Received: 9 August 2023 | Revised: 17 October 2023 | Accepted: 27 October 2023

DOI: 10.1002/ajmg.a.63472

AMERICAN JOURNAL OF medical genetics PART A WILEY

ORIGINAL ARTICLE

Growth, body composition, and endocrine-metabolic profiles of individuals with Kleefstra syndrome provide directions for clinical management and translational studies

Arianne Bouman[1,2] | Joyce M. Geelen[3] | Joost Kummeling[1,2] | Annette Schenck[1,2] | Yvonne G. van der Zwan[4] | Willemijn M. Klein[5] | Tjitske Kleefstra[1,2,6,7]

[1]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

[2]Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands

[3]Department of Pediatrics, Developmental and Genetic Pediatrics, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands

[4]Department of Pediatrics, Pediatric Endocrinology, Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands

[5]Department of Medical Imaging, Radboud University Medical Center, Nijmegen, The Netherlands

[6]Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands

[7]Center of Excellence for Neuropsychiatry, Vincent van Gogh Institute for Psychiatry, Venray, The Netherlands

Correspondence
Tjitske Kleefstra, Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands.
Email: t.kleefstra@erasmusmc.nl

Funding information
Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Numbers: 10250022110003, 91718310; ZonMw; Dutch Research Council, Grant/Award Numbers: 09150181910022, 1160.18.320, 015.014.036

Abstract

Mendelian neurodevelopmental disorders caused by variants in genes encoding chromatin modification can be categorized as Mendelian disorders of the epigenetic machinery (MDEMs). These disorders have significant overlap in molecular pathways and phenotypes including intellectual disability, short stature, and obesity. Among the MDEMs is Kleefstra syndrome (KLFS), which is caused by haploinsufficiency of *EHMT1*. Preclinical studies have identified metabolic dysregulation and obesity in KLFS models, but proper clinical translation lacks. In this study, we aim to delineate growth, body composition, and endocrine-metabolic characteristics in a total of 62 individuals with KLFS. Our results revealed a high prevalence of childhood-onset overweight/obesity (60%; 28/47) with disproportionately high body fat percentage, which aligns perfectly with previous preclinical studies. Short stature was common (33%), likely due to advanced skeletal maturation. Endocrine-metabolic investigations showed thyroid dysregulation (22%; 9/41), elevated triglycerides, and decreased blood ammonia levels. Moreover, hand radiographs identified decreased bone mineralization (57%; 8/14) and negative ulnar variance (71%; 10/14). Our findings indicate a high (cardio)metabolic risk in KLFS. Therefore, we recommend monitoring of weight and endocrine-metabolic profile. Supporting a healthy lifestyle and screening of bone mineralization is advised. Our comprehensive results support translational research and contribute to a better understanding of MDEM-associated phenotypes.

KEYWORDS
9q34.3, body composition, *EHMT1*, growth, Kleefstra syndrome, MDEM, metabolism

Abbreviations: BF%, body fat percentage; BHI, bone health index; BIA, bioelectrical impedance analysis; BMI, body mass index (kg/m^2); DEXA, dual-energy X-ray absorptiometry; HC, head circumference (cm); HipC, hip circumference (cm); KLFS, Kleefstra syndrome; MDEM, Mendelian disorder of the epigenetic machinery; MUAC, mid-upper arm circumference; NDD, neurodevelopmental disorder; SDS, standard deviation score; SMM(a), (appendicular) skeletal muscle mass; WC, waist circumference (cm)

1 | INTRODUCTION

The understanding of genetic variants underlying neurodevelopmental disorders (NDDs) has improved tremendously over the past decade and the number of diagnosed patients still increases. Over 1500 genes have been identified as related to Mendelian NDDs (Kochinke et al., 2016; Maia et al., 2021). Among these are two prominent groups based on pathophysiology: those involved in gene expression regulation (such as chromatin remodelers and transcription factors) and those involved in neuronal communication (Satterstrom et al., 2020). To the first class belong the Mendelian disorders of the epigenetic machinery (MDEMs), caused by gene variants that affect chromatin modification. These disorders share phenotypic characteristics with each other and with some of the classical imprinting disorders, including intellectual disability, developmental delay, autism spectrum disorder, short stature, and obesity (Fahrner & Bjornsson, 2014; Kochinke et al., 2016).

Clinical data collection in MDEMs so far has been primarily focused on neurodevelopmental features and somatic malformations. However, features that are less clinically evident and require dedicated and specialized measurements (e.g., biochemical assays to diagnose insulin resistance) are still largely uncharacterized. Comprehensive and longitudinal examinations are rarely done due to the fragmented follow up of individuals with such rare disorders, resulting in limited systematic longitudinal data.

Kleefstra syndrome (KLFS; OMIM #610253) is among the MDEMs (Fahrner & Bjornsson, 2014) and is caused by haploinsufficiency of the gene euchromatin histone methyltransferase 1 (*EHMT1*), resulting from either 9q34.3 microdeletions or intragenic variants (Kleefstra et al., 2006). Key characteristics of KLFS include intellectual disability, developmental delay, and autism spectrum disorder. Short stature and obesity are commonly observed in KLFS (Kleefstra & de Leeuw, 2010; Willemsen et al., 2012) as well as in other MDEMs, such as Kabuki syndrome and Wiedemann-Steiner syndrome (Fahrner & Bjornsson, 2014). However, the exact prevalence of these features in KLFS and how they progress over time remain uncertain, as no significantly representative cohort has been studied.

In preclinical models of KLFS including mouse and *Drosophila*, both the EHMT1 ortholog termed G9a-like protein (GLP) and the EHMT2 ortholog G9a, play a crucial role in fat homeostasis (Kramer, 2016; Ohno et al., 2013). Studies in mouse have shown that targeted knockout of *GLP* in differentiating brown adipocytes impairs brown fat differentiation and leads to obesity and insulin resistance (Ohno et al., 2013). *Drosophila* G9a mutants also have increased fat tissue and triglyceride levels, illustrating that metabolic regulation by the evolutionary conserved EHMT1/GLP/G9a protein family is not limited to brown fat (Riahi et al., 2019). G9a mutants also have increased glycogen stores, however, upon stress such as oxidative stress of infection, the Kleefstra fly model experiences an exaggerated metabolic shift that quickly exhausts sugar stores while fat stores cannot be assessed efficiently, causing reduced stress tolerance (Riahi et al., 2019). Whereas the metabolic dysregulation in response to stressors appears to be conserved in mammalian systems (Riahi et al., 2021), the clinical relevance of these findings remains unclear since no corresponding studies in a representative patient cohort have been performed to date.

Our aim therefore was to provide comprehensive insights into growth, body composition, and endocrine-metabolic profiles in KLFS. This systematic data collection is crucial for guiding patient management and studying potential clinical biomarkers. These biomarkers can also serve as outcome measures contributing to better diagnoses and as readouts for clinical trials. Ultimately, our study could guide both preclinical and clinical work into other MDEMs and contributes to the understanding of common phenotypes and underlying pathophysiological mechanisms in other MDEMs.

2 | METHODS

Prospective and retrospective data on growth, body composition, and biochemical investigations were collected from individuals with a molecular confirmed diagnosis of KLFS, known in the Radboud University Medical Center (Radboudumc) expert center for rare genetic NDDs. Individuals born before 32 weeks of gestational age were excluded. The project was approved by the Medical Ethics Committee of the Radboudumc, Nijmegen, The Netherlands (#2021-13018; #2021-13114; #2023-16406). Retrospective data were extracted from the Radboudumc biobank *Genetics and Rare disease* (#2018-4985).

2.1 | Growth parameters

Growth parameters were measured during individuals' Radboudumc outpatient clinic visit and were retrospectively assessed by review of medical records and *growth booklets* of the Dutch municipal health service, known as GGD. Included parameters were height, weight, head circumference (HC), mid-upper arm circumference (MUAC), waist circumference (WC), and hip circumference (HipC). During participants' outpatient visit, a comprehensive medical history and physical examination was conducted.

Only individuals of Dutch, Moroccan, Turkish, and Hindustani descent were included in the analyses to allow for comparison with sex-, age-, and ethnicity-matched references. The references include the outcomes from the latest *Fifth Dutch Growth study*: height for Dutch descent (Schonbeck et al., 2013), height for Moroccan and Turkish descent (Schonbeck et al., 2015), height for Hindustani descent (de Wilde et al., 2015), weight and body mass index (BMI, kg/m^2) (Schonbeck et al., 2011), HC (Fredriks et al., 2000), MUAC, WC, and HipC (Gerver & Bruin, 2001). *Z*-scores of measurements taken before 24 months of age were corrected for gestational age if born before 37 weeks (Bocca-Tjeertes et al., 2012). We collected parental height information anamnestically through our survey (Supplementary File 1). Target height was calculated, accounting for sex and ethnicity, when the height of both parents was known (van Zoonen, 2019).

