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Can Fetuin-A Be a Marker for Insulin Resistance and Poor Glycemic Control in Children with Type 1 Diabetes Mellitus?

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What is already known on this topic?

Hyperlipidemia and hyperglycemia lead to an increase in fetuin-A production.

What this study adds?

Fetuin-A levels could be useful in predicting complications such as hepatosteatosis and atherosclerosis in patients with type 1 diabetes mellitus.

Abstract

Objective: Metabolic impairment in type 1 diabetes mellitus (T1DM) with poor glycemic control causes insulin resistance, non-alcoholic fatty liver disease (NAFLD), atherosclerosis, and increased carotid intima-media thickness (CIMT). Fetuin-A has a protective effect in cardiovascular disorders and is increased in hepatosteatosis. We aimed to investigate the reliability of fetuin-A levels in early detection of diabetic complications in children with T1DM and to identify a cut-off value that may show poor metabolic control.
Methods: The study included 80 patients who had T1DM for at least 5 years and who had no chronic complications or an auto-immune disorder. Blood samples were drawn to measure hemoglobin A1c (HbA1c), biochemical parameters, and fetuin-A levels. Anthropometric parameters were also measured. Percent body fat was calculated. Hepatosteatosis and CIMT were assessed by sonography.
Results: Mean age of the patients was 13.5 years. Grade 1 hepatosteatosis was detected in 10%. Patients were stratified into 2 groups based on presence of NAFLD. Fetuin-A level was increased in patients with NAFLD. We identified a fetuin-A cut-off value (514.28 ng/mL; sensitivity: 47.34; specificity: 96.72) that may predict NAFLD. HbA1c and total cholesterol levels were found to be higher in patients with fetuin-A levels above higher the cut-off value.
Conclusion: Fetuin-A is a reliable parameter in the prediction of complications and poor glycemic control in patients with T1DM.
Keywords: Non-alcoholic fatty liver disease, fetuin-a, type 1 diabetes mellitus, complication

Introduction

Type 1 diabetes mellitus (T1DM) is more commonly seen at childhood. Children with T1DM are classically lean or of normal weight at the time of diagnosis. In recent years, in parallel to increased incidence of obesity, increased body weight and development of insulin resistance have become striking findings in patients with T1DM, particularly in those with poor glycemic control (1). Both glucotoxicity and metabolic imbalance caused by poor glycemic control lead to more severe complication at earlier ages, implying a need for a novel and practicable criterion for early diagnosis and monitoring of complications.

Hepatosteatosis is an important complication in children with T1DM as it indicates metabolic imbalance and development of peripheral insulin resistance. Lipoprotein is structurally impaired, and secondary to the hyperglycemia, glucose transporter 2-mediated glucose uptake from the circulation by the liver is increased. This results in increased fatty acid and lipoprotein synthesis, which is implied in the pathogenesis (2,3,4). Such hepatic disorders due to causes other than alcohol are termed as non-alcoholic fatty

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liver disease (NAFLD). In addition, hyperglycemia can also cause endothelial injury and activation of the coagulation system (5,6). In this regard, carotid intima-media thickness (CIMT) that reflects premature atherosclerotic changes is an important marker (7). Thus, hepatosteatosis and increased CIMT are complications which may herald poor metabolic control and chronic influences in children with T1DM (8).

Fetuin-A (Alpha-2 Heremans Schmid glycoprotein) is a negative acute phase reactant synthesized by the liver. This glycoprotein also causes insulin resistance by enhancing insulin receptor tyrosine kinase activity and insulin receptor auto-phosphorylation (9). The fact that fetuin-A production is increased by hyperlipidemia and hyperglycemia suggests that it can be a valuable parameter for predicting complications of T1DM as well as monitoring poor glycemic control (10).

This study aimed to investigate fetuin-A levels in predicting complications in patients with T1DM and to assess its relationship with clinical, radiological, and biochemical parameters. It also aimed to identify a cut-off value for fetuin-A that may indicate poor metabolic control and to assess its reliability.

Methods

The study was approved by the Erciyes University Local Ethics Committee (approval number: 2014/118) and informed consent was obtained from patients or relatives. The study included 80 patients (40 boys and 40 girls, mean age 13.5 years) without any chronic complications or comorbid auto-immune disorders and who had been followed with the diagnosis of T1DM for at least 5 years at the Pediatric Endocrinology Department of Erciyes University, Faculty of Medicine. Patients with alcohol consumption, smokers, those with positive serology for hepatitis, those with an infectious or comorbid systemic disorder, and those on drug therapy were excluded. In all patients, weight (kg), height (cm), waist circumference (cm), and neck circumference were measured by the same observer. Body mass index (BMI) was calculated by using the following formula: BMI = weight (kg) / height^2 (m^2). Patients with a BMI $>95^{th}$ percentile according to age and gender were considered as obese (11). The waist circumference was measured at the narrowest level between the costal arch and the processus spina iliaca anterior superior at the end of expiration in the patient standing upright (12). The neck circumference was measured at the level of the superior margin of the cricothyroid membrane in the patient standing upright with shoulders relaxed (13). Two blood pressure measurements with 15 minutes interval were performed by the same observer and the mean value was recorded.

Percentage of body fat (BF%) was analyzed by using Tanita BC480MA body composition analyzer. Blood samples were drawn after an 8-hour fast for determination of lipid profile [triglyceride, total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein], hepatic enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], gamma-glutamyl transferase (GGT), glycated hemoglobin A1c (HbA1c), serological hepatitis markers, systemic infection markers, and complete blood count (CBC). In addition, microalbumin: creatinine ratio was measured in spot urine samples of first morning void. Microalbumin: creatinine ratio of 0-30 µg/mg was defined as normal, while 30-300 µg/mg as micro-albuminuria and >300 µg/mg as proteinuria. Biochemical parameters were determined using Abbot Architect C8000 kits, while CBC was performed by using Siemens Advia 2120i hematology system.

Fundus examination and electromyography were performed in all patients. Children with diabetic micro-vascular complications (microalbuminuria-neuropathy-retinopathy) or comorbid auto-immune disorders were excluded.

Sonography was performed by the same observer in all patients. Internal carotid artery and the segment 1-2 cm proximal to the main carotid artery were screened and CIMT measurement was performed. To assess NAFLD, hepatic sonography was performed and rated as grade 1, 2, and 3 according to degree of hepatic echogenicity and appearance of right hemi-diaphragm and intra-hepatic vessels.

Blood samples (5 mL) were drawn into anticoagulant-free tubes for fetuin-A analysis. After allowing clotting over 20-30 minutes at room temperature, the samples were centrifuged at 5000 rpm for 10 minutes at 4±2 °C in a refrigerated centrifuge. The sera were stored at -80 °C until time of assay. Fetuin-A was measured using the enzyme-linked immunosorbent assay technique by taking the dilution factor into account. The detection limit was 0.35 ng/mL. Intra-assay and inter-assay coefficients of variation were 3.5% and 5.5%, respectively (14).

Statistical Analysis

The data were analyzed by using IBM SPSS Statistics version 22.0. Descriptive statistics were presented as count (n), percent (%), mean ± standard deviation, and median (minimum-maximum). In numeric variables, normal distribution was tested by Shapiro-Wilk normality test and Q-Q graphics. Comparisons between groups were performed by using the non-parametrical Mann-Whitney U test, while prevalence comparisons were performed by using chi-square test. A p-value <0.05 was considered as statistically significant. Receiver operating characteristics (ROC) analysis was used to determine cut-off value for fetuin-A and its reliability. The Youden index (represents the

maximum of sensitivity + specificity -1 for all cut points in the ROC curve) was used to determine the optimum cut-off value of fetuin-A for detection of hepatosteatosis.

Results

The study included 80 patients with T1DM (40 boys and 40 girls). Duration of DM ranged from 5 to 16 years. On sonography, grade 1 hepatosteatosis was detected in 8 patients (10%).

The patients were stratified into two groups based on presence or absence of hepatosteatosis, as NAFLD (+) and NAFLD (-). The groups were compared regarding age, gender, duration of DM, BMI, BF%, lean body mass (LBM), right and left CIMT, fetuin-A level, HbA1c level, lipid profile, and results of hepatic function tests. Mean blood pressure was assessed according to age- and sex-adjusted percentile curves. No patient had hypertension based on this assessment.

Mean age was 14 years (9-17 years) in NAFLD (+) patients and 13.5 years (6-18 years) in NAFLD (-) patients. There were 5 boys and 3 girls in the NAFLD (+) group, 35 boys and 37 girls in the NAFLD (-) group. There was no significant difference in age and duration of DM between the groups ($p = 0.37$ and $p = 0.089$; Table 1).

When groups were compared, BF% and right-left CIMT were found to be significantly higher in the NAFLD (+) group than in the NAFLD (-) group ($p = 0.037$, $p = 0.003$ and $p = 0.014$, respectively). No significant difference was detected in anthropometric measurements, BMI, and LBM between the two groups.

