birth HC in SDS (range)	-0.36 (-0.58 – 0.21)	-0.27 (-1.55 – 1.26)
Anthropometry at age one year		
Weight	**Boys (n=14)**	**Girls (n=22)**
Mean weight in gram	8235	7427
Mean weight in SDS (range)	-1.96 (-3.77 – 0.53)	-1.93 (-3.04 - -0.42)
Mean weight for height in SDS (range)	-0.71 (-2.20 – 1.49)	-1.25 (-2.59 - -0.34)
Height	**Boys (n=15)**	**Girls (n=19)**
Mean height in cm	72.20	70.4
Mean height in SDS (range)	-2.28 (-5.63 - -0.32)	-1.89 (-4.07 – 0.45)
Head Circumference	**Boys (n=9)**	**Girls (n=11)**
Mean HC in cm	44.89	42.4
Mean HC in SDS (range)	-2.08 (-4.26 - -0.04)	-3.64 (-4.98- -1.44)
Mean HC SDS for height SDS	-0.91 (-2.83 – 0.39)	-1.72 (-3.56 - -0.23)
Anthropometry at age > 18 years		
Weight	**Boys (n=4)**	**Girls (n=5)**
Mean weight in kg	45.29	61.11
Mean weight in SDS (range)	-2.09 (-1.77 – 0.92)	0.27 (-1.13 – 2.22)
Mean weight for height in SDS (range)	2.13 (-0.59 – 6.70)	5.52 (2.03 – 8.28)
Height	**Boys (n=5)**	**Girls (n=5)**
Mean height in cm	162.0	147.66
Mean height in SDS (range)	-2.16 (-2.99 - -1.08)	-3.86 (-5.57 - -1.47)

Figures I to IV show the height SDS of all subjects and the plotted anthropometric data for male and female subjects with KS compared to the Dutch growth charts. Seventy-five percent of the 12 subjects for whom a head circumference (HC) was accessible, had a HC below the 0 SDS at birth. The mean HC at birth for boys was -0.36 SDS (33.70 cm; n=4) and for girls was -0.27 SDS (33.2 cm; n=8). At about one year of age, all 20 subjects for whom a HC was accessible had a HC below the 0 SDS. Mean HC in boys was -2.08 SDS (44.89 cm; n=9) and in girls -3.64 SDS (42.4 cm; n=11).

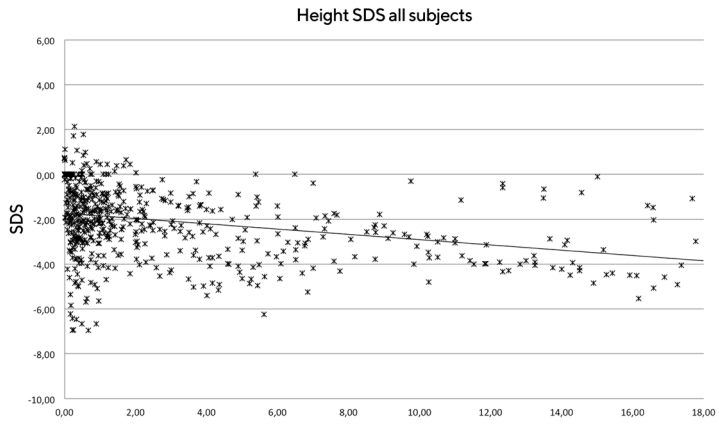

Figure I. Distribution of height in all subjects with Kabuki syndrome.

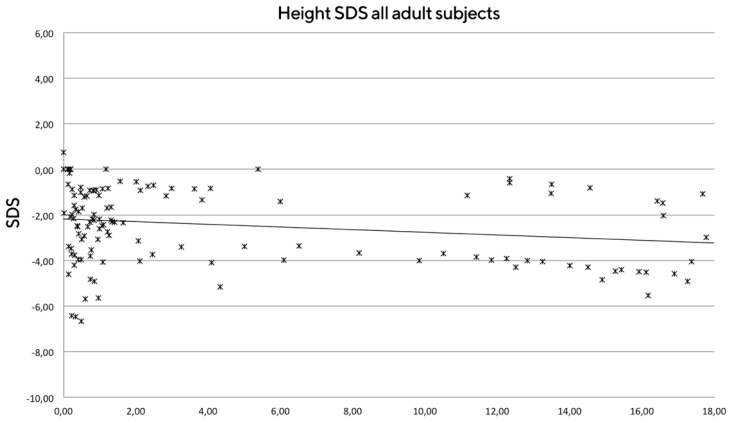

Figure II. Distribution of height in all adult subjects with Kabuki syndrome.

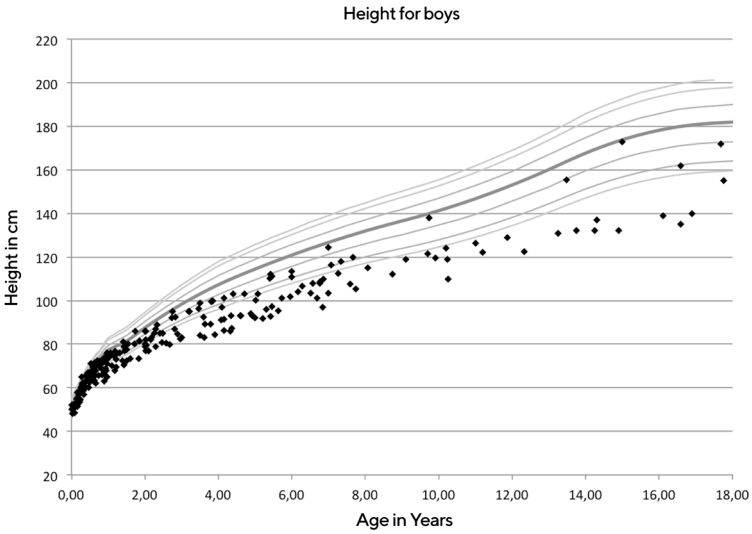

Figure III. Distribution of height in boys with Kabuki syndrome.

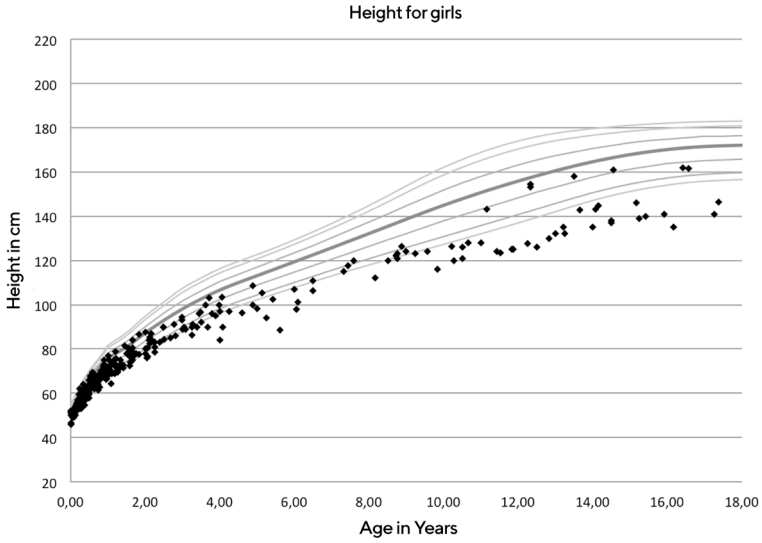

Figure IV. Distribution of height in females with Kabuki syndrome.

Table III shows the entire *KMT2D* gene mutations found in the 39 KS patients. None of the KS patients had a mosaic *KMT2D* mutation. There were two patients with a missense mutation and six patients had a RNA-splice mutation. A genotype-phenotype correlation regarding neither height nor weight was observed.

Table III. Genotype-Phenotype correlation of all the Kabuki syndrome patients.

Case no.	Sex	Age(y)	Mutation	Amino acid change	Height SDS	Weight SDS	BMI SDS
Frameshift mutation							
1	Male	0,0	c.2110delG	p.Asp704Thrfs*226	-0,54	2,59	
2	Male	2,2	c.2129delC	p.Pro710Hisfs*220	-2,21	-0,78	1,06
3	Male	14,3	c.2272delG	p.Glu758Serfs*172	-3,93	-3,4	-2,3
4	Male	2,7	c.3307_3310del	p.Cys1103Profs*15	-4,53	-1,66	2,12
5	Male	0,3	c.7471delG	p.Ala2491Leufs*52		-4,21	
6	Male	14,2	c.12515delC	p.Pro4172Leufs*43	-4,49	-2,78	0,07
7	Male	6,9	c.14778delC	p.Ser4927Valfs*68	-2,84	-2,9	-1,57
8	Female	15,2	c.1301delT	p.Leu434Glnfs*496	-3,36	-1,07	1,24
9	Female	8,8	c.2558_2559delCT	p.Pro853Argfs*3	-2,58	1,46	5,43
10	Female	6,1	c.3889delC	p.Arg1297Profs*15	-4,65	-2,85	-0,29
11	Female	17,3	c.4219_4222delTACT	p.Tyr1407Valfs*9	-4,9	-0,81	2,72
12	Female	9,8	c.5912delG	p.Ser1971Thrfs*76	-1,23	1,82	2,71
13	Female	5,1	c.9223dupT	p.Ser3075Phefs*3	-1,93	-0,97	0,38
14	Female	16,6	c.9329delG	p.Arg3110Profs*9	-1,5	2,22	3,77
15	Female	3,8	c.9770dupA	p.Lys3258Glufs*43	-2,16	-0,34	2,15
16	Female	16,2	c.12164_12165delCT	p.Pro4055Argfs*6	-5,53	-1,54	2,34
17	Female	4,0	c.12969dupA	p.Pro4324Thrfs*10	-1,56	-0,074	0,77
18	Female	1,6	c.13473_13476delTGAC	p.Asp4492Alafs*26	-2,46	-2,28	-0,08
Nonsense mutation							
19	Male	17,7	c.3904C>T	p.Gln1302*	-1,37	-1,27	-0,59
20	Male	6,9	c.8200C>T	p.Arg2734*	-5,49	-3,44	1,07
21	Male	16,6	c.11707C>T	p.Gln3903*	-2,02	-0,37	1,95
22	Male	15,0	c.11722C>T	p.Gln3908*	-0,09	-0,54	-0,53
23	Male	51,0	c.16360C>T	p.Arg5454*	-2,56	0,42	5,5
24	Female	2,4	c.6247C>T	p.Gln2083*	-2,25	-2,17	0,12
25	Female	19,8	c.7933C>T	p.Arg2645*	-3,3	-1,13	0,46

Case no.	Sex	Age(y)	Mutation	Amino acid change	Height SDS	Weight SDS	BMI SDS
26	Female	2,8	c.8311C>T	p.Arg2771*	-2,53	-2,83	-0,61
27	Female	3,7	c.8626C>T	p.Gln2876*	-0,32	-0,42	0,01
28	Female	5,6	c.11566C>T	p.Gln3856*	-6,24	-2,29	3,36
29	Female	7,3	c.12844C>T	p.Arg4282*	-2,43	0,12	2,68
30	Female	1,1	c.14798T>A	p.Leu4933*	-2,63	-2,82	-1,06
31	Female	13,7	c.15934G>T	p.Glu5312*	-2,87	-1,39	0,46
Splice-site mutation							
32	Male	4,4	UV, de novo. C.4871C>T	r.4870_4963del67; p.Ala1624Val	-1,57	0,65	3,83
33	Male	17,8	c.13999+5G>A	r.?	-3	-1,77	-0,09
34	Female	4,6	c.14076-2A>C	r.?	-2,89	-1,81	0,53
35	Female	14,5	c.14516-1G>C	r.14515_14522del8; p.Gly4840Lysfs*13	-4,15	-2,5	-0,47
36	Female	17,4	c.14644-2A>G	r.?	-4,04	1,45	5,44
37	Female	2,3	c.15921+1G>A	r.?	-2,45	-2,33	0,07
Missense mutation							
38	Male	5,5	c.4209C>G	p.Cys1403Trp	-1,23	-1,43	-0,54
39	Female	8,9	UV, de novo: c.16294C>T	p.Arg5432Trp	-1,65	-2,52	-2,05

DISCUSSION

There is a scarcity of literature reporting detailed anthropometric data relating to the physical growth of children with KS. In our study, we were able to collect growth data on 39 individuals. Birth weight, head circumference and length of the individuals in our cohort were found to be within the normal range. Mean height around the age of 12 months or beyond was below normal. It is noteworthy that a growth spurt according to puberty is lacking in all subjects, as is visualised in figures III and IV. Unfortunately, we have no information about their pubertal stage. In 1981, Niikawa et al. and Kuroki et al. independently studied KS (1,2). Since that time little has been published about the growth patterns in this population. Six studies reported small stature, of which only Niikawa's study

provides information about growth from birth to adolescence (11-15). He reported short stature in 73% of all his subjects (n=41) with KS in the range from -2.1 SD to -5.0 SD, none exceeded the +0.5 SD in the age range from 3 months to 22 years. Bögershausen et al. published a review providing genotype-phenotype correlations (15). They showed a significantly higher frequency of short stature in individuals with a *KMT2D* mutation than in subjects without the mutation ($p<0.0001$). Since our cohort consists of individuals with only a *KMT2D* mutation, this probably explains our subjects' lower height outcome in comparison to the reported data.

Somatic growth and maturation are influenced by a number of factors such as nutrition, genetic constitution and endocrine functions. Essential to normal growth and development are adequate levels of growth hormone (GH) and steroid hormones. During puberty the interaction of gonadal and adrenal steroid hormones with GH is essential for the normal adolescent growth spurt and sexual maturation (16). Patients with a selective deficiency of either hormone experience an attenuated growth spurt, as seen in our study of individuals with KS. Gonadal steroid hormones, primarily estradiol in both genders, increase bone mineral maturation and affect adult height by promoting epiphyseal fusion through direct effects on the growth plate (17-19). *KMT2D* is required for ligand-dependent estrogen receptor-α transactivation, one of the mediators of the biological effects of estrogen in estrogen responsive tissues. A study of estrogen receptor-α knockout mice demonstrated decreased longitudinal as well as radial skeletal growth associated with decreased serum levels of IGF-I. This might provide a potential biological mechanism for the cause of postnatal growth retardation and a diminished or absent pubertal spurt in KS. During puberty the GH/IGF-I axis undergoes a remarkable activation. Moreover, growth during childhood depends primarily on the GH/IGF-I axis and thyroid hormones. An effect of estrogen on the GH/IGF-I axis is supported by several clinical and experimental studies. The mechanism could occur through a direct action of testosterone on androgen receptors, or indirectly through the action of estrogens on the estrogen receptor-α as noted in a previous study showing a significant correlation between circulating levels of estrogen and GH secretion in men (17-21). These results suggest that testosterone stimulates GH secretion and action in adulthood, as in puberty, and lead to the proposition that this effect is dependent on the amount of aromatization of testosterone in estrogen. Since almost all KS individuals have a growth deflection during childhood and a

growth spurt deficiency, a defect in the GH/IGF-I axis might be a cause for the short stature. Whether there is a relation with the estrogen receptor-α due to a mutation in the *KMT2D* gene remains to be seen. Data on the hormonal status during adolescence in KS subjects is very scarce in the recent literature. Further research is required.

A possible correlation between the position of the *KMT2D* premature termination codon caused by the mutation and height SDS was assessed, but a significant difference could not be observed for the KS patients. The conclusions of this analysis are however limited by the fact that the different protein truncating mutations could be subject to different levels of nonsense-mediated RNA decay (NMD) *in vivo* (22). The dynamics of the NMD mechanism determines the residual level of truncated protein in cells. This level can however not be experimentally determined since neither RNA, nor protein samples from relevant tissues are available for our patients.

In the literature, five cases of growth hormone treatment in children with KS with (partial) growth hormone deficiency have been reported (23-26). Growth hormone treatment was shown to be beneficial in these children. In the KIGS (Pfizer International Growth Database) data, 21 children (5 girls and 16 boys) with KS are treated with growth hormone therapy. In 12 children, with a median age of 5.27 years, height SDS increased from -3.41 SDS (median) to -2.58 SDS after one year of treatment. All the reports of short stature and growth hormone treatment presented above were dated before 2010, when it was discovered that the majority of the cases are caused by mutations in the *KMT2D* gene (5). Therefore, the diagnosis of KS made in these subjects is based on clinical observations only and has not been confirmed genetically. Although it appears that rhGH treatment has a positive effect on linear growth acceleration, the main cause of growth retardation in KS children is still unknown.

CONCLUSION

The present study has some limitations. The small population of children with KS meeting entry criteria prevents the gathering of sufficient numbers to create a valid growth chart. Even so, we made a noteworthy insight into the longitudinal data. The number of girls enrolled was higher than boys; however, this sex differential could be a statistical aberration owing to the small sample size. Nevertheless, we are confident that the data provides more insight into growth patterns and trends for KS children in northern Europe.

The present study is the first report on growth data in a group of individuals with a genetically confirmed diagnosis of KS in northern Europe. Given the growth problems in KS, a defect in the GH/IGF-I axis is feasible.

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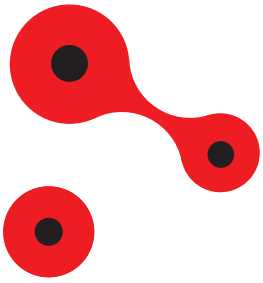

BODY PROPORTIONS IN CHILDERN WITH KABUKI SYNDROME

American Journal of Medical Genetics, Part A 2016;170A:610-614.

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ABSTRACT

Facial characteristics, short stature, and skeletal anomalies have been described for the clinical diagnosis of Kabuki Syndrome (KS) in children. However, no studies have investigated body proportions in KS. Knowledge of body proportions in KS may contribute to better insight into the growth pattern and characterization of this genetic disorder. Therefore, we compared body proportions of children with KS to normally proportioned controls to investigate if atypical body proportions are part of this genetic disorder. This study was designed and conducted within the setting of the Maastricht University Medical Centre (MUMC+), the official Dutch expert center for Kabuki syndrome. We conducted a cross-sectional study in 32 children (11 children with KS and 21 controls). Body proportions were determined by means of photogrammetric anthropometry, measurements based on digital photography. Body proportions, quantified as body ratios, differ significantly in children with KS from normally proportioned children. Children with KS have larger heads and longer arms proportional to their trunks and have been found to have longer upper arms proportional to their tibia length and feet. Based on deviations in body proportions it was shown possible to discern children with KS from normally proportioned controls.

INTRODUCTION

Children with Kabuki syndrome (KS) are characterized by a distinct facial appearance, mild to moderate intellectual disability, postnatal growth retardation, skeletal anomalies, and unusual dermatoglyphic patterns (1). Two genes have shown to be mutated in patients with KS, 55–80% present mutations in the *MLL2/KMT2D* gene and in 9–14% the *KDM6A* gene is mutated (2-4). The facial characteristics, which are used in the clinical diagnosis of KS, have been described extensively in recent literature, several studies also describe the presence of short stature and skeletal anomalies in KS (1,5-9). However, no studies have investigated body proportions in these children. Knowledge of body proportions in KS may contribute to better insight into the growth pattern and characterization of this genetic disorder. Therefore, we performed a cross-sectional study to compare body proportions of children with KS to normally proportioned controls.

METHODS

This study was designed and conducted within the setting of the Maastricht University Medical Centre (MUMC+), the official Dutch expert center for Kabuki syndrome. We work in close collaboration with the Dutch Kabuki Network.
Study Groups
In total, 11 children, between 3 and 16 years old, visiting the expert center for Kabuki syndrome in the Maastricht University Medical Centre were included in this study. KS was confirmed in all participating children based on the presence of gene mutations in *MLL2/KMT2D*. No mutations in *KDM6A* were found. For the control group, 21 children between 2 and 14 years old, visiting the outpatient clinic of Endocrinology and Growth of the Maastricht University Medical Centre were included. These children were all growth hormone deficient and received adequate growth hormone therapy. These children were all proportioned normally according to the Dutch reference values (10). Before inclusion, all patients and/or their parents had given informed consent.

Measurements
In a previous study, we developed a new method for taking anthropometric measurements, using photogrammetric anthropometry. It was shown to be a fast,

easy to use, validated method to take elaborate anthropometric measurements of the whole body, especially body proportions (11). Collected data used in this cross- sectional study are digital photographs taken of the children in underwear. Photographs are taken in a frontal and lateral position and measurements on these photographs are performed using the photometry software Paediatric Morphometrics designed by our research group. Digital photographs were taken conform the photogrammetric method described previously (11). Body proportions were determined according to the measurements of various anthropometric distances by selecting anatomical reference points in the photographs. These are the same reference points used in manual measurements (10). The measurements included: height (H), biacromial width (Biac), biiliacum width (Biil), upper arm length (UA), lower arm length (LA), hand length (HA), tibia length (Tibl), and foot length (FO). Additionally, head length (Hl) was determined on the photograph as the height difference between the top of the head and the chin. Trunk length (Trl) was determined as the height difference between biacromial width and biiliacum width. Arm length (ARM) was determined as the summation of upper arm length, lower arm length, and hand length.

Statistical Analysis

All data were exported to IBM SPSS Statistics for Windows version 20.0 for statistical analysis. Shapiro–Wilk tests were performed for all measurements to test for normality. Comparison of different groups was done using independent student t-test, Mann Whitney U test or Fisher's exact as appropriate.

RESULTS

Characteristics of the Study Participants

A total of 32 children were enrolled in this study (11 children and 21 controls). Both groups were comparable regarding age and gender. Significant differences between groups were seen in several proportional measurements. All characteristics are presented in Table I.

Table 1. Characteristics of the study participants.

	Children with KS	Controls	p-value
N	11	21	
Gender m / v (%)	58 / 42	76 / 24	0.433
Age	8.3 ± 3.4	10.2 ± 3.3	0.138
Age range	3.7 - 16.0	2.6 - 14.8	
Hl/H	0.20 ± 0.03	0.17 ± 0.02	<0.001
Hl/Trl	0.82 ± 0.07	0.55 ± 0.06	<0.001
Biac/Biil	1.25 ± 0.12	1.22 ± 0.07	0.363
Biac/H	0.22 [0.20-0.23]	0.23 [0.22-0.23]	0.457
Biil/H	0.17 [0.16-0.20]	0.18 [0.18-0.19]	0.123
Trl/H	0.25 [0.23-0.26]	0.30 [0.29-0.32]	<0.001
ARM/H	0.43 [0.38-0.45]	0.42 [0.41-0.43]	0.289
ARM/Trl	1.76 [1.68-1.88]	1.35 [1.31-1.47]	<0.001
UA/LA	1.24 ± 0.16	1.21 ± 0.07	0.565
UA/Tibl	0.97 ± 0.06	0.76 ± 0.05	<0.001
UA/FO	1.30 ± 0.09	1.09 ± 0.09	<0.001
Tibl/H	0.19 ± 0.02	0.23 ± 0.01	<0.001
Tibl/Trl	0.80 [0.77-0.84]	0.77 [0.70-0.79]	0.051
FO/Tibl	0.75 ± 0.07	0.70 ± 0.05	0.027

The eight ratios Hl/H, Trl/H, Hl/Trl, ARM/Trl, UA/Tibl, UA/FO, Tibl/H, and FO/Tibl show significant differences between children with KS and normally proportioned children. When presented for age, the ratios Hl/Trl, ARM/Trl, UA/Tibl and UA/FO show distinct distribution differences. These distributions are presented in Figures 1–4 where the black dots represent the control group and the white dots represent the KS group.

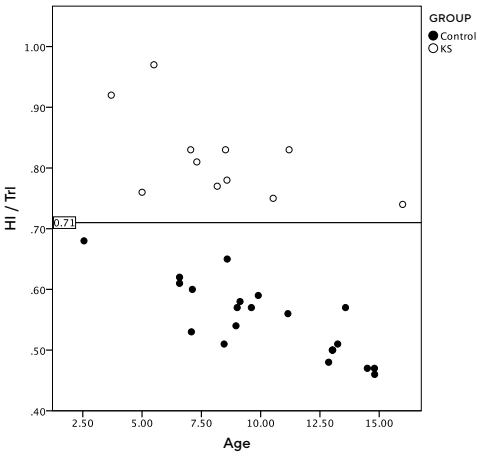

Figure 1. HI/TrI distribution for age; HI, head length; TrI, trunk length.

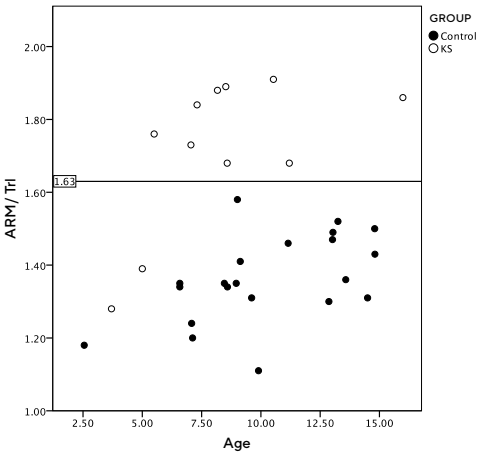

Figure 2. ARM/TrI distribution for age ARM, arm length; TrI trunk length.

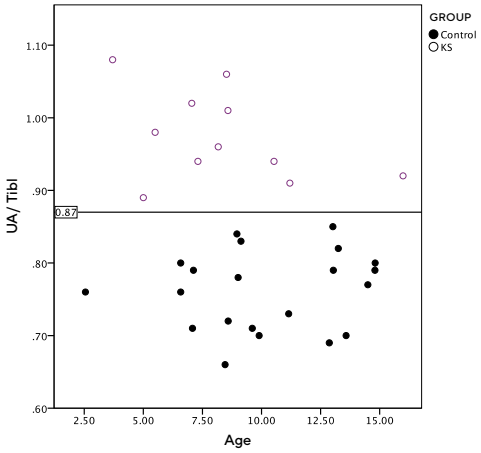

Figure 3. UA/TibI distribution for age UA, upper arm; TibI, tibia length.

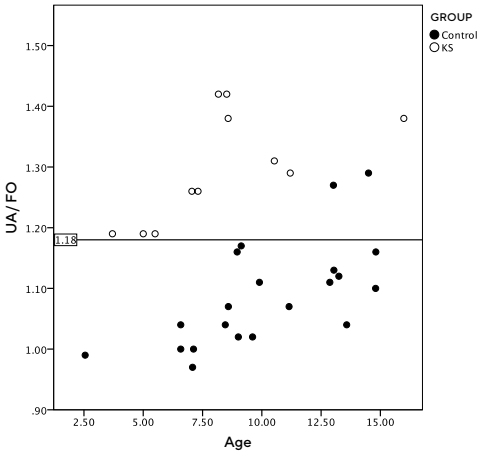

Figure 4. UA/FO distribution for age UA, upper arm; FO, foot.

