Follow-Up Study of Growth Hormone Therapy in Children with Kabuki Syndrome: Two-Year Treatment Results

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Keywords

Kabuki syndrome · Growth hormone · Cardiovascular risk factors

Abstract

Introduction: 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 catch-up growth and cardiovascular markers before and during growth hormone (rhGH) treatment in KS children. **Methods:** This prospective study included 18 children whose KS was genetically established. Each KS subject received rhGH for a period of 2 years. Several measurements were performed before and during treatment: anthropometry, glucose metabolism, lipid profile, markers for endothelial function, and low-grade inflammation. **Results:** This study found an increase in delta height standard deviation score (SDS) for the whole group of 1.1 SDS after 2 years of rhGH treatment. Baseline metabolic profiles showed no cardiometabolic abnormalities in these children. Although 4 out of 18 children were obese, there were no signs of the metabolic syndrome. During rhGH treatment, serum low-density lipoprotein cholesterol concentrations decreased

significantly (2.16–1.91 mmol/L, $p = 0.04$). Apolipoprotein B100 concentrations also showed a reduction after 24 months of treatment, but the other lipid and (apo)lipoprotein parameters did not change. While other endothelial function markers were stable, only vascular cell-adhesion molecule-1 concentrations increased (1,084–1,161 pg/mL, $p < 0.01$) during rhGH therapy. Furthermore, BMI and waist circumference improved during treatment. There were no signs of hypertension. **Conclusions:** At baseline and during rhGH therapy, there were no signs of the metabolic syndrome. This is the first study demonstrating that rhGH treatment in KS children is a safe and effective therapy and that it positively influences linear height without exerting adverse effects on a wide array of cardiovascular risk markers.

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Introduction

Kabuki syndrome (KS) is a rare genetic syndrome that is characterized by an unusual facial appearance, postnatal growth retardation, intellectual disability, and various other congenital anomalies [1, 2]. In 2010, exome sequencing revealed that heterozygous mutations in

KMT2D (also known as MLL2 gene, OMIM 602113) as a major cause of KS, explaining up to 75% of the cases [3, 4]. Additionally, mutations in KDM6A (OMIM, 159555) were discovered in 9–13% of the KS patients [5].

KS children are generally born with a normal birth weight and length. The short stature characterizing KS is mainly caused by postnatal growth delay. The origin for this growth delay is unknown; however, there are some cases of growth hormone (rhGH) deficiency reported in the literature [6–9]. Besides the previously noted features, patients may become very obese toward adolescence without endocrinological explanation for this phenomenon [10, 11]. Based on this observation, which is in line with the expert opinion of the clinicians of the Kabuki Expertise Centre in Maastricht University Medical Centre, we hypothesized that KS patients are at risk for developing metabolic syndrome.

The literature indicates positive long-term results of rhGH treatment on linear growth and improvement on metabolic risk markers for children with Prader-Willi syndrome [12, 13]. Moreover, several studies concerning adults treated with rhGH because of rhGH deficiency show beneficial effects on the metabolic outcome [14, 15]. To date, the effect of rhGH therapy in KS has been investigated in case studies involving rhGH-deficient KS children, but these studies were done without the benefit of understanding the genetic basis of KS [7–9, 16]. To address this deficiency, a study was conducted at Maastricht University Medical Centre on a cohort of 18 children with genetically proven KS. We recently published the 1-year treatment results of rhGH therapy on anthropometric variables in this study group [17]. 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 anthropometric variables after 2 years of treatment with rhGH and after discontinuation of treatment; and (3) to evaluate whether signs of the metabolic syndrome, low-grade inflammation, and endothelial dysfunction are present before the manifestation of clinical symptoms.

Subjects and Methods

Subjects

This study was performed as an open-label, prospective, non-randomized study of KS children (trial registration: NTR4722) at Maastricht University Medical Centre in Maastricht, the Netherlands. Participants were recruited between 2012 and 2014 after informed consent was obtained from their parents. Subjects with a genetically confirmed diagnosis of KS (KMT2D or

KDM6A gene mutation) and prepubertal status were included in the study.

Subjects with diabetes mellitus, an extremely low caloric intake, a previous or active malignancy, rhGH therapy, and children with signs of puberty were excluded. The Medical Ethics Committee of Maastricht University Medical Centre provided ethical approval of the study.