Data on clinical conditions that can affect growth, such as prenatal stressors, congenital disorders, food-related behavior, sports, and familial factors, were collected through a parent survey. This survey also included the request and explanation for performing body circumference measurements (HC, MUAC, WC, and HipC) (Supplementary File 1). HC was measured at the broadest point of the head, just above the eyebrows and ears. MUAC was measured with the arm in relaxed state, at the midpoint between the shoulder and elbow. WC was measured in an upright standing position, taken between the lower rib and the top of the iliac crest following a normal exhalation. HipC was determined as the widest circumference at the hip level. Body circumference measurements provided by parents were included only when measurements performed by medical professionals were not available. Overall, growth measurements were conducted by medical professionals at 89% (448/503) of the time points.

Up to one growth measure per month was included from birth until the age of 1 year. From age 1 until 21 years, a maximum of one measure per 6 months was included. If multiple measurements were available per defined period, the first one was included. For individuals above 21 years of age, only the most recent measurement was included. Measurements taken while using antipsychotic drugs were excluded for weight and BMI calculations. The following definitions were used: short stature = height $Z \leq -2$, microcephaly = HC $Z \leq -2$, overweight = BMI $Z \geq 1$, obesity = BMI $Z \geq 2$.

2.2 | Body composition

Body composition was evaluated using bioelectrical impedance analysis (BIA; InBody 770 Body Composition Analyzer). BIA measurement was performed while lightly dressed, after at least 3 h of fasting, and after toileting. BIA measurement and/or dual-energy X-ray absorptiometry scan results from routine clinical care (DEXA; Hologic Discovery A, software Horizon A 13.6.1.2) were used to evaluate body fat percentage (BF%), skeletal muscle mass (SMM), bone mineral density, and basal metabolic rate. BF% was considered to be in the normal range when body fat (kg) was above the 15%–23% of body weight (kg) (-Malina, 1988; Tahara et al., 2002; Wohlfahrt-Veje et al., 2014). Appendicular SMM of our individuals were compared to sex- and age-matched controls (McCarthy et al., 2014). Basal metabolic rate was estimated by use of the Schofield equation (Schofield, 1985).

2.3 | Radiological characteristics

As our findings showed striking trends in growth and body composition, we decided to conduct additional posteroanterior hand radiographs. This was done to enhance the interpretation and understanding of growth patterns and bone metabolism. Hand radiographs were made during patients' regular outpatient visit and retrospectively collected from medical records. Skeletal age and bone health index (BHI) were evaluated using the software BoneXpert 3.2.2 which includes sex- and age-matched reference data (BoneXpert, n.d.; Thodberg et al., 2016). Radiologist WK measured ulnar variance using the Hafner DIDI method below age 14 years and the adapted perpendicular method above age 14 years (Kox et al., 2020) and evaluated if any further ossal abnormalities were present.

2.4 | Endocrine-metabolic investigations

Biochemical investigations were performed during routine clinical care, or retrospective data were collected from medical records if blood sampling was not done at the Radboudumc in years 2021/2022. Only individuals' most recent measure was included, excluding measurements taken while using vitamin D, B12, or folic acid supplementation. All measurements were compared to sex- and age-matched controls (Supplementary File 2) (de Vries, 2015).

The analyzed biochemical markers were selected for their association with endocrine-metabolic dysregulation or their potential to affect endocrine-metabolic function. Sampling was performed through venipuncture. Investigations included hemoglobin (mmol/L), ALAT (U/L), thyroid function (free thyroxine 4 [fT4] pmol/L, thyroid-stimulating hormone [TSH] mIU/L), lipid profile (triglycerides mmol/L, cholesterol mmol/L, high-density lipoprotein cholesterol [HDL] mmol/L, low-density lipoprotein cholesterol [LDL] mmol/L), glucose (mmol/L), HbA1c (mmol/mol), lactate (mmol/L), pyruvate (μmol/L), LDH (U/L), 25-hydroxy vitamin D (25(OH)D, nmol/L), vitamin B12 (pmol/L), folic acid (nmol/L), ammonia (μmol/L), and creatinine (μmol/L). Samples of lactate, pyruvate, and ammonia were stored on ice until processing. Supplementary File 2 shows the used biochemical equipment.

Biochemical results of individuals with KLFS were compared to sex- and age-matched healthy controls who received a blood sampling in the Radboudumc in the past. The controls were anonymously identified and their biochemical results were collected using the software CTcue and Cliniquest.

Statistical analyses were performed using SPSS version 25 and GraphPad Prism 9, including unpaired *T*-tests, one-way ANOVA, and regression analyses. A p-value <0.05 was considered as significant.

3 | RESULTS

In this study, we included 62 individuals (23 males, 39 females) with KLFS. The mean age at inclusion was 18.96 years (range 2.0–57.0 years). KLFS was caused by a pathogenic variant in the *EHMT1* gene in 38 individuals and by a 9q34.3 deletion in 24 individuals. Patient characteristics and genotypes are included in Supplementary File 3.

3.1 | Growth parameters

Gestational age was known for 51 individuals, with a median gestational age of 40.0 weeks (IQR 3.57). Premature birth (<37 weeks) occurred in 10% of individuals (5/51), while 16% were born post-term (≥42 weeks, 8/51).

To optimize comparability with national growth charts, growth parameters were analyzed in Dutch individuals with a *EHMT1* pathogenic variant or 9q34.3 deletion smaller than 1.3 Mb. A cut-off of 1.3 Mb was chosen since no individuals in this study had a deletion between 1.3 and 2 Mb.

Subsequently, a cohort of 48 individuals (17 males, 31 females) was established, with a mean age of 20.5 years (range 2.3–57.0 years) at inclusion. At birth, mean weight *Z*-score of this cohort was −0.35 ± 1.01, corrected for gestational age. At the time of inclusion, mean height *Z*-score was −0.69 ± 1.37 ($N = 48$), mean BMI *Z*-score was 1.22 ± 1.35 ($N = 47$), and mean HC *Z*-score was −1.34 ± 1.29 ($N = 42$). Growth parameters at birth and throughout life are shown in Table 1, Figure 1, and Figure S1.

Mean height at inclusion of the cohort was smaller than average. This is also observed by comparison with the individuals' target height. Parental height was available in 37 individuals, showing that 68% of KLFS individuals had a height below their expected target height (25/37) at their last measure. Additionally, height was below the target height range in 11% of individuals (4/37). Height measurements in individuals above 21 years old indicated a short stature in 33% (6/18).

BMI gradually increased during childhood and adolescence. At inclusion, 38% of individuals had a normal weight (18/47), 32% were overweight (15/47), and 28% were obese (13/47); one individual was underweight (2%, age 21.9 years). The mean BMI *Z*-score of males and females was 1.11 and 1.27, respectively ($p = 0.70$). In the age category of 2–12 years, 33% were overweight (10/30) and 23% were obese (7/30). Three individuals below 21 years were using antipsychotic drugs at the time of one measurement, at which point they all had a normal BMI.

Head circumference was generally decreased compared to sex- and age-matched controls. Above the age of 21 years, 56% of individuals (9/16) had a microcephaly.

In correlation analyses, no significant differences were found for birthweight, height, BMI, and HC between males and females or between individuals with *EHMT1* variants and deletions smaller than 1.3 Mb. However, when this cohort was compared to individuals with deletions over 2 Mb ($N = 6$, range 2.1–3.2 Mb), the results indicated smaller height (mean *Z*-score −2.04 ± 1.62) and lower BMI (mean *Z*-score 0.39 ± 1.72) within the larger deletion group (height $p = 0.03$; BMI $p = 0.17$).

Several clinical conditions that might affect growth were identified (all results are included in Supplementary File 4). A congenital heart defect was present in 34% (13/38) of the individuals. Hyperphagia (50%; 16/32) and appetite for non-food substances (31%; 10/32) were common. Only a minority of the individuals exercised at least 1 h a day (33%; 12/36). Interestingly, none of the individuals' siblings were overweight or obese. Hypertension, diabetes, or menstruation occurring before the age of 10 years were not reported.

3.2 | Body composition

Body circumference measurements were performed in 36 individuals and compared to sex- and age-matched controls. As shown in Table 1

TABLE 1 Overview of longitudinal growth data of Dutch participants with a EHMT1 pathogenic variant or 9q34.3 deletion <1.3 Mb.