DISCUSSION

To our best knowledge, this is the first report on body proportions in children with Kabuki syndrome. We used photogrammetric anthropometry to estimate the body proportions of these children and compared the results to a reference group of normally proportioned children. We also used this method to determine if atypical body proportions are a characteristic feature in children with KS.
The ratios Hl/Trl, ARM/Trl, UA/Tibl, and UA/FO for age in children with KS clearly differentiate from the reference group. These ratios show that children with KS have larger heads and longer arms proportional to their trunks compared to normally proportioned children. Additionally, children with KS have been found to have longer upper arms proportional to their tibia length and feet. These differences can be quantified as proportional cut-off values which are independent of age. In all children with KS a Hl/Trl ratio >0.71 was found, opposed to the reference group which showed a Hl/Trl ratio <0.71. Similar results were found for ARM/Trl (>1.63 for KS), UA/Tibl (>0.87 for KS) and UA/FO (>1.18 for KS). Based on these deviations in body proportions it was shown possible to discern children with KS from normally proportioned controls. Analysis of these body proportions can be done accurately with photogrammetric anthropometry, which is a fast, low-cost, and easy to perform method.
In other conditions where short stature and skeletal anomalies are present, such as idiopathic short stature (ISS), SHOX gene (short stature homeobox-containing gene) defects, and Turner syndrome (TS), more research has been done on body proportions. In an extensive study of Malaquias et al. abnormal body proportions were observed in 88% of the children with SHOX-defects, 48% of the females with TS and 16% of children considered ISS (12). However, only Sitting height/Height ratio (SH/H) was investigated. In our study, it was not possible to accurately determine sitting height, neither manually, nor with the aid of photogrammetric anthropometry. Since children with KS often have difficulty with maintaining a certain pose for accurate manual measurement, sitting height was proven to be inaccurate. In a previous study, we investigated if sitting height could be determined on a photograph, however this lead to imperfect results (11). However, with the aid of photogrammetric anthropometry, we were able to examine other anthropometric ratios, that gave us an elaborate overview of the body proportions of these children.
It is known that in many skeletal dysplasia's, growth of the legs, and arms is often

more negatively affected than growth of the trunk (13). Interestingly, we found that in children with KS arm length was proportionately longer compared to trunk length. No significant difference was found between groups for tibia length proportional to trunk length.

When SHOX gene defects are present, shortening of the extremities is a main cause of short stature. In these children, the arm span and leg length is significantly reduced in comparison to the height (14,15). In children with KS we found no significant differences in arm length proportional to height compared to normally proportioned children, although tibia length was significantly shorter proportional to their height.

Body proportions in TS have between described in untreated girls with between 2–11 years of age. Since height is more affected in these children than other parts of the body, these girls have, on average, a relatively large trunk, large hands and feet, and relatively large biacromial width and biiliacum width proportional to their height (16). In the children with KS we found that they had, on average, a relatively small trunk compared to normally proportioned controls. Also, we found no significant differences in biacromial width and biiliacum width proportional to height between the children with KS and the reference group.

Apart from SH/H ratio, body proportions have not been elaborately described in children with idiopathic short stature. Therefore, comparison of body proportions to the children with KS in this study is not yet possible.

In future studies, we will compare body proportions in KS with other conditions in which short stature and skeletal anomalies are present. It will be interesting to determine if the proportional ratios presented in this study can also be used to differentiate various skeletal anomalies in children from each other.

CONCLUSION

To our best knowledge, this is the first report on body proportions in Kabuki syndrome. It is shown here that various body ratios in KS clearly deviate from normally proportioned children. The key differences in body proportions in KS are that these children have larger heads and longer arms proportional to their trunks and longer upper arms proportional to their tibia length and feet. Knowledge of these body proportions in KS is valuable, since it can strengthen the clinical diagnosis and provide the means to follow up on the body proportions during growth and development.

ACKNOWLEDGMENTS

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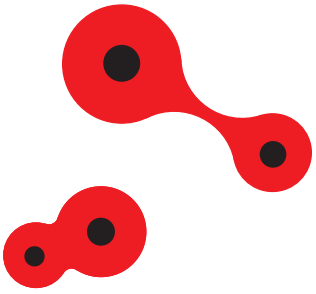

CHAPTER 4
GH TESTS

GROWTH HORMONE STIMULATION TESTS IN CHILDREN WITH KABUKI SYNDROME

Hormone Research Paediatrics, 2016;170(12):3172-3179.

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ABSTRACT

Kabuki syndrome is a multiple congenital malformation syndrome with a variety of clinical features including short stature. The cause of this postnatal short stature remains unknown.
Eighteen children with genetically proven Kabuki syndrome (8 boys and 10 girls; ages 3.3-9.9 with a mean of 6.7 years) who underwent GH stimulation test were evaluated in a prospective study. Two growth hormone stimulation tests were conducted, including IGF-I and IGFBP-3 serum levels. GH stimulation peak in relation to age, sex, height, BMI, IGF-I and IGFBP-3 SD scores were analyzed.
Five of the 18 children (27.8%) were biochemically GH deficient. This was not correlated with the BMI SD score. Of all patients, only one had an IGF-I below -2 SD and did not fulfil the GH deficiency criteria. Mean IGF-I was below normal (-0.8 SD). All subjects had normal IGFBP-3 levels.
The utility of performing GH stimulation tests on Kabuki syndrome children, as an indication of GH status in short stature is questionable. IGF-I levels did not correlate with the GH stimulation peak nor consequently the diagnosis GH deficiency.

INTRODUCTION

Kabuki syndrome (KS) (OMIM#147920) is a rare condition characterized by an intellectual disability, typical facial dysmorphology, short stature, and many possible congenital malformations such as heart defects or renal anomalies. Kabuki syndrome was first described by Niikawa and Kuroki independently of each other in 1981 (1,2). Causative dominant mutations in the *KMT2D* (formerly *MLL2*) and *KDM6A* genes have been identified in KS. They account for approximately 75% and 5% of the cases respectively (3,4).

Postnatal growth deficiency is a cardinal feature of the syndrome. Niikawa et al. were the first to describe the natural growth of male subjects, revealing that although birth weight and length are generally within the normal range, many patients showed postnatal growth retardation in their first year (5). The cause of growth impairment in KS remains poorly understood.

The prevalence in the general population of growth hormone deficiency (GHD) is estimated to be between 1:3,000 and 1:4,000, or one per cent in children who are below the third percentile for height. Some literature exists about the outcome of growth hormone stimulation tests in KS children diagnosed with GHD who received growth hormone treatment (6,7). However, no published studies examine growth hormone levels in general in Kabuki children or a possible cause of GHD.

In order to provide more insights into the role of growth hormone in the growth retardation of Kabuki patients, we determined the values of insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IFGBP-3) in relation to the outcome of growth hormone stimulation tests.

MATERIALS EN METHODS

Patients

In this prospective study, we were able to include 18 KS children (10 females and 8 males) with ages ranging from 3.3 to 9.9 years (mean 6.7 ± 2.2 years). Seventeen of the KS subjects had a *KMT2D* mutation and one had a *KDM6A* mutation. The height SDS ranged from -6.0 to 0.9 (mean -2.2 ± 1.7) and BMI SDS ranged from -2.0 to 4.4 (mean 0.7 ± 1.8). Subjects were eligible for inclusion in the study if the following criteria were met: a genetically confirmed (*KMT2D* or *KDM6A* mutation) diagnosis of KS and no current treatment with growth

hormones or other drugs that could influence growth. Furthermore, they had to be prepubertal with a chronological or bone age of less than 8 years for girls and 10 years for boys. Thus, the influence of puberty was ruled out. Also, all subjects needed normal results in standard laboratory tests, including the hormonal axis of the thyroid and adrenal gland. Almost all KS individuals underwent two stimulation tests: arginine and clonidine (ARG and CLO), according to the standard procedures after overnight fasting. Three subjects only had one test, two of which only CLO, and one ARG.
The project was approved by the regional Medical Ethics Committee and conducted according to the Declaration of Helsinki. Written permission was obtained from the parents.

Anthropometric measurements

Measurements were taken according to the internationally accepted methods described previously (8). Standing height was measured by a Harpenden stadiometer (Holtain Ltd., Crymych, Dyfed, UK) accurate to 0.1 cm. Body weight was measured using an electronic scale (Seca, model 701, Germany) accurate to 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. The growth data from the study population were compared to the reference values of the Dutch population (TNO The Netherlands 2010, with the 2001-2013 Growth Analyser BV, Rotterdam, The Netherlands).
Prepubertal status was established by the paediatric endocrinologist using the method of Tanner and Marshall (9) (10). In all patients, bone age (BA) was assessed by the same paediatric endocrinologist (DS) using the Greulich and Pyle atlas method (11).

Biochemical tests

All tests started in the early morning after an overnight fast. Most subjects underwent two growth hormone stimulation tests on two different days: CLO (Catapresan, Boehringer, Ingelheim, Germany) 0.15 mg/m^2 given orally and ARG ($_L$-arginine hydrochloride (10% solution)) 0.5 g/kg i.v. over 30 minutes at 0 min. Blood samples for IGF-I, IGFBP-3 and GH determination were obtained at baseline. Samples for GH were also taken at 30, 45, 60, 90 and 120 minutes after administration of ARG and CLO at 30, 60, 90, 120 and 150 minutes. If the maximum GH concentration of both tests were below the 7 µg/l, the patient

was diagnosed with GHD. The working group Endocrinology of the Dutch Foundation harmonizes the IGF-I, IGFBP-3 and GH levels in the Netherlands for Quality Medical Laboratory Technology (SKML). This is performed automatically by the IDS-iSYS program.

Serum analysis

Serum concentrations of GH, IGFBP-3 and IGF-I were measured by chemiluminiscence method in the IDS-iSYS Multi-Discipline Automated system by Immunodiagnostic Systems Holding, UK. The assay for GH was calibrated to the WHO international standard for somatropin from NIBSC, code 98/574. The sensitivity was limit of blank (LoB) 0.005 ng/mL, limit of detection (LoD) 0.015 ng/mL and limit of quantitation (LoQ) 0.049 ng/mL. Inter-assay coefficients of variation (CV) values were below 1.8% at 1.49 ng/mL, 3.5% at 9.87 ng/mL and 2.2% at 25.23 ng/mL (*n*=120). For converting the GH results from mE/l to µg/l, the conversion factor of 3 was used. Serum IGF-I assay was calibrated to the WHO international standard for IGF-I, code 02/254. The sensitivity was LoB 1.9 ng/mL, LoD 4.4 ng/mL and LoQ 8.8 ng/mL. Inter-assay CV values were below 2.9% at 21.9 ng/mL, 2.3% at 81.2 ng/mL, 1.9% at 163 ng/mL, 2.3% at 663 ng/mL and 1.4% at 304 ng/mL (n=400).
Analytical sensitivity of the IGFPB-3 assay was 0.1 µg/mL. At the mean IGFBP-3 concentration of 0.91 µg/mL, the intra-assay CV was 4.4% and total CV was 6.6%. At the mean of 8.83 µg/mL, the intra-assay CV was 4.8% and total CV was 5.2%. For comparison among children of different ages and genders, IGF-I and IGFBP-3 concentrations were expressed as an SD score (IGF-I SDS and IGFBP-3) according to reference data (12) (13) (14). Both IGF-I and IGFBP-3 levels were corrected for bone age.

Statistical analysis

Data were expressed as mean ± standard deviation. The peak serum GH response was used as the primary variable for analysis of stimulation tests. SD scores were calculated for IGF-I and IGFBP-3 based on previously reported normative data. The Pearson correlation was used to measure the strength of association between the variables. All statistical analyses were performed using the software SPSS version 22 for Mac (SPSS Inc, Chicago, IL, USA). A P-value of <0.05 was considered significant.

RESULTS

Table 1 lists the baseline characteristics of the 18 children, described according to their GH responses during GH stimulation tests. There were no significant difference in boys and girls concerning height and BMI SD scores, GH stimulation tests or IGF-I and IGFPB-3 levels.

Table 1. Characteristics of all KS patients according to their peak GH secretion (cut-offs) after the stimulation tests.

	Peak GH					
	Peak GH CLO in µg/l			Peak GH ARG in µg/l		
Cut-off value	<7	7-10	>10	<7	7-10	>10
n	9	3	5	7	4	4
Male/Female	4/5	1/2	3/2	1/6	3/1	2/2
Age (yr)	6.98	8.02	6	6.23	6.55	7.27
Height (SDS)	-2.41	-1.65	-2.29	-1.8	-2.16	-3.12
BMI (kg/m^2)	18.8	19.09	15.2	18.81	19.13	16.13
BMI (SDS)	1.35	1.23	-0.28	1.07	1.82	0.11
IGF-I (SDS)	-0.67	0.09	-1.57	-0.28	-0.67	-0.97
IGF-I BA (SDS)	-0.38	0.26	-1.42	0.37	-0.61	-0.78
IGFBP-3 (SDS)	0.75	0.13	0.01	1.05	-0.25	0.58

SDS, standard deviation score, BMI, body mass index, CLO, clonidine, ARG, arginine, BA, bone age.

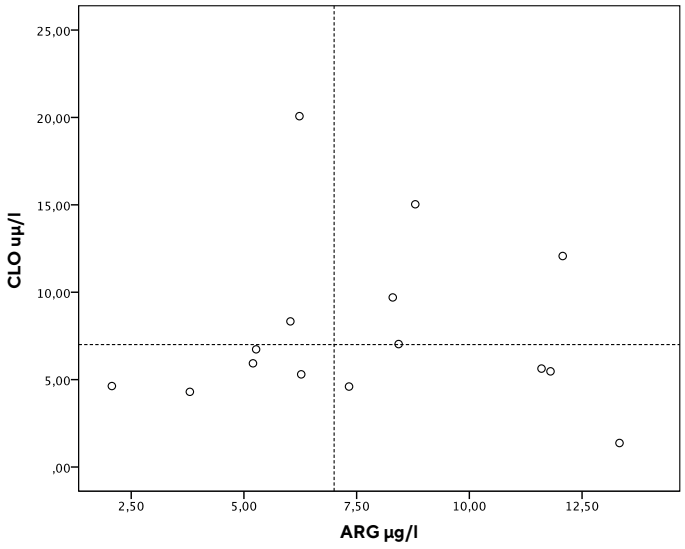

Fig. 1. Peak GH secretion after stimulation with ARG vs. CLO. A lack of GH response is indicated by the scattered line (GH <7 µg/l).

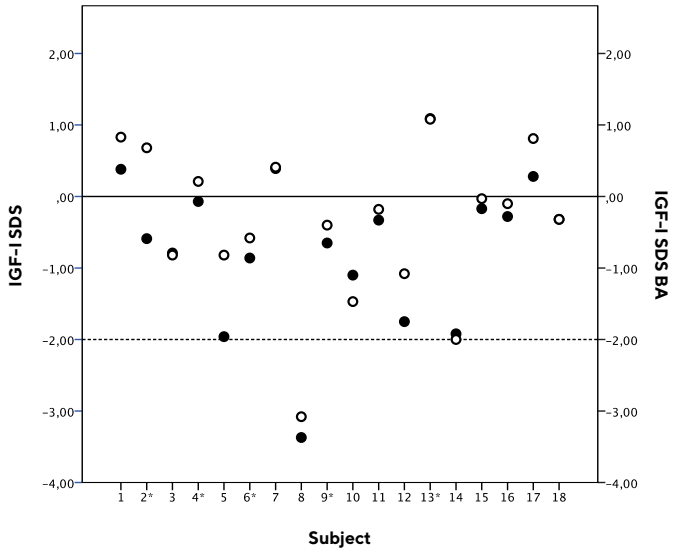

Fig. 2. Individual harmonized IGF-I SDS levels in 18 KS subjects. = IGF-I SDS not corrected for bone age; = IGF-I SD corrected for bone age. *Subjects with a lack of GH response.

The peak GH in both stimulation tests is shown in Fig. 1. The mean peak GH response to the ARG test was 8.3 µg/l (SD ± 3.7), with a range of 2.1 to 15.5. For the CLO test, the mean peak was 9.8 µg/l (SD ± 7.7), with a range of 1.4 to 31.9. Thus, clonidine was a stronger stimulus than arginine. The two tests revealed no statistically significant difference from each other. Clonidine and arginine tests showed low GH peak levels in 52.9% and 46.7% children respectively. The combination of these tests showed a GHD in 27.8% individuals.

Of all patients, only one had an IGF-1 below -2 SD (see Fig. 2). This patient did not fulfil the GHD criteria. The mean IGF-I SDS was -0.7 ± 1.1 (range -3.4 to 1.1) and for corrected bone age -0.4 ± 1.1 (range -3.1 to 1.1). The Pearson correlation for IGF-I vs. CLO and ARG was weak. Figure 3 shows the average maximum GH response of the CLO and ARG tests versus IGF-I SDS levels.

Table 2. Characteristics of the 18 KS patients

Sex	Age	Bone age	Height SDS	Height Velocity	BMI SDS	IGF-I	IGF-1 SDS	IFG-I BA SDS	IGFBP-3	CLOmax	ARGmax	Mean max GH
f	9.18	8	-1.1	5.65	2.81	237.12	0.38	0.83	6469.3	8.33	6.03	7.18
f	9.87	6.83	-2.63	2.1	2.9	191.23	-0.59	0.68	6260.7	4.63	2.07	3.35
m	8.59	8	-5.55	2.82	0.54	99.44	-0.79	-0.82	4347.7	1.37	13.33	7.35
f	6.48	5.75	-0.6	7.77	3.04	137.68	-0.07	0.21	4970	4.30	3.80	4.05
f	5.94	3	-1.83	6.52	-0.36	62.72	-1.96	-0.82	2080	12.07	12.07	12.07
f	5.69	5	-1.31	7.57	0.17	91.79	-0.86	-0.58	4834.6	6.73	5.27	6.00
m	7.05	7	-1.13	x	-0.92	140.74	0.39	0.41	2700	9.70	8.30	9.00
f	7.67	6.83	-5.84	4.5	0.35	45.89	-3.37	-3.08	5495.5	5.47	11.80	8.63
m	3.6	3	-2.76	7.2	0.23	51.25	-0.65	-0.4	1850	5.93	5.20	5.57
m	9.8	11	-1.8	x	-0.52	114.74	-1.1	-1.47	4971.8	31.93	x	x
m	3.84	3.5	-4.02	x	2.05	61.19	-0.33	-0.18	2900.8	15.03	8.80	8.80
m	5.62	4	-2.67	7.08	-1.98	45.89	-1.75	-1.08	4074.6	17.83	x	x
f	4.05	4	-2.67	6.49	-1.06	145.33	1.09	1.08	6616.6	5.30	6.27	6.30
f	4.79	5	-1.11	5.52	-0.58	53.54	-1.92	-2	5270.8	20.07	6.23	6.20
f	3.33	3	-1.95	4.78	-2.03	84.14	-0.17	-0.03	3925.1	x	15.47	x
m	7.49	7	-0.78	6.5	4.36	114.74	-0.28	-0.1	4859.6	4.60	7.33	5.97
m	9.47	8	0.85	x	1.58	191.23	0.28	0.81	5682	5.63	11.60	8.62
f	7.82	7.83	-2.72	x	1.8	152.98	-0.32	-0.32	6354.9	7.03	8.43	7.73
Mean	**6.68**	**5.93**	**-2.20**	**5.73**	**0.69**	**112.31**	**-0.67**	**-0.38**	**4648.00**	**9.76**	**8.25**	**7.14**
SD	**2.20**	**2.26**	**1.67**	**1.77**	**1.83**	**56.72**	**1.06**	**1.07**	**1482.90**	**7.66**	**3.71**	**2.16**

SD=Standard deviation. BMI=Body mass index. BA=Bone age. CLO=Clonidine. ARG=Arginine. X=missing data. Clonidine and arginine in µg/l. IGF-I and BP3 in ng/ml.

All subjects had normal IGFBP-3 levels, with a mean IGFPB-3 SDS of -0.3 ± 0.7 (range -1.8 to 0.5). The effect of BMI on the peak GH response or IGF-I was not statistically significant.

Bone ages were available for all KS children. The mean bone age to chronological age was 5.9 ± 2.3 (range 3 to 11), and, on average, the bone age was -0.8 ± 1.0 (range -3.0 to 1.2) years younger than the chronological age at the same period.

All of the 18 subjects' characteristics are presented in table 2.

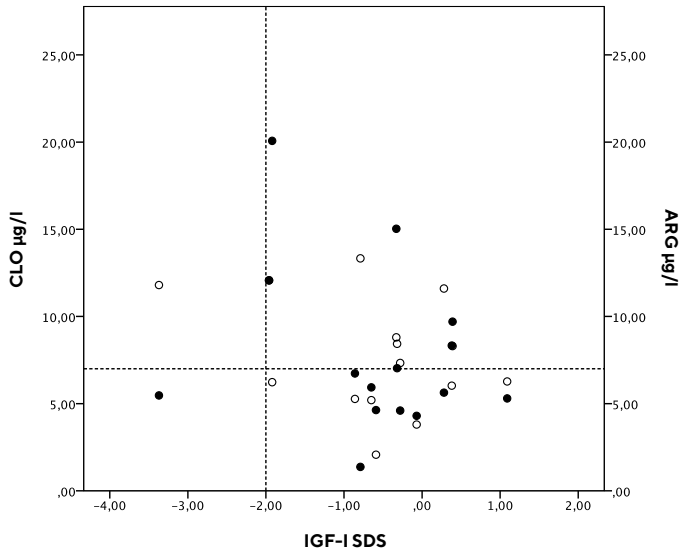

Fig. 3. The average maximum GH to the two tests plotted against uncorrected IGF-I SDS levels. = CLO; =ARG.

DISCUSSION

Short stature is a well-known feature of children and adults with Kabuki syndrome. Although birth weight and length are generally normal in KS children, growth delay often begins early in the first year of life (5). The biological basis of this impaired postnatal growth has not yet been clarified. In the present study 13 of the 18 prepubertal individuals displayed a normal GH response after stimulation testing with clonidine and/or arginine in KS children with a genetically proven mutation. According to the Dutch Growth Foundation, the diagnosis of GHD can be confirmed by two stimulation tests defined as the peak serum GH concentration below a cut-off value of 7 µg/l. Following this definition, only 5 KS subjects (27.8%) were diagnosed with GHD. However, a lack of adequate GH response is not always equal to GH deficiency. Since all children except one had normal IGF-I values. The exception concerned a KS child with an IGF-I value below -2 SD but with normal GH stimulation tests.
According to the Dutch Growth Research Foundation, the diagnosis of GHD is determined by two separate stimulation tests with a maximum GH peak of 7 µg/l. In the present study we used arginine and clonidine as stimuli for GH

secretion. These tests are the most widely used standard tests in the Netherlands for children who are suspected of GHD. In our population, we saw no significant difference between both stimulation tests. The CLO test showed that 9 out of 17 KS patients had a GH peak below 7 µg/l and 7 out the 15 patients for ARG. Although the assumption exists that a normal GH peak in one out of two performed stimulation tests is appropriate to exclude GHD, neurosecretory dysfunction might be missed.

Several cases of growth hormone treatment in KS with (partial) growth hormone deficiency have been reported in the literature. One case did not declare the cause of GHD (5); five others mention the GH test results (6,7,15-17). The patients ranged in age from 1 to 9 years (mean 4 yrs). In total, 7 GH stimulation tests were performed (3x arginine, 1x clonidine, 2x glucagon and 1x sleeping test), of which 2 tests were above 7 µg/l. The mean GH peak level was 4.83 µg/l. In all cases, it appears that rhGH treatment had a positive effect on linear growth acceleration. In our study, the assessment of GH stimulation tests demonstrated inconclusive results. Perhaps GH insufficiency or GH neurosecretory dysfunction plays a role. Although abnormalities in the central nervous system are often reported in this syndrome, malformations of the pituitary gland or hypothalamus have rarely been observed (18). In most genetic disorders, the cause of short stature is supposed to be based at the cellular level. Growth failure as part of many genetic conditions may occur due to a wide variety of mechanisms; and in many of these syndromes the underlying mechanisms are still unknown. There is usually no GH deficiency, but in some patients' pathology in the GH/IGF-1 axis can be detected.

We also determined IGF-I levels because they represent the endogenous GH secretion in healthy children and are fairly stable without circadian rhythm. This makes it a good diagnostic marker for screening GHD in short children (19,20). However, normal IGF-I levels do not exclude the diagnosis of GHD in about 30% of cases (21). Several studies also indicated inconsistent values of IGF-I serum concentrations with respect to the results of GH stimulation tests (14,22). The findings in this study do not support the evidence that IGF-I reflect the GH status of spontaneous endogenous secretion in KS children. None of the GHD KS patients had a low IGF-I level. Therefore, children with KS, short stature and normal IGF-I levels can still be at risk for GHD.

Abdominal adiposity is an important negative determinant of GH secretion (23,24). Studies point out that adiposity accounts for about 20% of the variability in peak GH response (25). Therefore, we examined the impact of BMI SDS on

stimulated GH secretion and the IGF-I. In our study there was no significant relation between the two; nevertheless, there was a trend towards lower GH peak levels with higher BMI levels. We found that the prevalence of low IGF-I levels tends to be higher in the non-obese (BMI around 0 SDS) than obese and lean KS subjects.

CONCLUSION

The present study is the first report on growth hormone stimulation tests in a group of individuals with a genetically confirmed diagnosis of Kabuki syndrome. A lack of growth hormone response was present in 5 of the 18 children. Furthermore, the IGF-I levels do not properly correspond with the GH test results. Apparently, growth hormone deficiency is not the only cause for small height in KS patients. Further research is necessary to determine the underlying cause of growth retardation in the majority of KS patients.

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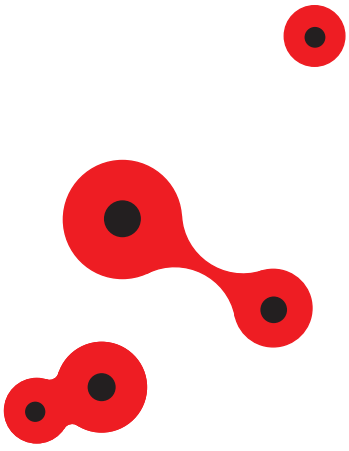

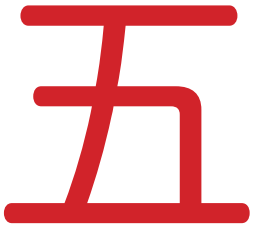

GROWTH HORMONE THERAPY IN CHILDREN WITH KABUKI SYNDROME: ONE-YEAR TREATMENT RESULTS

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ABSTRACT

Kabuki syndrome (KS) is a rare genetic malformation syndrome, resulting in characteristic features such as short stature. We investigate whether growth hormone treatment increases linear height and influences body proportions in Kabuki syndrome children.