As previously indicated, an arginine and clonidine rhGH-stimulation test was performed in all subjects at the start of the study to rule out rhGH deficiency. The stimulation tests were performed after overnight fasting according to the standard procedures. Most subjects underwent 2 GH stimulation tests on 2 different days: clonidine (Catapresan; Boehringer Ingelheim, Germany) 0.15 mg/m² given orally and arginine (L-arginine hydrochloride 10% solution) 0.5 g/kg given intravenously over 30 min at 0 min. Samples for IGF-I, IGFBP-3, and GH determination were obtained at baseline. Samples for GH assessment were also taken at 30, 45, 60, 90, and 120 min after the administration of arginine and clonidine at 30, 60, 90, 120, and 150 min. If the maximum GH concentration of both tests was below the 7 µg/L, the patient was diagnosed with GHD [18]. Subsequently, rhGH therapy with biosynthetic human rhGH (Genotropin®, Pfizer, New York, NY, USA) was initiated. It was administered daily just before bedtime, and the average dose given in our study was 1.0 mg/m²/day (equivalent to 0.035 mg/kg/day) for a duration of 24 months. KS children with a GH deficiency received a lower dose of rhGH (0.7 mg/m²/day).

Auxological Measurements

Upon entering the study and at 3-month intervals during the 2 years of treatment, all subjects underwent assessment of height, weight, and waist circumference (WC) and an assessment of the pubertal stages according to Tanner (D.A.S.) [19]. Additionally, anthropometric data were collected for a subgroup of 13 participants 1 year after rhGH therapy was stopped. Measurements were performed according to internationally accepted methods [20, 21]. 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 the lower rib) and the iliac crest (top of the pelvic bone) with the person in a standing position. 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 and diastolic blood pressures were determined 3 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. Systolic and diastolic blood pressures were expressed in percentiles referencing age- and sex-specific values [22].

Furthermore, the bone age was determined upon the start of the study and at 12 and 24 months. The latter was estimated for all children by the same pediatrician-endocrinologist (D.A.S.) using the Greulich and Pyle atlas method [23].

Biochemical Measurements

Fasting blood samples were collected before the start of rhGH treatment and at 6-month intervals during the therapy for analyz-

Table 1. Baseline data (mean $\pm$ SDS) and the auxological data after 1 year and 2 years of rhGH treatment of all 18 subjects with KS

	Pretreatment	One-year treatment	Two-year treatment
Chronological age	6.86 $\pm$ 2.07	7.97 $\pm$ 2.13	9.00 $\pm$ 2.16
Bone age	5.90 $\pm$ 2.14	7.28 $\pm$ 2.37	8.52 $\pm$ 2.46
HSDS	-2.40 $\pm$ 1.88	-1.69 $\pm$ 1.94*	-1.30 $\pm$ 1.88*
Height velocity, cm/year	5.96 $\pm$ 2.09	9.01 $\pm$ 2.03*	8.19 $\pm$ 1.65*
Height velocity SDS	0.41 $\pm$ 2.19	2.78 $\pm$ 1.55*	2.54 $\pm$ 1.46*
Parent-adjusted HSDS	-2.07 $\pm$ 1.66	-1.35 $\pm$ 1.61*	-0.95 $\pm$ 1.53*
Weight SDS	-1.34 $\pm$ 2.88	-1.16 $\pm$ 2.62	-0.74 $\pm$ 2.23*
BMI SDS	0.56 $\pm$ 1.79	0.19 $\pm$ 1.41	0.18 $\pm$ 1.36
Waist circumference SDS (<i>n</i> = 15)	0.10 $\pm$ 2.19	-0.12 $\pm$ 1.73	0.05 $\pm$ 1.43
WHtR (<i>n</i> = 15)	0.50 $\pm$ 0.06	0.47 $\pm$ 0.04	0.47 $\pm$ 0.04
Sitting HSDS	-1.90 $\pm$ 1.46	-1.10 $\pm$ 1.62*	-0.60 $\pm$ 1.55*
SLL/SH ratio SDS	-1.81 $\pm$ 1.84	-1.45 $\pm$ 1.56	-1.45 $\pm$ 1.49
SBP percentile	76 $\pm$ 23	60 $\pm$ 25	65 $\pm$ 26
DBP percentile	63 $\pm$ 29	61 $\pm$ 20	49 $\pm$ 25
IGF-SDS	-0.70 $\pm$ 1.07	1.41 $\pm$ 0.91*	1.60 $\pm$ 0.98*

SDS, standard deviation score; WHtR, waist-to-height ratio; SH, sitting height; SLL, subischial leg length; SBP, systolic blood pressure; DBP, diastolic blood pressure; KS, Kabuki syndrome; rhGH, growth hormone; HSDS, height SDS. * $p < 0.05$.

ing a panel of cardiovascular risk markers. Serum and plasma samples for analyzing IGF-I, glucose metabolism, and lipid profiles were separated after centrifugation (3,000 g for 15 min at 4°C) and stored at -80°C until the assays were performed. Plasma tubes were used for the cardiovascular markers (1,300 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 plasma amino acid and dimethylarginine (ADMA) determination, fasting blood samples were collected using lithium-heparin tubes (4,000 rpm, 10 min, 4°C). For ADMA analysis, heparin plasma was deproteinized with 6 mg of solid 5-sulfosalicylic acid (Sigma, St. Louis, MO, USA) per 100 μ L plasma and was kept frozen at -80°C until analysis.