Table 1. Assessment of patients according to hepatosteatosis

	NAFLD (-) n = 72		NAFLD (+) n = 8		p
	Median	Min-Max	Median	Min-Max	
Age (years)	13.50	6.00-18.00	14.00	9.00-17.0	0.370
DOD (years)	6.00	5.00-14.00	6.50	5.00-16.00	0.089
BMI (kg/m^2)	19.10	15.0-24.30	20.40	18.00-45.30	0.460
BF%	17.70	10.60-29.70	22.00	10.10-36.50	0.037
FFM (kg)	33.70	14.30-57.30	37.30	20.20-59.90	0.140
WC (cm)	66.00	54.0-83.00	69.00	54.00-88.00	0.195
NC (cm)	30.00	26.00-39.00	31.00	26.00-37.00	0.351
IMT-right (mm)	0.50	0.40-0.75	0.55	0.40-0.65	0.003
IMT-left (mm)	0.50	0.40-0.75	0.55	0.04-0.65	0.014

NAFLD: non-alcoholic fatty liver disease, DOD: the duration of diagnosis, BMI: body mass index, BF%: percentage of body fat, FFM: fat-free mass, WC: waist circumference, NC: neck circumference, IMT-right: intima-media thickness-right, IMT-left: intima-media thickness-left, Min: minimum, Max: maximum

Table 2. Biochemical parameters according to non-alcoholic fatty liver disease

	NAFLD (-) n = 72		NAFLD (+) n = 8		p
	Median	Min-Max	Median	Min-Max	
Fetuin (ng/mL)	377.12	133.24-577.16	434.20	102.64-586.88	0.024
HbA1c (%)	7.90	6.00-9.50	8.20	7.10-10.50	0.072
GGT (IU/L)	11.00	1.00-18.00	11.00	6.00-40.00	0.119
AST (IU/L)	19.00	10.00-88.00	17.00	9.00-62.00	0.847
ALT (IU/L)	13.00	7.00-52.00	17.00	10.00-83.00	< 0.001
AST/ALT ratio	1.46	1.06-2.57	1.00	0.16-1.88	< 0.001
Total cholesterol (mg/dL)	140.00	91.00-228.00	145.00	99.00-206.00	0.568
TG (mg/dL)	83.00	39.00-275.00	105.00	54.00-314.00	0.580
HDL (mg/dL)	58.00	28.00-98.00	61.00	28.00-92.00	0.233
LDL (mg/dL)	76.00	39.00-149.00	87.00	45.00-120.00	0.317

NAFLD: non-alcoholic fatty liver disease, HbA1c: hemoglobin A1c, GGT: gamma glutamyl transferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TG: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein, Min: minimum, max: maximum

When biochemical parameters were compared between groups, fetuin-A and ALT levels were found to be significantly higher in the NAFLD (+) group ($p = 0.024$ and $p = 0.072$, respectively; Table 2, Figure 1). AST: ALT ratio was significantly lower in the NAFLD (+) group. There was no significant difference in HbA1c, GGT, and lipid levels between the two groups.

The ROC analysis was used to assess usefulness of fetuin-A as a marker for hepatosteatosis development. In the ROC analysis, area under curve was estimated to be 0.672 [95% confidence interval (CI): 0.558-0.773; $p = 0.022$], indicating that fetuin-A is a reliable parameter. Again, in the ROC analysis, cut-off value for presence or absence of hepatosteatosis was calculated as 514.28 ng/mL (sensitivity: 47.34, 95% CI: 24.5-71.1 and specificity: 96.72, 95% CI: 86.6.99.5). Youden index was calculated as 0.51.

We also assessed the reliability of fetuin-A cut-off value as a marker for poor metabolic control. The statistical relationship between fetuin-A level above cut-off value and NAFLD positivity was assessed by chi-square test, revealing a significant difference (x^2: 20.476, $p < 0.001$).

When clinical findings between groups were analyzed by stratifying patients according to cut-off value, it was found that patients with a fetuin-A level above cut-off value had higher BF% value and increased right and left CIMT ($p = 0.001$, $p = 0.001$, and $p < 0.001$, respectively; Table 3; Figure 2). However, there was no significant difference in age, duration of DM, BMI, LBM, waist circumference (WC), and neck circumference between the two groups.

When biochemical parameters were analyzed by stratifying patients according to cut-off value, it was found that there were significant differences in HbA1c, GGT, and TC levels between patients with a fetuin-A level below and above cut-

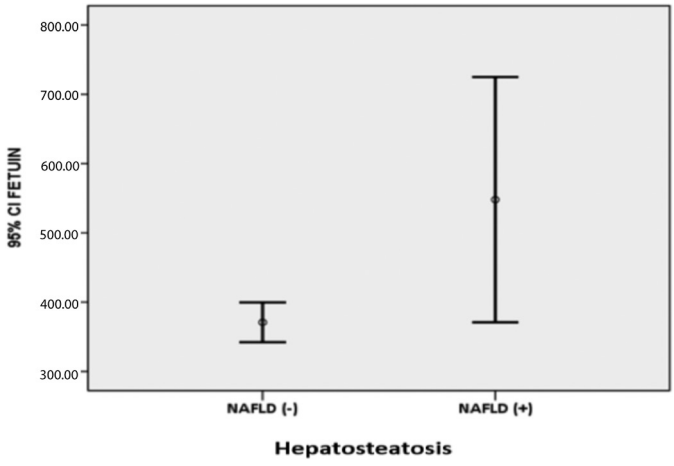

Figure 1. Relationship between fetuin-A and non-alcoholic fatty liver disease

NAFLD: non-alcoholic fatty liver disease, CI: confidence interval

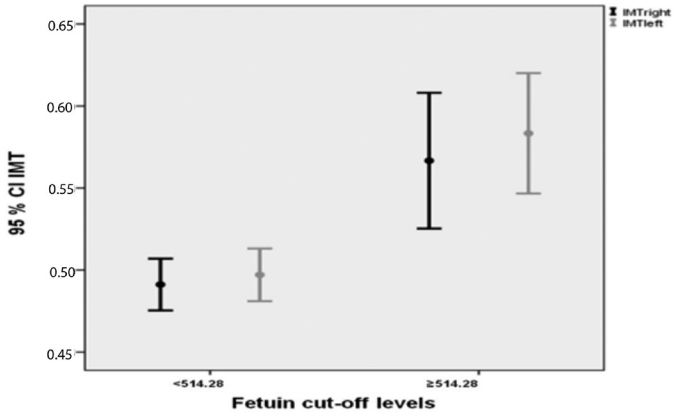

Figure 2. Relationship between fetuin-A and carotid intima-media thickness

CIMT: carotid intima-media thickness, IMT-right: intima-media thickness-right, IMT-left: intima-media thickness-left

Table 3. Anthropometric measurements according to fetuin-A cut-off levels

	Fetuin-A (ng/mL) < 514.28, n = 68		Fetuin-A (ng/mL) ≥514.28, n = 12		p
	Median	Min-Max	Median	Min-Max	
Age (years)	14.00	6.00-18.00	13.00	9.0-17.9	0.516
DOD (years)	6.00	5.0-16.0	6.75	5.00-11.00	0.131
BMI (kg/m²)	19.20	15.00-27.60	20.55	16.20-45.30	0.159
BF%	17.60	10.10-29.70	23.80	16.00-36.50	0.001
FFM (kg)	34.55	14.30-57.30	37.20	24.60-59.90	0.403
WC (cm)	67.00	54.0-83.0	69.50	54.0-88.0	0.462
NC (cm)	30.00	26.00-39.00	30.50	26.00-37.00	0.621
IMT-right (mm)	0.50	0.40-0.75	0.60	0.45-0.65	0.001
IMT-left (mm)	0.50	0.40-0.75	0.60	0.50-0.65	< 0.001

DOD: duration of diagnosis, BMI: Body mass index, BF%: percentage of body fat, FFM: fat-free mass, WC: waist circumference, NC: neck circumference, IMT-right: intima-media thickness-right, IMT-left: intima-media thickness-left, Min: minimum, Max: maximum

Table 4. Biochemical parameters according to fetuin-A cut-off levels

	Fetuin-A (ng/mL) < 514.28, n = 68		Fetuin-A (ng/mL) ≥514.28, n = 12		p
	Median	Min-Max	Median	Min-Max	
Fetuin (ng/mL)	371.72	102.64-510.40	584.90	514.28-586.88	< 0.001
HbA1c (%)	7.90	6.40-9.50	8.45	6.00-10.50	0.030
GGT (IU/L)	10.69	1.00-35.00	13.00	8.00-40.00	0.025
AST (IU/L)	18.50	9.00-88.00	25.50	11.00-62.00	0.080
ALT (IU/L)	14.00	7.00-83.00	16.00	10.00-60.00	0.099
AST/ALT	1.42	0.16-2.57	1.13	0.86-2.55	0.315
Total cholesterol (mg/dL)	140.00	91.00-228.00	167.00	99.00-198.00	0.027
TG (mg/dL)	83.00	39.00-275.00	106.00	57.00-314.00	0.609
HDL (mg/dL)	58.00	28.00-98.00	63.00	28.00-92.00	0.215
LDL (mg/dL)	76.00	39.00-149.00	84.50	45.00-115.00	0.370

HbA1c: Hemoglobin A1c, GGT: gama glutamil transferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TG: triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein, Min: minimum, Max: maximum

off value (p = 0.03, p = 0.025, p = 0.027, respectively), but there was no significant difference in ALT level nor in AST: ALT ratio (Table 4).

HbA1c, AST, GGT, BF%, and WC were detected to positively correlate with fetuin-A (r = 0.370, p = 0.001 – r = 0.274, p = 0.015 - r = 0.419, p < 0.001 - r = 0.485, p < 0.001 - r = 0.286, p = 0.010, respectively).

Discussion

The prediction of complications which determine mortality and morbidity in T1DM before onset of clinical findings, at a time when they are reversible, will increase success in the follow-up (15). In the present study, fetuin-A levels were investigated as a parameter to determine risk prediction.

In the literature, there is limited number of studies on association of NAFLD and poorly controlled T1DM. In a study from Egypt, NAFLD was detected in 4.5% of 692 children with T1DM (16). In a study on 202 patients with T1DM, Targher et al (8) detected hepatosteatosis in 44.4% of patients and reported higher HbA1c level, longer DM duration, and higher nephropathy rates in these patients. In that study, it was seen that mean age was higher in diabetic patients with NAFLD than in those without NAFLD (47 ± 12 years vs. 37 ± 12 years). In our study, there was NAFLD in 10% of 80 patients with T1DM; however, no significant difference was detected between patients regarding duration of DM. In addition, no micro- or macro- vascular complications were detected in the patients. Lack of significant difference in age and duration of DM and absence of complications may be attributed to selection of younger patients.

The most valuable marker of hepatic injury is elevated ALT levels and low AST: ALT ratio (17,18). In our study, it was found that ALT levels were higher, while AST: ALT ratio was significantly lower in patients with NAFLD.

In a study from a pediatric diabetes centre in Germany, 93 children and adolescents with T1DM were investigated for NAFLD using ultrasound, biochemical findings, and liver stiffness measurements (FibroScan® and acoustic radiation force imaging). Completely normal results were obtained in 88.1% of these patients, 1.1% fulfilled the criteria as potential NAFLD patients, and 10.8% showed some mild abnormality in at least one category (19). We obtained similar results in this present study, using a different technique to evaluate NAFLD.