In this prospective study, 18 genetically confirmed prepubertal KS children (9 females and 9 males) aged from 3.8 to 10.1 years (mean 6.8 ± 2.1 years) were treated with recombinant growth hormone for one year. Calculations for height, height velocity, BMI, sitting height and subischial leg length were made. Bone age, IGFBP-3 and, IGF-I were also measured.

This study showed an increase in height standard deviation score (SDS) for the whole group from -2.40 to -1.69 ($P<0.05$) after one year of rhGH treatment. For 10 subjects, the change in height SDS within one year was > 0.7 SDS and for 3 subjects > 0.5 SDS. The mean IGF-I SDS at the start of the study was -0.70 (± 1.07), which increased after 12 months to 1,41 (± 0.91) ($P<0.05$). KS children received rhGH at a younger age displayed significantly greater increases in height than those who started when they were older. The same was true for both gene mutation *KMT2D* versus *KDM6A* and for GH-deficiency versus non-GH deficiency KS children ($P<0.05$). Throughout the course of rhGH treatment, the subjects' body proportions remained normal.

All participants experienced catch-up growth during the year of rhGH treatment, but without influence on the body proportions.

INTRODUCTION

Kabuki syndrome (KS) is a rare autosomal dominant condition resulting in characteristic features such as a distinct facial appearance, intellectual disability and other medical manifestations (1,2). KS is linked to the *KMT2D* and *KDM6A* mutations therein account for 75% and 6% of the patients, respectively, which suggests additional heterogeneity of this syndrome (3,4).
One of the hallmarks of KS is short stature that originates from postnatal growth retardation. In a recent study, we confirmed this physical manifestation in the Dutch and Belgium KS population (5), where the mean adult height in men was 162 cm and that for women 147.7 cm. The cause of this growth retardation is unknown, but there are several reports of growth hormone deficiency in the literature (6-9). Growth hormone treatment was found to be beneficial in these children. However, this observation was only based on case reports, and without the benefit of knowing about the gene mutation for KS that was identified in 2010 (3). For this reason, a study was conducted at the Maastricht University Medical Centre in a group of 18 KS children with a proven mutation in the *KMT2D* or *KDM6A* gene. These children's growth responses after one year of recombinant human growth hormone (rhGH) treatment were studied along with possible changes in their body proportions.

SUBJECTS AND METHODS

Subjects

In this prospective study, we included 19 KS children. Subjects were qualified for inclusion in the study if the diagnosis of KS was genetically confirmed. Furthermore, they had to be prepubertal with a chronological or bone age (BA) of less than 8 years for girls and 10 years for boys. The paediatric endocrinologist confirmed the prepubertal status through employing Tanner (A1P1G1 or M1) and Marshall's method (10). In all patients, bone age was estimated by the same paediatric endocrinologist (DS) using the Greulich and Pyle atlas method (11). Moreover, all subjects required normal results in standard laboratory tests, including the hormonal axis of the thyroid and adrenal gland. Exclusion criteria were growth failure caused by other disorders, emotional deprivation and the use of drugs since that intervene with GH treatment.
The project was approved by the regional Medical Ethics Committee and

conducted according to the Declaration of Helsinki. Written permission was obtained from the parents.

Study Protocol

The study protocol is publicized under trial number NTR4722. Biosynthetic human GH (Genotropin, Pfizer, New York, NY) was given sc once daily at bedtime. All KS patients received rhGH at an average dose of 1 mg/m^2 (equivalent to 0.035 mg/kg/day) for 12 months. The initial dose of rhGH was altered based on IGF-I levels and/or growth hormone response.

Prior to any treatment all KS subjects underwent GH stimulation test. Almost all KS individuals underwent two stimulation tests: arginine and clonidine (ARG and CLO), according to the standard procedures after overnight fasting. Three subjects only had one test, two of which only CLO, and one ARG. A cutoff value for insufficient GH response was defined as a value below the 7 µg/l, using previously described assays at the local centre (12).

Concentrations of insulin-like growth factor (IGF-I) and IGF binding protein 3 (IGFBP3) were measured by the chemiluminiscence method through the IDS-iSYS Multi-Discipline Automated system from Immunodiagnostic Systems Holding, UK. For comparison among children of different ages and genders, IGF-I concentrations were expressed as an SD score (IGF-I SDS) according to reference data (13,14).

Measurements

At study entry and at three-months intervals during the first year of treatment, all subjects were assessed for height (H), sitting height (SH) and weight. Measurements were taken according to the internationally accepted methods mentioned previously (15) . Standing height and sitting height were measured with a Harpenden stadiometer (Holtain Ltd., Crymych, Dyfed, UK) and a sitting height table both accurate to 0.1 cm. Height velocity was calculated over whole year periods and expressed as cm/year. Body weight was measured using an electronic scale (Seca, model 701, Germany) accurate to 0.1 kg. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. To be informed about the body proportions, subischial leg length (SLL) was calculated as height minus sitting height. Parents' height was also measured and used to calculate the subjects' mid-parental height (16,17). Calculations were made as follows: $TH_{boys} = 44.5 + 0.376 * H_{father} + 0.411 * H_{mother}$ and $TH_{girls} = 47.1 +$

$0.334 * H_{father} + 0.364 * H_{mother}$.

All measurements were expressed in standard deviation scores, using Dutch reference values (TNO The Netherlands 2010, with the 2001-2013 Growth Analyser BV, Rotterdam, The Netherlands). For the sitting height and subischial leg length, the reference values of the Dutch population described in Paediatric Morphometrics were used (18).

Statistical analysis

Descriptive statistics were used and results are shown as mean ± standard deviation (SD). An independent T-test or a Mann-Whitney U-test was used to examine the difference between groups. Intra-group comparisons were made with the paired Student's t-test. The Pearson correlation was used to measure the strength of association between the variables. All statistical analyses were performed using the software SPSS version 22 for Mac (SPSS Inc, Chicago, IL, USA). A P-value of <0.05 was considered significant.

RESULTS

Of the 19 KS children recruited for the rhGH study, one child was excluded because of an identified tumor (rhabdomyosarcoma) in the abdomen. In total, 18 KS children (9 females and 9 males) ranging from 3.8 to 10.1 years of age (mean 6.8 ± 2.1 years) are described. Fifteen of the KS subjects had a *KMT2D* mutation and 3 had a *KDM6A* mutation. All patients were prepubertal during the study the entire study period.

The baseline characteristics of the subjects are given in Table 1. There were no significant differences between the subjects for any of the variables in Table 1.

At birth, the mean length and weight were within the normal range of ± 1 SD.

The final height prediction based on the anthropometric data of the parents was normal using the reference values from TNO. The calculated body proportions of the parents fell within the normal range of the Dutch population.

Table 1. Baseline characteristics of treatment naïve patients with KS (n = 18) and their parents' auxological data

Subject		Male	Female
Sex		9	9
Mutation	*KMT2D*	8	7
	KDM6A	1	2
Birth	Length (cm)	47.67 ± 3.28	49.56 ± 3.21
	Lenght SDS	-0.60 ± 1.26	0.10 ± 2.20
	Weight (g)	2877 ± 645	3204 ± 553
	Weight SDS	0.29 ± 1.23	0.08 ± 1.70
Midparental height (cm)		181.44 ± 7.44	168.50 ± 6.50
Father	Height SDS	-0.54 ±1.60	-0.81 ± 1.83
	SH/H ratio SDS	0.61 ± 0.98	1.10 ± 0.79
	SLL/SH ratio SDS	-0.58 ± 0.90	-1.03 ± 0.64
Mother	Height SDS	-0.35 ± 1.46	-0.14 ± 1.19
	SH/H ratio SDS	0.55 ± 1.07	0.08 ± 1.02
	SLL/SH ratio SDS	0.86 ± 0.05	0.88 ± 0.05

SH, sitting height. H, height. SLL, subischial leg lenght.

Treatment was started with a 'standard' rhGH dose 1 mg/m^2/day (close to 0.035 mg/kg/day), and the mean dose was 0.96 ± 0.17 mg/m^2/day (0.039 ± 0.010 mg/kg/day). Table 2 depicts all the results at baseline and after one-year of rhGH treatment. No statistical differences were seen betwee n baseline and one-year treatment results for BMI SDS, weight SDS and SLL/SH ratio SDS.

Table 2. Baseline data (mean ± SDS) and the auxological data after one-year of rhGH treatment of all 18 subjects with KS

	Pre-treatment	One-year treatment
Chronological age	6.86 ± 2.07	7.97 ± 2.13
Bone age	5.90 ± 2.14	7.28 ± 2.37
Height SDS	-2.40 ± 1.88	-1.69 ± 1.94 *
Height Velocity (cm/y)	6.55 ± 2.66	9.66 ± 2.04 *
Height Velocity SDS	0.29 ± 2.43	2.80 ± 1.56 *
Parent-adjusted Height SDS	-2.07 ± 1.66	-1.35 ± 1.61 *
Weight SDS	-1.34 ± 2.88	-1.16 ± 2.62
BMI SDS	0.56 ± 1.79	0.19 ± 1.41
Sitting Height SDS	-1.90 ± 1,46	-1,10 ± 1,62 *
SLL/SH ratio SDS	-1.56 ± 1.59	-1.45 ± 1.56
IGF-I SDS	-0.70 ± 1.07	1.41 ± 0.91 *

SDS, standard deviation score. SH, sitting height. SLL, subischial leg lenght. * P <0.05.

Table 3 shows all the individual subjects' characteristics. Seventy-two percent of the subjects displayed a good growth response to rhGH treatment. In 13 subjects, the change in height SDS within one year was > 0.5 SDS, of whom 10 subjects experienced growth of more than 0.7 SDS. Figure 1 and 2 shows the height before start and after one year rhGH treatment. There was a significant relationship between the age of commencing treatment and increases in linear growth at the younger the age, the better the catch-up growth. In 5 children, the height gain was less than 0.5 SDS. Of these, one was a female subject. She suffered from headache symptoms. Because this symptom was assumed to be a side effect of rhGH treatment, the dose of rhGH was lowered to approximately 0.017 ± 0.006 mg/kg per day. As a result, her height increased only 0.07 SDS after one year.

Her complaints turned out to be based on a tension headache. An MRI scan of her brain showed no abnormalities. Additionally, two out of the five poor responders experienced a normal growth in height before starting treatment, with a respective parental-adjusted height SDS of 0.35 and 0.44.
The mean growth velocity (cm/year) over one year of growth hormone therapy was 9.66. The HV SDS changed from 0.29 at start to 2.80 after the one-year therapy. The increase in height SDS of the non-GHD group was 0.62 versus 1.02 in the GHD group. With respect to gene mutation, the *KMT2D* height SDS increased by 0.74 SDS and for the *KDM6A* by 0.54 SDS, both statistically significant results. At the start of treatment, the height SDS for boys was less than for girls (0.43 SDS), but the increase in height SDS was nearly the same (0.7 SDS). There was short stature, defined by height SDS <-2.5, in 8 of the 18 subjects. The increase in height SDS between short stature and normal stature groups was almost the same.

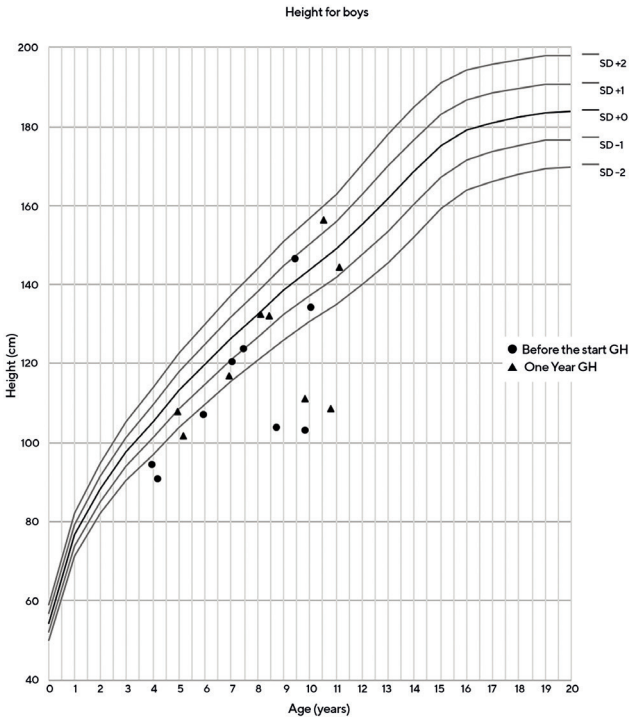

Figure 1. Height for age in boys. The • represent the subjects at the start and those with a ▲ after one-year of rhGH treatment.

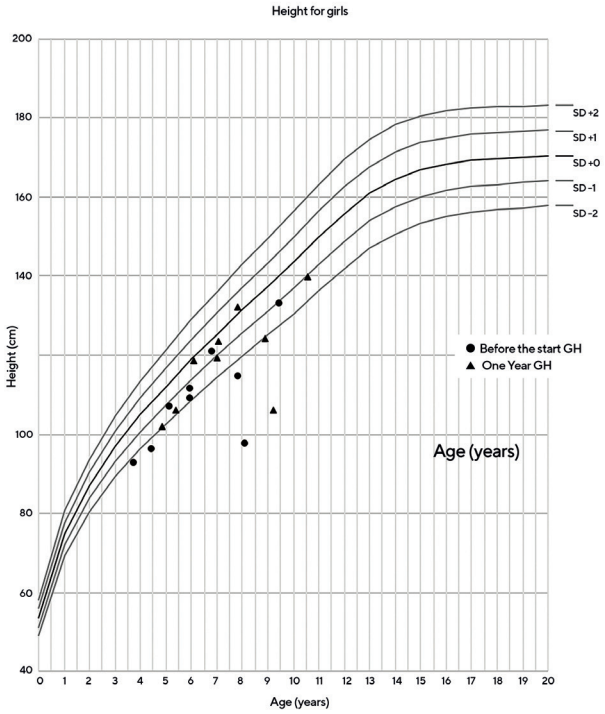

Figure 2. Height for age in girls. The • represent the subjects at the start and those with a ▲ after one-year of rhGH treatment.

At the start of the study, the subjects had a BA delay of approximately one year. During one-year of rhGH treatment, the BA advanced from mean 5.90 to 7.28 y, and thereby the difference between bone age and chronological age decreased from 0.96 to 0.69 y. This represents a significant difference. The mean ΔBA/ΔCA was 1.25 ± 0.45.

Serum IGF-I levels were measured at baseline, at 6 and at 12 months of rhGH treatment. At the start, the IGF-I SDS had a mean of -0.70 ± 1.07, which increased substantially after 6 months to 1.42 ± 1.17. At 12 months, the IGF-I levels remained the same (1.41 ± 0.91 SDS). Statistical analyses of subgroups showed that the *KMT2D* subjects had a significant increase of the IGF-I SDS ($P<0.001$). This increase was not observed in the *KDM6A* group ($P = 0.08$). For the subgroups defined by insufficient GH production and short stature, the IFG-I SDS also increased significantly. Five children had two GH stimulation test below the cutoff

Table 3. All individual subject's characteristics

Subject	Age	Sex	Mutation	Δ CA	Δ BA	Δ H_{SDS}	ΔSH/SLL_{SDS}	Δ IGF-I_{SDS}	PaH_{SDS}	$Effect_{GH}$	GHD
1	9.42	F	KMT2D	1.10	1.50	0.07	-0.58	0.81	-0.88	-	-
2	8.82	M	KMT2D	1.10	1.00	0.38	X	0.23	-3.14	-	-
3	6.80	F	KMT2D	1.03	1.08	0.85	0.45	2.45	-1.07	+	+
4	5.94	F	KMT2D	1.05	0.50	0.76	-0.86	3.09	-2.28	+	-
5	5.92	F	KMT2D	1.13	1.08	0.90	-0.76	1.67	-1.44	+	+
6	7.09	M	KMT2D	1.14	1.50	0.73	0.11	1.28	-0.89	+	-
7	8.07	F	KMT2D	1.13	1.00	0.57	1.53	4.88	-4.53	+	-
8	4.02	M	KMT2D	1.02	1.00	1.53	0.15	1.96	-3.92	+	+
9	10.07	M	KMT2D	1.11	2.50	0.72	-0.22	2.52	-1.57	+	-
10	4.17	M	KMT2D	1.01	1.50	1.05	1.41	2.07	-3.52	+	-
11	6.01	M	KMT2D	1.00	1.50	0.82	1.03	2.37	-2.73	+	-
12	4.43	F	KMT2D	1.00	2.00	0.78	1.05	1.60	-2.68	+	+
13	5.12	F	KMT2D	0.98	1.50	1.07	-0.22	3.01	-1.96	+	-
14	9.89	M	KDM6A	1.01	1.00	0.46	0.81	3.18	-5.72	-	-
15	3.78	F	KDM6A	1.11	1.17	0.52	X	0.97	-1.21	+	-
16	7.53	M	KMT2D	0.99	1.00	0.41	-0.79	1.22	0.35	-	-
17	9.47	M	KMT2D	1.13	2.00	0.46	-0.36	1.30	0.44	-	-
18	7.82	F	KDM6A	1.07	1.00	0.64	0.07	3.25	-0.42	+	-

Notes: Δ at one year rhGH minus start. X missing value.
CA, calender age in yr. BA, bone age in yr. H, height in SDS. SH, sitting height. SLL, subischial leg length. PaH, Parental-adjusted Height at start. GH, growth hormone. GHD, growth hormone deficiency.

point of 7 µg/l, off which 3 also had a height below the -2.5 SDS with retarded bone age. None of them had an IGF-I below the -2.5 SDS. It is remarkable that the IGF-I SDS at the start was lower in the 'normal GH responding' group in comparison to the 'insufficient GH responding' group. At the start, the IGF SDS in the normal GH responding subjects was -0.86 and increased to 1.29 after 12 months of treatment as opposed to -0.12 and 1.80 for the insufficient GH responding subjects. However, the relative increase was greater in the insufficient GH responding group. At baseline the IGFBP-3 were all within the normal range. Figure 3 and 4 shows the body proportions SLL versus SH for boys and girls. These figures demonstrate that KS children have normal body proportions that do not significantly change during GH treatment. Although the SH SDS increased slightly more than the height SDS, the SLL did not change significantly relative to the SH.

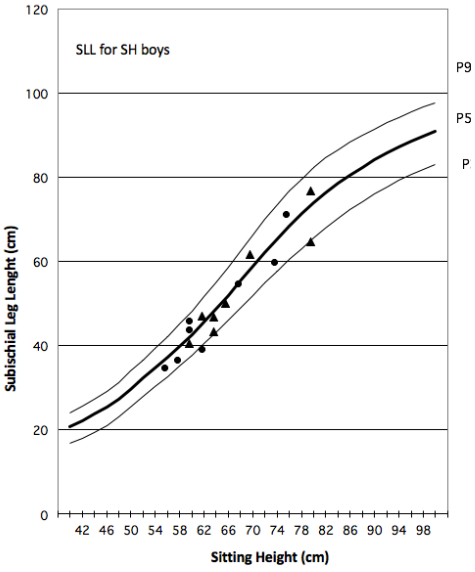

Figure 3. Subischial leg length for sitting height in boys. The • represent the subjects at the start and those with a after one-year of rhGH treatment.at the start and those with a ▲ after one-year of rhGH treatment.

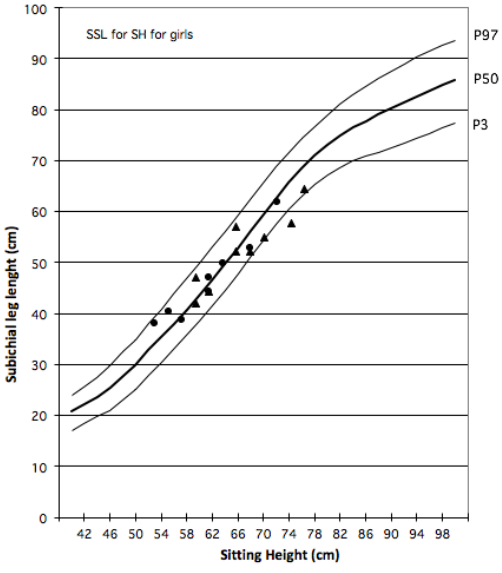

Figure 4. Subischial leg length for sitting height in girls. The • represent the subjects at the start and those with a ▲ after one-year of hGHtreatment.at the start and those with a after one-year of rhGH treatment.

DISCUSSION

We report on the effect of rhGH on the height of 18 children with a genetically confirmed diagnosis of Kabuki syndrome. rhGH therapy was initially used in children with GHD to normalize height during childhood (19). rhGH treatment has also be proven to be a safe and effective means to increase height across a range of growth disorders, including Small for Gestational Age (SGA), Idiopathic Short Stature (ISS), Prader-Willi syndrome (PWS), and Turner syndrome (TS) (20-23). Since KS children present many features similar to their TS and PWS peers (e.g., short stature, hypotonia and obesity), we hypothesized that KS children would experience the same positive growth effects of rhGH treatment as these other children. This expectation was confirmed by the results of the Kabi International Growth Hormone Study (KIGS- Pfizer international Growth Database) where 21 KS children treated with growth hormone therapy achieved an increase in height SDS from -3.41 to -2.58 SDS after one year of treatment (24). However, this is pre-2010 data, before the genetic basis for KS was known and thus not a proven syndrome in all cases. Ours is the first prospective study that evaluated the growth response of rhGH therapy on children whose KS is genetically validated. This study's results indicate a normalization of height during childhood in 72% of the KS patients, with a mean increased in height SDS from -2.40 to -1.69 for the entire study group.

Because KS is a very rare syndrome, it is difficult to conduct a large randomized study, especially regarding prepubertal KS children as we did. The only known prevalence is in the Japanese population where it affects 1 in 32.000 (25). We approached every family with a KS child in the Netherlands, as identified through the Dutch Kabuki Network and/or our database and asked them to join the study. Only one family declined. Given the paucity of KS, we decided to include all children who met the inclusion and exclusion criteria, regardless of their height. Obviously, KS children who were tall, according to the metric of mid-parental height, at the start of treatment remained tall, but their gain in height was relatively minor.

We previously described the growth pattern among Dutch and Belgium KS subjects with a *KMT2D* mutation (5). All of them showed a reduction in growth during childhood, but a genotype-phenotype correlation was not observed. In this study, not only children with a *KMT2D* mutation were included, three individuals with a *KDM6A* mutation also received rhGH therapy. There seems to

be a different facial dimorphism and some clinical features in *KMT2D* mutation-positive and -negative patients. Banka et al. report that the majority of typical KS patients (based on facial KS morphology scores) have *KMT2D* mutations, implying that the genetic heterogeneity of KS may be minimal (26). The conclusions by Paulussen et al. were similar, but also that the growth retardation (threshold -3 SD) was more pronounced in *KMT2D* positive patients (4). Since the *KDM6A* group is rather small, our conclusions require a degree of caution. Nevertheless, statistical analyses found a difference with respect to increase in height, with a variance in Δ Height SDS of 0.74 in the *KMT2D* group versus 0.54 for the *KDM6A*. Moreover, the IGF-I levels of both subgroups showed that the *KMT2D* subjects had a significant increase of the IGF-I SDS, which was not observed in the *KDM6A* group.

Normally IGF-I levels represent the endogenous GH secretion and serve as a good diagnostic marker for screening GHD in short children (27). It has a specificity of 95% but a low sensitivity of 70% (28). We reported earlier that in our KS subjects' IGF-I levels correlated neither with the GH stimulation peak nor with the diagnosis GH deficiency (12). In that study, 5 of the 18 children (27.8%) were biochemically GH deficient, but had normal IGF-I levels. During this study with rhGH treatment, the IGF-I levels increased significantly. Remarkably, though at baseline the good GH stimulation test responders had lower levels of IGF-I, but the increase during treatment was higher in the insufficient GH test responders. Previous research in girls with TS have shown that GH treatment moderately decreases the disproportion between sitting height and height (29). Schweizer et. al. described a higher gain in SH than in SLL in TS patients with rhGH therapy, particularly after puberty started, even though the body proportions showed no statistical change in comparison with the normal population (30). The same observation was made in our study. There was no disproportion before the start of GH treatment and the SLL/SH ratio stayed normal during GH therapy.

CONCLUSION

There was a positive effect of rhGH treatment on linear growth in KS children. This effect was stronger in the subjects with a *KMT2D* than in those with a *KDM6A* mutation. Therefore, rhGH treatment seems to be effective and justified in Kabuki syndrome children. Follow-up studies are needed to determine if improvement in height continues with long-term rhGH treatment.

ACKNOWLEDGEMENTS

We are grateful to the Dutch Kabuki Syndrome Network and to the individuals with Kabuki syndrome and their families/caregivers for their willingness to participate in this study. We also thank Robin Remmel and Berbel Kooger for their contributions to the collected data. The study was financially supported by Pfizer. The sponsor was not involved in the study design, collection, analysis and interpretation of data, writing of the report, or the decision to submit the manuscript for publication.

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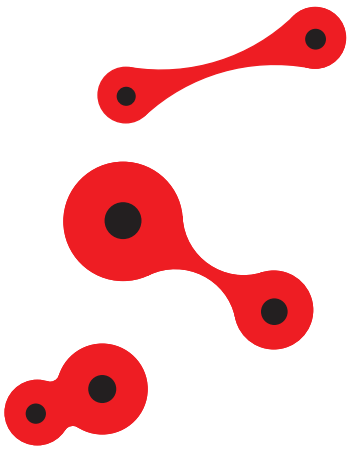

CHAPTER 6
ENERGY EXPENDITURE

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METABOLIC EFFECTS OF GROWTH HORMONE TREATMENT ON BODY COMPOSITION, ENERGY EXPENDITURE AND PHYSICAL ACTIVITY IN KABUKI SYNDROME

Submitted

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ABSTRACT

Kabuki Syndrome (KS) is a genetic disorder with characteristic facial dysmorphisms, moderate mental retardation, short stature, and obesity later in life.

Kabuki Syndrome (KS) is a genetic disorder with characteristic facial dysmorphisms, moderate mental retardation, short stature, and obesity later in life. This study evaluates body composition, energy expenditure and physical activity levels before and during GH-treatment in KS children.

In this prospective study, 18 KS children and 10 short stature control children were included. Each KS subject received GH at an average dose of 1 mg/m^2 (equivalent to 0.035 mg/kg/day). Before and during treatment several measurements were performed: fat mass, fat free mass, total energy expenditure (TEE), basal metabolic rate (BMR), and physical activity level (PAL).