As previously described, serum IGF-I was measured in venous blood samples with the chemiluminescence method in an IDS-iSYS Multi-Discipline Automated System by Immunodiagnostic Systems Holdings, UK [18]. Fasting plasma glucose concentrations were measured in venous blood samples with the enzymatic hexokinase method using a Roche cobas 8000 modular 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 calculated using the HOMA-IR formula = ((fasting insulin $\times$ fasting glucose)/22.5) [24]. 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).

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 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" $\times$ 0.453) + "HDL-cholesterol". If the 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 an ABX Pentra C400 (HORIBA Medical, Kyoto, Japan).

Apolipoprotein A-I (Apo-A-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 [25]. Serum Apo-C2 and Apo-C3 were measured with an ELISA kit (Randox). All samples from 1 subject were analyzed within 1 run at the end of the study.

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

Concentration of ADMA was determined in the plasma using the ultra-performance liquid chromatography separation module coupled to an electrospray ionization tandem mass spectrometry (Quattro Premier, Walters, Etten-Leur, the Netherlands) by the Department of Biomedical Genetics at Maastricht University as described by Waterval et al. [26].

Fig. 1. Height SDS at baseline, after 1 and 2 years of rhGH treatment and 1 year post treatment of all individual 18 subjects with KS. **a** Female. **b** Male. KS, Kabuki syndrome; SDS, standard deviation score; rhGH, growth hormone.

Table 2. Auxological data (mean $\pm$ SDS) after 2 years of rhGH treatment and 1 year after cessation rhGH treatment in 12 subjects with KS

	Before start treatment	One-year treatment	Two-year treatment	One year after cessation of treatment	<i>p</i> value 2-year vs. cessation
Chronological age	6.7 $\pm$ 1.99	7.9 $\pm$ 2.07	8.9 $\pm$ 2.08	9.85 $\pm$ 1.90	
HSDS	-2.55 $\pm$ 1.76	-1.82 $\pm$ 1.90	-1.32 $\pm$ 1.91	-1.60 $\pm$ 1.80	<0.001
Height velocity, cm/year	6.23 $\pm$ 2.38	9.19 $\pm$ 1.76	8.36 $\pm$ 1.58	4.50 $\pm$ 1.10	<0.001
Height velocity SDS	0.75 $\pm$ 2.45	2.95 $\pm$ 1.58	2.93 $\pm$ 1.46	-0.84 $\pm$ 1.56	0.001
Weight SDS	-1.74 $\pm$ 2.70	-1.54 $\pm$ 2.76	-1.05 $\pm$ 2.35	-0.74 $\pm$ 2.54	0.038
BMI SDS	0.14 $\pm$ 1.50	-0.09 $\pm$ 1.35	-0.14 $\pm$ 1.33	0.49 $\pm$ 1.67	0.026

SDS, standard deviation score; KS, Kabuki syndrome; rhGH, growth hormone; HSDS, height SDS.

Statistical Analysis

Results for continuous variables were expressed as mean ($\pm$ SDS) and 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 were, depending on normal

distribution, assessed either through Student's *t* test for paired samples or the Wilcoxon rank test for paired samples. Spearman and Pearson's correlation analyses were used to determine correlations between variables. Furthermore, multiple linear regression analyses were used to investigate the relationship between baseline parameters (e.g., age, height SDS [HSDS], and BMI SDS) and response to treatment by adjusting for multiple variables. All statisti-

Fig. 2. BMI SDS at baseline, after 1 and 2 years of rhGH treatment and 1 year post treatment of all individual 18 subjects with KS. **a** Female. **b** Male. KS, Kabuki syndrome; rhGH, growth hormone.

cal analyses were performed using the software SPSS version 25 (SPSS Inc, Chicago, IL, USA). A p value of <0.05 was considered significant.