The CIMT is used as an early marker of subclinical atherosclerosis, and it is reported that CIMT is higher in diabetic patients than in the normal population (20,21). Gökşen et al (20) conducted a study on 55 children with T1DM and 30 healthy children and found that CIMT values were significantly higher in diabetic patients when compared to healthy controls. In our study, there was no control group; however, it was found that bilateral CIMT value and BF% were higher in diabetic patients with NAFLD despite comparable BMI values, as well as comparable duration of DM and HbA1c levels.

Although it is unclear which factors regulate fetuin-A production, it was reported that free fat acids (FFAs) and hyperglycemia increase fetuin-A expression and induce insulin resistance (22). Ix et al (23) conducted a study to investigate the relationship between metabolic syndrome and fetuin-A on 711 patients with coronary artery disease having non-diabetic metabolic syndrome and found that the increase in fetuin-A level was associated to each component of metabolic syndrome. Authors suggested that this was due to increased lipolysis as a result of inhibition of tyrosine

kinase receptor by fetuin-A, increased release of FFAs from adipose tissue, and increased production of apolipoprotein B containing very LDL (24).

In a study on rats with induced T1DM, it was demonstrated that secondary insulin resistance developed immediately after deprivation of insulin reserve and that this was associated with fetuin-A levels (24). Although the number of clinical trials on this issue is limited, in the study by Gheissari et al (25), a significant decrease was observed in fetuin-A level after initiation of valsartan in T1DM patients with nephropathy. In another study in which patients with T1DM (n = 62) were compared with healthy controls, fetuin-A level and CIMT were found to be higher in the patient group (26). These findings have raised the question as to whether increased fetuin-A levels can predict development of findings of metabolic syndrome, such as insulin resistance, hyperlipidemia, NAFLD and increased CIMT values are added over time, in T1DM patients with poor glycemic control. In our study, both fetuin-A and CIMT values were found to be higher in patients with NAFLD. In the second stage of our study, using ROC analysis, a cut-off value of fetuin-A for NAFLD risk was calculated to be 514.28 ng/mL. The sensitivity and specificity were 47.34 (95% CI: 24.5-71) and 96.72 (95% CI: 86.6-99.5), respectively. When patients were stratified according to this cut-off value, it was seen that patients with fetuin-A levels above the cut-off value had poorer glycemic control and higher TC levels. However, there was no significant difference in ALT level and AST: ALT ratio between the two groups. These findings may indicate that fetuin-A levels may be a marker for hyperlipidemia, metabolic syndrome, and poor glycemic control before onset of hepatic injury in children with T1DM. Again, bilateral IMT values were significantly higher in patients with fetuin-A levels above the cut-off value; this finding supports the hypothesis that fetuin-A is a marker of early atherosclerotic changes.

Study Limitations

We acknowledge that the small number of T1DM patients diagnosed as hepatosteatosis in our study is a limitation. Also, our reason for using sonography instead of fine-needle biopsy, which is the accepted gold standard in the diagnosis of hepatosteatosis, was due to its being a non-invasive method.

Conclusion

In conclusion, our findings indicate that increased CIMT values and NAFLD can be accepted as markers of poor metabolic control in the follow-up of diabetic patients before detectible diabetic complications develop. The results also suggest that patients at risk can be identified by measuring fetuin-A level alone before onset of clinical and/or laboratory findings of chronic complications. Further comprehensive studies are needed in this area.

Ethics

Ethics Committee Approval: The study was approved by the Erciyes University Local Ethics Committee (approval number: 2014/118).

Informed Consent: Consent form was filled out by the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Medical Practices: Ülkü Gül Şiraz, Murat Doğan, Concept: Nihal Hatipoğlu, Selim Kurtoğlu, Sabahattin Muhtaroğlu, Design: Ülkü Gül Şiraz, Nihal Hatipoğlu, Selim Kurtoğlu, Data Collection or Processing: Ülkü Gül Şiraz, Murat Doğan, Analysis or Interpretation: Nihal Hatipoğlu, Sabahattin Muhtaroğlu, Literature Search: Ülkü Gül Şiraz, Murat Doğan, Writing: Ülkü Gül Şiraz.

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References

1. Bacha F, Klinepeter Bartz S. Insulin resistance, role of metformin and other non-insulin therapies in pediatric type 1 diabetes. Pediatr Diabetes 2016;17:545-558. Epub 2015 Nov 23
2. Vergès B. Lipid disorders in type 1 diabetes. Diabetes Metab 2009;35:353-360. Epub 2009 Sep 4
3. Im SS, Kang SY, Kim SY, Kim HI, Kim JW, Kim KS, Ahn YH. Glucose-stimulated upregulation of GLUT2 gene is mediated by sterol response element-binding protein-1c in the hepatocytes. Diabetes 2005;54:1684-1691.
4. Pacifico L, Nobili V, Anania C, Verdecchia P, Chiesa C. Pediatric nonalcoholic fatty liver disease, metabolic syndrome and cardiovascular risk. World J Gastroenterol 2011;17:3082-3091.
5. Viswanathan V. Preventing microvascular complications in type 1 diabetes mellitus. Indian J Endocrinol Metab 2015;19(Suppl 1):36-38.
6. Najafian B, Mauer M. Progression of diabetic nephropathy in type 1 diabetic patients. Diabetes Res Clin Pract 2009;83:1-8. Epub 2008 Dec 12
7. Fathi R, Marwick TH. Noninvasive tests of vascular function and structure: why and how to perform them. Am Heart J 2001;141:694-703.
8. Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Pichiri I, Sorgato C, Zenari L, Bonora E. Prevalence of non-alcoholic fatty liver disease and its association with cardiovascular disease in patients with type 1 diabetes. J Hepatol 2010;53:713-718.
9. Mathews ST, Rakhade S, Zhou X, Parker GC, Coscina DV, Grunberger G. Fetuin-null mice are protected against obesity and insulin resistance associated with aging. Biochem Biophys Res Commun 2006;350:437-443. Epub 2006 Sep 25

10. Takata H, Ikeda Y, Suehiro T, Ishibashi A, Inoue M, Kumon Y, Terada Y. High glucose induces transactivation of the alpha2-HS glycoprotein gene through the ERK1/2 signaling pathway. J Atheroscler Thromb 2009;16:448-456. Epub 2009 Aug 11

11. Senol V, Unalan D, Bayat M, Mazicioglu MM, Ozturk A, Kurtoglu S. Change in reference body mass index percentiles and deviation in overweight and obesity over 3 years in Turkish children and adolescents. J Pediatr Endocrinol Metab 2014;27:1121-1129.

12. Mazıcıoğlu MM, Hatipoğlu N, Oztürk A, Ciçek B, Ustünbaş HB, Kurtoğlu S. Waist circumference and mid-upper arm circumference in evaluation of obesity in children aged between 6 and 17 years. J Clin Res Pediatr Endocrinol 2010;2:144-150. Epub 2010 Dec 10

13. Mazicioglu MM, Kurtoglu S, Ozturk A, Hatipoglu N, Cicek B, Ustunbas HB. Percentiles and mean values for neck circumference in Turkish children aged 6-18 years. Acta Paediatr 2010;99:1847-1853.

14. Smith ER, Ford ML, Tomlinson LA, Rocks BF, Rajkumar C, Holt SG. Poor agreement between commercial ELISAs for plasma fetuin-A: An effect of protein glycosylation? Clin Chim Acta 2010;411:1367-1370. Epub 2010 Apr 24

15. Rosenbloom A, Silverstein J. Diabetes in the child and adolescent. in: Lifshitz F (ed). Pediatric Endocrinology. 4th ed. New York, Marcel Dekker, 2004; p. 611-651.

16. El-Karaksy HM, Anwar G, Esmat G, Mansour S, Sabry M, Helmy H, El-Hennawy A, Fouad H. Prevalence of hepatic abnormalities in a cohort of Egyptian children with type 1 diabetes mellitus. Pediatric Diabetes 2010;11:462-470.

17. Mansoor S, Yerian L, Kohli R, Xanthakos S, Angulo P, Ling S, Lopez R, Christine CK, Feldstein AE, Alkhouri N. The evaluation of hepatic fibrosis scores in children with nonalcoholic fatty liver disease. Dig Dis Sci 2015;60:1440-1447. Epub 2014 Dec 25

18. Yang HR, Kim HR, Kim MJ, Ko JS, Seo JK. Noninvasive parameters and hepatic fibrosis scores in children with nonalcoholic fatty liver disease. World J Gastroenterol 2012;18:1525-1530.

19. Kummer S, Klee D, Kircheis G, Friedt M, Schaper J, Häussinger D, Mayatepek E, Meissner T. Screening for non-alcoholic fatty liver disease in children and adolescents with type 1 diabetes mellitus: a cross-sectional analysis. Eur J Pediatr 2017;176:529-536. Epub 2017 Feb 17

20. Gökşen D, Levent E, Kar S, Ozen S, Darcan S. Serum adiponectin and hsCRP levels and non-invasive radiological methods in the early diagnosis of cardiovascular system complications in children and adolescents with type 1 diabetes mellitus. J Clin Res Pediatr Endocrinol 2013;5:174-181.

21. Çiftel M, Ertuğ H, Parlak M, Akçurin G, Kardelen F Investigation of endothelial dysfunction and arterial stiffness in children with type 1 diabetes mellitus and the association with diastolic dysfunction. Diab Vasc Dis Res 2014;11:19-25. Epub 2013 Oct 29

22. Dasgupta S, Bhattacharya S, Biswas A, Majumdar SS, Mukhopadhyay S, Ray S, Bhattacharya S. NF-kappaB mediates lipid-induced fetuin-A expression in hepatocytes that impairs adipocyte function effecting insulin resistance. Biochem J 2010;429:451-462.