Age (years)	Height Z-score ± SD	N	Head circumference Z-score ± SD	N	Weight Z-score ± SD	N	BMI Z-score ± SD	% overweight	% obesity	N	Waist circumference Z-score ± SD	N	Hip circumference Z-score ± SD	N
Birth	−0.99 ± 0.80	24	−0.64 ± 1.51	11	−0.19 ± 1.11	37	-	-	-	-	-	-	-	-
0–2	−0.27 ± 0.95	173	−1.11 ± 1.24	119	−0.20 ± 1.26	167	−0.03 ± 1.02	11	3	157	-	-	-	-
2–6	−0.15 ± 0.94	76	−0.85 ± 1.16	21	0.34 ± 1.11	70	0.64 ± 1.08	34	6	68	1.54 ± 0.70	6	1.20 ± 0.57	6
6–10	−0.08 ± 1.01	35	−0.18 ± 1.27	15	0.98 ± 1.50	38	1.17 ± 1.23	37	23	30	1.60 ± 1.91	5	0.91 ± 1.89	5
10–14	−0.38 ± 1.33	31	−0.66 ± 1.03	14	0.88 ± 1.59	27	1.35 ± 1.29	38	31	26	2.34 ± 1.49	5	1.39 ± 1.25	5
14–18	−0.65 ± 1.32	26	−0.94 ± 1.14	4	1.30 ± 1.75	21	1.90 ± 1.34	19	52	21	1.87 ± 1.24	3	0.72 ± 0.71	3
18–21	−1.02 ± 1.22	13	−1.95 ± 0.81	6	1.60 ± 2.19	14	1.89 ± 1.71	8	58	12	2.96 ± 1.81	3	1.23 ± 1.86	3
>21	−1.59 ± 1.17	18	−2.14 ± 1.41	16	−0.03 ± 1.58	18	0.83 ± 1.41	39	17	18	1.61 ± 1.64	14	0.59 ± 1.25	14

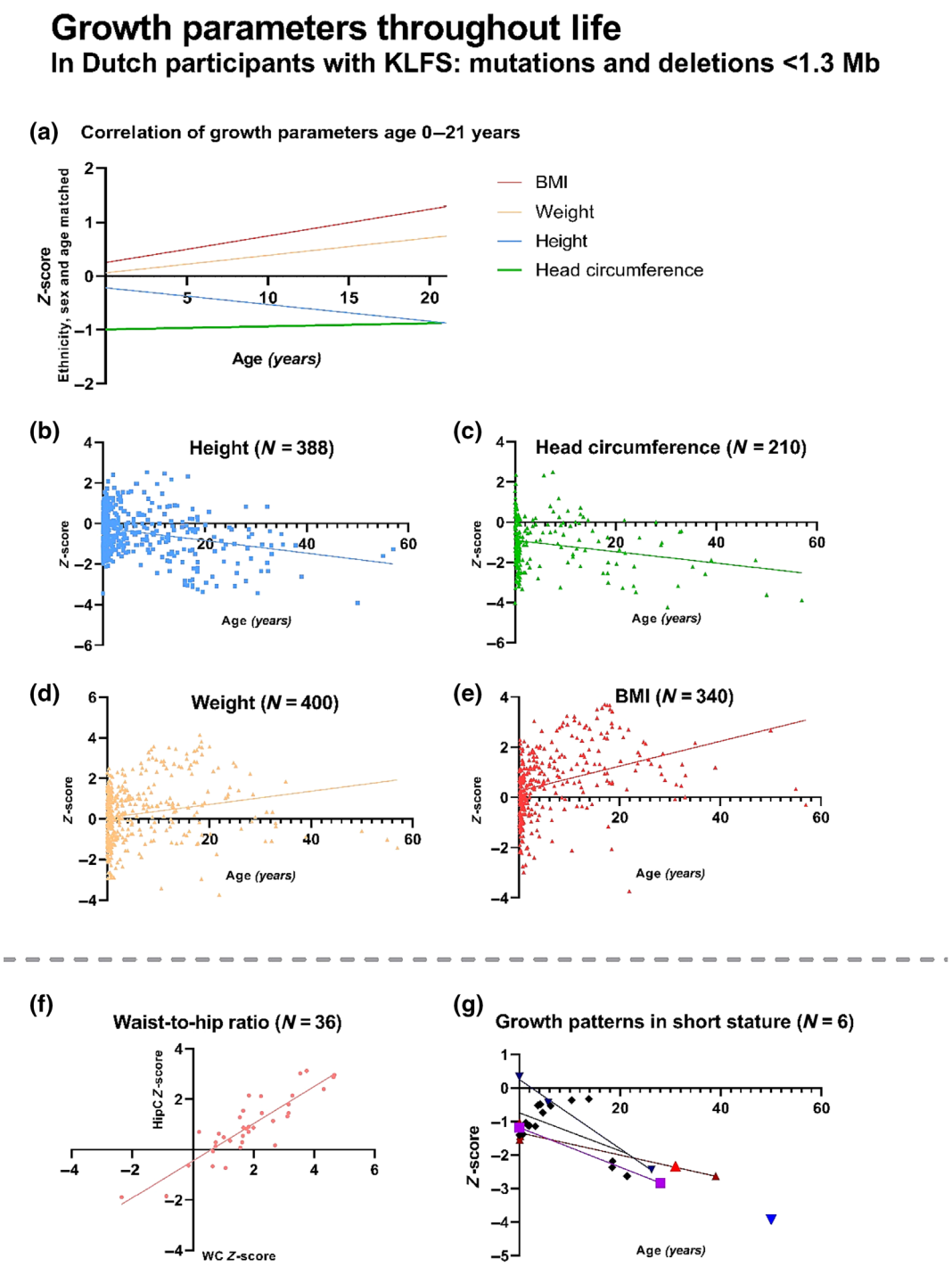

FIGURE 1 Growth parameters in 48 Dutch participants with Kleefstra syndrome (KLFS) caused by a *EHMT1* pathogenic variant or 9q34.3 deletion <1.3Mb. Graphs A. and B. show decrease in height *Z*-scores throughout childhood and adolescence. Graphs A. and C. show that the mean head circumference is lower than the population average, but remains stable over time. Graphs A., D., and E. show an increase in weight and BMI throughout childhood and adolescence. Graph F. shows a significant increase in waist circumference (WC) compared to hip circumference (HipC). Graph G. shows a large decrease in height *Z*-score in individuals who have a short stature above the age of 21 years.

and Figure 1, the results indicate significantly increased waist circumference (WC, *Z*-score 1.84 ± 1.49) compared to hip circumference (HipC, *Z*-score 0.91 ± 1.24), $p = 0.006$. The mean WC/height ratio was increased (0.54 ± 0.07). Mid-upper arm circumference (MUAC) measurements compared to sex- and age-matched controls were above the p90 in 72% of our individuals (26/36), while 6% (2/36) had an MUAC below the p10.

A BIA and/or DEXA scan was performed in seven female individuals to evaluate body fat percentage (BF%), skeletal muscle mass (SMM), and basal metabolic rate (Table 2). The amount of body fat (kg) was above

TABLE 2 Results of body composition measurements, investigated by BIA and/or DEXA scan.

	Individual (study ID)	19	32	45	52	53	58	99
Baseline	Age	15.4	12.8	4.9	7.5	11.4	8.8	13.3
	Weight (kg)	55.7	44.2	18.4	37	62.8	50.3	72.1
	BMI (kg/m^2)	22.19	18.44	16.1	21.6	26.6	24.6	27.68
	BMI *Z*-score	0.98	0.23	0.2	2.8	2.8	3.23	2.65
BIA	BF%	33.5	-	-	35.5	38	42.7	35.6
	Visceral fat area (cm^2)	88.4	-	-	69.6	123.9	128.8	125.8
	SMM total (kg)	20	-	-	12	20	15.1	25.6
	SMMa (kg)[a]	14.15		-	7.71	11.97	13.77	18.79
	SMMa (kg) in healthy individuals[b]	16.2 ± 2.6	-	-	6.1 ± 1.4	12.8 ± 3.0	9.1 ± 2.5	12.8 ± 3.0
	SMMa (% SMMa of weight)	25.4	-	-	20.8	19.1	27.4	26.1
	SMMa (%) in healthy individuals[b]	29.6 ± 3.8	-	-	27.1 ± 2.4	28.3 ± 4.3	27.1 ± 4.2	28.3 ± 4.3
	Skeletal muscle index (kg/m^2)	5.7			4.5	6.1	5.8	7.2
DEXA	BF%	-	37.6	39	-	44.2	-	-
	Visceral fat area (cm^2)	-	64.9	21.5	-	107	-	-
	Lean mass/height^2 *Z*-score	-	−2.3	-	-	0.4	-	-
	Skeletal muscle index (kg/m^2)		4.37	2.64		5.91		

Abbreviations: BF%, body fat percentage; BIA, bioelectrical impedance analysis; DEXA, dual-energy X-ray absorptiometry; SMMa, appendicular skeletal muscle mass.

[a]Appendicular skeletal muscle mass: skeletal muscle mass of arms and legs.

[b]Sex- and age-matched healthy controls, based on results of McCarthy et al. (2014).

the 15%–23% of body weight (kg) in all these individuals, resulting in a disproportionally high BF% for weight. Fat percentage was also above average in the three (3/7) individuals with a normal BMI.

Moreover, appendicular skeletal muscle mass (SMMa) was increased in 3/5 individuals with KLFS compared to matched controls. However, when SMMa was calculated as a percentage of weight, SMMa was as average in 2/5 individuals and below average in 3/5 individuals. One individual had a decreased lean mass (*Z-score* −2.3) as identified by DEXA scan. In individuals who underwent BIA-measurement, the basal metabolic rate (mean 1121 kcal, range 886–1374 kcal/day) was below the estimations of the Schofield equation.

3.3 | Radiological characteristics

A radiograph of the left hand was performed in 14 individuals who all had a height SDS in the normal range, with the aim to assess skeletal age, BHI, and ulnar variance (Table 3). The mean calendar age of the individuals was 9.2 years (range 3.0–20.0 years). Skeletal age was increased compared to calendar age, with a mean standard deviation score (SDS) of skeletal age of 0.5 ± 1.2.