Results

The baseline characteristics of the 18 included subjects are provided in Tables 1 and 2. Furthermore, the characteristics of all individual subjects are presented in the online supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000519963). In summary, 9 boys and 9 girls were recruited with a mean age of $7.5 (\pm 2.3)$ and $6.4 (\pm 1.8)$ years, respectively. Fifteen of the KS subjects had a KMT2D gene mutation, and 3 had a KDM6A gene mutation. Based on the results from rhGH stimulation tests, 4 patients were rhGH-deficient. For those patients, additional imaging of the hypothalamic-pituitary region was performed in the patient's region-

al hospital, and they were all normal. 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. rhGH treatment was, as mentioned above, initiated with a "standard" rhGH dose. The initial dose of rhGH was altered based on IGF-I levels and/or rhGH response. During 24 months of treatment, the mean dose was 0.96 ± 0.22 mg/m²/day (0.036 ± 0.010 mg/kg/day).

Anthropometry

The anthropometric measurements at baseline and after 1 and 2 years of rhGH treatment are displayed in Table 1 and Figures 1 and 2. Additionally, data of individual subjects are provided in the online supplementary table. HSDS improved from $-2.40 (\pm 1.88)$ at baseline to $-1.30 (\pm 1.88)$ after 2 years of treatment. Hence, an increase in Δ HSDS of 1.1 SDS occurred post-2 years of rhGH substitution.

Fig. 3. Bone age delay (years) at baseline and after 1 and 2 years of rhGH treatment of all individual 18 subjects with KS. KS, **a** Female. **b** Male. Kabuki syndrome; rhGH, growth hormone.

Individually, responsiveness to rhGH therapy, defined as 0.5 SDS change in height, was identified in 13/18 (72%) children after 1 year and 16/18 (89%) children after 2 years of treatment. Furthermore, all 16 subjects exceeded a height gain of 0.5 SDS, by surpassing 0.7 SDS. Two children did experience less growth response with an increase in HSDS of 0.28 and 0.36, respectively. One of them was suffering from headaches that were initially thought to be a side effect of rhGH therapy, and the rhGH dose was consequently lowered to about 0.017 mg/kg/day. The other child had already exhibited a normal growth pattern at the start of treatment and was somewhat older than the average age of the study group (9.47 years). Analysis of the results by multiple linear regression revealed that a younger age at the start of treatment was associated with a greater Δ HSDS adjusted for baseline HSDS, baseline BMI, and baseline IGF-I $\{(F [4, 13] = 5.15, p = 0.010), \text{ with an } R^2 \text{ of } 0.61. \text{ Subjects' predicted}$

Δ HSDS is equal to $2.30 - 0.115 (\text{age at onset in years}) + 0.01 (\text{BMI at onset}) - 0.009 (\text{HSDS at onset}) - 0.004 (\text{IGF-I SDS at onset})$. Both baseline IGF-I SDS and age were significant predictors of Δ HSDS ($p = 0.02$ and $p = 0.01$, respectively).

No statistically significant intergroup differences were seen in height gain SDS after the 2-year rhGH therapy for the subgroups KMT2D versus KDM6A or for short stature versus normal stature ($p = 0.44$ and $p = 0.77$). Corresponding numbers for the non-GH-deficiency and GH-deficiency subgroups were 1.02 and 1.42 Δ HSDS, respectively ($p = 0.15$). Nevertheless, all subgroups showed a significant increase in HSDS when compared to baseline. Boys and girls experienced almost the same increase in Δ HSDS, 1.07 and 1.15, correspondingly.

At the beginning of the study, the mean bone age delay of the subjects was approximately 1 year. After 2 years of rhGH therapy, the mean bone age advanced from 5.90 to

Table 3. Cardiovascular and metabolic parameters at baseline and after 12 and 24 months of rhGH treatment