23. Ix JH, Shlipak MG, Brandenburg VM, Ali S, Ketteler M, Whooley MA. Association between human fetuin-A and the metabolic syndrome: data from the Heart and Soul Study. Circulation 2006;113:1760-1767. Epub 2006 Mar 27

24. Jelenik T, Séquaris G, Kaul K, Ouwens DM Phielix E, Kotzka J, Knebel B, Weiß J, Reinbeck AL, Janke L, Nowotny P, Partke HJ, Zhang D, Shulman GI, Szendroedi J, Roden M. Tissue-specific differences in the development of insulin resistance in a mouse model for type 1 diabetes. Diabetes 2014;63:3856-3867. Epub 2014 Jun 10

25. Gheissari A, Javanmard SH, Shirzadi R, Amini M, Khalili N. Lowering effect of valsartan on fetuin-A in type 1 diabetes mellitus. Iran J Kidney Dis 2013;7:440-445.

26. Abd El Dayem SM, Battah AA, El Bohy Ael M, El Shehaby A. Evaluation of fetuin-A and carotid intima-media thickness in adolescent type 1 diabetic patients. J Pediatr Endocrinol Metab 2015;28:287-292.

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Effect of Maternal Obesity on Fetal Growth and Expression of Placental Fatty Acid Transporters

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What is already known on this topic?

Previous studies have investigated the association between maternal obesity and placental nutrient transporters, but results have been inconsistent.

What this study adds?

One of the most fundamental questions arising from the study is how diet-induced obesity and diet-induced obesity-resistant dams and their offspring differ in terms of their respective metabolic response to a high-fat diet.

Abstract

Objective: To explore the effects of maternal high-fat (HF) diet-induced obesity on fetal growth and the expression of placental nutrient transporters.

Methods: Maternal obesity was established in rats by 8 weeks of pre-pregnancy fed HF diet, while rats in the control group were fed normal (CON) diet. Diet-induced obesity (DIO) rats and diet-induced obesity-resistant (DIR) rats were selected according to body weight gain over this period. After copulation, the CON rats were divided into two groups: switched to HF diet (CON-HF group) or maintained on the CON diet (CON-CON group). The DIO rats and DIR rats were maintained on the HF diet throughout pregnancy. Pregnant rats were euthanized at day 21 gestation, fetal and placental weights were recorded, and placental tissue was collected. Reverse transcription-polymerase chain reaction was used to determine mRNA expression of placental nutrient transporters. Protein expression was determined by Western blot.

Results: Average fetal weight of DIO dams was reduced by 6.9%, and the placentas of CON-HF and DIO dams were significantly heavier than the placentas of CON-CON and DIR dams at day 21 of gestation ($p < 0.05$). The fetal/placental weight ratio of DIO dams was significantly reduced compared with the fetal/placental weight ratio of CON-CON dams ($p < 0.05$). The mRNA expression of *GLUT-1* and *SNAT-2* were not significantly different between groups. The mRNA and protein expression levels of *CD36*, *FATP-1*, and *FATP-4* in DIO dams were decreased significantly ($p < 0.05$).

Conclusion: Maternal obesity induced by a HF diet led to intrauterine growth retardation and down-regulated the expression of placental fatty acid transporters.

Keywords: Obesity, placenta, nutrient transport, high-fat diet, intrauterine growth

Introduction

Maternal obesity may lead to serious health complications for mother and fetus. It increases the incidence of maternal pregnancy complications such as gestational diabetes and preeclampsia (1,2,3,4). In addition to maternal health complications, there is also a considerable increase in the risk of fetal complications. These include stillbirth (3,5,6), spontaneous abortions (5) and fetal asphyxia (7), as well as increased risk of delivery of small for gestational age or large for gestational age babies (8,9,10). In the long-term, the offspring of obese mothers have a higher risk of

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obesity in adult life, and this is likely due to the combined effects of genetics and environment. Given these factors, understanding how maternal obesity might have an impact on offspring health is of major public health importance.

Pregnancy is a critical period of physiological change for both the mother and the fetus. The placenta, the interface between the maternal and fetal blood circulations (11), is responsible for the maternal-to-fetal transfer of nutrients which are essential for fetal growth and development. Fetal growth is directly related to maternal nutrient availability and the placental ability to transport these nutrients from maternal circulation to the fetus. Glucose, amino acids, and fatty acids are essential macronutrients for adequate fetal growth. All of these traverse the placental syncytiotrophoblasts (SCTB) mediated by specific transporters. Placental glucose transport occurs by facilitated diffusion along a concentration gradient through members of the glucose transporter (GLUT) family (12,13). Amino acid transport across the human placenta is a complex process because more than 20 different amino acid transporters with overlapping specificities are expressed in the SCTB. For example, system A is a sodium-dependent accumulative transport system which mediates the uptake of neutral amino acids (both essential and nonessential) with short and unbranched side chains, mostly L-alanine, glycine, L-serine, L-methionine, and L-glutamine (14,15). In recent years, a gene family of sodium-coupled neutral amino acid transporters [(SNAT); (*SCL38* gene family)] coding for proteins that possess the classically described system A transport activities (in terms of their functional properties and patterns of regulation) has been cloned (16). SNAT-2 is widely expressed in rat tissues, and its mRNA concentration increases in cultured cells during adaptive regulation or with the addition of cAMP (17). The proteins associated with fatty acid transport include fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPpm), and other FABP (18,19). FATPs, six members (FATPs1-6), are integral membrane proteins that are of importance for the uptake of long-chain fatty acids (18). Five members (FATPs1-4, and 6) have been identified in placental trophoblasts (18). FATP-1 and FATP-4 have been frequently studied in placental tissue as their expression correlates with docosahexaenoic acid levels in maternal plasma, cord blood, and placental phospholipids, suggesting an important role in the transfer of long-chain polyunsaturated fatty acids (20). Of note, previous studies have investigated the association between maternal obesity and placental nutrient transporters, but results have been inconsistent. Reynolds et al (21) have reported that maternal high-fat (HF) consumption induces sex-specific nutrient transport in the rat placenta (21). In this study, maternal HF consumption was found to be associated with increased placental CD36 mRNA expression only in female placentas, and placental GLUT1, GLUT4, and SNAT2 mRNA expression significantly increased only in HF male placentas. This sex difference may be driven by sexually dimorphic placental alterations which occur as a result of maternal gestational diets. Farley et al (22) reported that maternal obesity was accompanied by decreased placental SNAT activity although the offspring grew normally. In a Canadian study in humans, it was found that maternal obesity was associated with increased placental CD36 mRNA and protein expression and decreased FATP-4 mRNA and protein and FABP3 protein expression (23). However, Zhu et al (24), working with sheep, showed that maternal obesity enhanced the mRNA expression and protein content of FATPs in the placenta. These complex and conflicting results raise significant doubt as to whether placental nutrient transport is associated with maternal obesity. Therefore, in this study, we developed a model of maternal obesity in which rats are fed a HF diet and used this model to determine how maternal obesity influenced fetal growth, placental weight, as well as placental gene and protein expression of nutrient transporters.

Methods

All animal procedures were approved by the Animal Ethics Committee of Anhui Medical University (approval number: 20131188). Female Sprague-Dawley rats, 6 weeks old, were obtained from the Experimental Animal Center of Anhui Medical University. The rats were maintained in controlled temperature (23-25 °C), light (12-hr light - dark cycle), and humidity (55 ± 5 %) conditions with access to food and water *ad libitum*. Subsequent to a seven day adaptation period, animals were given *ad libitum* access to either a control (CON; n = 40) diet of standard rodent chow (3.435 kcal/g; 12 % energy as fat, Jiangsu Xie Tong Biological Engineering Co., Ltd.) or a HF; (n = 53) diet which contained 70 % normal chow, 10 % lard, 1 % cholesterol, 3 % casein, 10 % egg yolk powder, and 6 % whole-milk powder (4.487 kcal/g; 45 % energy as fat, Jiangsu Xie Tong Biological Engineering Co., Ltd.). Body weight of each dam was monitored weekly. After 7 weeks of feeding, rats fed the HF diet exhibited varying somatic weight change in response to the diet. According to the approach undertaken in previous studies (25,26), 18 rats with the highest body weight gain were designated as diet-induced obesity (DIO) rats, while 18 rats with lowest body weight gain were designated as diet-induced obesity-resistant (DIR) rats. During the 8th week, the food intakes of the CON, DIO, and DIR rats were measured. At the end of

the 8th week, the CON, DIO, and DIR rats were mated with age-matched Sprague-Dawley male rats fed the CON diet. Copulation was confirmed by the presence of sperm in a vaginal flush; the day of copulation was designated gestational day (GD) 0. After copulation, the CON rats were divided into two group: half were switched to the HF diet (CON-HF group, n = 10), while half were maintained on the CON diet (CON-CON group, n = 10). The DIO (n = 10) rats and DIR (n = 10) rats were maintained on the HF diet throughout pregnancy. Food intakes during pregnancy were measured. Body weights were monitored weekly. At the end of GD21, all rats were fasted overnight prior to being euthanized with chloral hydrate which was also used in previous studies (27,28,29). Blood samples of rats were obtained from the common abdominal aorta for measurements of insulin levels. Fetuses in each litter were counted and weighed, and then crown-rump length was measured. Fetal blood samples were also collected for measurements of insulin level. Maternal and fetal blood glucose levels were measured immediately using an Accu-Chek blood glucose monitor (Accu-Chek; Roche Diagnostics, Mannheim, Germany) (30,31). Serum insulin level was determined by radioimmunoassay (Beijing North Biotechnology Research Institute, Beijing, China). Placental weights were recorded, and placentas were snap frozen in liquid nitrogen and stored at -80 °C until further analysis.

Isolation of Total RNA and Real-time Polymerase Chain Reaction

Total RNA was extracted from placental tissues, and real-time polymerase chain reaction (rt-PCR) performed as previously described (32). Total RNA was extracted using TRI reagent (Molecular Research Center). Ribonuclease-free deoxyribonuclease-treated total RNA (1.0 μg) was reverse-transcribed with Aves myeloblast leukemia virus reverse transcriptase (Promega). RT-PCR was performed with a LightCycler 480 SYBR Green I kit (Roche Diagnostics GmbH) using gene-specific primers as listed in Table 1. Specific primers were synthesized by Shanghai Sangon Biological Engineering Technology (Shanghai, China). The amplification reactions were carried out on a LightCycler 480 Instrument (Roche Diagnostics GmbH) with an initial hold step (95 °C for 5 minutes) and 50 cycles of a three-step PCR (95 °C for 15 seconds, 60 °C for 15 seconds, 72 °C for 30 seconds). The comparative cycle threshold method was used to determine the amount of target, normalized to an endogenous reference (*GAPDH*) and relative to a calibrator using the LightCycler 480 software (version 1.5.0; Roche).