In the KS children, TEE showed a significant increase (P<0.01), rising from 5.19 (±2.00) MJ before the start of GH treatment to 7.94 (±2.76) MJ during treatment. Children with a reduced GH secretion during GH stimulation test had the highest increase in TEE. Further, the BMR increased significantly (P=0.001), rising from 4.05 (±0.90) MJ to 4.73 (±1.03) MJ during 6-weeks of GH treatment. Similar increases were also evident with the FFM rising from 15.9 (±6.4) kg to 17.1 (±5.8) kg (P = 0.01) and the PAL rising from 1.25 (±0.26) to 1.66 (±0.39) (P <0.01). There was a significant difference in all the above-mentioned measurements for the short stature control group, even after 6-weeks of GH treatment in the KS group.

Our study shows that GH treatment in KS children significantly improves height, body composition and energy expenditure. Nevertheless, the healthy control group displayed higher energy expenditure levels without GH treatment.

INTRODUCTION

Kabuki Syndrome (KS) is a genetic disorder with mild to moderate mental retardation, characteristic facial dysmorphisms, postnatal growth retardation, and skeletal anomalies (1,2). Clinical findings also include early hypotonia, joint laxity, persistent fetal fingertip pads, obesity, endocrine anomalies, and a variety of structural defects (3,4). The prevalence of the syndrome varies between 1:32.000 (Japan) and 1:86.000 (New Zealand and Australia) (3,5). In KS patients, the major genetic cause is a heterozygous mutation in the *KMT2D* or *KDM6A* gene in 56% to 75% of the cases (6-8). There are some familial cases with an autosomal dominant inheritance, but most of the mutations are de novo. Nineteen to 57% of the KS patients will develop obesity during adolescence (5). Dietary habits and diminished physical activity are hypothesized to play a significant role in the development of obesity in these subjects. In the literature, most KS patients presenting as overweight or obese were diagnosed by means of the Body Mass Index (BMI) method. BMI is expressed as weight/height^2 and, due its simplicity, it is a popular measurement for overweight and obesity worldwide. However, especially in children, the test is not as reliable as it seems (9). For example, based on the formula weight/height2, tall people with the exact same body composition as smaller people, have a lower BMI. Its reliability is even worse in individuals with the unusual body proportions frequently seen in syndromes (10). Besides, the BMI is often used as an indication of body composition, but the BMI measurement makes no discrimination between body fat mass and lean mass. A better tool is the use of the doubly labeled water (DLW) method what is regarded as the 'gold standard' for the assessment of not only body composition but also for total energy expenditure (TEE) in free-living conditions.
Growth hormone (GH) treatment is known to have a positive effect on TEE and body composition through anabolic effects evident throughout increased nitrogen retention (11,12). Increased nitrogen retention reflects increased protein synthesis and thereby an increase of fat free mass, which is associated with a higher energy expenditure. Previous studies in children have shown a strong relation between these early metabolic changes after start of GH treatment with long term changes in growth (13). The same might be visible in children with Kabuki syndrome (14,15). Therefore, the primary aim of the present study was to determine body composition and energy expenditure in KS children receiving GH treatment compared to healthy control children without GH treatment. The

second aim was to measure the physical activity level (PAL) and body movement by using a DirectLife triaxial accelerometer for movement registration ($Tracmor_D$).

SUBJECTS AND METHODS

Subjects

In this prospective study, 18 KS children (9 females and 9 males) were included. Fifteen of the KS persons had a *KMT2D* mutation and three had a *KDM6A* mutation. All were in a state of good health, without significant cardiovascular or respiratory disease. None of the subjects had thyroid disease or were using medications that could influence their growth, body composition or energy expenditure.

Biosynthetic human GH (Genotropin, Pfizer, New York, NY, USA) was given subcutaneously once daily at bedtime. All KS patients received GH at an average dose of 1 mg/m^2 (equivalent to 35 µg/kg/day). The initial dose of GH was altered based on IGF-I levels and/or growth response.

Prior to any treatment all KS subjects underwent an arginine and/or clonidine test, as previously described (16). The test abnormal when the peak serum GH concentration of both stimulation tests were below the cut-off value of 7 µg/l, including GH deficiency through GH stimulation tests.

Also, ten control children in age range 3.4 - 9.8 years were selected. These children were healthy and free from any medical condition (including GH deficiency) that could restrict their physical activity. To compare both groups, especially for their body composition, the control children were selected based on short stature. The control children underwent the same protocol for measurements of height, weight, basal metabolic rate (BMR) and DLW method as the KS children did.

The project was approved by the regional Medical Ethics Committee and conducted according to the Declaration of Helsinki. Written permission was obtained from the parents. The study protocol is publicized under trial number NTR4722.

Anthropometric measurements

The anthropometric measurements were taken according to the internationally accepted methods described previously (17). Height and weight were measured at the start of therapy, at 6 weeks, and then every 3 months, up to a total of 1 yr.

Standing height and sitting height were measured with a Harpenden stadiometer (Holtain Ltd., Crymych, Dyfed, UK) and a sitting height table, both being accurate to 0.1 cm. Height velocity (HV) was calculated over whole year periods and expressed as cm/y. Body weight was measured using an electronic scale (Seca, model 701, Germany) accurate to 0.1 kg. BMI was calculated from the formula weight (kg)/height^2 (m^2). All measurements were expressed in standard deviation scores, using the Dutch reference values of the Netherlands (TNO The Netherlands 2010, with the 2001-2013 Growth Analyser BV, Rotterdam, The Netherlands).

Doubly labeled water method (DLW)

Human total energy expenditure (TEE) consist of four components: sleeping metabolic rate (SMR), arousal, diet-induced energy expenditure (DEE), and the energy cost of physical activity (AEE). Basal metabolic rate (BMR) is defined as SMR and the energy cost of arousal.

TEE was measured using the DLW method before and at 6 weeks after the start of GH therapy according to the Maastricht protocol (18). A weighed dose of water with a measured enrichment of about 5 at % ^{2}H and 10 at % ^{18}O was ingested during the evening preceding the BMR measurement. This dose results in an initial excess body water enrichment of ~ 115 p.p.m. for ^{2}H and ~ 2200 p.p.m. for ^{18}O. A baseline urine sample was collected just before dosing. Additional urine samples were collected in the mornings (from second voiding) and evenings of days 1, 8 and 15. Samples were analysed on an isotope-ratio mass spectrometer (Optima; VG Isogas, Middlewich, Cheshire, UK). The hydrogen isotope is eliminated from the body as water, while the oxygen isotope is eliminated as both water and carbon dioxide. The difference between the two elimination rates is therefore a measure of carbon dioxide production. TEE was calculated, using Weir's equations (19), from carbon dioxide production assuming a respiratory quotient of 0.85.

Basal metabolic rate (BMR)

BMR was measured by an open-circuit, ventilated hood system (Omnical, Maastricht University, The Netherlands) in the morning after a 12-hour overnight fast, before and at 6 weeks after the start of GH therapy. The subjects were transported to the lab by wheelchair. The BMR took place in a quiet, thermo neutral room while the subject rested in a supine position for 30 minutes. The

subjects were awake, resting quietly and watching age-appropriate television. A transparent, ventilated hood was placed over the subject's head for sampling respiratory gases at 1-minute intervals. The first 10 minutes of each study were excluded to allow EE to stabilize and reach basal levels. Measurements of BMR obtained immediately after periods of movement, which altered the BMR, were excluded. The 1-minute BMR measurements, from a minimum of 15 minutes, were averaged and used as the BMR. Oxygen consumption and carbon dioxide production were calculated from the flow through the hood and the oxygen and CO_2 concentrations in the incoming and outgoing air. Weir's equations was used to calculate BMR (19).

Physical activity level (PAL)

Knowing TEE and BMR, physical activity level (PAL) can be calculated as TEE/BMR. Habitual physical activity was also assessed before and at 6 weeks after the start of GH therapy using a DirectLife triaxial accelerometer for movement registration ($Tracmor_D$) (Philips New WellnessSolutions). The $Tracmor_D$ was secured at the lower back with an elastic belt, and the subjects were instructed to wear the accelerometer during waking hours for two weeks. At the end of the monitoring period, the $Tracmor_D$ was connected to a personal computer and the recorded data was downloaded using dedicated software. $Tracmor_D$ output was expressed as activity counts/minute. The $Tracmor_D$ activity counts/minute were calculated over the entire monitoring period and divided by the number of monitoring days to determine the average $Tracmor_D$ counts per day (Cnts/d) (20).

Body composition

While the subjects were fasting, their weight and height were measured as described earlier. Total body water was measured by the deuterium dilution technique before and at 6 weeks after the start of GH therapy as described by Westerterp et al. (18). The deuterium dilution space was divided by 1.04 to derive total body water. Because the water percentage in children is different from adults, we used the water percentages by Lohman formula; FFM = TBW / water percentage Lohman (21).

Statistical analysis

Descriptive statistics are shown as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used to test for normal distribution. Intra-group comparisons were made with the paired Student's t-test. For inter-group comparisons, the independent Student's t-test was employed. The Pearson and the Spearman rank correlation was used to measure the strength of association between the variables. All statistical analyses were performed using the software SPSS version 22 for Mac (SPSS Inc, Chicago, IL, USA). A P-value of <0.05 was considered significant.

RESULTS

Details of the 18 subjects are given in Table 1. Age, height, weight, and BMI between the males and females were not significantly different. At the start of GH treatment, the mean age was 6.9 ± 2.1 years (ranging from 3.8 to 10.1 years). All patients were prepubertal during the entire study period. At baseline, the mean height SDS for the entire group was -2.40 (±1.88), with the males slightly shorter at the start than their female counterparts.

The fat free mass was significantly higher in the male group (P = 0.01). The DLW method failed in one female subject (*KDM6A* mutation) and was not included in the calculations of TEE, FFM and body composition.

Fourteen of the 18 KS children displayed a normal GH response after stimulation testing with clonidine and/or arginine using a cut-off value of 7 µg/l. Four KS subjects (22.2%) had a GH response less than 7 µg/l.

There was also a control group of ten healthy children with a mean age 6.45 ± 1.76 years. The independent Student's t-test showed no difference between the KS children and the control group for age, height SDS, weight SDS and BMI SDS at baseline. However, there were significant differences energy expenditure at baseline measurements (Table 2). This significant difference was persistent after 6 weeks of GH treatment in the KS group versus the control group without GH therapy.

Table 1. Baseline characteristics (mean ± SDS) of treatment naïve patients with KS (n = 18).

Subject		Male	Female
Sex		9	9
Mutation	*KMT2D*	8	7
	KDM6A	1	2
Reduced growth hormone tests		1	3
Age start treatment (yr)		7.45 ± 2.33	6.37 ± 1.83
Height SDS		-2.61 ± 2.23	-2.18 ± 1.55
Weight SDS		0.29 ± 1.23	0.08 ± 1.70
BMI SDS		0.64 ± 2.01	0.48 ± 1.65
Mid parental height (cm)		181.44 ± 7.44	168.50 ± 6.50
Mid parental height SDS		-0.54 ± 1.60	-0.81 ± 1.83
Height velocity (cm/year)		6.92 ± 2.82	6.26 ± 2.67
Height velocity SDS		1.03 ± 3.34	-0.29 ± 1.36
TEE (MJ)		5.16 ± 1.71	5.22 ± 2.42
TEE/FFM		0.38 ± 0.13	0.31 ± 0.06
BMR (MJ)		4.18 ± 1.12	3.92 ± 0.65
BMR/FFM		0.18 ± 0.03	0.20 ± 0.02
FFM (kg)		17.52 ± 7.91	14.11 ± 4.00
%FFM		80.22 ± 7.47	63.81 ± 10.71
FM (kg)		4.64 ± 3.05	7.93 ± 6.12
%FM		19.80 ± 7.49	32.21 ± 10.77
PAL		1.37 ± 0.20	1.12 ± 0.27

BMI = body mass index, SDS = standard deviation score, TEE = total energy expenditure, BMR = basal metabolic rate, FFM = fat free mass, FM= fat mass, PAL=physical activity level.

Table 2. Baseline characteristics (mean ± SDS) of Kabuki syndrome children versus control group children.

	Kabuki group	Control group	*P*-value
Sex (M/F)	9/9	2/8	0.11
Age (yr)	6.91 ± 2.11	6.45 ± 1.76	0.57
Height SDS	-2.40 ± 1.88	-2.33 ± 1.80	0.93
Weight SDS	-1.34 ± 2.88	-1.32 ± 1.36	0.99
BMI SDS	0.56 ± 1.79	-0.12 ± 0.96	0.28
TEE (MJ)	5.19 ± 2.00	8.46 ± 1.43	<0.01*
TEE/FFM	0.35 ± 0.11	0.61 ± 0.09	<0.01*
BMR (MJ)	4.05 ± 0.90	4.03 ± 0.47	0.11
BMR/FFM	0.19 ± 0.03	0.30 ± 0.05	<0.01*
%FFM	70.2 ± 20.7	76.1 ± 7.6	0.68
PAL	1.25 ± 0.26	2.09 ± 0.18	<0.01*

SDS = standard deviation score, BMI = body mass index, TEE = total energy expenditure, BMR = basal metabolic rate, FFM = fat free mass, PAL = physical activity level, * = statistical significant.

Body Mass Index and Fat Mass

Four out of the 18 KS children were obese, with a BMI above the 2 SDS. Their mean BMI was 3.01 SDS (ranging from 4.31 to 2.05) before the start of treatment. After one year, a significant reduction was evident with a mean of 1.97 SDS (range 2.61 to 0.78). Overall, the BMI reduced from 0.56 to 0.19 SDS ($P = 0.06$) in all KS children. Notably, the FM% in non-obese KS children decreased from 22.58% to 18.07%, a difference of -4.51%. However, the FM% of the obese children increased from 35.60% to 37.75%, a gain of 2.15%. Their weight SDS displayed the opposite effect, with an increase in the non-obese group from -2.29 SDS to -1.75 (difference of 0.53 SDS) and for the obese children from 1.99 SDS to 0.53 SDS (difference of -1.07 SDS). There was no significant difference in BMR, TEE and PAL at baseline and during GH treatment between obese and non-obese KS children. None of the children in the control group were obese.

Total Energy Expenditure (TEE)

TEE as measured by double-labeled water, showed a significant increase ($P<0.0001$) from 5.19 (±2.00) MJ before the start of GH treatment to 7.94 (±2.76) MJ during treatment (table 3). Since FFM of the KS subjects altered across the two testing phases (before and at 6 weeks GH treatment) and the control group,

it was deemed crucial to appropriately adjust BMR with regard to these FFM fluctuations. A common method of adjusting for FFM changes is by dividing TEE by FFM.

Compared with the GH treated KS group, the TEE/FFM was remarkably higher (*P* <0.01) with regard to the baseline of the control group. So, the energy expenditure still was higher in the control group versus the GH treated KS group. Figure 1 shows the TEE/FFM before the start of therapy and during 6-weeks GH treatment. All participants improved the TEE/FFM during treatment. Figure 2 displays the TEE/FFM in the KS group against the control group. Even after six weeks of GH treatment (Table 2 and 3), the control group showed a higher basal TEE 8.46 (±1.43) MJ than the KS children. This difference was significant (<0.05). There was no difference between the KS children who were obese, had short stature or low GH stimulation tests.Body Mass Index and Fat Mass

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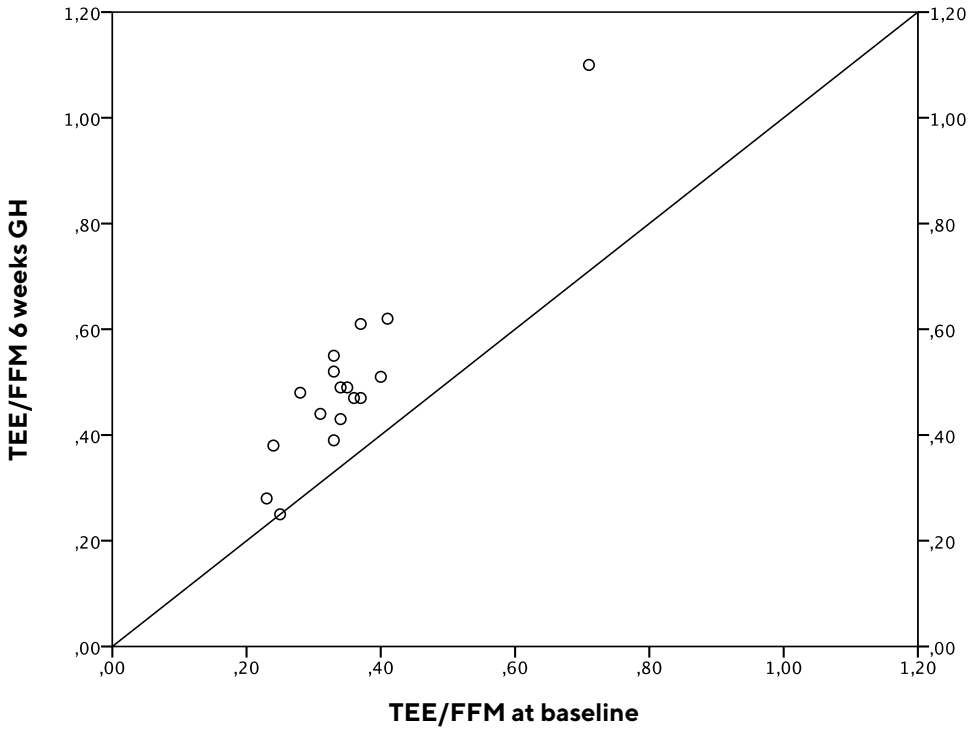

Figure 1. TEE/FFM in Kabuki syndrome children before and after 6 weeks of GH treatment. All participants' showed improvement in TEE.

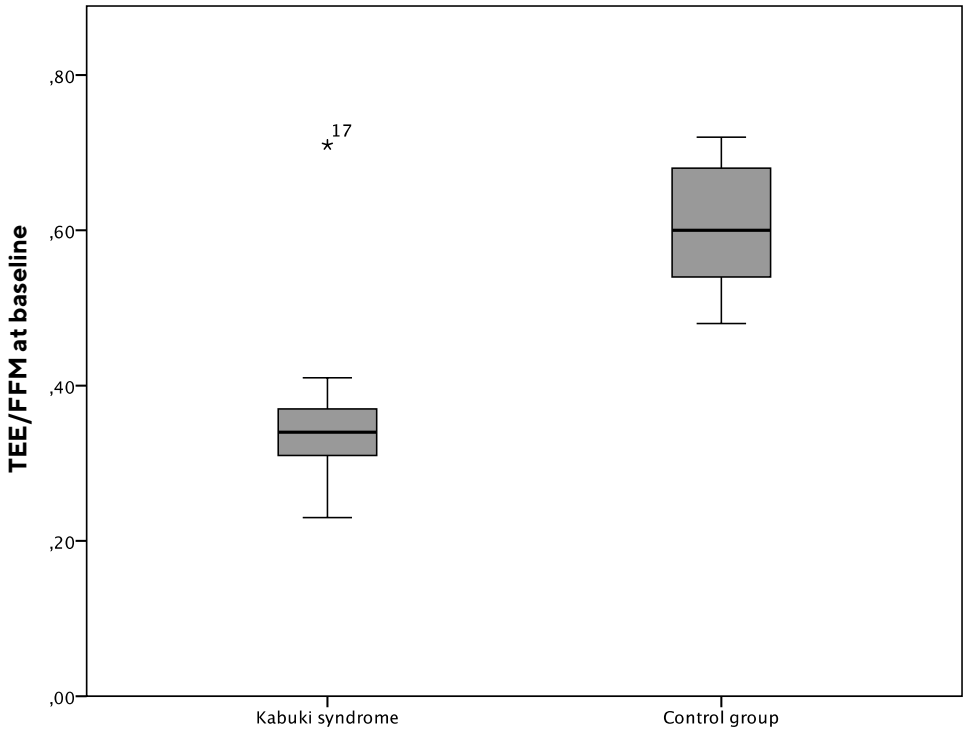

Figure 2. TEE/FFM in Kabuki syndrome children versus healthy control group children. The difference between the groups is significant.

Basal Metabolic Rate (BMR)

Table 3 shows that the BMR also increased significantly (*P*<0.0001) in the course of GH treatment. This ranged from 4.05 (±0.90) MJ before the start to 4.73 (±1.03) MJ after the 6-weeks of GH treatment. The children with a reduced GH stimulation test, obesity and *KMT2D* mutation showed the largest increase in BMR. Also, when the resting energy expenditure was divided by fat free mass (BMR/FFM), there was a significant increase (P=0.02). The BMR was significantly higher in the control group compared to the KS group at baseline, which benefited the control group (Figure 3).

Fat Free Mass (FFM)

To determine whether the fat free mass (FFM) improved during GH treatment in the KS group, the DLW data were used to calculate the body composition. The FFM increased significantly from 15.9 (±6.43) kg to 17.1 (±5.79) kg ($P = 0.01$) during GH treatment (table 3). There was a large difference between the two mutations. The mean Δ FFM for the *KMT2D* mutation was 1.75 (±0.91); the *KDM6A* mutation had a mean of 0.13 (±0.22). No difference was found after 6-weeks of GH treatment regarding the percentage of FFM (FFM%) to weight. There was also no difference between the control group and the KS group at baseline.

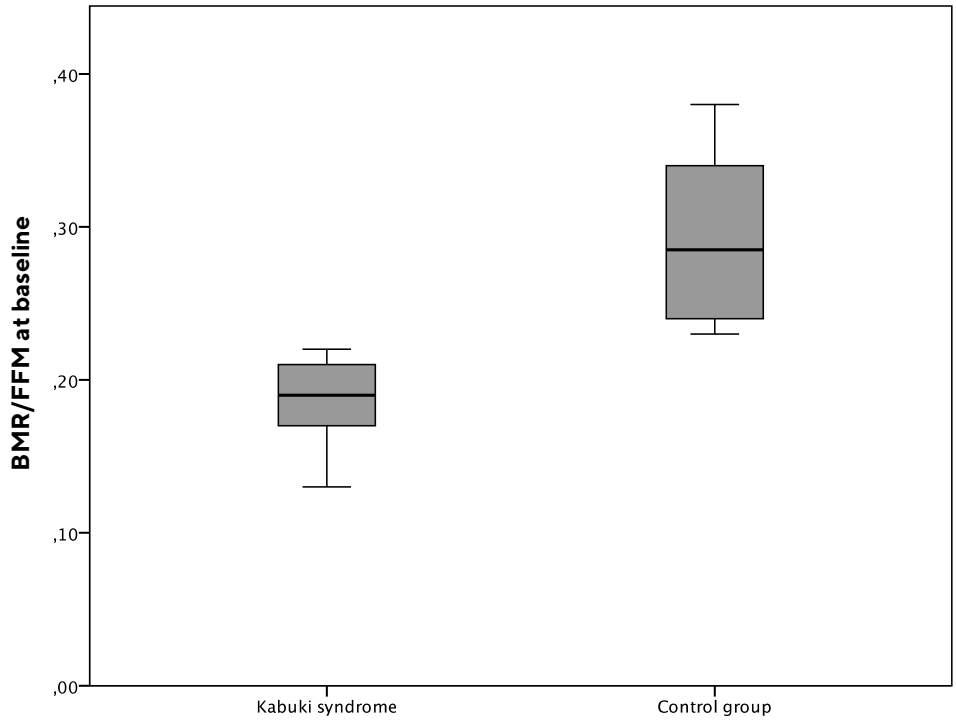

Figure 3. BMR/FFM in Kabuki syndrome children versus healthy control group children. The difference between the groups is significant.

Physical Activity Level (PAL)

The physical activity level (PAL) was calculated by TEE/BMR and showed a significant increase (P <0.01) from 1.25 (±0.26) before the start of GH treatment to 1.66 (±0.39) during treatment for the entire KS study group (Table 3). There was a strong correlation between TEE and BMR at baseline (R^2 = 0.76). However, in 4 children the PAL was less than 1. Since BMR is a component of TEE, this value indicates a measurement error. Even after removing these children from the calculation, the difference still is significant at baseline of 1.36 (±0.17) to 1.70 (±0.37) (P <0.01) during treatment.

When compared with the control group, there was a significantly higher PAL in the control group 2.09 (±0.18) at baseline and during GH treatment (P <0.03) alongside the KS group.

Table 3. Baseline data (mean ± SD) and the data during rhGH treatment of all 18 subjects with KS.

	Pre-treatment	One-year treatment	*P*-value
Chronological age	6.86 ± 2.07	7.97 ± 2.13	
Height SDS	-2.40 ± 1.88	-1.69 ± 1.94	<0.01*
Height Velocity (cm/y)	6.55 ± 2.66	9.66 ± 2.04	<0.01*
Height Velocity SDS	0.29 ± 2.43	2.80 ± 1.56	<0.01*
Parent-adjusted Height SDS	-2.07 ± 1.66	-1.35 ± 1.61	<0.01*
Weight SDS	-1.34 ± 2.88	-1.16 ± 2.62	0.47
BMI SDS	0.56 ± 1.79	0.19 ± 1.41	0.06

	Pre-treatment	Six weeks treatment	*P*-value
TEE (MJ)	5.19 ± 2.00	7.94 ± 2.76	<0.01*
TEE/FFM	0.35 ± 0.11	0.50 ± 0.18	<0.01*
BMR (MJ)	4.05 ± 0.90	4.73 ± 1.03	<0.01*
BMR/FFM	0.19 ± 0.03	0.20 ± 0.38	0.04*
FFM (kg)	15.91 ± 6.43	17.10 ± 5.79	0.01*
%FFM	74.4 ± 10.9	73.12 ± 22.3	0.08
TBW	12.23 ± 4.88	13.13 ± 4.39	0.02*
PAL	1.25 ± 0.26	1.66 ± 0.37	<0.01*
TracmorD (counts/min)	3124 ± 827	3251 ± 916	0.32

SDS = standard deviation score, BMI = body mass index, TEE = total energy expenditure, BMR = basal metabolic rate, FFM = fat free mass, TBW = total body water, PAL = physical activity level, TracmorD = DirectLife activity monitor output, * = statistical significant.