	Baseline		12 months GH		24 months GH		<i>p</i> value ^a	<i>p</i> value ^b
	<i>n</i>	mean ± SD	<i>n</i>	mean ± SD	<i>n</i>	mean ± SD		
Glucose (3.1–7.8 mmol/L)	18	4.21±0.73	18	4.60±0.72	18	4.52±0.37	0.065	0.157
Insulin (12–150 pmol/L)	18	19.30±16.22	17	39.70±54.49	18	24.06±20.07	0.161	0.450
HOMA-IR	18	0.55±0.51	17	1.24±1.81	18	0.71±0.63	0.147	0.396
Hb1Ac (25–44 mmol/mol)	18	29.67±5.59	18	30.83±5.07	18	31.61±4.88	0.096	0.294
TC (3–5.5 mmol/L)	18	3.96±0.81	18	3.77±0.71	18	3.67±0.72	0.076	0.071
HDL (>0.9 mmol/L)	18	1.53±0.56	18	1.56±0.48	18	1.47±0.48	0.816	0.444
LDL (3.5–4.4 mmol/L)	18	2.16±0.73	18	1.94±0.67	18	1.91±0.69	0.014*	0.039*
TG (0.4–1.6 mmol/L)	18	0.61±0.17	18	0.60±0.24	18	0.62±0.23	0.984	0.754
ADMA (0.42–1.10 µM)	15	0.63±0.13	14	0.56±0.12	–	–	0.118	–
E-selectin, ng/mL	17	26.6±10.2	18	24.8±7.9	14	26.09±5.81	0.322	0.269
P-selectin, ng/mL	17	53.9±17.4	18	48.9±23.6	14	46.97±28.34	0.390	0.011*
ICAM-1, pg/mL	17	603±101	18	594±102	14	601±112	0.551	0.624
ICAM-3, ng/mL	17	0.88±0.29	18	0.74±0.32	18	0.68±0.26	0.071	0.005*
VCAM-1, pg/mL	17	1,084±166	18	1,161±194	14	1,193±162	0.006*	0.135
MCP-1, pg/mL	17	155±75	18	122±30	14	121±43	0.076	0.015*
IL-6, pg/mL	16	0.99±0.93	18	0.81±0.56	14	0.77±0.53	0.469	0.667
IL-8, pg/mL	17	5.45±2.39	17	3.74±1.06	14	3.78±1.60	0.008*	0.092
TNF-α, pg/mL	17	2.80±0.60	18	2.51±0.58	14	2.67±1.10	0.329	0.666
TNFR-1, pg/mL	17	3,002±1,349	18	3,129±1,965	14	3,671±3,395	0.622	0.313
TNFR-2, pg/mL	17	5,064±1,783	18	4,909±1,798	14	5,340±3,561	0.631	0.642
SAA, pg/mL	17	3,764±5,552	18	2897±4,776	13	4,953±1,239	0.668	0.606
CRP, fg/mL	17	178±183	18	164±296	14	216±339	0.828	0.354
TM, ng/mL	17	6.0±1.8	18	6.3±2.2	14	7.0±3.0	0.444	0.555
Apo-A-1, g/L	17	1.17±0.30	18	1.21±0.23	14	1.17±0.24	0.238	0.824
ApoB100, g/L	17	0.72±0.18	18	0.68±0.15	14	0.66±0.15	0.130	0.026*
Apo C-2, mg/mL	17	3.01±1.36	18	3.45±1.38	14	3.55±0.93	0.064	0.408
Apo C-3, mg/mL	17	6.37±2.08	18	6.88±2.23	14	7.23±2.05	0.087	0.118
Apo B-48, ng/mL	17	10,408±4,135	18	10,537±4,214	14	10,432±4,776	0.701	0.523

^a*p* value 12 months compared to baseline; ^b*p* value 24 months compared to baseline. SDS, standard deviation score; *n*, number; HOMA-IR, homeostatic model assessment-insulin resistance; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; ADMA, plasma amino acid and dimethylarginine; ICAM, intercellular adhesion molecule; VCAM, vascular cell-adhesion molecule; MCP, monocyte chemoattractant protein; IL, interleukin; TNF, tumor necrosis factor; TNFR, TNF receptor; SAA, serum amyloid A; CRP, C-reactive protein; TM, thrombomodulin; Apo, apolipoprotein; rhGH, growth hormone.

8.52 years, as is shown in Figure 3. This advance in the bone age resulted in a significant decrease in the difference between BA and CA (chronological age) from approximately 1 year to 6 months. The mean ΔBA/ΔCA was 1.25 ± 0.45 and 1.26 ± 0.31 after 1 and 2 years of treatment, respectively.

During treatment, no significant changes in body proportions were measured. A significant difference in the sitting height was observed which was greater than the difference in height. Nevertheless, no significant alteration in the subischial leg length/sitting height ratio was noted. This ratio decreased in the first year of treatment from –1.81 SDS to –1.45 SDS and remained stable during further treatment.

Throughout this study, every participant received rhGH treatment for a duration of 24 months. After this period, only 3 participants had an indication for continuing rhGH substitution according to the current guidelines of the Dutch Growth Research Foundation. These guidelines declare that rhGH treatment is indicated when patients (1) suffer from short stature, (2) have a delayed bone age, (3) other causes than rhGH deficiency for growth delay are ruled out, and (4) rhGH deficiency is demonstrated by 2 rhGH stimulation tests. Subsequently, in 15 participants, rhGH treatment was ended after 24 months. Data on linear growth were obtained for 12 participants in this subgroup 1 year after cessation of rhGH treatment. In this subgroup, HSDS declined significantly

from -1.32 to -1.60 ($p < 0.001$). In addition, a significant decrease in the height velocity was also observed ($p < 0.001$), which is displayed in Table 2 and Figure 1.