Western Blot

Total lysate from rat placentas was prepared by homogenizing 50 mg placenta tissue in 300 μL lysis buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride) supplemented with a cocktail of protease inhibitors (Roche). Protein concentrations were determined using bicinchoninic acid (BCA) protein assay reagents (Pierce, Rockford, IL) according to manufacturer's instructions. The levels of CD36, FATP1, and FATP4 in placental tissue were quantified using Western blot, as previously described (32). Briefly, an amount of protein (40 ~ 80 μg) was separated electrophoretically by SDS-PAGE and transferred to a polyvinylidene fluoride membrane. Membranes were blocked in 5% non-fat milk in tris buffered saline (TBST) (137 mM NaCl, 2.7 mM KCl, 25 mM Tris-Cl, pH 8.0) supplemented with 0.1% Tween-20 overnight at 4 °C. The membranes were incubated for 2 hours with the following antibodies: CD36 (1:500; Abcam Inc, Cambridge, MA), FATP1 (1:500; Abcam Inc, Cambridge, MA), and FATP4 (1:1000; Abcam Inc, Cambridge, MA). β-actin (1:2000; Santa Cruz Biotechnologies, CA, USA) was used as a loading control. After being washed in TBST containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with secondary antibody (goat anti-rabbit IgG or goat anti-mouse IgG; both from Santa Cruz Biotechnologies, CA, USA) for 2 hours. The membranes were then washed for four times in TBST containing 0.05% Tween-20 for 10 min each. Finally, enhanced chemiluminescence solution (ECL kit; Pierce Biotechnology, USA) was added, and Fine-do X6 visualizer was used for the photographing (Tanon, Shanghai, China).

Statistical Analysis

All statistical analysis was performed using the SPSS 13.0 software (SPSS Inc, Chicago, IL). For animal experiments, the litter was considered the unit for statistical analysis among different groups. For fetal weight and crown-rump length, the means were calculated per litter. All values are expressed as the mean ± standard deviation (SD). Comparisons between CON and HF groups were compared

Table 1. Oligonucleotide sequences and size of primers

Genes	Sequences (5' to 3')	Sizes (bp)
GAPDH	Forward: CTCAGTTGCTGAGGAGTCCC Reverse: ATTCGAGAGAAGGGAGGGCT	120
CD36	Forward: CCTCGGATGGCTAGCTGATT Reverse: AGAGCACTTGCTTCTTGCCA	189
FATP-1	Forward: CTTCTGGGACTTCCGTGGAC Reverse: GTGTCGTCGTAGCTCTAGCC	159
FATP-4	Forward: CATGCGGCCTGATGACATTG Reverse: CCGGGAGGCTGAAAACTTCT	130
GLUT-1	Forward: GCTGTGGCTGGCTTCTCTAA Reverse: CCGGAAGCGATCTCATCGAA	185
SNAT-2	Forward: CTCCTCGCTGGTTCTTCTGG Reverse: CCAAGGCTCGTGGTTTTTGG	123

using two independent sample t-test. One-way ANOVA was used to determine differences among three or more groups and further comparison between two groups was assessed with least significant difference (LSD) post hoc test. A p-value < 0.05 was considered statistically significant.

Results

Effect of HF Diet on Body Weight, Food Intake, Energy Intake, Fasted Blood Glucose, and Serum Insulin of Dams

After two weeks, dams fed HF diet were significantly heavier than CON dams ($t = 2.254$, $p = 0.029$, Figure 1A). After 7 weeks of feeding, there was a significant difference in body weight among CON rats, DIR rats, and DIO rats ($F = 39.864$, $p < 0.001$, Figure 1B); the weight of DIO rats was significantly higher than that of CON rats (21.2%, $p < 0.05$). There was a significant difference in food intake and energy intake among the three groups ($F = 7.477$, $p = 0.002$, Figure 1C; $F = 28.412$, $p < 0.001$, Figure 1D). However, there was no difference in weight between CON and DIR rats at this time. There was no significant difference in fasted blood glucose or serum insulin among the three groups (Figure 1E and 1F).

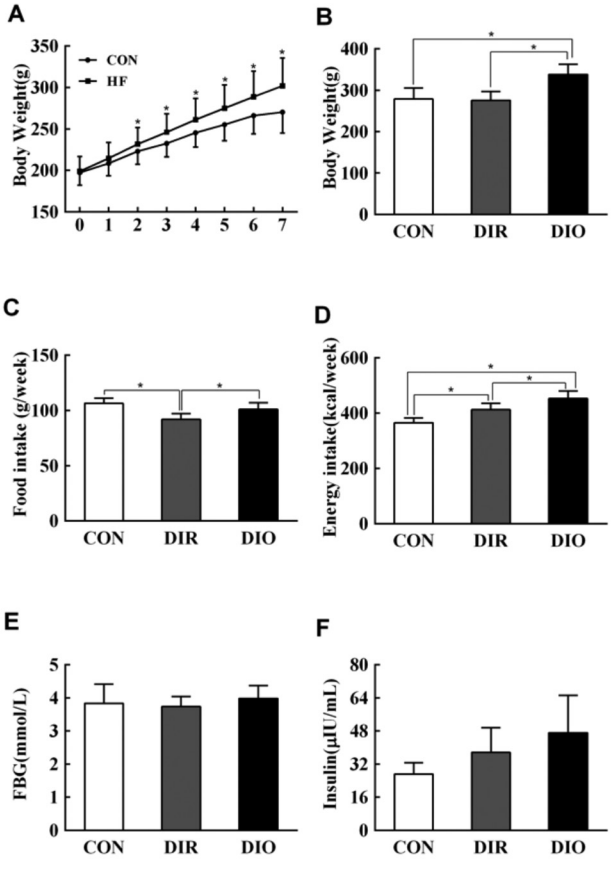

Figure 1. Body weight, food intake, energy intake, fasted blood glucose, and serum insulin analysis in rats. A) body weight in rats that were fed either a control or high-fat diet, B) body weight at 8 weeks, C) food intake during the 8th week, D) energy intake during the 8th week, E) fasting blood glucose at 8 weeks, F) serum insulin after 8 weeks feeding

Data are mean + standard deviation

*Indicates $p < 0.05$

FBG: fasting blood glucose, DIR: diet-induced obesity-resistant, DIO: diet-induced obesity, HF: high-fat

Weight Gain, Food Intake, and Energy Intake During Pregnancy, Fasted Blood Glucose and Serum Insulin of Dams at GD21

There was no significant difference in weight gain during pregnancy among the four groups (CON-CON: 116.78 ± 17.58 g, CON-HF: 114.21 ± 18.93 g, DIR: 109.77 ± 15.93 g, DIO: 125.69 ± 20.24 g; $F = 1.353$, $p = 0.273$; Figure 2A). However, fasting blood glucose and serum insulin levels at GD21 were not significantly different (Figure 2B, 2C). There were significant differences in food intake and energy intake during pregnancy among the four groups ($F = 3.547$, $p = 0.028$, Figure 2D; $F = 9.848$, $p < 0.001$, Figure 2E). The food intake of DIR rats was less than that of CON-CON rats and DIO rats during pregnancy (Figure 2D). The energy intake during pregnancy was more in DIO rats and CON-HF rats than in CON-CON rats and DIR rats (Figure 2E).

Effect of Maternal Obesity on Fetal Growth, Placental Weight, and Fetal to Placental Weight Ratio

Average fetal weight of DIO dams was reduced by 6.9% (DIO: 4.88 ± 0.17 g, CON-CON: 5.23 ± 0.31 g, $p < 0.05$; Figure 3A), while the average fetal weight of DIR dams and CON-HF dams was similar to those of CON-CON dams. Mean fetal weight

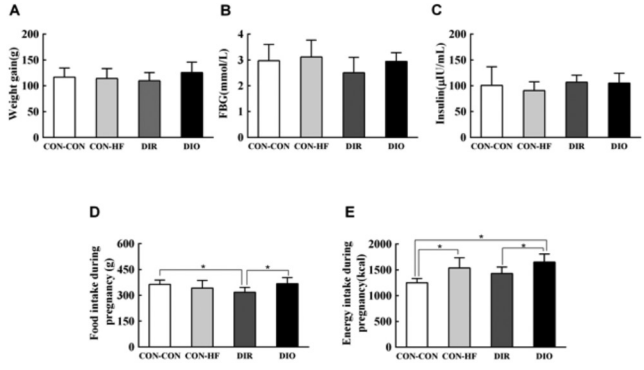

Figure 2. Weight gain, food intake, energy intake during pregnancy, and fasting blood glucose and serum insulin at gestational day 21. A) weight gain, B) fasting blood glucose, C) serum insulin, D) food intake, E) energy intake during pregnancy (n = 10 per group)

Data are mean ± standard deviation

*Indicates $p < 0.05$

FBG: fasting blood glucose, DIR: diet-induced obesity-resistant, DIO: diet-induced obesity, HF: high-fat

was significantly lower in DIO offspring compared with DIR offspring. However, there was no significant difference in offspring crown-rump length between the four groups (Figure 3B). At GD21, the placentas of CON-HF and DIO dams were heavier than those of CON-CON and DIR dams (Figure 3C). Figure 3D shows that the fetal to placental weight ratio of DIO dams was significantly lower compared with the fetal to placental weight ratio of CON-CON and DIR dams.