Physical activity with the Accelerometer

We also determined physical activity levels with an accelerometer (Tracmor$_D$). Since there was a large variability in wearing time, the following cut-off values were used: a minimum wearing time of 10 hours a day for at least 3 or more days. The children that met these cut-off values had a mean wearing time of 8.5 days. Although not significant (table 3), GH treatment resulted in increased physical activity in 6 out of 10 participants, as measured from 3124 (±827) counts/min to 3251 (±916) count

DISCUSSION

GH treatment is known to benefit body composition and energy expenditure not only in GH deficient children but also in patients with Prader-Willi, Turner or Silver Russell syndrome (22,23). However, little information exists concerning the effects in KS. This is the first study describing body composition and energy expenditure in KS children before and during treatment with rhGH. Already after six weeks of GH treatment, we found significant increases in TEE/FFM, BMR/FFM, PAL and FFM. These effects are important in the prevention of obesity as often seen in Kabuki children.

Metabolic studies with GH treatment have shown several positive anabolic effects. Specifically, an increase in energy expenditure was visible within 48 hours after start of treatment, reaching a maximum effect within two weeks (24). This results from an increase in metabolic activity of lean mass due to improved protein turnover. However, there have been no previous reports concerning energy expenditure and fat free mass in KS patients before and during GH treatment. The present study demonstrates that before treatment there was a significant difference in TEE and PAL between the short stature control group and the KS children while there was no difference in height SDS and weight SDS. Moreover, the difference in TEE and PAL became less but was still significantly different after 6 weeks of GH treatment in the KS group compared to the short stature group. Weight gain is largely based on the balance of two factors: food intake and energy expenditure. Several physiological mechanisms regulate this process (25). Only a small disturbance, or imbalance, is sufficient to cause obesity in the long-term (26). Of the daily energy expenditure, BMR is the largest component accounting for 60-70% (27) of TEE. Therefore, the ability to estimate

BMR is integral to assessing the risk of obesity. As several previous studies revealed a positive relationship between energy expenditure and adiposity, the difference between the two study groups in energy expenditure could explain the obesity attached to KS later in life. Since the control group had a higher PAL, they must be more active than the KS children. The higher TEE also lends support to this premise.

Patients with GHD have an adverse body composition characterized by increased visceral fat mass and reduced lean body mass. Prader-Willi syndrome patients are also known for their increased FM and reduced FFM or lean body mass, resembling persons with GHD (28,29). Several previous studies indicate that GH treatment is beneficial for children with PWS in terms of linear growth, body composition, physical strength, and resting energy expenditure (30). In our group of KS children, only four out of the eighteen were obese with a BMI above 2 SDS. This can be explained by the fact that our population consisted only of prepubertal children and that the problem of obesity arises mainly during adolescence in KS. All our subjects showed a significant decrease in BMI during GH treatment. Remarkably, the FM% decreased in the non-obese group while that of the obese group increased. Even though the weight loss SDS in the obese group was evidently higher (+1.6 SDS difference), there was no significant difference in energy expenditure between the groups. Correcting BMR and TEE for FFM, both parameters still improved significantly after 6 weeks of GH treatment.

Previously, we published a positive effect of GH treatment on linear growth in KS children (31). Gregory et al. showed that early changes in BMR and TEE in response to GH treatment may predict the long term effects on growth (14). A study by Hoos et al. revealed the same results after six weeks of GH treatment (15). Our study observed similar metabolic changes, but there was no significant correlation with the response on catch-up growth and TEE. However, FFM, TBW, and PAL measured with BMR/TEE showed a significant relation with an increased linear growth of more than 0.7 SDS height.

GH treatment in GHD adults have shown increased physical activity (32). To get more insight into the physical activity of our patients, the accelerometer was used. Accelerometer output is used to estimate activity energy expenditure (AEE). Moreover, accelerometers' output is linearly related to the physical activity level (33). We used the Tracmor$_D$ as accelerometer, an instrument whose accuracy is established (34). In this study, there was no correlation between the

PAL measured with the DLW method versus the accelerometer. It is known that the accuracy of the Tracmor$_D$ relies on the wearing time, which turned out to be a challenge for our population. However, 10 out of the 18 subjects carried the accelerometer adequately, of whom only six KS subjects showed an increase in physical activity. A reasonable supposition is that the energy expended by these subjects was invested in catch-up growth, not a more active lifestyle as might be indicative of an adult population.
The DLW method is the 'golden standard' for measuring energy expenditure in adults and children. However, in 4 children the PAL was less than 1. PAL can be calculated as TEE/BMR. Since BMR is a component of TEE, PAL can never be smaller than one. Therefore, we looked at the correlation between TEE and BMR, and those were significantly related before and after 6-weeks of GH treatment. As all four children with an abnormal PAL were not potty dry, we theorize that the urine collection (with cotton balls) for the measurements of TEE was not accurately executed. Nevertheless, when we performed the calculation without the four children, the results remained the same.

CONCLUSION

In conclusion, we have demonstrated that the effects of GH treatment have been beneficial on total energy expenditure, basal energy expenditure, body composition, and increase in free fat mass after six weeks of treatment in KS children. Furthermore, there was a relation with the long-term growth response to GH treatment and the PAL, all signs of improved metabolic changes in KS children treated with GH. Therefore, GH treatment not only shows catch-up growth but also improves anabolic status in KS children. These are important observations with respect to the considerable health risks faced by adolescent KS patients (5).

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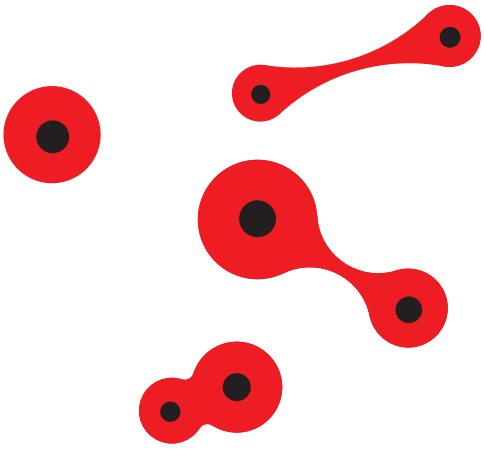

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CARDIOVASCULAIR MARKERS IN KABUKI SYNDROME CHILDEREN BEFORE AND AFTER 12-MONTH GROWTH HORMONE TREATMENT

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ABSTRACT

Kabuki Syndrome (KS) is a genetic disorder with characteristic facial dysmorphisms, short stature, hypertension, and obesity later in life. The aim of this study was to evaluate cardiovascular markers before and during GH-treatment in KS children.

This prospective study included 18 children whose KS was genetically established. Each KS subject received GH at an average dose of 1 mg/m^2/day (equivalent to 35 µg/kg/day) for a period of 12 months. Several measurements were performed before and during treatment: anthropometry, glucose metabolism, lipid profile, markers for endothelial function, and low-grade inflammation.

Baseline metabolic profiles showed no cardiometabolic abnormalities in these children. Although several children were obese, there were no signs of the metabolic syndrome. During GH treatment, both serum LDL cholesterol concentrations (2.16 to 1.94 mmol/l, P=0.01) and the inflammation marker IL-8 (5.45 to 3.74 pg/mL, P<0.01) decreased significantly. While other endothelial function markers were stable, only VCAM-1 concentrations increased (1084 to 1161 pg/mL, P<0.01) during GH therapy. The apolipoproteins (Apo B-100, C2 and C3) showed a reduction after 6 months treatment, but after 12 months this was no longer significant. Furthermore, BMI and waist-circumference improved during treatment. There were no signs of hypertension.

This is the first study demonstrating that GH treatment in KS children is a safe therapy that positively influences lipid and (apo) lipoprotein profiles. Furthermore, at baseline and during GH therapy there were no signs of the metabolic syndrome.

INTRODUCTION

Kabuki syndrome (KS, OMIM 147920) is a multiple anomaly syndrome first described in 1981 separately by Niikawa and Kuroki in Japan (1,2). The estimated incidence of Kabuki syndrome is around 1 in 32.000 of the Japanese population (3). Patients are mainly characterized by facial features, including long palpebral fissures, everted lower eyelids, arched eyebrows with sparse or dispersed lateral one-third, a depressed nasal tip and prominent ears (1). A key feature of Kabuki syndrome is short stature, a consequence of postnatal growth retardation (4). Furthermore, mild to moderate mental retardation, joint laxity, hypotonia, cardiovascular and urogenital anomalies, short fifth fingers, dermatoglyphic abnormalities, hypodontia, and fingertip pads are diversely described characteristics in Kabuki syndrome (3,5,6). Mutations in two genes have been reported
to be responsible for KS: lysine-specific methyltransferase 2D (*KMT2D*; OMIM 602113), the major gene involved in about 70% of patients, 4,5 and lysine-specific demethylase 6A (*KDM6A*; OMIM 159555), occurring in less than 5% of patients. Mutations in two genes have been reported as responsible for KS. The major gene is the *KMT2D* gene (also known as *MLL2* gene, OMIM 602113) (7). In approximately 70% of clinically diagnosed Kabuki syndrome patients, a *KMT2D* mutation has been identified. The second gene is the *KDM6A* gene (OMIM, 159555) which occurs in about 9-13% of Kabuki syndrome patients (8).
Besides the aforementioned features, obesity and hypertension are important characteristics of Kabuki syndrome, as they are in Turner syndrome and Prader-Willi syndrome (PWS) children. The literature indicates positive long-term results of growth hormone (GH) treatment on linear growth and improvement on metabolic risk markers for these children (9). It is known that GH has effects on bone metabolism, insulin sensitivity, protein, and lipid metabolism, as well as body composition in adults (10,11). In children, these metabolic effects can be positive, but adverse effects are also known.
Therefore, the aims of this study were: 1) to evaluate possible aberrations in anthropometric variables, biochemical parameters and cardiovascular markers in KS children at baseline; 2) to evaluate whether there are already signs of the metabolic syndrome, low-grade inflammation and endothelial dysfunction before clinical symptoms become manifest; 3) to evaluate if one year of GH treatment influenced these parameters in KS children.

METHODS

Study design

This study was performed as an open-label, prospective, non-randomized study of KS children (trial registration: NTR4722) at the Maastricht University Medical Centre Maastricht, The Netherlands. Participants were recruited between 2012-2014. The Medical Ethics Committee of the Maastricht University Medical Centre provided ethical approval of the study. Informed consent was obtained from the subjects' parents before the start of the study. Eighteen patients diagnosed with Kabuki syndrome and a proven mutation for KS (*KMT2D* or *KDM6A*) were included. Subjects with diabetes mellitus, an extremely low caloric intake, a previous or active malignancy, growth hormone therapy, and children with signs of puberty were excluded.

All subjects received biosynthetic human GH (Genotropin, Pfizer, New York, NY), administered sc once daily at bedtime. The average dose was 1 mg/m^2/day GH (equivalent to 35 µg/kg/day) for 12 months. The initial dose of GH was altered based on IGF-I levels and/or growth hormone response. Prior to any treatment, all KS subjects underwent a previously described arginine and/or clonidine GH stimulation test, to rule out GH deficiency (12).

Methods

Upon entering the study and at three-month intervals during the first year of treatment, all subjects underwent assessment of height, weight and waist circumference (WC). Measurements were performed according to internationally accepted methods (13,14). Body mass index (BMI) was calculated as follows: Weight (kg)/(Height (m))2. Height, weight and BMI were expressed in standard deviation scores (SDS), using the reference values of The Netherlands (TNO The Netherlands 2010, with the 2001-2013 Growth Analyser BV, Rotterdam, The Netherlands). The WC was measured at the part of the trunk located midway between the lower costal margin (bottom of lower rib) and the iliac crest (top of pelvic bone) with the person in standing position, with feet about 25-30 cm apart. The observer stood beside the subject, fitting the tape snugly, yet without compressing any underlying soft tissues. The circumference was measured to the nearest 0.5 cm at the end of a normal expiration. The waist to height ratio (WHtR) was also calculated as a screening tool for the prediction

of cardiovascular disease.
The brachial systolic (SBP) and diastolic blood pressure (DBP) were determined three times in the sitting position using a cuff size corresponding to arm size. An automated device (Dinamap, Pro 300, GE Healthcare, UK) was employed for these readings. SBP and DBP were expressed in percentiles referencing age- and sex-specific values (15).

Fasting blood samples were collected before the start of GH treatment and after six and twelve months of GH treatment. Serum and plasma for IGF-I, glucose metabolism and lipid profile were separated after centrifugation (3000 × *g* for 15 min at 4 °C) and were stored at −80°C until the assays were performed. Plasma tubes were used for the cardiovascular markers (1300 x g for 15 min at 4°C within 30 min after blood sampling). After centrifugation, plasma samples were immediately portioned into aliquots and stored at -80°C until analyses at the end of the intervention study. For the ADMA determination, fasting blood samples were collected using lithium-heparin tubes (4000 rpm, 10 min, 4°C). For ADMA analysis, heparin plasma was deproteinized with 6 mg of solid 5-sulfosalicylic acid (SSA; Sigma, St. Louis, MO, USA) per 100 µL plasma, and was kept frozen at -80°C until analysis.

IGF-I analysis

Serum IGF-I was measured in venous blood samples with chemiluminiscence method in the IDS-iSYS Multi-Discipline Automated system by Immunodiagnostic Systems Holding, UK, previously described (12).

Glucose metabolism

The fasting plasma glucose concentrations were measured in venous blood samples with the enzymatic hexokinase method using the Roche Cobas 8000 Molecular Analyzer (Roche Diagnostics, Rotkreuz, Switzerland). Serum insulin was quantified on the Immulite 2000 Xpi Immunoassay system (Siemens Healthcare Diagnostics, Munich, Germany). Homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the HOMA-IR calculation = ((fasting insulin x fasting glucose)/22.5) (16). A HOMA-IR value > 3 was chosen as an indicator of reduced insulin sensitivity. Plasma HbA1c was determined by ion-exchange high-performance liquid chromatography (VARIANT II, Bio-Rad, Hercules, USA).

Lipid profile

Serum total cholesterol was measured using the Cholesterol Gen. 2 set as reagents (Roche, Cat. no. 05168538). The color intensity of the formed quinone-imine complex was an indirect photometric measurement of the cholesterol concentration. Serum high-density lipoprotein (HDL) cholesterol concentrations were measured using HDL-Cholesterol plus third generation as reagents. Triglycerides (TG) concentrations were measured using standardized triglycerides set (Roche, Cat. no.05171407) as reagents. Low-density lipoprotein (LDL) cholesterol concentrations were calculated using the 'Friedewald-formula': 'total cholesterol' - ('triglycerides' x 0.453) + 'HDL-cholesterol'). If triglyceride concentration was > 4,5 mmol/L and/or total cholesterol < 1,3 mmol/L, the LDL concentration was not calculated, but instead measured on a ABX Pentra C400 (Horiba Medical, Kyoto, Japan).
Apolipoprotein A-I (apoA-I) and apolipoprotein B100 (apoB100) were measured in fasting serum samples using an immunoturbidimetric reaction (Horiba ABX, Montpellier Cedex, France). Serum Apo-B48 concentrations were measured with a sandwich ELISA kit (Shibayagi) containing a specific anti-Apo-B48 antibody, as described previously (17). Serum Apo-C2 and Apo-C3 were measured with an ELISA kit (Randox). All samples from one subject were analysed within one run at the end of the study.

Markers for endothelial function and chronic low-grade inflammation

Fasting EDTA-plasma samples were used for measurement of biomarkers for low-grade inflammation [IL-6, IL-8, TNF-α ρC-reactive protein (CRP), serum amyloid A (SAA)] and endothelial function [soluble vascular cell adhesion molecule (sVCAM), soluble intercellular adhesion molecule 1 (sICAM-1), soluble endothelial selectin (sE-selectin and sP-selectin)] by using a multi array detection system based on electrochemiluminescence technology (SECTOR Imager 2400; Meso Scale Discovery). All samples from one subject were analyzed within one run at the end of the study.

Plasma Amino Acid and Dimethylarginine (ADMA) Analysis

Concentration of ADMA was determined in plasma using the ultra-performance liquid chromatography (UPLC) separation module coupled to an electrospray ionization tandem mass spectrometry (ESI-MS/MS, Quattro Premier, Walters, Etten-Leur, The Netherlands) by the Department of Biomedical Genetics at

Maastricht University as described by Waterval et al. (18).

Statistics

Results for continuous variables were expressed as mean (±SDS); when variables were not normally distributed, as median (interquartile range). Binominal data are shown numerically (n). Normal distribution was determined using the Kolmogorov-Smirnov test. Between-group differences in comparing metabolic risk markers at different time points (t = 0, 6,12 months) were, depending on normal distribution, assessed either through the Student's t-test for paired samples or the Wilcoxon rank test for paired samples. Spearman correlation analysis was used to determine correlations between variables. All statistical analyses were performed using the software SPSS version 22 for Mac (SPSS Inc, Chicago, IL, USA). A P-value of <0.05 was considered significant.

RESULTS

Baseline characteristics of the subjects are given in Table 1. In total, 18 KS children (9 females and 9 males) with ages ranging from 3.8 to 10.1 years (mean 6.8 ± 2.1 years) were included. Fifteen of the KS subjects had a *KMT2D* gene mutation and three had a *KDM6A* gene mutation. All patients remained prepubertal during the study period. Thus, sex hormones did not influence the parameters. At baseline, no significant differences were identified between the boys and girls.

Anthropometry

Baseline height SDS was -2.40 ± 1.68 and after one-year GH treatment this improved significantly to -1.69 ± 1.94 (P<0.01). The weight SDS at start was -1.34 ± 2.88 and after one-year therapy -1.16 ± 2.62 (P=0.47).

Four out of the 18 KS children were obese with a BMI above the 2 SDS. Their mean BMI was 3.01 SDS (ranging from 4.31 to 2.05) before starting treatment, with a reduction of almost statistical significance after one year to a mean of 1.97 SDS (range 2.61 to 0.78) (P=0.06). Overall, BMI reduced from 0.56 to 0.19 SDS (P=0.06). Interestingly, there was a nearly significant association between the decrease in BMI and the increase in IGF-I concentrations after one-year GH treatment (P=0.06).

Waist circumference was significantly (P<0.01) higher in the obese KS children, with a mean of -0.83 ± 1.55 for the non-obese KS children and for the obese

group 1.92 ± 2.66 at start of GH treatment. After one-year of therapy this was -0.60 ± 1.47 and 1.60 ± 1.65 respectively. This still marked a significant difference, despite the subjects' reduction in BMI. The mean WHtR at baseline was 0.50 ± 0.06 and decreased to 0.47 ± 0.04 after one year of GH treatment (P<0.01). For

Table 1. Baseline data and after one year of GH treatment of all 18 subjects with KS (mean ± SDS)

Subject		Male	Female	All
Sex		9	9	18
Mutation	*KMT2D*	8	7	15
	KDM6A	1	2	3
Age (yr)	Baseline	7.45 ± 2.33	6.37± 1.83	6.86 ± 2.07
Birth	Length	-0.60 ± 1.26	0.10 ± 2.20	-0.25 ± 1.78
	Weight	0.29 ± 1.23	0.08 ± 1.70	0.19 ± 1.44
Height (SDS)	Baseline	-2.61 ± 2.23	-2.18 ± 1.55	-2.40 ± 1.88
	One-year GH	-1.88 ± 2.29*	-1.51 ± 1.63*	-1.69 ± 1.94*
BMI (SDS)	Baseline	0.64 ± 2.01	0.48 ± 1.65	0.56 ± 1.79
	One-year GH	-0.01 ± 1.45	0.38 ± 1.42	0.19 ± 1.41
IGF-I (SDS)	Baseline	-0.64 ± 0.63	-0.75 ± 1.42	-0.70 ± 1.07
	One-year GH	1.15 ± 1.01*	1.66 ± 0.79*	1.41 ± 0.91*
WC (SDS)	Baseline	0.76 ± 2.55	-0.85 ± 1.64	-0.10 ± 2.19
	One-year GH	0.09 ± 2.03	-0.32 ± 1.48	-0.12 ± 1.73
WCHt ratio	Baseline	0.50 ± 0.07	0.51 ± 0.05	0.50 ± 0.06
	One-year GH	0.46 ± 0.04	0.48 ± 0.05*	0.47 ± 0.04*
P_{BPsys} (%)	Baseline	73 ± 24	80 ± 24	76 ± 23
	One-year GH	66 ± 25	60 ± 33	60 ± 25
P_{BPdia} (%)	Baseline	65 ± 26	56 ± 24	63 ± 29
	One-year GH	66 ± 19	56 ± 21	61 ± 20

Abbreviations: GH, growth hormone; SDS, standard deviation score; WC, waist circumference; WCHt, waist circumference/ Height ratio; P, Percentile; BP, bloodpressure; sys, systolic; dia, diastolic. * significant (*P*<0.05) between baseline and one-year GH.

Blood pressure

Blood pressure values were all within the normal range, before and after one year of GH treatment. There was no significant difference between boys and girls, and there was no association with BMI and WC. Furthermore, GH treatment did not change blood pressure.

Glucose metabolism

At baseline, fasting glucose, insulin, and HbA1c concentrations as well as the HOMA-IR, were all within the normal range. After 12 months of GH treatment there were no significant changes in HbA1c, glucose, insulin and HOMA-IR. However, there was a trend towards the development of insulin resistance after 12 months (Table 2) since fasting glucose as well as insulin concentrations slightly increased. In terms of disaggregating effects between genders, only girls presented a significant increase in fasting glucose concentrations after one year of GH treatment (P=0.02). Effects on parameters reflecting glucose metabolism did not relate to baseline BMI and changes in BMI.

Table 2 - Cardiovascular and metabolic parameters at baseline and after 6 months and 12 months of growth hormone treatment.

	baseline		6 months GH		12 months			
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	*P* value[a]	*P* value[b]
Height SDS	18	-2.40 ± 1.86	18	-1.94 ± 1.92	18	-1.69 ± 1.94	0.549	0.000*
Weight SDS	18	-1.34 ± 2.88	18	-1.44 ± 2.93	18	-1.16 ± 2.62	0.924	0.470
BMI SDS	18	0.56 ± 1.79	18	0.12 ± 1.82	18	0.19 ± 1.41	0.415	0.057
WC SDS	15	-0.10 ± 2.19	18	0.00 ± 1.86	18	-0.12 ± 1.73	0.190	0.839
SBP percentile	18	76 ± 23	14	68 ± 31	18	63 ± 29	0.477	0.148
DBP percentile	18	60 ± 25	14	52 ± 22	18	61 ± 20	0.431	0.841
Glucose (3.1-7.8 mmol/L)	18	4.21 ± 0.73	15	4.63 ± 0.71	18	4.60 ± 0.72	0.037*	0.065
Insulin (12-150 pmol/L)	18	19.30 ± 16.22	17	44.34 ± 51.25	17	39.70 ± 54.50	0.056	0.161
HOMA-IR	18	0.55 ± 0.51	14	1.61 ± 1.93	17	1.24 ± 1.81	0.059	0.147
Hb1Ac (25-44 mmol/mol)	18	29.67 ± 5.59	18	30.83 ± 5.55	18	30.83 ± 5.07	0.063	0.104
TC (3-5.5 mmol/L)	18	3.96 ± 0.81	17	3.71 ± 0.67	18	3.77 ± 0.71	0.042*	0.076
HDL (>0.9 mmol/L)	18	1.53 ± 0.56	17	1.49 ± 0.54	18	1.56 ± 0.48	0.413	0.816
LDL (3.5-4.4 mmol/L)	18	2.16 ± 0.73	17	1.83 ± 0.57	18	1.94 ± 0.67	0.013*	0.014*
TG (0.4-1.6 mmol/L)	18	0.61 ± 0.17	17	0.85 ± 0.64	18	0.60 ± 0.24	0.145	0.984
ADMA (0.42-1.10 µM)	15	0.63 ± 0.13	18	0.58 ± 0.09	14	0.56 ± 0.12	0.088	0.118
E-selectin (ng/mL)	17	26.6 ± 10.2	17	28.7 ± 11.7	18	24.8 ± 7.9	0.063	0.322
P-selectin (ng/mL)	17	53.9 ± 17.4	17	57.1 ± 31.6	18	48.9 ± 23.6	0.815	0.390
ICAM-1 (pg/mL)	17	603 ± 101	17	628 ± 135	18	594 ± 102	0.066	0.551
ICAM-3 (ng/mL)	17	0.88 ± 0.29	17	0.85 ± 0.22	18	0.74 ± 0.32	0.420	0.071
VCAM-1 (pg/mL)	17	1084 ± 166	17	1209 ± 229	18	1161 ± 194	0.001*	0.006*
MCP-1 (pg/mL)	17	155 ± 75	17	140 ± 40	18	122 ± 30	0.363	0.076
Il-6 (pg/mL)	16	0.99 ± 0.93	17	0.92 ± 0.59	18	0.81 ± 0.56	0.952	0.469
Il-8 (pg/mL)	17	5.45 ± 2.39	17	4.05 ± 1.61	17	3.74 ± 1.06	0.006*	0.008*
TNF-α (pg/mL)	17	2.80 ± 0.60	17	2.77 ± 0.68	18	2.51 ± 0.58	1.000	0.329
TNFR-1 (pg/mL)	17	3002 ± 1349	17	3329 ± 1822	18	3129 ± 1965	0.168	0.622
TNFR-2 (pg/mL)	17	5064 ± 1783	17	5311 ± 1979	18	4909 ± 1798	0.416	0.631
SAA (pg/mL)	17	3764 ± 5552	17	2597 ± 2362	18	2897 ± 4776	0.513	0.668
CRP (fg/mL)	17	178 ± 183	17	128 ± 141	18	164 ± 296	0.414	0.828
TM (ng/mL)	17	6.0 ± 1.8	17	6.7 ± 2.0	18	6.3 ± 2.2	0.013*	0.444
Apo A-1 (g/L)	17	1.17 ± 0.30	17	1.18 ± 0.27	18	1.21 ± 0.23	0.326	0.238
Apo B-100 (g/L)	17	0.72 ± 0.18	17	0.67 ± 0.18	18	0.68 ± 0.15	0.042*	0.130
Apo C-2 (mg/mL)	17	3.01 ± 1.36	17	3.77 ± 1.42	18	3.45 ± 1.38	0.004*	0.064
Apo C-3 (mg/mL)	17	6.37 ± 2.08	17	7.32 ± 2.51	18	6.88 ± 2.23	0.031*	0.087
Apo B-48 (ng/mL)	17	10408 ± 4135	16	11533 ± 4679	18	10537 ± 4214	0.375	0.701

← Abbreviations: SDS, standard deviation score; n, number; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment-insulin resistance; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, triglycerides; ADMA, plasma amino acid and dimethylarginine; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; MCP, Monocyte Chemo-attractant Protein; IL, interleukin; TNF, tumor necrosis factor; TNFR, TNF-receptor; SAA, serum amyloid A protein; CRP, C-reactive protein; TM, thrombomodulin; Apo, Apolipoprotein.
P value[a and b] compared to baseline values.