Before initiating rhGH treatment, overall BMI SDS was 0.56 ± 1.79 , with a mean of 0.64 (boys) and 0.48 (girls). Overall BMI SDS decreased to 0.19 ± 1.41 after 1 year of rhGH treatment and remained stable at this level after the second year. However, BMI increased significantly to 0.49 ± 1.67 1 year after discontinuation of rhGH therapy, as is demonstrated in Table 2 and Figure 2. At start of the study, 4 patients were obese, defined as BMI >2 SDS. Their BMI SDS decreased from 3.01 to 1.74 after 2 years of treatment, representing an ongoing nearly significant effect ($p = 0.05$). The WC and WHtR changed, respectively, from -0.10 to 0.05 SDS ($p = 0.44$) and 0.50 to 0.47 ($p < 0.01$), respectively. The WHtR ratio decreased from 0.58 to 0.51 in the obese group which is not statistically significant ($p = 0.145$).

Blood Pressure

Blood pressure values were all within the normal range at baseline, as well as after 1 and 2 years of rhGH therapy. There was no significant difference between boys and girls, and there was no association with BMI and WC.

Glucose Metabolism

At baseline, fasting glucose, insulin, and HbA1c concentrations, as well as the HOMA-IR, were all within the normal range. After 2 years of rhGH treatment, there were no significant changes in HbA1c, glucose, insulin, and HOMA-IR. However, there was a trend toward the development of insulin resistance after 12 months (Table 3) since both fasting glucose and insulin concentrations increased slightly. This tendency completely disappeared after 2 years of treatment. In terms of disaggregating effects between genders, only girls presented a significant increase in fasting glucose concentrations after 1 year of rhGH treatment ($p = 0.02$).

Igf-I

Favorable results were observed for serum IGF-1 levels. These levels significantly increased from a mean of -0.70 ± 1.07 at the start to 1.60 ± 0.98 after 24 months of rhGH therapy. Additionally, serum IGF-1 SDS levels >2 were seen in 6 patients during treatment. rhGH dosage was adjusted for these patients which resulted in a lowering of serum IGF-1 SDS levels below 2.

Lipid and Lipoprotein Metabolism

All parameters associated with lipid and lipoprotein metabolism were within normal ranges at baseline and

after 12 and 24 months of rhGH therapy. A significant decrease was seen for LDL cholesterol concentrations during the whole study period when compared to baseline (Table 3). This decline was not correlated with the decrease in BMI or the WC. In line with LDL, a statistically significant decrease of ApoB100 concentrations was observed at 24 months. The ApoB100/Apo-A1 ratio, a known predictor for the risk of cardiovascular disease, significantly decreased from 0.66 at baseline to 0.58 after 1 year of treatment ($p = 0.03$). However, after 2 years of treatment, the ApoB100/Apo-A1 ratio increased to 0.61 and therefore no longer represented a significant difference.

Markers for Endothelial Function and Chronic Low-Grade Inflammation

Endothelial function was evaluated by measuring ADMA, intercellular adhesion molecule-1, VCAM-1, intercellular adhesion molecule-3 (ICAM-3), E and P-selectin, and monocyte chemoattractant protein-1 (MCP-1) levels. With the exception of VCAM-1, all of these remained stable during the first year of rhGH treatment. Significant alterations were evident for P-selectin, ICAM-3, and MCP-1 after 2 years. P-selectin levels decreased significantly after 24 months of rhGH treatment. Furthermore, a declining trend in ICAM-3 and MCP-1 levels was already seen at 12 months of therapy and reached significance after 24 months of rhGH therapy.

The presence of a low-grade inflammatory state was evaluated by measurements of TNF- α , TNF receptors, IL-6, IL-8, C-reactive protein, and serum amyloid A concentrations. With one exception, no changes in these parameters were found before and after 12- and 24-month rhGH treatments. The chemokine IL-8 initially decreased significantly; however, this effect was no longer perceived after the 2-year rhGH treatment.

Discussion

In addition to the well-known congenital anomalies such as distinctive facial features, postnatal growth retardation is one of the main clinical characteristic of children with KS. This study provides the first long-term analysis of the effects of rhGH therapy on catch-up growth and cardiovascular risk markers. After 2 years of rhGH treatment, a significant increase on overall linear growth was seen with an increase in the mean height from -2.40 SDS to -1.30 SD and with a responsiveness to treatment of 89%. In addition to growth promotion, improvements in

BMI and WCHt were noticed without signs of cardiovascular abnormalities.