Effect of Maternal Obesity on Placental mRNA and Protein Expression of Nutrient Transporter

The mRNA expression of *GLUT-1* and *SNAT-2* was not significantly different between the groups (Figure 4A and 4B). The mRNA expression of placental fatty acid transporters *CD36*, *FATP-1,* and *FATP-4* in DIO dams was

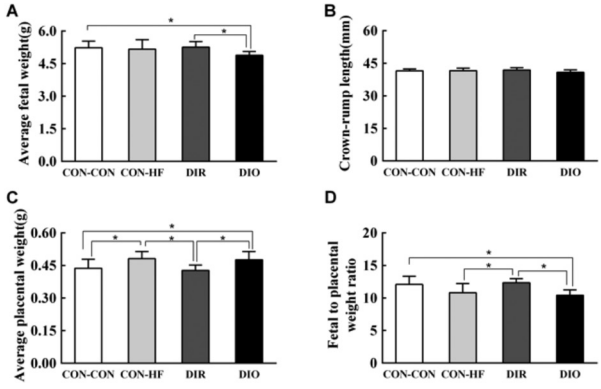

Figure 3. Effect of maternal obesity on fetal growth, placental weight, and fetal to placental weight ratio. A) average fetal weight, B) crown-rump length, C) placental weight, D) fetal to placental weight ratio. n = 10 per group

Data are mean ± standard deviation

*Indicates $p < 0.05$

DIR: diet-induced obesity-resistant, DIO: diet-induced obesity, HF: high-fat

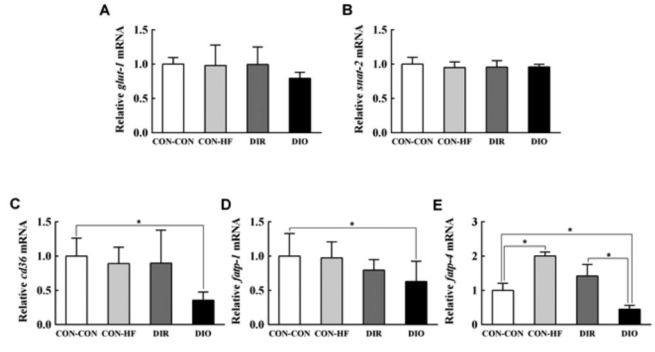

Figure 4. Effect of maternal obesity on placental mRNA expression of nutrient transporters. A) *GLUT-1*, B) *SNAT-2*, C) *CD36*, D) *FATP-1*, E) *FATP-4*, n = 6 per group.

Data are mean ± standard deviation

*Indicates $p < 0.05$.

DIR: diet-induced obesity-resistant, DIO: diet-induced obesity, HF: high-fat

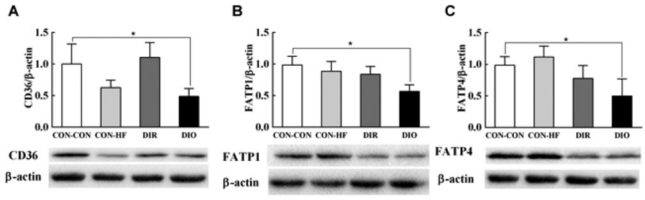

Figure 5. Effect of maternal obesity on placental fatty acid transporters protein expression. A) *CD36*, B) *FATP-1*, C) *FATP-4*. n = 6 per group

Data are mean + standard deviation

*Indicates $p < 0.05$

DIR: diet-induced obesity-resistant, DIO: diet-induced obesity, HF: high-fat

decreased by 35.7%, 62.9%, and 45.0%, respectively (Figure 4C, 4D, 4E). *FATP-4* mRNA expression in DIO dams was significantly reduced compared with that in DIR dams. The mRNA expression of *CD36*, *FATP-1,* and *FATP-4* in the CON-HF group was not significantly reduced compared with that in CON-CON group. As shown in Figure 5, placental fatty acid transporter protein expression in DIO dams was significantly decreased compared with that in CON-CON dams. Protein expression of *CD36*, *FATP-1,* and *FATP-4* in DIO dams was decreased by 48.7%, 57.2%, and 50.4%, respectively (Figure 5A, 5B, 5C). Protein expression of *CD36*, *FATP-1,* and *FATP-4* was not significantly reduced in CON-HF group compared with the CON-CON dams.

Discussion

We report the effect of maternal HF, DIO on fetal growth and placental nutrient transport. We found correlation between maternal HF, DIO and reduced fetal growth. However, in animals resistant to induced obesity, no reduction in fetal weight was observed. Meanwhile, maternal HF, DIO down-regulated the expression of placental fatty acid transporters (CD36, FATP-1 and FATP-4).

Obesity results from complex interactions of environmental and genetic components which facilitate the development of an obese phenotype (33). Diet is the most important environmental factor leading to obesity, and models of HF DIO are commonly used in studies. After feeding with a high fat diet, rats exhibited different phenotypes in response to the diet. Therefore, we selected the DIO and DIR rats according to weight gain after 7 weeks of HF feeding. We found that food intake and energy intake of DIR rats were lower than those of DIO rats, consistent with previous reports (34). Liu et al (35) reported that DIR rat has the ability to sense and respond to energy imbalance accurately, while the ability in DIO is blunted. Despite having the same feeding conditions as the DIO group, DIR rats are sensitive

to the energy balance system and can adjust their energy expenditure to maintain a normal weight depending on the level of energy intake.

Previous studies have investigated the association between maternal obesity and fetal growth, but results have been contradictory. Increased risks for both fetal macrosomia and intrauterine growth restriction (IUGR) have been reported (8,9,10,36,37). In the current study, fetal weight of DIO dams was significantly reduced, and a clear association between maternal obesity and an increased the risk for IUGR were shown. Furthermore, DIO dams had normal blood glucose and serum insulin levels in our study, suggesting the absence of gestational diabetes mellitus despite the obese phenotype. In previous studies, obese mothers often developed abnormal glucose homeostasis (10,38). Thus, reports show inconsistencies which may be due to study design (contents of HF diet, time of HF diet feeding), degree of obesity in subjects, or species difference. Our results also demonstrate that fetal growth is different in DIO and DIR dams. Despite having the same feeding conditions as the DIO group, the offspring of DIR rats seem to be healthier compared with DIO offspring, consistent with previous reports (34). In a future study, the focus will be on the issue of how the DIR rats are protected from the deleterious effects of a HF diet.

The fetal to placental weight ratio has been considered to be a marker of placental nutrient transporter efficiency (39,40). A lower fetal to placental weight ratio may indicate below average placental nutrient transport efficiency. In our study, fetal to placental weight ratio was lower in DIO dams indicating that placental nutrient transport efficiency in DIO dams was decreased.

Fetal growth is mainly dependent on fetal nutrient availability, which is determined by the capacity of the placenta to transport nutrients. The transport of placental fatty acids is critical for fetal growth, particularly in late gestation (41). A previous study found that maternal obesity was associated with decreased FATP-4 mRNA and protein expression, whereas CD36 expression was increased (23). We found that the fatty acid transporters (*CD36*, *FATP-1*, and *FATP-4*) mRNA and protein expression were down-regulated in the DIO placenta. Thus, maternal obesity was associated with decreased placental fatty acid transporter mRNA and protein expression. It has been reported that several placental transport functions are altered in pregnancies complicated by IUGR (42). Placental fatty acid transporters may have an important role to play in the process of IUGR induced by maternal obesity. Our results are inconsistent with previously reported results (21,22,23,24), which may be due to species difference (rat, sheep, human, etc.), degree of obesity in subjects, study design, or gestational age studied (mid-gestation, late pregnancy, delivery, etc.).

Study Limitations

There are several limitations of the current study. Due to the nature of our obesity model, we are limited by the number of rats in our study, which left us underpowered to thoroughly assess differences between male and female fetuses. Thus, though we did not detect any trends, we cannot conclusively rule out the effect of fetal sex on these relationships. In addition, some metabolic factors (i.e. leptin, inflammation, lipids and fatty acids levels) in maternal circulation were not measured in current study. We are currently unable to ascertain how maternal obesity affected placental fatty acid transport. Further studies will be designed to explore the mechanism.

Conclusion

In summary, the current study indicates that maternal HF, DIO led to fetal growth retardation. Moreover, maternal obesity inhibits placental nutrient transport efficiency. In particular down-regulation of the fatty acid transporters (*CD36*, *FATP-1*, and *FATP-4*) mRNA and protein expression may have an important role in the development of IUGR in the offspring of obese mothers. One of the most fundamental questions arising from the study is how DIO and DIR dams and their offspring differ in terms of their respective metabolic response to a HF diet. In further studies, the metabolic difference between the DIO and DIR dams should be investigated comprehensively. Growth and development differences in their offspring should be also explored. These differences may be fundamental to future understanding of the effect of diet on obesity and health.

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Ethics

Ethics Committee Approval: The study was approved by the Animal Ethics Committee of Anhui Medical University (approval number: 20131188).

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Kui Ye, Dan Zhang, Yi Li,

Concept: Li Li, Chuan-Lai Hu, Design: Li Li, Chuan-Lai Hu, Kui Ye, Data Collection or Processing: Kui Ye, Dan Zhang, Yi Li, Hai-Qing Wang, Han-Lin Lai, Analysis or Interpretation: Kui Ye, Li Li, Dan Zhang, Yi Li, Literature Search: Kui Ye, Dan Zhang, Yi Li, Writing: Kui Ye, Li Li.