The BHI SDS was in 57% of individuals below −1 SDS (8/14; mean −0.8 ± 1.1 SDS). In a 4-year-old female with a BHI SDS of −1.2 and sclerotic bone lesions based on the X-hand, a DEXA scan was performed which revealed osteoporosis.

Negative ulnar variance (*shortness/hypoplastic aspect of the distal ulna*) was present in 71% of individuals (10/14). No other ossal abnormalities were observed in any of the individuals.

3.4 | Endocrine-metabolic investigations

Biochemical investigations were conducted in 44 individuals with KLFS, including tests for hemoglobin, ALAT, thyroid function, lipid profile, lactate, pyruvate, LDH, glucose, HbA1c, 25(OH)D, vitamin B12, folic acid, ammonia, and creatinine. Results are presented in Table 4 and Figure 2.

Thyroid investigations were performed in 41 individuals (17 males, 24 females) with the mean age of 14.6 years (range 2.0–45.8 years). Mean fT4 was 13.9 ± 2.6 pmol/L ($N = 39$) and mean TSH was 3.81 ± 3.80 mIU/L ($N = 41$). Thyroid dysregulation was observed in 22% (9/41) of individuals and classified as follows:

1. Subclinical hypothyroidism: Six individuals had (sub)normal fT4 levels (10.5–16.2 pmol/L) and elevated TSH levels (4.29–7.46 mIU/L). Four of the six individuals had a normal BMI, while two had obesity. Levothyroxine treatment was initiated in two individuals at ages 2.5 and 32.7 years.
2. Thyroiditis: One individual, aged 12.8 years, was diagnosed with thyroiditis. TSH was elevated (17.7 mIU/L) while fT4 was in the low-normal range (10 pmol/L). TPO and TSH-receptor antibodies were absent. Thyroid ultrasound showed a pattern suggestive for thyroiditis (granulomatous reflection pattern with lobulated contours and some hyperemia). Levothyroxine treatment was started.
3. Auto-immune thyroiditis: One individual, aged 15.5 years, was diagnosed with auto-immune thyroiditis with TPO (thyroid peroxidase) antibodies. Levothyroxine treatment was prescribed, but the individual showed persistent hypothyroidism at age 20 (fT4

TABLE 3 Radiological bone parameters investigated by X-hand, BIA, and DEXA scan.

			X-hand						BIA	DEXA	
Age	Sex	Height SDS	Skeletal age	Skeletal age SDS (compared to calendar age)	Bone health index	Bone health index SDS	Negative ulnar variance, yes/no (mm)	Ulnar variance (mm) in healthy individuals[a]	Bone mineral content (kg) compared to BIA reference range for sex and age	Bone mineral density: lumbar vertebrae (Z-score)	Bone mineral density: femoral neck (Z-score)
3.0	M	−1.15	2.9	−0.3	3	−1.5	No (−3.5)	−2.0 ± 1.8	-	-	-
4.1	F	−1.12	3.3	−1.3	3.4	−1.2	Yes (−3.6)	−2.1 ± 1.4	-	−2.6	−4.7
4.2	F	−0.57	5.1	1.3	3.6	−1.7	Yes (−5.7)	−2.1 ± 1.4	-	-	-
4.6	F	−0.22	4.4	−0.5	4.2	0.0	Yes (−4.6)	−2.1 ± 1.4	-	-	-
4.9	F	−0.99	4.7	−0.4	3.4	−2.2	No (−3.4)	−2.1 ± 1.4	-	0.2	-
5.0	F	0.07	-	-		-	-	-	-	−0.4	-
7.5	F	0.44	8.1	0.7	4.4	−0.3	Yes (−6.1)	−2.3 ± 1.3	1.4 (high)	1.1	−0.3
8.8	F	1.22	10.4	1.9	4.3	−2.8	Yes (−7.9)	−2.7 ± 2.5	1.4 (low)	-	-
9.5	F	0.07	11.9	2.9	4.4	−1.4	Yes (−4.8)	− 1.9 ± 1.4	-	-	-
9.7	M	−1.05	10.3	0.9	4.2	−1	Yes (−3.8)	−1.6 ± 1.8	-	-	-
10.4	F	−0.02	9.4	−0.6	4.5	−0.2	Yes (−8.0)	−1.9 ± 1.5	-	-	-
11.0	F	0.00	-	-		-	-	-	2.2 (high)	1.8	2.4
12.8	F	−0.70	12.1	−0.5	5.3	0.1	Yes (−4.4)	−1.0 ± 2.0	-	−0.2	−1.6
13.7	F	−0.15	14.9	1.3	5.0	−1.0	No (−2.3)	− 1.8 ± 1.7	2.6 (high)	-	-
15.5	F	−0.17	16.4	1.0	5.9	1.3	No (−2.6)	−1.0 ± 1.8	2.3 (normal)		
20.0	F	−0.07	17.5	N/A	5.7	0.1	Yes (−5.0)	− 1.4 ± 1.2	-	-	-

Abbreviations: BIA, bioelectrical impedance analysis; DEXA, dual-energy X-ray absorptiometry; SDS, standard deviation score.
[a]Matched for method, sex, and skeletal age (Kox et al., 2020).

TABLE 4 Comparison of endocrine-metabolic biochemical results between our cohort and healthy controls.

	Kleefstra syndrome					Healthy controls		
Measurement	*N* (male %)	Mean age (range)	Mean ± SD	% increase (*N*)[a]	% decrease (*N*)[a]	*N* (male %)	Mean age (range)	Mean ± SD
Hemoglobin	41 (39%)	13.8 (0–48)	8.3 ± 0.9 mmol/L	2% (1/41)	7% (3/41)	33 (45%)	15.5 (5–25)	8.5 ± 2.5 mmol/L
ALAT	36 (36%)	14.6 (2–41)	23 ± 9 U/L	3% (1/36)	-	26 (50%)	16.0 (2–25)	19 ± 15 U/L
fT4	39 (44%)	13.9 (2–46)	13.9 ± 2.6 pmol/L	-	18% (7/39)			
TSH	41 (41%)	14.6 (2–46)	3.81 ± 3.80 mIU/L	20% (8/41)	-	31 (48%)	14.9 (4–25)	2.66 ± 1.49 mE/L
Triglycerides**	33 (36%)	13.9 (2–45)	1.62 ± 0.93 mmol/L	15% (5/33)	3% (1/33)	34 (47%)	14.3 (2–25)	1.01 ± 0.49 mmol/L
Cholesterol	34 (35%)	14.4 (2–45)	4.0 ± 0.7 mmol/L	-	6% (2/34)	31 (42%)	14.5 (2–25)	3.75 ± 0.71 mmol/L
HDL	33 (36%)	13.9 (2–45)	1.16 ± 0.24 mmol/L	-	-	33 (42%)	14.4 (2–25)	1.23 ± 0.34 mmol/L
LDL	33 (36%)	13.9 (2–45)	2.13 ± 0.57 mmol/L	-	12% (4/33)	33 (42%)	14.4 (2–25)	2.04 ± 0.70 mmol/L
Lactate (venous blood gas)	13 (54%)	11.3 (2–27)	1.51 ± 0.47 mmol/L	-	-	35 (60%)	16.5 (9–25)	1.76 ± 0.97 mmol/L
Lactate	19 (26%)	9.9 (2–27)	1.36 ± 0.54 mmol/L	-	5% (1/19)			
Pyruvate	17 (24%)	9.8 (2–27)	63 ± 26 μmol/L	-	29% (5/17)	35 (63%)	17.0 (9–25)	61 ± 39 μmol/L
L/P ratio	17 (24%)	9.8 (2–27)	20.64 ± 5.67	29% (5/17)	-			
LDH	15 (27%)	13.4 (2–33)	241 ± 134 U/L	7% (1/15)	-	23 (57%)	17.8 (9–25)	182 ± 51 U/L
Fasting glucose	14 (29%)	23.4 (1–47)	4.9 ± 0.7 mmol/L	-	7% (1/14)			
Non-fasting glucose	22 (32%)	12.4 (1–32)	5.2 ± 0.9 mmol/L			34 (53%)	15.6 (2–25)	5.37 ± 2.6 mmol/L
HbA1C	20 (30%)	13.7 (2–34)	34 ± 3 mmol/moL	-	-			
25(OH)D	12 (25%)	10.8 (3–22)	53 ± 20 nmol/L	-	25% (3/12)	35 (49%)	14.0 (2–25)	63.4 ± 31.8 nmol/L
Vitamin B12	16 (19%)	11.7 (3–27)	446 ± 265 pmol/L	13% (2/16)	-	40 (53%)	14.2 (3–25)	440 ± 220 pmol/L
Folic acid	13 (8%)	12.7 (4–27)	18.2 ± 7.7 nmol/L	-	8% (1/13)	39 (54%)	13.9 (3–25)	24.0 ± 13.7 nmol/L
Ammonia****	15 (20%)	10.5 (2–27)	18 ± 6 μmol/L	-	73% (11/15)	34 (44%)	14.8 (2–25)	34 ± 12 μmol/L
Creatinine	26 (38%)	17.4 (2–45)	59 ± 23 μmol/L	15% (4/26)		34 (47%)	15.7 (5–25)	50 ± 25 μmol/L

Note: This table shows the results of biochemical endocrine-metabolic investigations in Kleefstra syndrome (KLFS) compared to sex- and age-matched healthy controls. The whole standardized set of biochemical investigations could not be evaluated in all participants; the second column shows the number of studied KLFS participants. In KLFS, triglycerides were significantly increased ($p < 0.005$) and ammonia was significantly decreased ($p < 0.0001$) compared to matched controls.