The cause of the short stature in KS remains unclear. Niikawa et al. [6] reported about postnatal growth retardation in male subjects. Their finding was validated in a northern European study on 39 individuals with a genetically proven diagnosis of KS [27]. A genotype-phenotype correlation was not observed. Further research was done on the GH-IGF-I axis in a prospective study on 18 genetically confirmed KS individuals [18]. A combination of both arginine and clonidine rhGH stimulations tests demonstrated a lack of GH response in 27.8% of the KS individuals. However, IGF-I levels did not correspond to the GH stimulations tests, while none of the GHD KS patients had a low IGF-I level. These results indicate that GHD is not the only cause of short stature in KS patients. Therefore, we conclude that the utility of performing GH-stimulation tests on KS children as an indication of GH status in short stature is questionable.

Nevertheless, rhGH treatment in KS children produced significant catch-up growth. Furthermore, a significant association was seen between the age of initiating treatment and the growth response. In this study, KS patients received rhGH therapy for a period of 2 years. This exerted a positive effect on linear growth, which partially diminished when rhGH therapy was stopped. One year after the end of rhGH treatment, HSDS deflected to -0.23 . Results about the effect of discontinuing rhGH treatment before achieving final height are scarce. The limited literature about this subject concerns study groups with patients suffering of other syndromes. Nevertheless, these studies report comparable results on linear growth after discontinuation of rhGH treatment. Additionally, they report about metabolic deterioration after cessation of rhGH therapy [28–31]. However, the metabolic effect of discontinuing rhGH was not included in this study, but the decline in linear growth provides another argument that rhGH is an effective treatment in KS children.

Bone age measurements are an important step in diagnosing growth-related diseases in patients with short-stature and are delayed in patients with GHD, SGA, hypothyroidism, malnutrition, and chronic illness [32]. GH and IGF-I are traditionally considered potent stimulators of bone growth. After GH treatment, increased serum IGF-I levels stimulate growth plate development, resulting in BA progression and impact final height. During 2 years of rhGH treatment, our study showed a bone age progression from approximately 1 year to 6 months. Studies with similar conditions to ours (prepubertal at start of rhGH treatment, same dosing, and 2 years of

treatment) by Cohen et al. [33] and Kang et al. [34] in GHD children reported 1.2 and 1.28 years of BA progression/year of CA respectively, which were consistent with our findings (1.25 of BA progression/year of CA). Our results suggest that the 2-year GH treatment benefits on HSDS for CA are most likely not to affect the final height gain.

During the first year of rhGH treatment in our study, the increase in sitting height was slightly greater than the increase in height. However, this did not result in a significant alteration in the subischial leg length/sitting height ratio. Furthermore, this ratio did not change at all during the second year of rhGH treatment, thereby representing normal body proportions during rhGH therapy.

To our knowledge, no past research reported on metabolic markers in KS patients, and little is known about the phenotype of adult KS patients. However, KS children tend toward obesity during adolescence [10, 11]. Furthermore, fat distribution is described as mainly truncal in KS [11]. Therefore, we hypothesized that KS patients are predisposed to an elevated risk for developing metabolic syndrome in adulthood. While our study population was prepubertal, only 4 patients were suffering from obesity. Nevertheless, we found a reduction in BMI after 2 years of rhGH treatment from 0.56 SDS to 0.18 SDS in the total study population, with a nearly significant ($p = 0.05$) decrease in the obesity subgroup ($n = 4$). Additionally, there was a significant correlation between the BMI, WC, and the WHtR in the total study population, confirming that the fat distribution in our population was truncal. Studies demonstrated that rhGH treatment induced reductions in visceral adipose tissue and improved insulin sensitivity [35, 36]. Indeed, there was a reduction in the WC and WHtR on rhGH in our KS children population. Despite the small study size, this indication is clinically important with regard to the prevention of truncal obesity and therefore the eventual risk on the development of the metabolic syndrome. Next to obesity, 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 cholesterol concentrations [37]. Since there is, at best, scant understanding about the MS or cardiovascular complications in KS subjects, blood pressure and cardiovascular parameters were also studied. At baseline and after 24 months of rhGH therapy, blood pressure remained normal, and there were no significant correlations with BMI or the WC. The same was true for dyslipidemia. Notably, the LDL cholesterol concentrations significantly decreased during rhGH treatment. Since ApoB100 concen-

trations were also lowered, it can be suggested that not only LDL cholesterol concentrations but also the number of LDL particles was reduced. A recent meta-analysis by Giagulli et al. [38] revealed an overall reduction in LDL levels in GHD patients, which echoes the findings in our study. This finding points to a preventative intervention against cardiovascular problems later in life.