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References

1. Thrift AP, Callaway LK. The effect of obesity on pregnancy outcomes among Australian Indigenous and non-Indigenous women. Med J Aust 2014;201:592-595.
2. Vinturache A, Moledina N, McDonald S, Slater D, Tough S. Pre-pregnancy Body Mass Index (BMI) and delivery outcomes in a Canadian population. BMC Pregnancy Childbirth 2014;14:422.
3. Crane JM, Murphy P, Burrage L, Hutchens D. Maternal and perinatal outcomes of extreme obesity in pregnancy. J Obstet Gynaecol Can 2013;35:606-611.
4. Bautista-Castaño I, Henriquez-Sanchez P, Alemán-Perez N, Garcia-Salvador JJ, Gonzalez-Quesada A, García-Hernández JA, Serra-Majem L. Maternal obesity in early pregnancy and risk of adverse outcomes. PLoS One 2013;8:e80410.
5. Aune D, Saugstad OD, Henriksen T, Tonstad S. Maternal body mass index and the risk of fetal death, stillbirth, and infant death: a systematic review and meta-analysis. JAMA 2014;311:1536-1546.
6. Yao R, Ananth CV, Park BY, Pereira L, Plante LA, Perinatal Research C. Obesity and the risk of stillbirth: a population-based cohort study. Am J Obstet Gynecol 2014;210:457-459. Epub 2014 Mar 25
7. Persson M, Johansson S, Villamor E, Cnattingius S. Maternal overweight and obesity and risks of severe birth-asphyxia-related complications in term infants: a population-based cohort study in Sweden. PLoS Med 2014;11:e1001648.
8. Tenenbaum-Gavish K, Hod M. Impact of maternal obesity on fetal health. Fetal Diagn Ther 2013;34:1-7. Epub 2013 Jun 11
9. Catalano PM, McIntyre HD, Cruickshank JK, McCance DR, Dyer AR, Metzger BE, Lowe LP, Trimble ER, Coustan DR, Hadden DR, Persson B, Hod M, Oats JJ; HAPO Study Cooperative Research Group. The hyperglycemia and adverse pregnancy outcome study: associations of GDM and obesity with pregnancy outcomes. Diabetes Care 2012;35:780-786. Epub 2012 Feb 22
10. Hayes EK, Lechowicz A, Petrik JJ, Storozhuk Y, Paez-Parent S, Dai Q, Samjoo IA, Mansell M, Gruslin A, Holloway AC, Raha S. Adverse fetal and neonatal outcomes associated with a life-long high fat diet: role of altered development of the placental vasculature. PLoS One 2012;7:e33370. Epub 2012 Mar 19
11. Fowden AL, Forhead AJ, Coan PM, Burton GJ. The placenta and intrauterine programming. J Neuroendocrinol 2008;20:439-450. Epub 2008 Feb 8
12. Baumann MU, Deborde S, Illsley NP. Placental glucose transfer and fetal growth. Endocrine 2002;19:13-22.
13. Day PE, Cleal JK, Lofthouse EM, Hanson MA, Lewis RM. What factors determine placental glucose transfer kinetics? Placenta 2013;34:953-958. Epub 2013 Jul 22
14. Jansson T. Amino acid transporters in the human placenta. Pediatr Res 2001;49:141-147.
15. Cleal JK, Lewis RM. The mechanisms and regulation of placental amino acid transport to the human foetus. J Neuroendocrinol 2008;20:419-426. Epub 2008 Feb 8
16. Mackenzie B, Erickson JD. Sodium-coupled neutral amino acid (System N/A) transporters of the SLC38 gene family. Pflugers Arch 2004;447:784-795. Epub 2003 Jul 4
17. Hatanaka T, Huang W, Martindale RG, Ganapathy V. Differential influence of cAMP on the expression of the three subtypes (ATA1, ATA2, and ATA3) of the amino acid transport system A. FEBS Lett 2001;505:317-320.
18. Kazantzis M, Stahl A. Fatty acid transport proteins, implications in physiology and disease. Biochim Biophys Acta 2012;1821:852-857.
19. Cunningham P, McDermott L. Long chain PUFA transport in human term placenta. J Nutr 2009;139:636-639. Epub 2009 Feb 18
20. Larque E, Demmelmair H, Klingler M, De Jonge S, Bondy B, Koletzko B. Expression pattern of fatty acid transport protein-1 (FATP-1), FATP-4 and heart-fatty acid binding protein (H-FABP) genes in human term placenta. Early Hum Dev 2006;82:697-701. Epub 2006 Mar 30
21. Reynolds CM, Vickers MH, Harrison CJ, Segovia SA, Gray C. Maternal high fat and/or salt consumption induces sex-specific inflammatory and nutrient transport in the rat placenta. Physiol Rep 2015;3. pii: e12399.
22. Farley DM, Choi J, Dudley DJ, Li C, Jenkins SL, Myatt L, Nathanielsz PW. Placental Amino Acid Transport and Placental Leptin Resistance in Pregnancies Complicated by Maternal Obesity. Placenta 2010;31:718-724. Epub 2010 Jul 7
23. Dube E, Gravel A, Martin C, Desparois G, Moussa I, Ethier-Chiasson M, Forest JC, Giguere Y, Masse A, Lafond J. Modulation of fatty acid transport and metabolism by maternal obesity in the human full-term placenta. Biol Reprod 2012;87:1-11.
24. Zhu MJ, Ma Y, Long NM, Du M, Ford SP. Maternal obesity markedly increases placental fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe. Am J Physiol Regul Integr Comp Physiol 2010;299:1224-1231. Epub 2010 Sep 15
25. Cifani C, Micioni Di Bonaventura MV, Pucci M, Giusepponi ME, Romano A, Di Francesco A, Maccarrone M, D'Addario C. Regulation of hypothalamic neuropeptides gene expression in diet induced obesity resistant rats: possible targets for obesity prediction? Front Neurosci 2015;9:187.
26. Lin N, Cai DL, Jin D, Chen Y, Shi JJ. Ginseng panaxoside Rb1 reduces body weight in diet-induced obese mice. Cell Biochem. Biophys 2014;68:189-194.
27. Rastelli VM, Akamine EH, Oliveira MA, Nigro D, Passaglia Rde C, Carvalho MH, Fortes ZB. Influence of insulin on the microvascular response to inflammatory mediators in neonatal streptozotocin diabetic rats. Inflamm Res 2005;54:173-179.
28. Lee HC, Curry DL, Stern JS. Tonic sympathetic nervous system inhibition of insulin secretion is diminished in obese Zucker rats. Obes Res 1993;1:371-376.
29. Zhou F, Chen H, Wang X, Yu P, Hu Y. Hypoxia-induced regulation of placental REDD1 and mTOR was impaired in a rat model of estrogen-induced cholestasis. Arch Gynecol Obstet 2016;294:1219-1226. Epub 2016 Aug 22
30. Wang Y, Peng GQ, Jiang XQ, Dong LZ, Song WG, Zhang QL. Comparison between Diabetic Rats' Glucoses Measured by Two Types of Glucose Meters and the Full Automatic Biochemical Analyzer. Laboratory Animal and Comparative Medicine 2015;35:411-413.
31. Subhasree N, Kamella A, Kaliappan I, Agrawal A, Dubey GP. Antidiabetic and antihyperlipidemic activities of a novel polyherbal formulation in high fat diet/streptozotocin induced diabetic rat model. Indian J Pharmacol 2015;47:509-513.

32. Chen YH, Zhao M, Chen X, Zhang Y, Wang H, Huang YY, Wang Z, Zhang ZH, Zhang C, Xu DX. Zinc supplementation during pregnancy protects against lipopolysaccharide-induced fetal growth restriction and demise through its anti-inflammatory effect. J Immunol 2012;189:454-463. Epub 2012 Jun 1

33. Perusse L, Bouchard C. Gene-diet interactions in obesity. Am J Clin Nutr 2000;72(5 Suppl):1285-1290.

34. Ricci MR, Levin BE. Ontogeny of diet-induced obesity in selectively bred Sprague-Dawley rats. Am J Physiol Regul Integr Comp Physiol 2003;285:610-618. Epub 2003 May 29

35. Liu JM, Wang JX, Zheng L, Lian WG, Xu ZN, Liu SF. Establishment of obesity prone and obesity resistant rat models and their metabolic study. Acta Nutrimenta Sinica 2041;36:184-187.

36. Crew RC, Waddell BJ, Mark PJ. Maternal obesity induced by a 'cafeteria' diet in the rat does not increase inflammation in maternal, placental or fetal tissues in late gestation. Placenta 2016;39:33-40. Epub 2016 Jan 6

37. Aye IL, Rosario FJ, Powell TL, Jansson T. Adiponectin supplementation in pregnant mice prevents the adverse effects of maternal obesity on placental function and fetal growth. Proc Natl Acad Sci U S A 2015;112:12858-12863. Epub 2015 Sep 28

38. Rosario FJ, Kanai Y, Powell TL, Jansson T. Increased placental nutrient transport in a novel mouse model of maternal obesity with fetal overgrowth. Obesity (Silver Spring) 2015;23:1663-1670.

39. Wallace JM, Horgan GW, Bhattacharya S. Placental weight and efficiency in relation to maternal body mass index and the risk of pregnancy complications in women delivering singleton babies. Placenta 2012;33:611-618. Epub 2012 Jun 12

40. Lager S, Samulesson AM, Taylor PD, Poston L, Powell TL, Jansson T. Diet-induced obesity in mice reduces placental efficiency and inhibits placental mTOR signaling. Physiol Rep 2014;2:e00242.

41. Larque E, Ruiz-Palacios M, Koletzko B. Placental regulation of fetal nutrient supply. Curr Opin Clin Nutr Metab Care 2013;16:292-297.

42. Ortega-Senovilla H, Alvino G, Taricco E, Cetin I, Herrera E. Enhanced circulating retinol and non-esterified fatty acids in pregnancies complicated with intrauterine growth restriction. Clin Sci (Lond) 2009;118:351-358.

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Plasma Amino-Terminal Propeptide of C-Type Natriuretic Peptide Concentration in Normal-Weight and Obese Children

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What is already known on this topic?

Results confirmed the previously described relationship between plasma amino-terminal propeptide of C-type natriuretic peptide (NT-proCNP) concentration and growth velocity. Plasma NT-proCNP concentration showed a negative correlation with age, weight, and height in children. Gender was not a factor that alters the age-dependent plasma NT-proCNP concentration until puberty.

What this study adds?

In contrast to what has been suggested before, plasma NT-proCNP concentration of children with overweight/obesity was not significantly lower than that of children with normal weight in age groups analyzed in a Turkish population. Thus, it is too early to conclude that CNP is a potential biomarker in childhood obesity. Further studies are necessary to address this question.

Abstract

Objective: In studies on the relationship between amino-terminal propeptide of C-type natriuretic peptide (NT-proCNP) concentration and height velocity in children, CNP has been implicated as an emerging new growth marker during childhood. It has been reported that besides its well-studied role in growth, plasma CNP levels are reduced in overweight and/or obese adolescents, suggesting CNP as a potential biomarker in childhood obesity. The primary goal of this study was to test this hypothesis in a Turkish population.