[a]Compared to sex- and age-matched reference values (Supplementary File 3).

$p < 0.005$.**$p < 0.0001$.

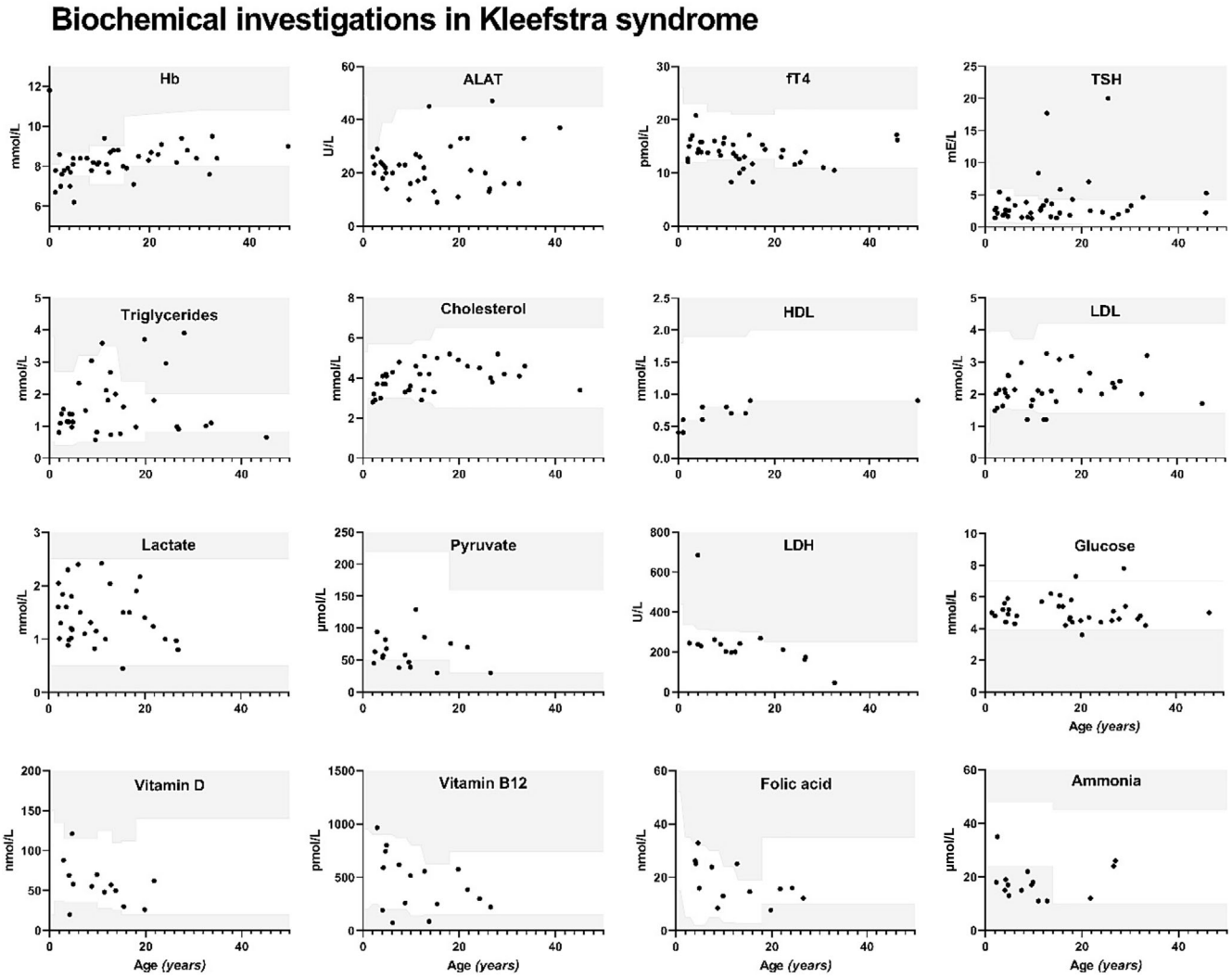

FIGURE 2 Results of biochemical endocrine-metabolic evaluations and its distribution per age in Kleefstra syndrome (KLFS). The white area in the graphs shows the reference range for sex and age in the healthy population.

5.4 pmol/L, TSH 17.3 mIU/L, TPO antibodies 224 U/mL), likely due to non-compliance with therapy.

4. Central hypothyroidism: One individual, aged 11.0 years, was diagnosed with isolated central hypothyroidism based on low fT4 (8.3 pmol/L) and elevated TSH (8.38 mIU/L). MRI ruled out pituitary gland malformation. A subsequent measure showed comparable values after which levothyroxine was prescribed and values normalized (fT4 16.8 pmol/L, TSH 1.3 mIU/L).

We retrospectively analyzed MRI cerebrum results of 19 individuals from prior clinical care. While a pituitary cyst was identified in one individual, no pituitary gland abnormalities were found in the remaining individuals.

Lipid spectrum analyses were performed in 33 individuals, including 23 children. Triglycerides were significantly increased in KLFS (mean 1.62 ± 0.93 mmol/L) compared to healthy controls (mean 1.01 ± 0.49 mmol/L), $p < 0.005$. Two children with a normal BMI showed triglyceride levels above the reference range for age and sex (6.81 and 3.59 mmol/L, respectively), which persisted during follow-up.

Simultaneous measurements of fasting glucose and insulin levels were obtained from three females (ages 26.6, 17.7, and 21.8 years). All participants had normal glucose levels, but the last two individuals showed elevated insulin levels (15.4 mIU/L with a glucose level of 4.6 mmol/L, and 24 mIU/L with a glucose level of 4.7 mmol/L). These findings are indicative of insulin resistance, as revealed by HOMA-IR values of 3.2 and 5.0, respectively.

Blood ammonia levels were found to be significantly lower in KLFS compared to healthy controls (18 ± 6 µmol/L and 34 ± 12 µmol/L respectively, $p < 0.0001$), with 73% (11/15) of individuals with KLFS exhibiting results below the reference range. Ammonia levels were decreased independent of age ($r = 0.005$).

Vitamin D levels were evaluated in 12 individuals and 25% (3/12) were found to have a vitamin D level ≤30 nmol/L (females, aged 4.2, 15.4, and 19.8 years) after which supplementation was started. Two out of three individuals with vitamin D deficiency had a BMI within

the normal range (BMI *Z*-score between −1 and 1). Five individuals above the age of 4 years were excluded from analyses due to vitamin D supplementation.

One participant had a folic acid deficiency (1/13; female, aged 19.8 years). In another participant (female, aged 26.6 years) the results of homocysteine and methylmalonic acid indicated a folate deficiency, despite having normal folic acid levels.

4 | DISCUSSION

In this first comprehensive study on growth, body composition, and endocrine-metabolic status in Kleefstra syndrome (KLFS), we collected data from a relatively large rare disease cohort comprising 62 individuals. Our obtained insights might be relevant to optimize health surveillance, understand pathogenic mechanisms, support translational research, and determine treatment targets (McPartland, 2016). We introduced comprehensive study methods that can be performed within regular clinical care and applied to characterize other MDEMs as well.

4.1 | BMI and body composition

We collected growth parameters of Dutch individuals with KLFS, which allowed comparison with ethnicity-matched controls. Our results showed a high prevalence of individuals who were overweight or obese (overall 59%), consistent with previous reports on KLFS (Kleefstra & de Leeuw, 2010; Willemsen et al., 2012) and other MDEMs (Fahrner & Bjornsson, 2014). Interestingly, we found gradual increase in BMI during childhood and adolescence and 56% of individuals who were overweight or obese in the age category of 2–12 years. This 56% prevalence greatly exceeds the 15% prevalence in the age-matched Dutch population (Centraal Bureau voor de Statistiek, 2022). Furthermore, although a previous Dutch study found increased risk and high rates of overweight (23%–25%) and obesity (10%–15%) in children aged 2–18 years with intellectual disability (Wouters et al., 2020), our study also shows significantly higher rates in individuals with KLFS compared to this population.

Interestingly, hyperphagia was frequently present (50%), while overweight in siblings was absent. Moreover, our results showed disproportionally high body fat percentages (Wohlfahrt-Veje et al., 2014), truncal fat distribution and a waist circumference/height ratio above 0.5. Taken together, these results mark a high (cardio)metabolic risk in KLFS (Browning et al., 2010) and indicate that overweight/obesity in KLFS is a consequence of haploinsufficiency of the chromatin regulator EHMT1.

The high body fat percentage perfectly matches previous preclinical studies in mouse and *Drosophila* models of KLFS (Kramer, 2016; Ohno et al., 2013; Riahi et al., 2019). In the latter, the authors demonstrated normal food intake and limited access to lipid stores upon conditions of increased energy demands (Riahi et al., 2019), strongly suggesting that the high prevalence of obesity is a direct consequence of evolutionary conserved transcriptional and metabolic dysregulation that occurs upon deficiency of members of the EHMT protein family (Riahi et al., 2019; Riahi et al., 2021).