In children, it is known that high IGF-I levels and treatment with rhGH are associated with increased insulin levels and correlate with growth response in some cases [39, 40]. In this study, the IGF-I levels significantly increased during rhGH therapy. Fasting glucose, insulin, HbA1c concentrations, and the HOMA-IR were all within the normal range at baseline and remained normal after 2 years of rhGH treatment despite the association between rhGH administration and insulin resistance.

RhGH deficiency in adults is associated with an increase in visceral adiposity, adverse serum lipid profiles, vascular endothelial dysfunction, and reduced exercise capacity [41–43]. It is known that obesity is accompanied by chronic inflammation of adipose tissue. While data suggest that GH plays an inhibitory role on TNF- α release, current studies are inconclusive [44]. However, PWS positive associations were found between TNF- α and body weight. Furthermore, rhGH treatment decreased the levels of the inflammatory markers, albeit not to a statistically significant level [45]. We observed a similar trend with the reduction of TNF- α levels in the first year, and only after 2 years of treatment, this disappeared. The same was true for IL-8.

ADMA is a competitive inhibitor of nitric oxide synthase, which is responsible for most vascular nitric oxide production. A high prevalence of elevated plasma ADMA is observed in patients with hypercholesterolemia, hypertension, renal failure, diabetes mellitus, cardiovascular disease, and many other clinical disorders, even among apparently healthy persons. Andrade et al. [46] studied ADMA levels in healthy children and made a biochemical profile for ADMA levels with a mean of 0.61 μ M (0.42–1.10). Our study group had the same mean level at baseline, and during rhGH treatment, the levels even exhibited a slight decline. Therefore, we can conclude that our population of KS children has normal ADMA levels and does not seem to have any signs of cardiovascular problems.

Studies of endothelial function in GHD adults using biochemical methods revealed that 12 months of rhGH treatment led to a decrease of these biochemical parameters similar to that experienced by healthy controls. In the literature, a strong correlation exists between the observed changes in IGF-1 and these parameters [47]. In

patients with the acromegaly serum, ICAM and VCAM levels are significantly higher than those in the control group [48]. Also, in our group, the VCAM-1 concentration was significantly elevated during the first year of treatment. The other parameters remained stable, although ICAM-3 concentrations exhibited a significant tendency toward lower values. In conclusion, we can say that there are inconsistent outcomes regarding endothelial function when employing biochemical methods in KS children during rhGH replacement.

In conclusion, we demonstrated that there was a convincing positive effect of rhGH treatment on linear growth in KS children. Furthermore, there were no severe cardiometabolic abnormalities in KS children at baseline or during rhGH treatment. Moreover, serum LDL cholesterol and ApoB100 concentrations were lowered. Biomarkers for endothelial function and inflammation did not change during therapy. Additionally, no important side effects were reported. Therefore, the results from this study demonstrate that rhGH treatment in KS children is a safe and effective means of promoting catch-up growth during a 2-year therapeutic approach. Long-term follow-up will be mandatory to evaluate the effect of rhGH on final height, the tendency toward obesity during adolescence, and the eventual risk on metabolic syndrome.

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Statement of Ethics

This study was performed as an open-label, prospective, non-randomized study of KS children (trial registration: NTR4722, study approval reference number: 2012-000432-26) at Maastricht University. The Medical Ethics Committee of the Maastricht University Medical Centre provided ethical approval of the study. Written informed consent was obtained from all parents.

Conflict of Interest Statement

All authors report no conflicts of interest to declare.

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Author Contributions

Drs. Lieke van Monfort was responsible for data analysis and writing the manuscript. Dr. Willem-Jan Gerver contributed to the acquisition and design of the study as well as both critically revising the article in terms of important intellectual content and in providing final approval of the version to be submitted. Drs. Berbel Kooger preformed analysis of biomarker data. Prof. Jogchum Plat contributed to the acquisition, analysis, and interpretation of cardiovascular biomarker data and in critically revising the article in terms of important intellectual content. Dr. Jorgen Bierau contributed to the acquisition and analysis of the ADMA data.

Prof. Constance Stumpel was responsible for the genetic content of the final version to be submitted. Dr. Dina Schott was responsible for study design, data curation, literature review, supervision, drafting and critically revising the article, as well as providing final approval of the version to be submitted.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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