Lipid and lipoprotein metabolism

At baseline, all parameters related to lipid and lipoprotein metabolism were within normal ranges and also remained normal during GH treatment after 6 and 12 months. Serum lipid and lipoprotein concentrations at baseline and changes after one year were not correlated with BMI and WC. More specifically, serum LDL cholesterol levels significantly decreased after one year of GH treatment. In line with the effects on serum LDL concentrations, Apo B-100 concentrations showed a significant decrease in the first 6-month of treatment. Furthermore, the Apo-B/Apo-A1 ratio was significantly reduced from 0.66 to 0.58 (P=0.03) after one year. However, the apolipoprotein-B48 (Apo-B48) concentrations, a marker for the number of intestine derived chylomicrons, remained unchanged during GH treatment. The concentrations of both Apolipoprotein-C2 (Apo-C2) and Apolipoprotein-C3 (Apo-C3) as respective activator and inhibitor of lipoprotein lipase increased significantly during the first 6 months of treatment, slightly decreased in the second period of 6 months, but remained not significant elevated after 12 months relative to baseline values. The ratio Apo C3/C2 remained constant during GH treatment and there were no associations with serum TG concentrations. However, there was a significant association between Apo-C2 and TG (P=0.04).

Markers for endothelial function and chronic low-grade inflammation

Endothelial function was evaluated by measuring intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-3 (ICAM-3), E and A-selectin, and Monocyte Chemo-attractant Protein-1 (MCP-1) levels. With the exception of VCAM-1, all of these remained stable during GH treatment. VCAM-1 concentrations significantly increased from 1084 to 1161 pg/mL (P<0.01). The thrombomodulin (TM) notably increased during the first 6 months of treatment with GH, but this was no longer the case at 12 months.
The presence of a low-grade inflammation state was evaluated by measurements

of TNF-α αTNF receptors, Il-6, Il-8, CRP, and SAA concentrations. Though no changes in these parameters were found before and after one-year GH treatment, except for the chemokine Il-8, which decreased significantly from 5.45 to 3.74 pg/ml (P<0.01).

DISCUSSION

Reports regarding metabolic risk profiles in KS mainly describe obesity and the prevalence of hypertension in adolescence and at later life stages. This is the first report describing cardiovascular and metabolic risk profiles in children with KS as well as the specific effects of growth hormone treatment on this risk profile. We found that one year of GH treatment reduced BMI, WC and WHtR in combination with an improved lipid and lipoprotein profile. Because KS patients are predisposed to an elevated risk for obesity and the metabolic syndrome in adulthood, these findings are clinically relevant and significant.

We reported earlier that GH treatment in KS children exerted a positive effect on linear growth (19). The height SDS increased from -2.40 at baseline to -1.69 after one year of treatment for the entire study group. This effect was stronger in subjects with a *KMT2D* versus *KDM6A* mutation. The *KMT2D* height SDS increased by 0.74 SDS and for the *KDM6A* by 0.54 SDS, both statistically significant results. Also, the IGF-I levels revealed significant increase during GH therapy. In children, it is known that high IGF-I levels and treatment with GH are associated with increased insulin levels and correlated with growth response in some cases (20,21). Therefore, levels of fasting glucoses, insulin and HbA1c were measured, including the calculations of the HOMA-IR. The fasting glucose levels increased significantly during the first 6 months of GH treatment, but remained stable in the following 6 months. The same was true for the HbA1c, insulin levels and HOMA-IR, but this was not significant. There were no correlations with these parameters and catch-up growth. Though many studies cite evidence of adverse effects of GH on carbohydrate metabolism in line with our observation, these effects are generally regarded as of no clinical importance. Usually, insulin resistance is also reflected by an increase in circulating TG and decreased in HDL concentrations, which was not the case in our study. Hence, it is not possible to predict the net effect of GH on carbohydrate metabolism, and there is a risk of a negative influence on the cardiovascular markers. Therefore, further long-term study on the safety of GH treatment on carbohydrate metabolism in general, and

more specifically in KS, is needed.
Hypertension and dyslipidemia are typical components of the metabolic syndrome, as defined by elevated serum total and LDL cholesterol and/or triglycerides and decreased HDL (22). To our knowledge, no past research has reported on blood pressure measurements in Kabuki syndrome patients. At baseline and after 12 months of GH therapy the blood pressure remained normal, and there were no significant relations with BMI or WC. The same was true for the dyslipidemia. Given the risk to develop MS in KS patients it is especially relevant and noteworthy that the LDL cholesterol concentrations significantly decreased during GH treatment. Since also apoB100 concentrations were lowered, it can be suggested that not only of LDL cholesterol concentrations but also the number of LDL particles was reduced. A recent meta-analysis by Giagulli et al. revealed an overall reduction in LDL levels in GHD patients during GH treatment, which is also seen in our study (23). Whether this LDL reduction can be explained by a lower hepatic LDL production or a faster clearance of Apo-B containing lipoproteins cannot be concluded from our data. What can be found in literature are suggestions that GH induces peripheral and hepatic TG uptake by increasing lipoprotein lipase (LPL) and/or hepatic lipase (HL), and induces inhibition of intrahepatic lipase TG lipolysis storage (24). However, the primary molecular mechanism underlying the reduction in LDL remains to be clarified.
Only a few reports have mentioned levels of apolipoproteins during GH treatment (25,26). Apo-A1 is the major protein component of HDL in plasma, an anti-artherogenic lipoprotein (27). While Apo-B100 is the primary apolipoprotein of LDL. The ratio Apo-B100/Apo-A1 has been reported as a strong predictor for the risk of myocardial infarction (28). GH therapy in adult survivors of childhood acute lymphoblastic leukemia displayed significant positive changes in the ratio Apo-B100/Apo-A1, and consequent reduction in the prevalence of the MS (29). Yildiz et al. found the same positive results with long-term GH therapy in GHD children (30). Similarly, our study found a significant reduction of the Apo-B/Apo-A1 ratio in KS children after one year of GH treatment. This can be associated with a lower risk for cardiovascular disease. Moreover, TG concentrations are also an established CVD risk factor. Even so, we did not observe any changes in serum TG concentrations. This was somewhat unexpected since there was a significant increase in circulating Apo-C2 and C3 concentrations during GH treatment.
In general, Apo-C3 inhibits lipoprotein lipase and hepatic lipase. Various studies

indicate that elevations of Apo-C3 may predispose patients to non-alcoholic fatty liver disease (31). In GH studies, Apo-C3 increased significantly (32). Apo-C2 activates lipoprotein lipase, which reduces triglycerides, cholesterol and chylomicrons. As noted, both parameters increased significantly in the first six months of GH therapy in KS children, which might have translated into elevated TG concentrations. However, since the ratio C3/C3 remained stable during GH therapy, LPL activity probably also stayed constant, explaining its lack of effect on TG concentrations. However, this result is inconsistent with data suggesting that GH stimulates TG uptake by enhancing LPL expression (24). LPL activity is also relevant in the clearance of chylomicron particles and, if indeed LPL activity was unchanged, it might explain why Apo-B48 protein concentrations also did not change (33). Apo-B48, produced as a protein component of chylomicrons in the gut, is known to be the only specific marker of circulating chylomicrons (34). Growth hormone deficiency in adults is associated with an increase in visceral adiposity, adverse serum lipid profiles, vascular endothelial dysfunction, and reduced exercise capacity (35-37). Obesity is known to be associated with chronic inflammation of fatty tissue. Adipocytes, macrophages and endothelial cells, the three major cell types in adipose tissue, are all immunologically active. Endothelial cells are known to produce a whole range of cytokines, such as TNF-α and a broad array of chemokines, including IL-6 and IL-8 (38-40). While data suggest that GH plays an inhibitory role on TNF-α release, current studies are inconclusive (41). However, in PWS positive associations were found between TNF-α and body weight. Furthermore, GH treatment decreased the levels of the inflammatory markers, albeit not to a statistically significant level (42). We observed a similar trend with the reduction of TNF-α levels. TNF-α exerts its regulatory effects by binding one of two TNF receptors, TNFR-1 and TNRF-2. To our knowledge, these receptors have never been analyzed in GH studies. We have measured the TNFR-1 and TNRF-2 receptors before and during GH treatment in KS children, but there was no noticeable difference. Regarding the inflammatory markers, our results only displayed a significant reduction of IL-8. Rotter et al. described that IL-8 gene expression, among IL-6 and TNF-α was markedly increased in cells from non-obese but insulin-resistant subjects (43). In addition to TNF-α and IL-8, we have also studied hsCRP and IL-6, of which GH replacement is known to be beneficial in GHD patients. We found, however, no changes in hsCRP and Il-6 after one year of GH treatment. The same was true for the SAA. MCP-1 is produced mainly by macrophages and endothelial

cells. Similar to how E-selectin might play an important role in the development of vulnerable plaque, elevated MCP-1 concentrations have been shown to predict future cardiovascular risk. (44). Studies using pharmacological inhibitors suggested that there is a positive effect of GH on MCP-1 (45). In our study, MCP-1 levels indeed decreased during GH therapy, although not significantly.
ADMA is a competitive inhibitor of nitric oxide synthase, which is responsible for most vascular nitric oxide (NO) production. NO is an important mediator of vascular tone and structure in normally functioning endothelial cells. ADMA levels have been linked with age, blood pressure, glucose resistance and thickness of the carotid intima-media (46). A high prevalence of elevated plasma ADMA is observed in patients with hypercholesterolemia, hypertension, renal failure, diabetes mellitus, cardiovascular disease and many more other clinical disorders, even in apparently healthy persons. Andrade et al. studied ADMA levels in healthy children and made a biochemical profile for ADMA levels with a mean of 0.61 µM (0.42-1.10) (46). Our study group had the same mean level at baseline, and during GH treatment the levels even dropped a little bit. Therefore, we can conclude that our population of KS children has normal ADMA levels. Studies of endothelial function in GHD adults using biochemical methods revealed that 12 months of GH treatment led to a decrease of these biochemical parameters similar to healthy controls.
In the literature, a strong correlation exists between the observed changes in IGF-1 and these parameters (47). In patients with acromegaly serum, ICAM and VCAM levels are significantly higher compared to the control group (48). Also in our group, the VCAM-1 concentration was significantly elevated. The other parameters remained stable, although ICAM-3 concentrations even showed a trend toward lower values. Furthermore, GH therapy showed a tendency in significant correlation with improvement in the marker of TM. In conclusion, we can say that there are inconsistent outcomes regarding for the endothelial function using biochemical methods in KS children during GH replacement.
The fat distribution in KS is descripted in earlier studies as mainly truncal, which means mostly higher VAT (visceral adipose tissue). In our group of KS children, four out of the eighteen were obese, that is, with a BMI above 2 SDS. This low number is little because obesity is common during adolescence in KS, but our population consisted of only prepubertal children. However, there was a significant correlation between the BMI, WC and the WHtR in these children, so that confirms that the fat distribution in our children was truncal. Studies

demonstrated that GH treatment induced reductions in VAT and improved insulin sensitivity (49,50). Indeed, GH reduces the WC and WHtR in our cohort of children. Though the number is small, this sign is clinically important with regard to the prevention of truncal obesity and the risk of metabolic syndrome.

In conclusion, we demonstrated that, although several children were obese, there are no severe cardiometabolic abnormalities in KS children at baseline. There were no clear signs for the metabolic syndrome. Furthermore, after 12 months of GH treatment there were no undesirable effects on the cardiovascular risk markers, despite an elevation in VCAM-1 levels and slight signs of insulin resistance, of which the importance is not yet clarified. Meanwhile, serum lipid and lipoprotein profiles improved. Biomarkers for endothelial function and inflammation did not changes during therapy. Moreover, no important side effects were reported. Therefore, the results from this study demonstrate that GH treatment in KS children is a safe and effective means of promoting catch-up growth during one-year therapy.

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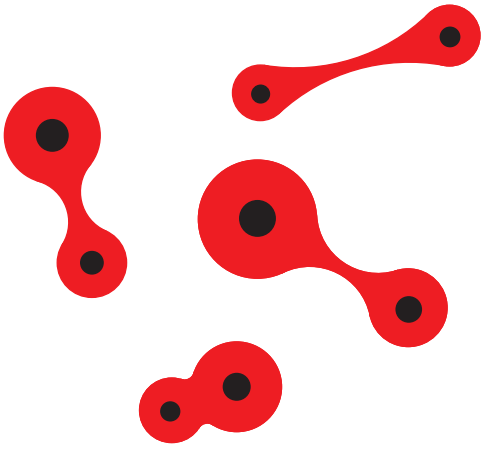
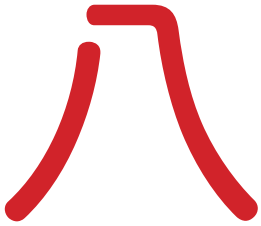


CHAPTER 8
JOINT LAXITY



HYPERMOBILITY IN INDUVIDUALS WITH KABUKI SYNDROME: THE EFFECT OF GROWTH HORMONE TREATMENT

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ABSTRACT

Kabuki syndrome (KS) is a multiple congenital malformation syndrome which has been described across all ethnic groups. Diagnostic criteria are based on dysmorphic features and a characteristic pattern of congenital anomalies. Most KS patients possess two genetic subtypes: *KMT2D*-associated, autosomal-dominant KS type 1 (KS1; OMIM 147920); and *KDM6A*-associated, X-linked-dominant KS type 2.

Generalized joint hypermobility is one feature of Kabuki syndrome, but its exact incidence and pattern is not well-described in the literature. As part of our prospective study on the metabolic and growth effect of GH treatment, we assessed children from our Dutch Kabuki cohort who were eligible for growth hormone therapy. We assessed severity and pattern of joint hypermobility, both before and after 24 months of growth hormone replacement therapy.

The prevalence of hypermobility was 31% in boys and 14% in girls using the Beighton score and 69% in boys and 57% in girls using the Bulbena score. This varies from the general population where girls are more affected. After 2 years of growth hormone treatment, there was a statistically significant decrease in the presence of joint hypermobility to 6% using the Bulbena score and none with respect to the Beighton score. We hypothesized that this result suggests a direct effect of growth hormone on connective tissue in patients with KS. Further research is needed to elucidate this mechanism.

INTRODUCTION

Kabuki syndrome (KS; OMIM 147920) is a multiple congenital malformation syndrome that was independently described by Niikawa et al. and Kuroki et al. (1,2). Although there are no scientific reports on the overall incidence of KS, it has been reported across almost all ethnic groups and its prevalence outside Japan presumably approximates that seen in the Japanese population, i.e. 1 in 32,000 (3,4). Clinical features of KS include developmental delay or intellectual disability, hypotonia, postnatal growth retardation, presence of fetal fingertip pads, and characteristic facial features. These features include long palpebral fissures with eversion of the lateral portion of the lower eyelid; broad, arched eyebrows with lateral sparseness; short columella with depressed nasal tip; and large, prominent or cupped ears (5). In 2010, Ng et al. performed whole exome sequencing in 10 cases clinically diagnosed with KS and discovered the cause as mutations in the *KMT2D* gene (formerly *MLL2*, MIM# 602113; NM 003482.3) (6,7). Since then, *KMT2D* mutations have been identified in approximately 56-70% of the clinically diagnosed individuals (8,9). In the same period, a second gene was determined to be responsible for a subgroup of KS patients: *KDM6A* (formerly *UTX*; MIM# 300128; NM 021140.3), a partner of *KMT2D* in its pathway (10). This led to the definition of two subtypes of KS: *KMT2D*-associated, autosomal-dominant KS type 1 (KS1; OMIM 147920); and *KDM6A*-associated, X-linked-dominant KS type 2 (KS2; OMIM 300867). There is some debate as to whether the phenotype of KS type 2 can be distinguished from KS type 1 (9,11).

One of the frequently reported features of Kabuki syndrome is joint hypermobility, which, in combination with hypotonia, has a negative impact on an already delayed motor development. A prevalence of 50-80% for joint hypermobility (usually described as generalized in children with KS) is mentioned; however, this has never been formerly assessed within a KS population (12). Therefore, we decided to assess hypermobility in children with Kabuki syndrome and to evaluate whether growth hormone treatment affects hypermobility in patients with a proven mutation causing Kabuki syndrome by re-assessing hypermobility scores after 12 and 24 months of growth hormone replacement therapy. This research is part to our Dutch KS Center prospective study on the metabolic and growth effect of GH treatment in children with Kabuki syndrome (13).

METHODS

This study was performed as an open-label, prospective, non-randomized study of KS children (trial registration: NTR4722) at the Maastricht University Medical Centre Maastricht, The Netherlands. Participants were recruited between 2012-2014. In total 27 KS children (13 males and 14 females) were included. Their mean age was 8 years (2-18 yr). Subjects with previous growth hormone therapy were excluded. Twenty-four of the KS individuals had a *KMT2D* mutation and three had a *KDM6A* mutation. The Medical Ethics Committee of the Maastricht University Medical Centre provided ethical approval of the study. Informed consent was obtained from the subjects' parents before the start of the study. Of the 27 subjects, 18 KS children (9 females and 9 males) were also included in a growth hormone study. Not all 27 subjects could receive growth hormone treatment because of the influence of pubertal hormones, as has been described in earlier publications on this study [Schott et al. 2016]. Subjects with diabetes mellitus, an extremely low caloric intake, a previous or active malignancy, and children with signs of puberty were excluded. Biosynthetic human GH (Genotropin, Pfizer, New York, NY, USA) was given subcutaneously once daily at bedtime. The average dose was 1 mg/m^2/day GH (equivalent to 35 µg/kg/day) for 12 months. The initial dose of GH was altered based on IGF-I levels and/or growth hormone response described previously (13,14).

Generalized joint laxity was assessed using the modified Beighton 9-point scoring system by trained clinicians (MK and JS) in the research clinic for children with Kabuki Syndrome. As described by Junge et al., each joint was assessed separately (15). With a score of 7 or more a child was considered as having generalized hypermobility. Because the Beighton scoring system only includes a limited number of joints, the Bulbena et al. scoring criteria were also employed to assess several joints separately, although this score has not been validated in large groups of children. Generalized hypermobility of the joints is present when a score of 5 is obtained in females and 4 in males (16).

Descriptive statistics are shown as mean ± standard deviation (SD). The Kolmogorov-Smirnov test was used to test for normal distribution. Intra-group comparisons were made with the paired Student's t-test. For inter-group comparisons, the independent Student's t-test was employed. All statistical analyses were performed using the software SPSS version 22 for Mac (SPSS Inc, Chicago, IL, USA). A P-value of <0.05 was considered significant.

RESULTS

Twenty-seven KS children were assessed using both scores, before starting growth hormone replacement therapy. They were between ages 2 and 18 years old at the time of the first assessment and their mean age was 8.0 years (Table 1). Twenty-five children were Caucasian, one subject was Asian and another one was from Northern Africa. The parents of 21 (78%) of them believed their child was hypermobile, based on their own observations and information they read in literature.

Table 1. Baseline characteristics of all 27 KS subjects, including hypermobility scores.

		Male	Female	All
Sex		13	14	27
Age	(mean ± SDS)	8.6 ± 4.3	7.4 ± 3.7	8.0 ± 3.9
Mutation	KMT2D	12	12	24
	KDM6A	1	2	3
Positive test	Beighton	4	2	6
	Bulbena	9	8	17
	Beighton (Mean age ± SDS)	9.2 ± 5.1	4.8 ± 1.6	7.7 ± 4.6
	Bulbena (Mean age ± SDS)	8.9 ± 5.1	5.6 ± 2.4	7.3 ± 4.3
Beighton score	(mean ± SDS)	4.7 ± 2.2	4.2 ± 2.4	4.4 ± 2.3
Bulbena score	(mean ± SDS)	5.8 ± 2.9	5.2 ± 2.0	5.5 ± 2.5

Using the Beighton score the prevalence of hypermobility was 31% in boys and 14% in girls. For the Bulbena score it was 69% in boys and 57% in girls respectively. The mean age for a positive score in the Beighton group was 7.7 ± 4.6 years and 7.3 ± 4.3 years in the Bulbena group respectively. The mean age for a negative score in the Beighton group was 8.0 ± 3.9 years and the Bulbena group 9.1 ± 3.2 years. There was no statistically significant difference between boys and girls. Joints most often affected included the hip, knee and fifth finger (Fig. 1). No significant gender differences in affected joint pattern of joints were discovered.

Prevalence of hypermobility in different joints (bulbena)

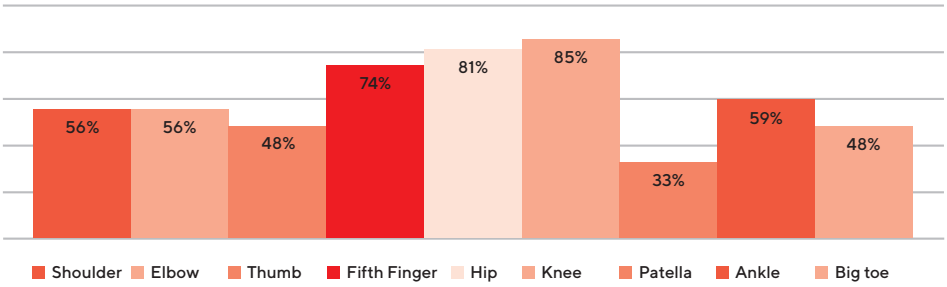

Figure 1. Pattern of hypermobility of separate joints (Bulbena score)

Three of the 27 children had a *KDM6A* mutation and had the same hypermobility as the *KMT2D* subjects. Only one of the KS children had a patellar luxation in the previous history and was not considered hypermobile using both scores. The children with other orthopedic anomalies in their history, such as hip dislocations, were all considered to be hypermobile using both scores (Table 2).

Table 2. Previous history of connective tissue problems

Condition	Percentage (% of total)
Hip dysplasia	19%
Patella dislocation	4%
Subluxation other joints	7%
Scoliosis	0%
Abnormal scarring	4%
Abnormal stretching of skin	4%
Hematomas	7%

Eighteen children (67%, 9 males and 9 females) were also assessed in a growth hormone study. Age, height, and BMI between the males and females were not significantly different. At the start of GH treatment, the mean age was 6.9 ± 2.1 years (ranging from 3.8 to 10.1 years). Four KS individuals were GH deficient, but this had no influences on hypermobility. Baseline characteristics are shown in Table 3. Serum IGF-I levels were measured at baseline, and at 12 months of GH therapy. At the start, the IGF-I SDS had a mean of -0.70 ± 1.07, which increased significant after 12 months of GH treatment (1.41 ± 0.91 SDS). The height also significantly increased from -2.40 to -1.69 (+0.7) SDS for the entire study group after one year of treatment.

Both Beighton and Bulbena assessments were performed at start and after 12 and 24 months of GH therapy. Details of the hypermobility of all 18 subjects at these three points in time are given in Table 4. Only in 4 individuals both scores were not assessed after 24 months of GH treatment and are not included in Table 4. After 2 years of growth hormone replacement therapy there was a statistically significant decrease in the presence of joint hypermobility from 61% to 6% using the Bulbena score for assessment ($P = 0.03$, P-value < 0.05). Using the

Table 3. Baseline data (mean ± SDS) and the auxological data after one-year of GH treatment of all 18 subjects.

	Pre-treatment	One-year treatment
Chronological age	6.86 ± 2.07	7.97 ± 2.13
Bone age	5.90 ± 2.14	7.28 ± 2.37
Height SDS	-2.40 ± 1.88	-1.69 ± 1.94*
Height Velocity (cm/y)	6.55 ± 2.66	9.66 ± 2.04
Height Velocity SDS	0.29 ± 2.43	2.80 ± 1.56
Parent-adjusted Height SDS	-2.07 ± 1.66	-1.35 ± 1.61*
Weight SDS	-1.34 ± 2.88	-1.16 ± 2.62*
BMI SDS	0.56 ± 1.79	0.19 ± 1.41
IGF-I SDS	-0.70 ± 1.07	1.41 ± 0.91*

SDS = Standard deviation score. *Statistically significant (P<0.05) relative to baseline.

Table 4. Hypermobility characteristics of GH treated KS children at baseline, one-year and two-years results (n = 18).

		Baseline	One-year	Two-years
Positive test for all subjects	Beighton	6	3	0*
	Bulbena	11	6	1*
Beighton score	(mean ± SDS)	4.7 ± 2.3	3.2 ± 2.3	3.1 ± 1.8
Bulbena score	(mean ± SDS)	5.3 ± 2.9	4.2 ± 2.3	3.2 ± 1.3*
Male (Positive test)	Beighton	3	1	0
	Bulbena	5	4	0*
Female (Positive test)	Beighton	3	2	0
	Bulbena	6	2*	1*

SDS = Standard deviation score. *Statistically significant (P<0.05) relative to baseline.

Beighton score, there was a decrease from 33% to 0% (P = 0.01). This effect of GH treatment on the hypermobility was equal in males and females. No differences were observed in mutation, GH deficiency or catch-up growth.

DISCUSSION

This is the first paper to specifically describe hypermobility in children with genetically confirmed Kabuki syndrome. As reported previously generalized hypermobility is one of the key features of KS. This study also confirms the presence of hypermobility in these children. However, with hips and knees most affected, our work found the pattern less generalized than previously thought. Furthermore, we demonstrate that growth hormone treatment has a positive effect on this hypermobility.