Body composition analyses showed that appendicular skeletal muscle mass (SMMa) in KLFS was either as average or below average when compared to controls (McCarthy et al., 2014). Previous findings in the Kleefstra mouse model (Ohno et al., 2013) have demonstrated that the loss of *EHMT1* in brown adipose tissue and its consequent H3K9 demethylation of the muscle-selective gene promoters induce muscle differentiation (Ohno et al., 2013). Whether this mechanism is also present in brown adipose tissue derived from KLFS individuals needs to be confirmed but our initial data does not support this observation. Our findings are in line with the typically observed correlation between increased BMI and decreased SMM in healthy individuals, Down syndrome, and Prader-Willi syndrome (Abramowitz et al., 2018; Khan et al., 2018; Lin & Wuang, 2012). Therefore, decreased muscle mass in KLFS could serve as an additional risk factor for the development of overweight.

4.2 | Growth and radiological characteristics

At maturity, short stature and microcephaly were present in 33% and 56% of individuals, respectively. These findings are consistent with previous reports on MDEMs, associating short stature and/or microcephaly with 58% (41/70) of the MDEMs, including Kabuki syndrome and Wiedemann-Steiner syndrome (Fahrner & Bjornsson, 2014, 2019). Although in some MDEMs the mechanisms underlying growth retardation have been explored (e.g., altered balance between cellular proliferation and differentiation), the exact mechanisms are unclear (Fahrner & Bjornsson, 2019).

In KLFS, various mechanisms may contribute to short stature. Our findings indicate that individuals with a higher BMI during childhood were more likely to have an advanced skeletal age, resulting in adult height being reached at younger ages. Conversely, individuals with a normal BMI could reach average height. Based on our growth data, individual growth patterns, and skeletal ages, we conclude that advanced skeletal maturation appears likely to be the primary cause of short stature in KLFS.

In several MDEMs, short stature is associated with an insufficient growth spurt (Schott, Blok, et al., 2016) and/or growth hormone deficiency (Schott, Blok, et al., 2016; Schott, Gerver, & Stumpel, 2016; Sheppard et al., 2021). However, we observed a sufficient growth spurt in most individuals with KLFS. Based on the individual growth charts of our participants, we consider IGF-1 deficiency unlikely in KLFS. Further work is crucial to expand data collection on growth parameters to validate if our results are internationally consistent and to further explore underlying mechanisms of short stature. Additionally, it is essential to develop syndrome-specific growth charts to monitor health and disease in clinical practice.

Radiological investigations showed decreased BHI at all ages in 57% of individuals, suggestive of decreased bone mineral density (Leijten et al., 2019). Anti-epileptic drugs (affecting calcium and bone

metabolism) were not used in any of the participants. While most participants were walking independently, the majority of them had daily exercise times below the Dutch recommendations of at least 1 h a day (Gezondheidsraad, 2017), which could potentially have a negative impact their bone quality (Mergler et al., 2016). The decreased bone mineralization in KLFS has not been observed before. However, EHMT1 is expressed in osteocytes and osteoblasts (Balemans et al., 2014), supporting a role for the chromatin remodeler in bone mineralization. Since decreased bone mineral density is clinically relevant and sufficient treatment is available, this clinical aspect required further attention and investigation in KLFS.

Furthermore, negative ulnar variance was found in 71% of individuals. Although ulnar variance is rarely studied in NDDs, negative variance has been reported in Rett syndrome with a prevalence of 41%–79% (Glasson et al., 1998; Leonard et al., 1995). The clinical relevance and underlying mechanism of this finding is unclear but it appears to be a clinical feature of KLFS as well.

4.3 | Endocrine-metabolic investigations

In endocrine biochemical studies, we observed thyroid dysregulation in KLFS in 22% of individuals. This prevalence exceeds the population prevalence of 5% (Chiovato et al., 2019). We identified various factors that can contribute to thyroid dysregulation, highlighting the importance of comprehensive diagnostic screening. One individual had isolated central hypothyroidism without pituitary gland abnormalities. Central hypothyroidism has so far not been associated with KLFS, however, some case reports have described hypothalamic dysfunction (e.g., hypogonadotropic hypogonadism) (Higuchi et al., 2018; Torga et al., 2018). Therefore, further research is required to evaluate pituitary etiology and its implications in KLFS.

Triglycerides were significantly increased in KLFS compared to sex- and age-matched controls, while there were no differences in cholesterol, HDL, and LDL. Triglycerides remain significantly increased compared to an age- and BMI-matched Kabuki syndrome cohort (van Montfort et al., 2021) (Supplementary File 5). With the inclusion of individuals having a BMI *Z*-score within the range of −1 to 1, the mean triglyceride levels in the KLFS group are 1.22 ± 0.65 mmol/L, whereas they are 0.61 ± 0.19 mmol/L in the Kabuki syndrome ($p < 0.03$). Interestingly, increased triglycerides in KLFS align with findings obtained in the *Drosophila* KLFS model, which shows elevated triglyceride levels at steady state and impaired triglyceride mobilization under oxidative stress (Riahi et al., 2019). Impaired mobilization of fat stores, together with altered expression of genes involved in lipid metabolism, could contribute to obesity in KLFS (Riahi et al., 2019).

Fasting insulin and glucose measurements were performed in three individuals, of whom two individuals showed a high HOMA-IR, indicating insulin resistance. These individuals had normal blood glucose levels and no medical history of diabetes mellitus. In none of our other participants diabetes mellitus was present. Insulin resistance has been previously observed in mouse models with *EHMT1* deficiency in brown adipose tissue (Ohno et al., 2013). Although this previous study also associated *EHMT1* haploinsufficiency with liver steatosis (Ohno et al., 2013), we could not confirm this association with liver steatosis based on normal ALAT levels in 94% of our participants.

A noteworthy finding from our biochemical evaluations is significantly decreased ammonia in KLFS, which has not been previously associated with the condition. The clinical relevance of hypoammonia in general and in KLFS specific remains unknown, and we do not expect a different dietary protein intake in these individuals. Additional studies on amino acid metabolism and urea cycle metabolism are necessary to determine the significance of decreased ammonia.

To illustrate the clinical importance of endocrine-metabolic dysregulation, we present the case of a 20-year-old female participant (BMI *Z*-score 0.50 at last examination aged 15.5 years) who experienced major depression and severe fatigue, commonly associated with KLFS (Vermeulen et al., 2017). Results of biochemical investigations revealed auto-immune thyroiditis (fT4 5.4 pmol/L), folate deficiency (7.71 nmol/L), and vitamin D deficiency (26 nmol/L), which may explain her symptoms. Considering our findings of a high prevalence of thyroid dysregulation, folate deficiency, and vitamin D deficiency independently of BMI, we recommend routine biochemical assessments in these patients, as they might report their complaints differently due to cognitive impairment.

To what extent growth and endocrine-metabolic profiles contribute to neurodevelopmental and cognitive phenotypes associated with KLFS remains an important question for future research.

5 | CONCLUSION AND RECOMMENDATIONS

In conclusion, this study has shown that KLFS is associated with being overweight/obese, a high body fat percentage, insulin resistance, and increased triglycerides, resulting in a high risk for (cardio)metabolic disease. Further biochemical investigations highlighted significantly decreased ammonia levels, thyroid dysregulation, and vitamin deficiencies. Based on hand radiographs and DEXA scans, the bone density was generally decreased. This research contributes to a detailed phenotypic characterization of KLFS, provides novel biomarkers, and emphasizes the potential of clinical biomarkers in the translation of preclinical studies, both in KLFS and in the broader group of MDEMs.

Based on our findings, we have established five key recommendations: (1) We recommend lifelong surveillance of weight and waist circumference and the support of a healthy lifestyle. (2) We suggest to evaluate bone mineralization through hand radiograph or DEXA scan at the onset of puberty and when multiple or unexpected fractures occur, with follow-up according to local protocols. (3) Moreover, we recommend screening of thyroid function at puberty onset and awareness of the potential central origin of hypothyroidism. Therefore, evaluate both fT4 and TSH levels and consider an MRI of the pituitary gland when central hypothyroidism is suspected. (4) In addition, based on our biochemical findings, we advise screening for glucose metabolism (glucose,

insulin), lipid spectrum, and vitamin status (vitamin D, B12, and folic acid) at the onset of puberty. For individuals with obesity starting from age 8 to 10 years, regular monitoring of glucose metabolism (glucose, insulin), lipid spectrum, and vitamin status (vitamin D, B12, and folic acid) is advised, with a frequency set at once every 1–3 years. (5) Lastly, consider the possible coexistence of somatic and psychiatric morbidity, including screening on hypothyroidism, and vitamin status before prescribing psychiatric drug treatment.