Joint hypermobility means the ability to move joints beyond the normal range of movement without pain. It can occur in only one joint or in almost all joints of an individual with general hypermobility. Joint hypermobility is a normal variability in the population, usually present as an autosomal dominant trait in families. However, is can also indicate an underlying connective tissue disease. Then, it is usually accompanied by weakness and / or abnormal stretching of connective

tissue in skin and vascular wall.
Joint hypermobility is frequently described as a feature of monogenic syndromes or chromosomal anomalies without being part of an obvious connective tissue disease. Although no data exist, it is our experience that in those cases the pattern of hypermobility is more variable, with often only a few joints affected. Sometimes surgery is needed, but this frequently leads to unsatisfactory results, as has been described in other children with connective tissue disorders (17).
In the literature, hypermobility is described in up to 75% of individuals with Kabuki syndrome. There is no specific literature on the aetiology of joint hypermobility in KS, although Philip et al. suggested that KS might be a connective tissue disorder. However, no other research supports this assertion. Indeed, given the function of the genes responsible for KS, this finding is suspect (18). A high prevalence of congenital dislocation of the hip was already reported in the first publications by Kuroki and Niikawa et al. in 1981 (1,5). Philip et al. reported on sixteen patients outside of Japan. Of these, ten patients had hypermobile joints including hip dislocation, a finding not previously reported in the Japanese literature (18). The researchers suggested the frequency of hip dislocation in the Japanese patients might reflect an unrecognized general joint laxity. Schrander-Stumpel et al. also reported joint hyperlaxity in 90 percent of the individuals from the Dutch Kabuki Center, none of whom are included in the current study. Hip dislocation occurred in 28 percent of their cohort (5). Kurosawa et al. reported three patients with recurrent dislocation of the patella (19). All three patients had generalized ligamentous laxity. Kawame et al. reported patients with patellar dislocation, congenital hip dysplasia and recurrent shoulder dislocations (20). One patient in the literature was reported with cutis laxa as well as hyperextensible joints (21). We did not find patients with cutis laxa in our cohort nor is it mentioned as a Kabuki feature in any of the other studies as a phenotypical description of the syndrome.
Kabuki syndrome is one of the syndromes in which hypermobility is considered a main feature, although the exact prevalence remains unknown. Therefore, we assessed all children eligible for the study from our cohort at the Dutch Kabuki Expertise center for the effects of growth hormone (13). Within this group of 27 children with KS between the ages of 2 and 18 years old, we found that 14-69% were considered to have general hypermobility using both the Beighton and Bulbena scoring systems. There was no difference between boys and girls. This is in contrast to the general population in which the prevalence of general

hypermobility is considered to be higher in girls (15).
Both the Beighton and Bulbena scores were used for assessment of hypermobility and both have been validated for use in children, although the Bulbena score has not been validated in large cohorts (15,22). In our experience, the Beighton score is less useful in children with syndromal anomalies since it only includes a limited number of joints, notably not the ones most often affected in these populations. This applies both to our cohort of Kabuki patients for whom the hips are most often affected and in children who do not fulfil the criteria for general hypermobility from both scoring systems. This localized hypermobility also accounts for the higher percentage of hypermobility found with the Bulbena score compared to the Beighton score, given the fact that the Bulbena score includes those joints most often affected in Kabuki syndrome (e.g. hip, knee and ankle, all not included in the Beighton score). All studies performed with these scores were focused on healthy children. Therefore, it is unclear whether these scoring systems apply to a population of children with an underlying genetic anomaly not directly affecting connective tissue but in whom the hypermobility might also be caused, at least in part, by a neuromuscular component.
In addition to a description of incidence of hypermobility we also assessed hypermobility after one and two years of growth hormone replacement therapy, since it is known that growth hormone can have a number positive effects on growth and development. Several syndromes (e.g. Turner syndrome, Prader Willi Syndrome) without the diagnosis of growth hormone deficiency have benefited from growth hormone treatment at supraphysiological doses by obtaining a higher final height than expected (23). However, more beneficial effects than those on final height involve changes in body composition and the increase in muscle mass (24) (25). We hypothesize that children with KS present more negative effects of hypermobility on their motor development because of the fact that their muscle mass is also low, and they have hypotonia, which may be severe. Indeed, in all children that could be assessed after two years of treatment, hypermobility decreased and even disappeared in most cases. Furthermore, nearly all parents spontaneously reported significant improvements in motor skills. Given our study's design, it was not possible to pinpoint the mechanism exactly, but it seems likely that growth hormone might have a direct effect on connective tissue. It is known that an excess of growth hormone can lead to stiffness and joint pain, as seen in patients with acromegalic arthropathy (26). It is also known that joint stiffness is considered an adverse event in growth

hormone treatment. However, in patients with hypermobility this joint stiffness effect might be considered a desirable effect. A possible mechanism by which growth hormone induces joint stiffness is the induction of decorin, a structural protein in the skeletal muscle extracellular matrix that regulates genes for muscle growth and repair. Through this mechanism, growth hormone treatment stimulates muscle and tendon collagen synthesis (27). Further research on the exact mechanism by which growth hormone replacement therapy improves hypermobility in Kabuki syndrome is needed.

CONCLUSION

Hypermobility is indeed a feature of Kabuki syndrome as described in the literature. We are the first to formerly assess a Kabuki cohort on this feature and its distribution. Growth hormone replacement therapy has many beneficial effect on children with Kabuki syndrome, including a significant improvement in joint hypermobility, but the exact mechanism for this improvement is not yet known.

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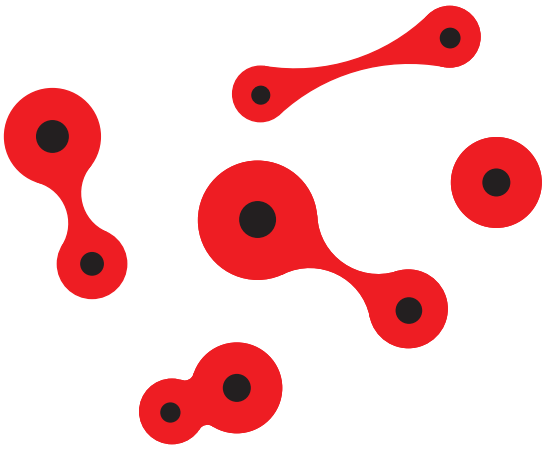

CHAPTER 9
DISCUSSION

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GENERAL DISCUSSION

Dina A. Schott

GENERAL

Kabuki (歌舞伎) is a traditional, highly-stylized Japanese theatrical dance-drama renowned for the elaborate make-up worn by its performers. The individual Japanese characters mean sing (歌), dance (舞), and skill (伎). Kabuki is therefore sometimes translated as "the art of singing and dancing". The history of Kabuki began in 1603 when Izumo no Okuni began performing a new style of dance drama in the dry riverbeds of Kyoto (1).

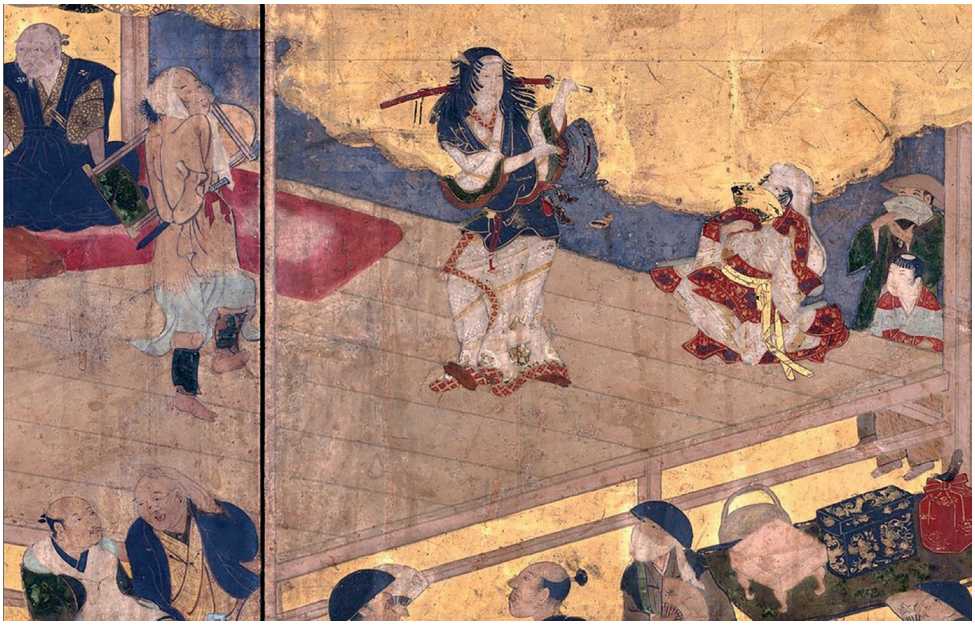

Figure I. The earliest portrait of Izumo no Okuni, the founder of Kabuki (1600s)

When two Japanese physicians encountered children with a similar appearance as these dancers-actors, it is not surprising that they named the syndrome after Kabuki-makeup (2,3). Later on, the term "makeup" was dropped and the preferred term for the disorder became Kabuki syndrome (KS). Although KS has been identified for decades, research has focused mainly on genetic aspects and clinical aspects in case reports. Research into the genetic origins of KS led to the

discovery of two genes, *KMT2D* in 2010 and *KDM6A* in 2012 (4,5). In the present study, children with one of those two gene mutations were included. It was speculated that Kabuki is a heterogeneous syndrome, meaning that additional genes could be involved. Indeed, this is the case in a limited number of cases, see introduction.

As we continue to learn more about the genetics of KS, it is crucial to learn also about specific deficits that children with KS struggle with every day. KS is a multisystem disorder characterized by multiple abnormalities including distinctive facial features, growth delays, varying degrees of intellectual disability, skeletal abnormalities, and a wide variety of additional symptoms affecting multiple different organ systems (6,7). Intellectual disability and behavioral abnormalities are currently important topics for research all over the world, including the collaboration between our expertise center in Maastricht and our colleagues at Radboud University Hospital in Nijmegen.

Many medical issues associated with KS arise early in a child's life and medical intervention often leads to positive outcomes. However, more longitudinal follow-up studies are necessary before we can fully understand typical lifespan issues for an individual with KS. Further research will not only help us better understand affected children and their families but will also provide insight into therapeutic options. One possible option came from the Dutch KS network, originating from the question of whether KS children benefit from GH treatment. Positive effects of GH treatment are well-known in children and adults with Prader-Willi and in females with Turner syndrome, although these children generally lack GH deficiency. Therefore, we initiated this study to investigate the growth pattern, genetic relation to growth and body proportions of a group of Dutch prepubertal KS children. Research into their endocrine background and growth retardation was done in the context of observing the effect of GH treatment on energy expenditure and following catch-up growth. A cardiovascular risk profile was prepared and monitored for safety reasons.

Short stature and growth hormone treatment

While short stature or postnatal growth delay is mentioned in many case reports, to our knowledge no research has ever been conducted into the cause of this growth retardation. Literature searches revealed case studies about GHD in KS children, but no fundamental basis was mentioned or considered. As discussed in the introduction, somatic growth is influenced by a number of factors.

Consequently, we first investigated the genetic constitution and the endocrine status of KS children in order to find an explanation for their short stature.

The starting point was the assumption that GH treatment would be beneficial for the KS children based on the effects of a number of imprinting syndromes. Imprinted genes are of fundamental importance in normal growth and development. Their expression are in a parent-of-origin specific manner by epigenetic modifications, including DNA methylation and histone modification (8). A number of imprinting syndromes arising from aberrant expression of imprinting genes have been described. These include Silver-Russell syndrome, Beckwith-Wiedemann syndrome, Prader-Willi syndrome, and pseudohypoparathyreoidism type 1b and others. Thought this list represents diverse phenotypes, there are common features associated with disordered growth. On a molecular basis, each may be caused by aberrant methylation at a differentially methylated region (9). KS can be caused by mutations in the *KMT2D* gene or the *KDM6A* gene. Both gene mutations are the cause of disruption of normal histone methylation and impair proper activation of certain genes in many of the body's organs and tissues, resulting in abnormalities of development and functional characteristic of KS. Since the abnormal methylation is related to short stature in the previously referenced syndromes, it seems valid that in KS the same mechanism is causative for the postnatal growth retardation. Notably, most of these syndromes show a good response to GH therapy, even though they do not meet the criteria of GHD.
Another potential biological mechanism for the cause of postnatal growth retardation in KS is a defect in the estrogen receptor. *KMT2D* is required for ligand-dependent estrogen receptor-α transactivation, one of the mediators of the biological effects of estrogen in estrogen responsive tissues. A study of estrogen receptor-α knockout mice demonstrated decreased longitudinal as well as radial skeletal growth associated with decreased serum levels of IGF-I. Examining the IGF-I levels in our study revealed no abnormalities, and therefore this mechanism is questionable.

To investigate the GH status of the KS subjects, all included KS subjects were tested by GH provocation tests before starting GH treatment. Investigation among our group revealed that 4 patients were biochemically GHD. Since many of the growth-promoting actions of GH are mediated by IGF-I, which is

secreted by the liver and locally by target tissues, we also examined the level of IGF-I. None of our 4 biochemically GHD children had an abnormal IGF-I level. So, none of the KS children in our study had GHD according to the criteria in the international guidelines. Therefore, we cannot explain the general cause of short stature in KS by GHD.
Nevertheless, a positive response in catch-up growth was experienced from providing GH treatment in our KS subjects. The mean catch-up growth after one year of treatment for the whole group was 0.7 SDS, which is considered a good response. Since growth response during the first year of GH therapy is a strong predictor of final height, GH treatment in KS appears justified (10). These data are consistent with those recorded in the Kabi International Growth Study (KIGS), where 21 KS children treated with GH showed an increase of 0.83 SDS height after one year of treatment. However, these are pre-2010 data, which were generated before the genetic basis for KS was known.
Our knowledge about the genetic background of the Dutch KS patient group allows for some considerations. Catch-up growth was stronger in subjects with a *KMT2D* (0.75 SDS) than for those with a *KDM6A* (0.51 SDS) mutation. Only 3 subjects had the *KDM6A* mutation, and therefore it is difficult to derive conclusions concerning the effectiveness of GH treatment from such a small group.
Furthermore, bone age accelerates during GH treatment in KS. The average bone age delay was 0.98 years before start of GH treatment and after one year of treatment this was decreased to 0.69 years. This progression in bone age can affect the final height. Since all children were still prepubertal during GH therapy, this acceleration was not mediated by sex steroids. GH treatment in GHD, SGA, Turner syndrome and other conditions also shows bone age acceleration (11). Despite this advancement, bone age still remained delayed or age-appropriate in these groups of patients. Because the bone age advancement in KS is minor (0.29 years), the prognosis for final height appears encouraging.
All biochemically GHD KS children exhibited a good response on GH therapy, despite normal IGF-I levels at the start of treatment. No correlation was found between pretreatment GH, IGF-I, and IGF-BP3, and first year response to GH therapy, supporting the hypothesis that these laboratory parameters do not generally predict first-year growth response to GH therapy.

While short stature is a key feature of KS, an examination of body proportions

provides further insights into the condition. Few specific genes for human body proportions are known. *Hox* genes and homeobox sequences together with a growing number of growth and signaling factors are known to regulate the growth of body segments (12). There is evidence that *Hox* expression is linked with forearm, hand, and digit length differences in the apes (13). The short stature homeobox (SHOX) is another genomic region that may be relevant to human body proportions. It is responsible for a significant proportion of long-bone growth (14). Some studies find that legs are disproportionately affected, but other studies find no disproportion (15). In an analysis of two human subjects, Livshits et al. estimate that between 40% and 75% of inter-individual variation in the body proportions that they studied (adjusted for age and sex) are attributable to "genetic effects" (16). Another area of research is epigenetic regulation of body growth (e.g., DNA methylation and histone modification) which may play a role in determination of human size and shape (17). A review of the literature indicates that there is evidence that adults with disproportions, especially high sitting height (short legs), are at greater risk for cardiovascular disease via hypercholesterolaemia, impaired glucose and insulin regulation, increased pulse pressure and blood pressure, and higher fibrinogen levels (18-21). Some cancers are associated with relatively long legs (22). In KS children, we did not find such abnormalities in sitting height versus height using the conventional anthropometric measurements and the photogrammetric anthropometry. However, KS children had larger heads and longer arms proportional to their trunk and tibia length, feasibly an effect of DNA methylation. Remarkably, GH treatment did not change the sitting height ratio, as is the case in Turner syndrome. Untreated patients with Turner syndrome have relatively short legs, a condition which improves somewhat during GH therapy (23).

Well-being and prevention

Beside the direct effect of GH on the growth of KS, there are important psychological consequences. GH studies revealed that the quality of life and psychological well-being are restored when GH therapy in GHD is administered (24). All short children share the central concern of wanting to be taller. From a social perspective, shortness can be a problem independent of the cause. In many societies, there are advantages associated with taller stature. Research suggests that some elements of life are easier for taller people because height is a socially desirable asset (25). Short children can suffer from physical, social

and psychological problems (26). While it is clinically proven that GH therapy has a positive effect on height, the impact on psychological factors is less established. Christensen et al. showed that height in adult life is correlated with HRQoL (Health-related Quality of Life) and that short stature in adult life may be associated with a significant reduction in HRQoL in the general UK population (27).
Although we did not measure the quality of life, almost everybody in the study group mentioned spontaneously that their child was fitter after starting GH treatment and displayed better concentration or school performance. It was even more remarkable that in the majority of subjects these positive traits diminished after discontinuation of GH treatment. In addition, a number of parents who toilet trained their children for years, realized that their child had suddenly become clean. These observations are worthy of further investigate.

Concomitants of hypermobility may differ in children from trivial to serious problems of nonarticular and musculoskeletal properties (28). This may influence the quality-of-life of an individual, which could cause an economic burden to the society (29). Because, joint hypermobility is a frequently reported feature in KS, this aspect was also evaluated in this study. In KS a prevalence of 50-80% for joint hypermobility (usually described as generalized in children with KS) is mentioned; however, this has never been formerly assessed within a KS population. Our study revealed a prevalence of hypermobility of 22% using the Beighton score and 63% using the Bulbena score. Reports indicate that GH has arthritogenic involvement as evidenced by articular manifestation in acromegalic patients. Reduction of the GH levels in acromegalic arthropathy revealed less joint pain and stiffness (30). This GH induced stiffness could have a positive influence on hypermobility in KS children. Indeed, after 2 years of growth hormone treatment, there was a statistically significant decrease in the presence of joint hypermobility to 6% using the Bulbena score and none with respect to the Beighton score.

Human GH has been known to improve energy expenditure and physical capacity, which are worth considering an important additional aspect for the patient. Regular physical activity can help in thinking, learning, reducing the risk of depression and may benefit better sleeping. Our study showed that body composition, energy expenditure and physical activity improved significantly

during GH treatment. GH treatment resulted not only in catch-up growth but, more importantly, better body composition with a higher lean body mass. Healthy body composition means having a relatively higher level of muscle tissue, which leads to the higher metabolism reflected by the higher energy expenditure in our study group.
Even more important is a reduction in visceral fat. This lowers the risk of diseases like diabetes, metabolic syndrome, osteoporosis, chronic inflammation, hypertension, and some forms of cancers to include colorectal and breast cancers. The fat distribution in KS is likewise truncal and after one year of GH treatment there was indeed a decrease in visceral fat. Although there were no signs of cardiovascular disease in our KS children, GH treatment resulted in a significant reduction in serum lipid and lipoprotein profiles.

Risks and side effects

It is often discussed that GH treatment is potentially a risk factor for cancer. GH raises concentrations of IGF-I, which can be mitogenic and antipoptotic. This can potentially provoke development of cancers. However, retrospective analysis of national databases found no evidence of an increased risk of developing neoplasm in GH-treated patients. Nevertheless, we must be careful in KS patients whose genetic mutations are associated with some kind of cancers. However, based on case reports of cancer in KS patients, there has been no increased incidence so far. Nonetheless, during this study we sadly lost a KS child who eventually died from the complications of treatment of a rhabdomyosarcoma. Because the patient had just been included into the study, GH treatment was not the trigger for the origin of the cancer's development. Though, we have strictly monitored the IGF-I levels during the study and adjusted the GH dose when the IGF-I level surpassed +2.5 SDS. Studies in Prader-Willi syndrome children, who, like those with KS, also have an underlying methylation problem as in KS, still have not shown an increased risk of developing cancer during GH treatment.
Short term GH treatment does not appear to have clinically significant adverse effects. Data show that blood glucose, hemoglobinA1c and insulin remain within normal limits during GH treatment. However, there were minimal signs of insulin resistance, which is identified during GH treatment.

Future directions

Although the study has now been completed and the results are good, it is important to keep a perceptiveness on the risk factors in the future. Important is the follow-up of the final height and the metabolic status in KS. Since Maastricht is recognized as expertise center for KS, it is obvious that follow-up research will take place there.

Conclusions

GH treatment is effective in increasing growth velocity and it is also highly probable that it increases adult height in KS children. GH also accelerates bone age and exerts favorable effects on energy expenditure and body composition. Safety data with GH therapy in KS are reassuring, with no indication of a tendency for adverse effects on cardiovascular risk markers. Data on malignancy during GH treatment give no cause for concern. Even so, attention to possible risks should occur throughout GH therapy during appropriate follow-ups.

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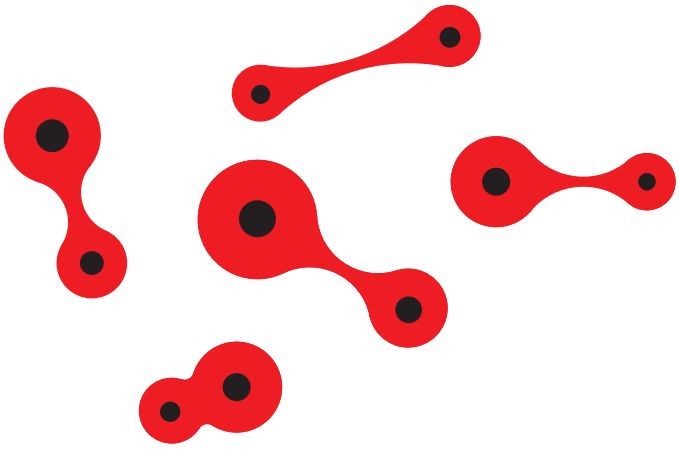
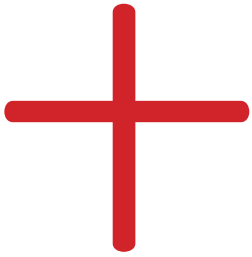


CHAPTER 10
VALORISATION



CLINICAL RELEVANCE

Dina A. Schott

Introduction

Human growth hormone is naturally produced in the pituitary gland and plays a vital role in cell regeneration, growth and maintaining healthy human tissue, including that of the brain and various vital organs. The study of human growth hormone dates to a little more than a century. Since the introduction of recombinant human growth hormone (rhGH) in 1985, the indications for GH treatment have widened. Besides growth hormone deficiency, it is currently licensed for use in Prader-Willi syndrome, Turner syndrome, Leri-Weill syndrome, Silver-Russel syndrome, as well as for patients with chronic renal failure and children small for gestational age. Although most of these children are neither evidently GH deficient nor have end-organ GH resistance, they nonetheless benefit from GH treatment.

Does the same apply for children with KS? This question arose after an inspiring presentation on one of the Kabuki network days from a father with a Prader-Willi syndrome (PWS) child. (PWS has many overlapping features with KS.) During his presentation he also talked about GH treatment which his child received and the great improvements he had observed through this therapy. Research established that GH treatment in children and adults with PWS has positive effects on height and, most especially, metabolism.

If the same holds true for KS children, this treatment would have an important social and scientific relevance.

Target population, clinical and social relevance

The primary goal of this work was to research whether GH therapy in children with KS contributes to the reduction of disabilities, improves metabolic status, gauge its effectiveness on height gain, and determine its safety. Presently, there is a growing list of benefits of GH treatment in children, adolescents and adults. Besides growth stimulation, human growth hormone has been known to improve the physical capacity of individuals through stimulating collagen synthesis in the skeletal muscle and tendons, increasing muscle strength and, as a result, improving exercise performance. In GH-deficient adults, participants who were administered long-term GH treatment experienced normalization of muscle strength, increased exercise capacity, and improved thermoregulation and body composition (1). Decreases in abdominal and visceral adipose tissue along with diastolic blood pressure are other favorable benefits of human growth hormone (2). Furthermore, quality of life and psychological well-being improves upon

administration of growth hormone therapy in growth hormone-deficient adults. As a result, cognitive function and mood significantly increased according to mood scales (3). Many of these benefits are noted in our study.
One of the hallmarks of KS is short stature that originates from postnatal growth retardation. In our KS population, a good response in catch-up growth was experienced from providing GH treatment at an early age, as described in Chapter 5 of this thesis. Perhaps even more important than the resultant height improvement, GH treatment was beneficial for total energy expenditure, basal energy expenditure, and increases in free fat mass (see Chapter 6). It is known that early administration of GH enhances the synaptic activity in neural networks and circuits, helping improve the subject's cognitive and adaptive functions (4). Although not quantified, parents mentioned better school performance and improved neurological functioning of their child. In conclusion, we can state that GH therapy in KS has a proven medical indication.
But are there risks and adverse effects from using GH therapy in non-GHD children? In Chapter 7 safety issues are discussed. There is a slightly elevated risk for increased insulin resistance. Also, GH is expensive, with the annual cost of GH treatment around €10.000 per year per patient. Therefore, one must demand the highest standards of ethical and clinical safety when treating children with no biological disorder in the GH-axis. The question of whether it is the preferred treatment for the KS person is especially difficult to answer in the case of children. Since GH treatment is not mandatory in KS, adherence to treatment can be problematic in children. It has been observed that the duration of a treatment is inversely related to its compliance in chronic diseases in general. The need for daily subcutaneous injections can further aggravate treatment compliance since injectable therapies are perceived as painful and difficult to administer. A good degree of compliance requires an effective and efficient treatment plan. Devices used for treatment administration have to be easy to use (5). In our study group, the parents were highly motivated, and the effectiveness of GH treatment was primarily monitored by height, growth rate, and levels of IGF-I. All these markers showed improvement and the conclusion was that the adherence was good. However, some parents mentioned that their child had trouble with the daily injections. High patient/parent motivation and thorough training on the GH administration technique help achieve high adherence to GH therapy. For young children, the parents/legal guardians should consult with the treating physician in deciding whether GH therapy is preferable based on the positive and negative

consequences of the treatment.

Innovation and implementation in clinical practice

The clinical effectiveness of GH therapy with rhGH was proven repeatedly in practice, as seen in this first study of GH treatment in children with the very rare Kabuki syndrome. The results of this thesis were presented at the Dutch GH advice group. During this meeting, all of the paediatrician-endocrinologists present agreed that GH therapy in KS children is effective and safe. Consequently, GH treatment should be indicated for KS children. The goal of implementing this practice is ongoing. Currently, debate exists on whether this treatment should be based only on short stature, or, similar to PWS children, on its positive metabolic effects.

In addition, the agreement has been made that all KS children in the Netherlands who qualify for rhGH therapy should be treated at the MUMC+, the Centre of Expertise for KS. This policy confirms expertise and ensures documentation and publication of the follow-up results. Already, several newly diagnosed children with KS have requested GH therapy at our Centre of Expertise in the MUMC+ and have been placed on a waiting list.

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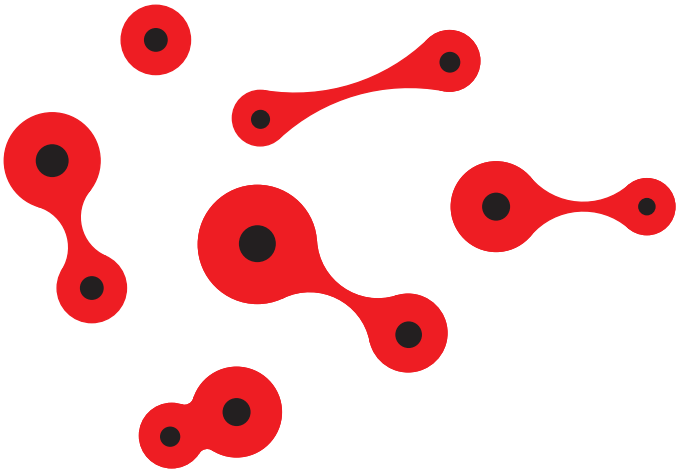
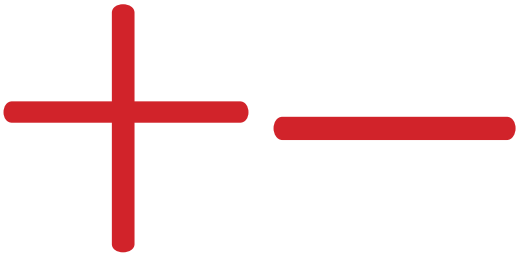


CHAPTER 11
SUMMARY



SUMMARY

Dina A. Schott

SUMMARY

The focus in this thesis is on the growth pattern, body proportions and growth hormone treatment in children with Kabuki syndrome. In addition, we studied the metabolic effects of growth hormone on the energy expenditure, body composition and cardiovascular biomarkers.

Chapter 1 provides an overview of the characteristics of Kabuki syndrome with specific attention to the genetics aspects of the syndrome. The endocrine status of these children is described, with emphasis on growth and growth hormone deficiency. The effects of growth hormone on regulating body composition, energy, physical activity and cardio-vascular metabolism is portrayed. The introduction also provides the aims of the performed studies and describes the outline of the thesis.

Chapter 2 describes the growth pattern in KS patients with a proven *KMT2D* gene mutation. Postnatal growth retardation was a clinic feature in all cases. Furthermore, a diminished pubertal spurt was observed. A possible explanation for this growth deflection is supposed by a defect in the estrogen receptor. The *KMT2D* gene is required for ligand-dependent estrogen receptor-α transactivation. Because estradiol promotes growth, in both genders, by epiphyseal stimulation in the growth plate, a defect in this estrogen receptor could potentially cause growth problems. A genotype-phenotype relation was not observed.

Chapter 3 is looking more into detail at the growth pattern in KS children by investigation of the body proportions. The key difference in these children, relative to the normal population, is that they have larger hands and longer arms proportional to their trunk.

In ***Chapter 4*** the cause of the growth retardation is further investigated by means of growth hormone stimulation tests in relation to the IGF-I values. Twenty-eight percent of the KS children had a lack of GH response, but none of these children had an abnormal IGF-I level. Although, GHD in KS is described in the literature, this seems not to be the main course of the short stature.

Chapter 5 reveals the growth response to GH treatment in prepubertal KS children. GH was given irrespective their hormonal status and the results evaluated after one year of GH treatment. There was an increase in height standard deviation score (SDS) for the whole group from -2.40 to -1.69 ($P<0.05$).

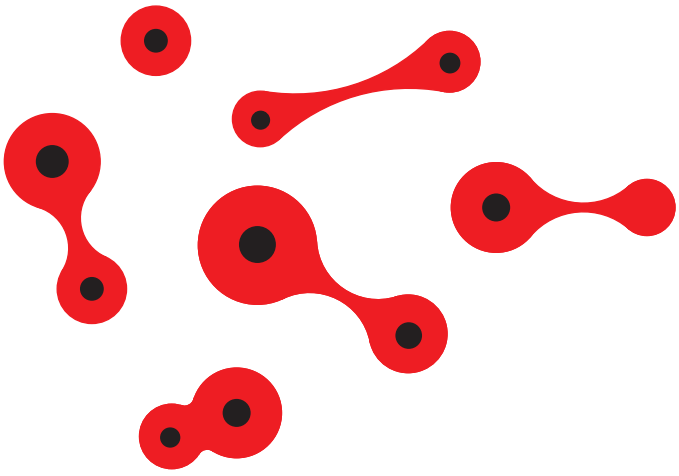
The mean IGF-I SDS increased from -0.70 (± 1.07) to 1,41 (± 0.91) (P<0.05) after 12 months. KS children who received GH at a younger age displayed significantly greater increases in height than those who started when they were older. The same was true for both gene mutation *KMT2D* versus *KDM6A* and for GH-deficiency versus non-GH deficiency KS children (P<0.05). Throughout the course of GH treatment, the subjects' body proportions remained normal.

Chapter 6 describes the metabolic effects of GH treatment. TEE showed a significant increase (P<0.01) already after 6 weeks of treatment. The same was true for the BMR. Children with a reduced GH secretion during GH stimulation tests had the highest increase in TEE. There was also a significant increase in FFM and in the activity level (PAL). The rise in PAL was relatively less than TEE, so the increase in TEE was predominantly an improvement of the BMR. Notably, there was a significant difference in all the above-mentioned measurements compared to a short stature control group, even after 6-weeks of GH treatment in the KS group.

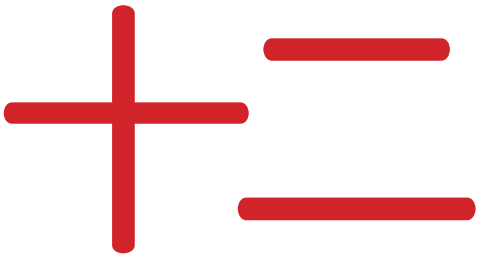
Description of the cardiovascular markers before and during GH treatment are revealed in ***Chapter 7.*** Baseline metabolic profiles showed no cardiometabolic abnormalities in KS children. Furthermore, after 12 months of GH treatment there were no undesirable effects on the cardiovascular risk markers, despite an elevation in VCAM-1 levels and slight signs of insulin resistance, of which the importance is not yet clarified. During GH treatment, both serum LDL cholesterol concentrations and the inflammation marker IL-8 decreased significantly and BMI and waist-circumference improved. Moreover, no important side effects were reported. Therefore, the results demonstrate that GH treatment in KS children is safe.

In ***Chapter 8*** hypermobility was analyzed. Hypermobility is indeed a feature of Kabuki syndrome as described in the literature. The prevalence of hypermobility was 31% in boys and 14% in girls using the Beighton score and 69% in boys and 57% in girls using the Bulbena score. This varies from the general population where girls are more affected. After 2 years of growth hormone treatment, there was a statistically significant decrease in the presence of joint hypermobility to 6% using the Bulbena score and none with respect to the Beighton score.

Finally, in ***Chapter 9*** the main findings of this thesis are discussed. In addition, clinical implications are emphasized, and some future research is suggested.



CHAPTER 12
SAMENVATTING



SAMENVATTING

Dina A. Schott

SAMENVATTING

In dit proefschrift onderzochten we de groeipatronen, lichaamsproporties en het effect van groeihormoonbehandeling bij kinderen met het Kabuki syndroom (KS). Daarnaast hebben we de metabole effecten van groeihormoon op het energieverbruik, lichaamssamenstelling en cardiovasculaire biomarkers bestudeerd.

Hoofdstuk 1 geeft een overzicht van de kenmerken van het KS weer met speciale aandacht voor de genetische aspecten van dit syndroom. De endocriene status van deze kinderen wordt beschreven, met de nadruk op groei en groeihormoondeficiëntie. De effecten van groeihormoon op de lichaamssamenstelling, energiewisseling, lichaamsbeweging en cardiovasculair metabolisme worden weergegeven. De inleiding beschrijft ook de doelen van de uitgevoerde onderzoeken en de opzet van het proefschrift.
Hoofdstuk 2 beschrijft de groeipatronen bij KS-patiënten met een bewezen *KMT2D*-genmutatie. Postnatale groeiachterstand was in alle gevallen een klinische kenmerk. Bovendien werd een verminderde puberale groeispurt waargenomen. Er wordt verondersteld dat een defect in de oestrogeenreceptor een mogelijke verklaring kan zijn voor deze verminderde groeispurt. Het *KMT2D*-gen is namelijk vereist voor ligandafhankelijke oestrogeenreceptor-α transactivering. Omdat estradiol de groei bevordert door epifysaire stimulatie in de groeiplaat in beide geslachten, kan een defect in deze oestrogeenreceptor groeibeperkingen veroorzaken. Een genotype-fenotype relatie werd niet waargenomen.
Hoofdstuk 3 gaat dieper in op het groeipatroon van KS-kinderen door onderzoek naar de lichaamsproporties. Het belangrijkste verschil tussen deze kinderen, ten opzichte van de normale populatie, is dat ze grotere handen hebben en langere armen ten opzichte van hun romp.
In ***Hoofdstuk 4*** wordt de oorzaak van de groeiachterstand verder onderzocht door middel van groeihormoonstimulatietesten in relatie tot de IGF-I-waarden. Achtentwintig procent van de KS-kinderen had een gebrek aan groeihormoon (GH) respons, maar geen van deze kinderen had een abnormaal IGF-I-niveau. Hoewel groeihormoon deficiëntie in KS in de literatuur wordt beschreven, lijkt dit niet de hoofdoorzaak van de kleine lengte te zijn.
Hoofdstuk 5 beschrijft de groeirespons in prepuberale KS-kinderen met

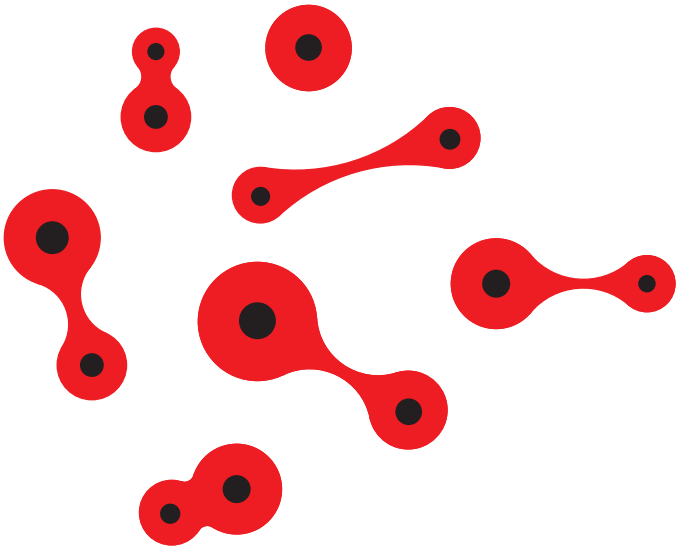
GH-behandeling. GH werd ongeacht hun hormonale status gegeven en de resultaten werden geëvalueerd na een jaar behandeling met GH. Er was een lengte groei toename van de standaardafwijkingsscore (SDS) voor de hele groep van -2,40 tot -1,69 (P <0,05). Het gemiddelde IGF-I SDS nam toe van -0,70 (± 1,07) tot 1,41 (± 0,91) (P <0,05) na 12 maanden behandeling. KS-kinderen die op jongere leeftijd GH kregen, vertoonden aanzienlijk grotere lengtetoenames dan degenen die op latere leeftijd begonnen. Hetzelfde gold voor zowel gen mutatie *KMT2D* versus *KDM6A* als voor GH-deficiëntie versus niet-GH-deficiëntie KS-kinderen (P <0,05). Gedurende de loop van de GH-behandeling bleven de lichaamsverhoudingen van de proefpersonen normaal.

Hoofdstuk 6 beschrijft de metabole effecten van GH-behandeling. TEE vertoonde al na 6 weken behandeling een significante toename (P <0,01). Hetzelfde betrof de BMR. Kinderen met een verminderde GH-afscheiding tijdens GH-stimulatietests hadden de hoogste toename in TEE. Daarnaast was er ook een aanzienlijke toename in FFM en in het activiteitenniveau (PAL). De toename in PAL was relatief minder dan TEE, dus de toename in TEE was voornamelijk een verbetering van de BMR. Opmerkelijk was dat er een significant verschil was in alle bovengenoemde metingen vergeleken met een gezonde controlegroep, zelfs na 6 weken behandeling met GH in de KS-groep.

Beschrijving van de cardiovasculaire markers voor en tijdens de GH-behandeling worden weergeven in ***Hoofdstuk 7.*** Baseline metabole profielen toonden geen cardio-metabole afwijkingen bij kinderen met KS. Bovendien waren er geen ongewenste effecten op de cardiovasculaire risicomarkers na 12 maanden behandeling met GH. Wel was er een verhoging van de VCAM-1-spiegels en lichte tekenen van insulineresistentie, waarvan het klinisch belang nog niet is te duiden. Tijdens de GH-behandeling namen zowel de LDL-cholesterolconcentraties in serum als de ontstekingsmarker IL-8 significant af en verbeterde de BMI en tailleomtrek. Verder werden geen belangrijke bijwerkingen gemeld. Concluderend tonen de resultaten aan, dat GH-behandeling bij KS-kinderen veilig is.

In ***Hoofdstuk 8*** werd de hypermobiliteit geanalyseerd. Hypermobiliteit is inderdaad een kenmerk van het Kabuki-syndroom zoals beschreven in de literatuur. De prevalentie van hypermobiliteit was 31% bij jongens en 14% bij meisjes met de Beighton-score en 69% bij jongens en 57% bij meisjes met de Bulbena-score. Dit varieert van de algemene bevolking waar meisjes normaal gesproken meer aangedaan zijn. Na 2 jaar behandeling met groeihormoon was

er een statistisch significante afname van de aanwezigheid van gezamenlijke hypermobiliteit tot 6% met behulp van de Bulbena-score en geen enkele met betrekking tot de Beighton-score.
Ten slotte worden er in ***Hoofdstuk 9*** de belangrijkste bevindingen van dit proefschrift besproken en bediscussieerd. Bovendien worden de klinische implicaties benadrukt en wordt enig toekomstig onderzoek gesuggereerd.



CHAPTER 13

十三

LIST OF ABBREVIATIONS
DANKWOORD
CURRICULUM VITAE
LIST OF PUBLICATIONS

Dina A. Schott

LIST OF ABBREVIATIONS

AEE	Activity related Energy Expenditure
ADMA	Asymmetric Dimethylarginine
Apo	Apolipoprotein
ARG	Arginine
ARM	Arm Length
BA	Bone Age
BC	Body Composition
Biac	Biacromial width
Biil	Biiliacum width
BP	Blood Pressure
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CA	Calendar Age
CAMs	Cellular Adhesion Molecules
CLO	Clonidine
CRP	C-reactive Protein
DBP	Diastolic Blood Pressure
DEE	Diet-induced Energy Expenditure
DEXA-scan	Dual Energy X-ray Absorptiometry scan
DIT	Diet Induced Thermogenesis
DLW	Doubly Labelled Water
EE	Energy Expenditure
EDTA	Ethylenediaminetetra-acetic Acid
EMEA	Agency for the Evaluation of Medical Products
FGFR	Fibroblast Growth Factor Receptors
FFM	Fat Free Mass
FM	Fat Mass
FO	Foot Length
GH	Growth Hormone
GHD	Growth Hormone Deficiency
GHIH	GH Inhibiting Hormone
GHRH	GH Releasing Hormone
GLU	Glucagon
H	Height

HA	Hand Length
HC	Head Circumference
HDL	High-Density Lipoprotein
HL	Head Length
HOMA-IR	Homeostatic Model Assessment-Insulin Resistance
ICAM	Intercellular Adhesion Molecule
IGF-I	Insulin-like Growth Factor I
IGFBP-3	Insulin-like Growth Factor-binding Protein 3
IL	Interleukin
IMP	Investigational Medicinal Product
INS	Insuline
ITP	Immuun Trombocytopenische purpura
KIGS	Pfizer Growth Study Program
KS	Kabuki Syndrome
LA	Lower Arm length
LDL	Low-Density Lipoprotein
METC	Medical research ethics committee (MREC); in Dutch medisch ethische toetsing commisie
MCP-1	Monocyte Chemo-attractant Protein-1
MS	Metabolic Syndrome
MUMC	Maastricht University Medical Centre
MLL2	Myeloid/lymphoid or mixed-lineage leukemia 2
NS	Noonan Syndrome
NO	Nitric Oxide
PAL	Physical Activity Level
PWS	Prader-Willi Syndrome
rhGH	recombinant human Growth Hormone
RNA	Ribonucleic Acid
SAA	Serum Amyloid A-protein
SBP	Systolic Blood Pressure
SH	Sitting Height
SDS	Standard déviation score = z-score
SHP2	Scr Homology region 2-containing protein tyrosine Phosphatase

SHOX	Short Stature Homeobox gene
SLL	Subischial Leg Length
STAT5b	Signal Transducer and Activator of Transcription 5b
TBW	Total Body Water
TC	Total Cholesterol
TEE	Total Energy Expenditure
TG	Triglycerides
Tibl	Tibia length
TM	Trombomodulin
TNF-a	Tumor Necrosis Factor alpha
TNFR	TNF-receptor
Trl	Trunk length
TS	Turner Syndrome
UA	Upper Arm length
VAT	Visceral Adipose Tissue
VCAM	Vascular Cell Adhesion Molecule
VH	Ventilated Hood
WC	Waist Circumference
WCHt	Waist Circumference/Height ratio

DANKWOORD

Na vele inspanningen is het dan zo ver, mijn proefschrift is klaar. Deze studie opzetten en uitvoeren zou niet mogelijk zijn zonder de hulp en inspanning van vele mensen. Ik wil dan ook iedereen hartelijk danken die betrokken is geweest bij de totstandkoming van dit proefschrift!

Een aantal mensen wil ik graag in het bijzonder bedanken:

Allereerst alle kinderen en hun ouders die hebben deelgenomen aan deze studie. Ik blijf het ontzettend bijzonder vinden dat er op één na, iedereen mee heeft willen doen aan deze studie. Ik heb bewondering gehad voor de meerdere reizen voor velen van jullie naar het verre zuiden met een nuchter kind achter in de auto. Door de polibezoeken, opnames en Kabuki-netwerkdagen heb ik jullie beter leren kennen. Nooit een keer heb ik een wanklank gehoord en waren jullie allemaal zeer positief. Ik hoop dan ook van harte dat deze mooie resultaten en al jullie inspanningen beloond mogen worden met een indicatiestelling voor groeihormoonbehandeling voor alle kinderen met Kabuki syndroom.

Mijn promotor, Prof. dr. C.T.M.C. Stumpel. Beste Connie, ik heb veel bewondering voor je altijd optimistische en enthousiasmerende houding. Nooit een keer heb ik jou horen klagen over wat dan ook. Je was altijd bereid mij te helpen waar nodig en supersnel in het beantwoorden van e-mails of corrigeren van manuscripten. Dat je op een dag naar mij toekwam met de vraag of Kabuki kinderen geen groeihormoon behandeling kon krijgen, was de start voor deze fantastische studie.

Mijn co-promotor, Dr. W.J.M. Gerver. Lieve Willem-Jan, je hebt mij het vak geleerd. Samenwerkend als kinderarts in opleiding op de polikliniek heb je mij geënthousiasmeerd voor het mooie vak van endocrinologie. Ik mocht jouw endocrinologie poli draaien en jij nam mijn spoedpoli over, erg bijzonder. Door deze fijne samenwerking kwam een fellowship kinderendocrinologie tot stand, en niet geheel zonder hindernissen. Uiteindelijk samen met Nijmegen werd het een mooie opleiding met start van dit promotietraject. Als ik weer eens in de put zat, wist jij mij er moeiteloos uit te halen met je nuchtere blik en onvergetelijke humor. Maar endo-Maastricht is niks zonder de steun van je vrouw Angèle. Jullie

zijn een hecht team waar ik fijn mee heb samengewerkt. Angèle bedankt voor het kritisch nakijken van dit manuscript.

Prof. dr. L. Zimmermann, mede promotor. Beste Luc, dank voor de plezierige samenwerking en mogelijkheid die je mij hebt gegeven om te kunnen promoveren.

Alle studenten die hebben meegewerkt aan dit onderzoek. Yoni Caspers, jij was de eerste student die in de Kabuki trein stapte, in een periode dat de hele studie nog van start moest gaan. Jij hebt met mij de eerste stap gezet naar de groeicurves van Kabuki kinderen in Nederland. Nicole Cramers, Dianne Ackermans en Juul Coenen hebben dit gedeelte van je overgenomen. Dit was de opzet voor het eerste artikel, waar ik jullie dankbaar voor ben. Tevens hebben Dianne en Juul een start gemaakt met het informeren van ouders en kinderen en het opzetten van een draaiboek, het pionierswerk ging jullie goed af. Pippa Staps en Vivianne de Vries zijn heel Nederland doorgereisd om kinderen te includeren voor de studie en met succes! Door jullie inspanningen wilde iedereen mee doen. Jullie werden overal warm ontvangen, een hecht tweetal waren jullie. Pippa heeft blijkbaar het onderzoek virus overgenomen en doet nu zelf een promotietraject in het Radboud, veel succes. Lotte Oosterbeek nam het stokje over en moest de uitdagingen aan van opnames voor groeihormoontesten en ventilated hood metingen. Door je enthousiasme lukte dit altijd, ondanks de hindernissen die je tegenkwam. Even was er na jou geen opvolger i.v.m. de zomervakantie, maar jij wist een uitweg: Lieke van Montfort, een vriendin, wilde in de zomervakantie wel wat bijleren. Inmiddels ken ik Lieke al een tijdje, naast het meehelpen met de studie en het schrijven van een artikel, hebben we elkaar met name in de kliniek leren kennen. Je bent een doorzetter en komt er wel als kinderarts. Dat je in je vrije tijd een studie onderzoek wilde doen, geeft dat wel aan. Als bescheiden Belgische meid kwam Eva Janssens, inmiddels in opleiding tot kinderarts in Antwerpen. Een absoluut stille kracht was jij, die veel werk heeft verzet. Berbel Kooger kwam als eerste A-KO student een combinatie stage doen. Een deel onderzoeksstage en een deel kinderkliniek in het MUMC+. Door je voor opleiding biomedische wetenschappen merkte ik direct dat je meer ervaring had op het gebied van onderzoek. Naast de studie hebben we ook samen in de kliniek gewerkt en met veel plezier, hetgeen ook geleid heeft tot een opleidingsplek als kinderarts, proficiat. Robin Remmel heeft de studie afgesloten. Jij hebt veel werk

verzet t.a.v. de statistiek omtrent de uitslagen van het dubbel gelabeld water onderzoek. Samen hebben we de resultaten hiervan gepresenteerd in Parijs op de ESPE, spannend vond je dat maar je hebt het super goed gedaan. Dank aan jullie allen, zonder jullie inzet was ik niet zo ver gekomen.

Sommige onderzoeken vielen buiten mijn expertise als kinderarts-endocrinoloog, en heb dan ook veel steun gehad aan onderzoekers van het MUMC+. In het bijzonder wil ik de afdeling humane biologie danken, Guy Plasqui en Loek Wouters voor hun deskundigheid op het gebied van de Tracmor en DLW-analyses. Jörgen Bierau en Jogchum Plat voor hun kennis op het gebied van de biomarkers.

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Leden van de promotiecommissie, hartelijk dank voor het beoordelen van mijn proefschrift. In het bijzonder Prof. Dr. N.C. Schaper, beste Nicolaas dank voor je kritische en stimulerende feedback.

Mijn collega's en vrienden van het Zuyderland MC. Tijdens de start van dit onderzoek heb ik veel steun gehad van mijn collega kinderartsen en arts-assistenten uit het Heerlense. Halverwege zijn er de Sittardse collega's bijgekomen hetgeen niet zonder slag of stoot is verlopen. Nu we samen onderweg zijn, voel ik dat we meer naar elkaar groeien, en dat dit een opstap is naar een mooie vakgroep met vele mogelijkheden. Ik zie het als een promotietraject, lang en moeizaam, maar het eindresultaat is/wordt prachtig.

De firma Pfizer Nederland voor de plezierige samenwerking en de financiële ondersteuning van deze studie.

Mijn paranimfen Monique Engel en Merel Klaassens. Monique weet als geen

ander hoe moeizaam een promotietraject kan zijn, we hebben hierover altijd lekker samen kunnen mopperen. Jij moet nog heel even, maar komt er zeker ook, succes. Merel met jou heb ik het afsluitende deel van dit proefschrift geschreven. Als kinderarts erfelijke en aangeboren aandoeningen kon ik fijn met je sparren.

Mijn geweldige ouders en broer. Lieve pa en ma, jullie zeiden altijd: "dat maken wij niet meer mee", nou dus wel! Bedankt voor jullie steun en het feit dat jullie altijd voor me klaar staan. Jos dank voor je ondersteuning en creatieve inbreng voor de cover van dit proefschrift.

CURRICULUM VITAE

Dina Antina Schott (Nina) was born in Veendam, the Netherlands, on December 30th, 1969. After she passed her secondary school, she started the study chemistry at the university of Groningen because of drawing of lots. In 1991, she started medical school at the university of Gent, Belgium, which was continued in Groningen. Upon completion of her degree she took up a post as a paediatric resident in st Lucas Hospital in Winschoten and Medisch Spectrum Twente in Enschede. Thereafter she started her Pediatric training at the department of Pediatrics in Maastricht University Medical Center (Prof.dr. L. Zimmermann) and Viecuri in Venlo. From 2008-2010 she was fellow paediatric endocrinology at the Maastricht University Medical Center (Dr. W.J.M. Gerver) and Radboud University Hospital (Dr. B.J. Otten). During this period she started her research project on metabolic effects of growth hormone treatment in small for gestational age children. Later on switching to metabolic effects of growth hormone treatment in Kabuki syndrome children. This thesis is the result of this research project. At this moment she is working as a paediatric endocrinologist at the department paediatrics of Zuyderland Medical Center.

LIST OF PUBLICATIONS

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- **Schott N, Penders B, Gerver WJ, Stumpel CT.** Body proportions in children with Kabuki syndrome. Am J Med Genet A. 2016;170:610-4.

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- **Schott DA, Gerver WJM, Stumpel C.** Growth Hormone Therapy in Children with Kabuki Syndrome: 1-year Treatment Results. Horm Res Paediatr 2017; 88:258-264

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- **Hoevenaren IA, Schott DA, Otten BJ, Kroese-Deutman HC.** Prepubertal unilateral gynecomastia: a report of two cases. Eur J Plast Surg. 2011;34:395-398.

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Kabuki (歌舞伎) is a classical Japanese dance-drama. Kabuki theatre is known for the stylization of its drama and for the elaborate make-up worn by some of its performers.
The individual kanji, from left to right, mean:

sing: 歌 , dance: 舞 , and skill: 伎.

Kabuki is therefore sometimes translated as:
“the art of singing and dancing”.

When two Japanese physicians encountered children with a similar appearance as these dancers-actors, it is not surprising that they named the syndrome after Kabuki-makeup.