AUTHOR CONTRIBUTIONS

Conceptualization and methodology: Arianne Bouman, Joyce M. Geelen, and Tjitske Kleefstra. *Recruitment of participants*: Arianne Bouman, Joost Kummeling, and Tjitske Kleefstra. *Retrospective data collection*: Arianne Bouman and Joost Kummeling. *Growth and biochemical investigations*: Arianne Bouman, Joyce M. Geelen, Yvonne G. van der Zwan, and Tjitske Kleefstra. *Radiological investigations*: Arianne Bouman and Willemijn M. Klein. *Writing—original draft*: Arianne Bouman, Joyce M. Geelen, and Tjitske Kleefstra. *Writing—review and editing*: Arianne Bouman, Joyce M. Geelen, Joost Kummeling, Annette Schenck, Yvonne G. van der Zwan, Willemijn M. Klein, and Tjitske Kleefstra.

ACKNOWLEDGMENTS

The authors are grateful to all patients and their caregivers for participation. We thank Bregina Kersten for the availability of the InBody 770 body composition analyzer. We also thank Dr. Nina Schott for the availability of lipid spectra and BMI of the Kabuki syndrome study cohort.

FUNDING INFORMATION

AB and TK received funding from the Netherlands Organisation for Health Research and Development PSIDER—project BRAINMODEL (10250022110003). TK received a grant from the Dutch Research Council (015.014.036 and 1160.18.320), and the Netherlands Organisation for Health Research and Development (91718310). AS was supported by a Dutch Research Council grant (09150181910022). This work was funded by a Catalyst Grant from United for Metabolic Diseases (UMD-CG-2021-014) which is funancially supported by Metakids.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationship that would be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Arianne Bouman https://orcid.org/0000-0002-1138-8026

REFERENCES

Abramowitz, M. K., Hall, C. B., Amodu, A., Sharma, D., Androga, L., & Hawkins, M. (2018). Muscle mass, BMI, and mortality among adults in the United States: A population-based cohort study. *PLoS One*, *13*(4), e0194697. https://doi.org/10.1371/journal.pone.0194697

Balemans, M. C., Ansar, M., Oudakker, A. R., van Caam, A. P., Bakker, B., Vitters, E. L., van der Kraan, P. M., de Bruijn, D. R., Janssen, S. M., Kuipers, A. J., Huibers, M. M., Maliepaard, E. M., Walboomers, X. F., Benevento, M., Nadif Kasri, N., Kleefstra, T., Zhou, H., Van der Zee, C. E., & van Bokhoven, H. (2014). Reduced euchromatin histone methyltransferase 1 causes developmental delay, hypotonia, and cranial abnormalities associated with increased bone gene expression in Kleefstra syndrome mice. *Developmental Biology*, *386*(2), 395–407. https://doi.org/10.1016/j.ydbio.2013.12.016

Bocca-Tjeertes, I. F., van Buuren, S., Bos, A. F., Kerstjens, J. M., Ten Vergert, E. M., & Reijneveld, S. A. (2012). Growth of preterm and full-term children aged 0-4 years: Integrating median growth and variability in growth charts. *The Journal of Pediatrics*, *161*(3), 460–465.E1. https://doi.org/10.1016/j.jpeds.2012.03.016

BoneXpert. (n.d.). Bone health index. https://bonexpert.com/what-is-bone-age/#bonehealthindex

Browning, L. M., Hsieh, S. D., & Ashwell, M. (2010). A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. *Nutrition Research Reviews*, *23*(2), 247–269. https://doi.org/10.1017/S0954422410000144

Centraal Bureau voor de Statistiek. (2022). *Jaarrapport Landelijke Jeugdmonitor 2022*. Ministerie van Volksgezondheid, Welzijn en Sport.

Chiovato, L., Magri, F., & Carle, A. (2019). Hypothyroidism in context: Where we've been and where we're going. *Advances in Therapy*, *36*-(Suppl 2), 47–58. https://doi.org/10.1007/s12325-019-01080-8

de Vries, T. (2015). Laboratoriumdiagnostiek bij kinderen. *Een praktische handleiding*.

de Wilde, J. A., van Dommelen, P., van Buuren, S., & Middelkoop, B. J. (2015). Height of South Asian children in the Netherlands aged 0-20 years: Secular trends and comparisons with current Asian Indian, Dutch and WHO references. *Annals of Human Biology*, *42*(1), 38–44. https://doi.org/10.3109/03014460.2014.926988

Fahrner, J. A., & Bjornsson, H. T. (2014). Mendelian disorders of the epigenetic machinery: Tipping the balance of chromatin states. *Annual Review of Genomics and Human Genetics*, *15*, 269–293. https://doi.org/10.1146/annurev-genom-090613-094245

Fahrner, J. A., & Bjornsson, H. T. (2019). Mendelian disorders of the epigenetic machinery: Postnatal malleability and therapeutic prospects. *Human Molecular Genetics*, *28*(R2), R254–R264. https://doi.org/10.1093/hmg/ddz174

Fredriks, A. M., van Buuren, S., Burgmeijer, R. J., Meulmeester, J. F., Beuker, R. J., Brugman, E., Roede, M. J., Verloove-Vanhorick, S. P., & Wit, J. M. (2000). Continuing positive secular growth change in the Netherlands 1955-1997. *Pediatric Research*, *47*(3), 316–323. https://doi.org/10.1203/00006450-200003000-00006

Gerver, W. J. M., & Bruin, R. (2001). *Paediatric morphometrics: A reference manual* (Vol. 2). Datawyse/Universitaire Pers Maastricht.

Gezondheidsraad. (2017). *Beweegrichtlijnen* (Vol. 2017/08). Gezondheidsraad.

Glasson, E. J., Bower, C., Thomson, M. R., Fyfe, S., Leonard, S., Rousham, E., Christodoulou, J., Ellaway, C., & Leonard, H. (1998). Diagnosis of Rett syndrome: Can a radiograph help? *Developmental Medicine and Child Neurology*, *40*(11), 737–742. https://doi.org/10.1111/j.1469-8749.1998.tb12341.x

Higuchi, S., Takagi, M., Takeda, R., Yoshihashi, H., Narumi, S., & Hasegawa, T. (2018). An association with hypopituitarism and 9q subtelomere deletion syndrome. *Clinical Case Reports*, *6*(12), 2371–2375. https://doi.org/10.1002/ccr3.1591

Khan, M. J., Gerasimidis, K., Edwards, C. A., & Shaikh, M. G. (2018). Mechanisms of obesity in Prader-Willi syndrome. *Pediatric Obesity*, *13*(1), 3–13. https://doi.org/10.1111/ijpo.12177

Kleefstra, T., Brunner, H. G., Amiel, J., Oudakker, A. R., Nillesen, W. M., Magee, A., Genevieve, D., Cormier-Daire, V., van Esch, H., Fryns, J. P., Hamel, B. C., Sistermans, E. A., de Vries, B. B., & van Bokhoven, H. (2006). Loss-of-function mutations in euchromatin histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion syndrome. *American Journal of Human Genetics*, *79*(2), 370–377. https://doi.org/10.1086/505693

Kleefstra, T., & de Leeuw, N. (2010). Kleefstra syndrome. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews*(®). University of Washington.

Kochinke, K., Zweier, C., Nijhof, B., Fenckova, M., Cizek, P., Honti, F., Keerthikumar, S., Oortveld, M. A., Kleefstra, T., Kramer, J. M., Webber, C., Huynen, M. A., & Schenck, A. (2016). Systematic phenomics analysis deconvolutes genes mutated in intellectual disability into biologically coherent modules. *American Journal of Human Genetics*, *98*(1), 149–164. https://doi.org/10.1016/j.ajhg.2015.11.024

Kox, L. S., Jens, S., Lauf, K., Smithuis, F. F., van Rijn, R. R., & Maas, M. (2020). Well-founded practice or personal preference: A comparison of established techniques for measuring ulnar variance in healthy children and adolescents. *European Radiology*, *30*(1), 151–162. https://doi.org/10.1007/s00330-019-06354-x

Kramer, J. M. (2016). Regulation of cell differentiation and function by the euchromatin histone methyltransferases G9a and GLP. *Biochemistry and Cell Biology*, *94*(1), 26–32. https://doi.org/10.1139/bcb-2015-0017

Leijten, A. D., Hampsink, B., Janssen, M., Klein, W. M., & Draaisma, J. M. T. (2019). Can digital X-ray radiogrammetry be an alternative for dual-energy X-ray absorptiometry in the diagnosis of secondary low bone quality in children? *European Journal of Pediatrics*, *178*(9), 1433–1441. https://doi.org/10.1007/s00431-019-03425-5

Leonard, H., Thomson, M., Bower, C., Fyfe, S., & Constantinou, J. (1995). Skeletal abnormalities in Rett syndrome: Increasing evidence for dysmorphogenetic defects. *American Journal of Medical Genetics*, *58*(3), 282–285. https://doi.org/10.1002/ajmg.1320580316

Lin, H. C., & Wuang, Y. P. (2012). Strength and agility training in adolescents with Down syndrome: A randomized controlled trial. *Research in Developmental Disabilities*, *33*(6), 2236–2244. https://doi.org/10.1016/j.ridd.2012.06.017

Maia, N., Nabais Sa, M. J., Melo-Pires, M., de Brouwer, A. P. M., & Jorge, P. (2021). Intellectual disability genomics: Current state, pitfalls and future challenges. *BMC Genomics*, *22*(1), 909. https://doi.org/10.1186/s12864-021-08227-4

Malina, R. M. (1988). Human growth: Selected aspects of current research on well nourished children. *Annual Review of Anthropology*, *17*, 187–219.

McCarthy, H. D., Samani-Radia, D., Jebb, S. A., & Prentice, A. M. (2014). Skeletal muscle mass reference curves for children and adolescents. *Pediatric Obesity*, *9*(4), 249–259. https://doi.org/10.1111/j.2047-6310.2013.00168.x

McPartland, J. C. (2016). Considerations in biomarker development for neurodevelopmental disorders. *Current Opinion in Neurology*, *29*(2), 118–122. https://doi.org/10.1097/WCO.0000000000000300

Mergler, S., de Man, S. A., Boot, A. M., Heus, K. G., Huijbers, W. A., van Rijn, R. R., Penning, C., & Evenhuis, H. M. (2016). Automated radiogrammetry is a feasible method for measuring bone quality and bone maturation in severely disabled children. *Pediatric Radiology*, *46*(7), 1017–1022. https://doi.org/10.1007/s00247-016-3548-4

Ohno, H., Shinoda, K., Ohyama, K., Sharp, L. Z., & Kajimura, S. (2013). EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. *Nature*, *504*(7478), 163–167. https://doi.org/10.1038/nature12652

Riahi, H., Brekelmans, C., Foriel, S., Merkling, S. H., Lyons, T. A., Itskov, P. M., Kleefstra, T., Ribeiro, C., van Rij, R. P., Kramer, J. M., & Schenck, A. (2019). The histone methyltransferase G9a regulates tolerance to oxidative stress-induced energy consumption. *PLoS Biology*, *17*(3), e2006146. https://doi.org/10.1371/journal.pbio.2006146

Riahi, H., Fenckova, M., Goruk, K. J., Schenck, A., & Kramer, J. M. (2021). The epigenetic regulator G9a attenuates stress-induced resistance and metabolic transcriptional programs across different stressors and species. *BMC Biology*, *19*(1), 112. https://doi.org/10.1186/s12915-021-01025-0

Satterstrom, F. K., Kosmicki, J. A., Wang, J., Breen, M. S., De Rubeis, S., An, J. Y., Peng, M., Collins, R., Grove, J., Klei, L., Stevens, C., Reichert, J., Mulhern, M. S., Artomov, M., Gerges, S., Sheppard, B., Xu, X., Bhaduri, A., Norman, U., … Buxbaum, J. D. (2020). Large-scale exome sequencing study implicates both developmental and functional changes in the neurobiology of autism. *Cell*, *180*(3), 568–584. e23. https://doi.org/10.1016/j.cell.2019.12.036

Schofield, W. N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition. Clinical Nutrition*, *39*-(Suppl 1), 5–41.

Schonbeck, Y., Talma, H., van Dommelen, P., Bakker, B., Buitendijk, S. E., Hirasing, R. A., & van Buuren, S. (2011). Increase in prevalence of overweight in Dutch children and adolescents: A comparison of nationwide growth studies in 1980, 1997 and 2009. *PLoS One*, *6*(11), e27608. https://doi.org/10.1371/journal.pone.0027608

Schonbeck, Y., Talma, H., van Dommelen, P., Bakker, B., Buitendijk, S. E., HiraSing, R. A., & van Buuren, S. (2013). The world's tallest nation has stopped growing taller: The height of Dutch children from 1955 to 2009. *Pediatric Research*, *73*(3), 371–377. https://doi.org/10.1038/pr.2012.189

Schonbeck, Y., van Dommelen, P., HiraSing, R. A., & van Buuren, S. (2015). Trend in height of Turkish and Moroccan children living in The Netherlands. *PLoS One*, *10*(5), e0124686. https://doi.org/10.1371/journal.pone.0124686

Schott, D. A., Blok, M. J., Gerver, W. J., Devriendt, K., Zimmermann, L. J., & Stumpel, C. T. (2016). Growth pattern in Kabuki syndrome with a KMT2D mutation. *American Journal of Medical Genetics. Part A*, *170*(12), 3172–3179. https://doi.org/10.1002/ajmg.a.37930

Schott, D. A., Gerver, W. J., & Stumpel, C. T. (2016). Growth hormone stimulation tests in children with Kabuki syndrome. *Hormone Research in Pædiatrics*, *86*(5), 319–324. https://doi.org/10.1159/000449221

Sheppard, S. E., Campbell, I. M., Harr, M. H., Gold, N., Li, D., Bjornsson, H. T., Cohen, J. S., Fahrner, J. A., Fatemi, A., Harris, J. R., Nowak, C., Stevens, C. A., Grand, K., Au, M., Graham, J. M., Jr., Sanchez-Lara, P. A., Campo, M. D., Jones, M. C., Abdul-Rahman, O., … Quintero-Rivera, F. (2021). Expanding the genotypic and phenotypic spectrum in a diverse cohort of 104 individuals with Wiedemann-Steiner syndrome. *American Journal of Medical Genetics. Part A*, *185*(6), 1649–1665. https://doi.org/10.1002/ajmg.a.62124

Tahara, Y., Moji, K., Aoyagi, K., Tsunawake, N., Muraki, S., & Mascie-Taylor, C. G. (2002). Age-related pattern of body density and body composition of Japanese men and women 18-59 years of age. *American Journal of Human Biology*, *14*(6), 743–752. https://doi.org/10.1002/ajhb.10091

Thodberg, H. H., Bottcher, J., Lomholt, J., Kreiborg, S., Wolf, G., & Pfeil, A. (2016). A new implementation of digital X-ray radiogrammetry and reference curves of four indices of cortical bone for healthy European adults. *Archives of Osteoporosis*, *11*, 17. https://doi.org/10.1007/s11657-016-0267-2

Torga, A. P., Hodax, J., Mori, M., Schwab, J., & Quintos, J. B. (2018). Hypogonadotropic hypogonadism and Kleefstra syndrome due to a pathogenic variant in the EHMT1 gene: An underrecognized association. *Case Reports in Endocrinology*, *2018*, 4283267. https://doi.org/10.1155/2018/4283267

van Montfort, L., Gerver, W. J. M., Kooger, B. L. S., Plat, J., Bierau, J., Stumpel, C., & Schott, D. A. (2021). Follow-up study of growth hormone therapy in children with Kabuki syndrome: Two-year treatment results. *Hormone Research in Pædiatrics*, *94*(7–8), 285–296. https://doi.org/10.1159/000519963

van Zoonen, R. (2019). *JGZ-richtlijn lengtegroei*. TNO. http://resolver.tudelft.nl/uuid:ec6a3440-93d5-4cbc-9f3b-45513a1ca520

Vermeulen, K., de Boer, A., Janzing, J. G. E., Koolen, D. A., Ockeloen, C. W., Willemsen, M. H., Verhoef, F. M., van Deurzen, P. A. M., van Dongen, L., van Bokhoven, H., Egger, J. I. M., Staal, W. G., & Kleefstra, T. (2017). Adaptive and maladaptive functioning in Kleefstra syndrome compared to other rare genetic disorders with intellectual disabilities. *American Journal of Medical Genetics. Part A*, *173*(7), 1821–1830. https://doi.org/10.1002/ajmg.a.38280

Willemsen, M. H., Vulto-van Silfhout, A. T., Nillesen, W. M., Wissink-Lindhout, W. M., van Bokhoven, H., Philip, N., Berry-Kravis, E. M., Kini, U., van Ravenswaaij-Arts, C. M., Delle Chiaie, B., Innes, A. M., Houge, G., Kosonen, T., Cremer, K., Fannemel, M., Stray-Pedersen, A., Reardon, W., Ignatius, J., Lachlan, K., … Kleefstra, T. (2012). Update on Kleefstra syndrome. *Molecular Syndromology*, *2*(3–5), 202–212. https://doi.org/10.1159/000335648

Wohlfahrt-Veje, C., Tinggaard, J., Winther, K., Mouritsen, A., Hagen, C. P., Mieritz, M. G., de Renzy-Martin, K. T., Boas, M., Petersen, J. H., & Main, K. M. (2014). Body fat throughout childhood in 2647 healthy Danish children: Agreement of BMI, waist circumference, skinfolds with dual X-ray absorptiometry. *European Journal of Clinical Nutrition*, *68*(6), 664–670. https://doi.org/10.1038/ejcn.2013.282

Wouters, M., Evenhuis, H. M., & Hilgenkamp, T. I. M. (2020). Physical fitness of children and adolescents with moderate to severe intellectual disabilities. *Disability and Rehabilitation*, *42*(18), 2542–2552. https://doi.org/10.1080/09638288.2019.1573932

SUPPORTING INFORMATION

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How to cite this article: Bouman, A., Geelen, J. M., Kummeling, J., Schenck, A., van der Zwan, Y. G., Klein, W. M., & Kleefstra, T. (2023). Growth, body composition, and endocrine-metabolic profiles of individuals with Kleefstra syndrome provide directions for clinical management and translational studies. *American Journal of Medical Genetics Part A*, 1–14. https://doi.org/10.1002/ajmg.a.63472