Methods: Consent was taken from 317 children [ages 0-18 (158 girls, 159 boys)] and their parents. All subjects were physically examined; anthropometric measurements were obtained. Body mass index was calculated. During routine blood work, 1 mL extra blood was taken. Plasma NT-proCNP concentration was measured by enzyme-linked immunosorbent assay.

Results: Results confirmed the previously described relationship between plasma NT-proCNP concentration and growth velocity. Plasma NT-proCNP concentration showed a negative correlation with age, weight, and height in children. Gender was not a factor that alters the age-dependent plasma NT-proCNP concentration until puberty.

Conclusion: Unlike previous reports, plasma NT-proCNP concentration of overweight/obese children was not significantly lower than that of children with normal weight in age groups analyzed in a Turkish population. Thus, it is too early to conclude that CNP is a potential biomarker in childhood obesity. Further studies are necessary to address this question.

Keywords: C-type natriuretic peptide, amino-terminal propeptide of C-type natriuretic peptide, obesity, overweight, growth, biomarker

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Introduction

The amino-terminal propeptide of C-type natriuretic peptide (NT-proCNP) has mainly been implicated as a paracrine/endocrine factor involved in regulation of endochondral growth (1,2,3,4,5,6,7,8,9,10,11,12,13,14, 15). The relationship between plasma CNP concentration and height velocity in children has been demonstrated, and CNP has been implicated as an emerging new growth marker during childhood (5,12,13). Besides its well-studied role in growth, recent investigations also relate CNP and the signaling pathway induced by this peptide with obesity (16,17,18,19). These studies have shown that plasma CNP levels are reduced in adolescents with overweight and/or obesity, suggesting CNP as a potential biomarker in childhood obesity.

The identification of CNP was based on its structural similarity to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (20). Despite their structural similarity, natriuretic peptides are functionally distinct hormones (8). The first two, ANP and BNP, are produced by the atrium and the ventricle, respectively. They act mainly as cardiac hormones (6). CNP, on the other hand, is detected in tissues of a wide variety of systems in the body including the skeletal, central nervous, cardiovascular, urogenital, and immune systems (21). Recent investigations are focusing on the fact that CNP may be used as a potential biomarker related to disease conditions of at least some of these tissues and systems in the body (21). In this context, two recent studies which were performed by the same group analyzed plasma CNP concentration of adolescents with normal weight versus overweight and/or obesity in an Italian population (18,19). They observed lower plasma CNP levels in children with overweight/obesity, suggesting "a defective natriuretic peptide system in these patients" (18).

Childhood obesity is a continuously growing health problem, being considered as a major risk factor for dyslipidemia, hypertension, and damaged glucose metabolism, early onset of endothelial dysfunction, atherosclerosis, and cardiovascular diseases (22,23,24). Based on this knowledge and previous reports (18,19), it was considered important to evaluate CNP as a potential biomarker in early detection of obesity-related disease conditions during child growth. However, the half-life of CNP in the circulation is very short, approximately 2 minutes (21). It has been shown that the proCNP is also secreted from the cell in equal molar concentrations as CNP (25,26). In addition, proCNP is more stable in the circulation, allowing accurate measurement and estimation of CNP concentration (12). It is also known that degradation of proCNP in the circulation starts from the carboxyl terminal of the peptide (12) and N-terminal-directed antibodies against proCNP increase specificity for the propeptide (21). Thus, NT-proCNP is considered as an accurate target for the measurement of circulating CNP concentration and as a potential biomarker in growth and/or human diseases (12,21,27).

The first two studies relating the plasma CNP concentration with obesity have been performed on early adolescents (18,19). Almost all studies relating CNP with growth, on the other hand, have been performed on a variety of age groups of children (5,12,13,28,29). The primary goal of the present study was to analyze plasma NT-proCNP concentration in healthy Turkish normal-weight and overweight/obese children in a broad spectrum of age groups and evaluate the value of NT-proCNP as a potential biomarker in childhood obesity.

Reports within the last decade indicate that weight gain is an important and growing childhood problem also in Turkey (30,31,32,33). Since previous studies have demonstrated the importance of use of population-specific data for the evaluation of age-related changes in growth parameters during childhood (34,35,36), the existence of recently updated Turkish population-specific growth charts was an important advantage for this study (30,31,32,33).

Methods

Subjects

Subjects were children of ages between 0 and 18 years (158 girls and 159 boys) recruited from those attending the Outpatient Clinic of the Pamukkale University Hospital Pediatric Endocrinology Unit in Denizli, Turkey and the Outpatient Clinics of Ankara University Children's Hospital Social Pediatrics and Pediatric Endocrinology and Adolescent Departments in Ankara, Turkey. The study was approved by the Institutional Clinical Ethics Review Board of Pamukkale University Faculty of Medicine, Denizli, Turkey (decision dated 27.05.2014 and numbered 2014/08, approval number: 1). Written consent was taken from all participants and/or parents/legal guardians of the participating children.

Establishment of the Age Groups Studied

Since previous studies have correlated plasma CNP/NT-proCNP concentration primarily with height velocity, the age groups in this study were established according to the general knowledge on height velocity changes during childhood (34,35,36,37). In addition, Turkish population-specific age-dependent height velocity changes were also analyzed from the existing growth charts of Turkish children (31,32,33). Age groups established on the basis of these

sources were: 1) 0-1 month (newborns), 2) 1-12 months, 3) 1-4 years, 4) 4-10 years, 5) 10-12.5 years, 6) 12.5-14.5 years, and 7) 14.5-18 years. The study model established by these age groups well represented the age-dependent change in height velocity in Turkish children.

All age groups except newborns were further divided into subgroups according to body mass index (BMI) percentiles (38,39,40,41). The statistical percentiles were used to identify overweight (≥95th percentile) up to 2 years of age and obesity (≥95th percentile), overweight (85th to 95th percentile), and normal-weight (5th to 85th percentile) groups in the 2-18 years age range (38,39,40,41). The statistical percentiles of Turkish children published previously were used in all these procedures as the population-specific guidelines (30,31,32). Inclusion criteria of healthy children for this study were as described previously (12,13).

Study Procedures

All children were seen in the participating outpatient clinics; family and medical histories were obtained. A physical examination was performed in all subjects. Anthropometric measurements including length/height (length by recumbent stadiometer for subjects younger than 2 years old, height by Harpenden stadiometer) and weight (by electronic scale) were obtained (12) in all subjects. BMI (kg/m^2) was calculated. Percentiles and standard deviation scores were determined according to the Turkish population-specific growth charts (32).

Analysis of Plasma Amino-Terminal Propeptide of C-Type Natriuretic Peptide Concentration

Venous blood (1 mL) was drawn into tubes containing ethylenediaminetetraacetic acid and processed within two hours. Plasma was isolated by centrifugation of the blood for 10 min at 2000 g. Plasma samples in which hemolysis was observed were excluded from the study. The plasma samples were stored at -80 °C in aliquots until assayed. Each sample was assayed at least twice, and mean value was calculated for each sample. New aliquots were used for each assay. Commercially available "Enzyme Immunoassay for the Quantitative Determination of Human NT-proCNP in Plasma and Serum" (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria, Cat. No. BI-20872) was used according to the manufacturers protocol and as reported previously (12).

The normal-weight group (abbreviated as NW-group for the rest of this report) consisted of 146 children (76 girls and 70 boys) in the established 7 different groups. The data on this group were used to analyze the age-dependent changes in plasma NT-proCNP concentration (Figure 1), and comparison of plasma NT-proCNP concentration based on gender in each age group (Table 1). Plasma NT-proCNP concentrations in the NW-group [136 children (71 girls and 65 boys)] were compared with those of children with overweight/obesity [abbreviated as the OW/O-group for the rest of this report; 171 children (82 girls and 89 boys)] in all groups except for the newborns [10 children (5 girls and 5 boys)] (Table 2).

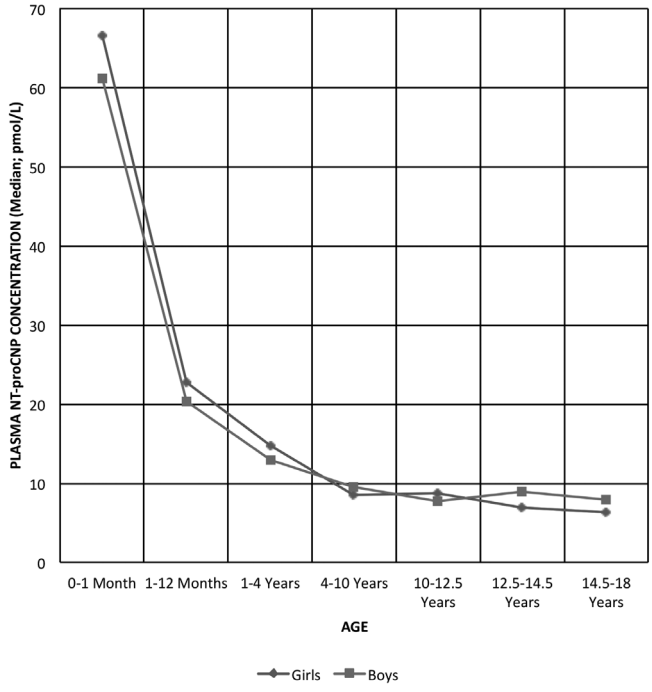

Figure 1. Age-dependent changes in plasma amino-terminal propeptide of C-type natriuretic peptide (NT-proCNP) concentration. Children of normal weight [146 children (76 girls, and 70 boys)] in the 7 different age groups were used to analyze the age-dependent changes in plasma NT-proCNP concentration

Table 1. Amino-terminal propeptide of C-type natriuretic peptide concentration in children of normal weight based on gender in each age group

Age groups	Female	Male	p
0-1 month	62.93 ± 7.76	63.02 ± 11.32	0.69
1-12 months	23.82 ± 5.67	22.05 ± 6.17	0.265
1-4 years	15.57 ± 5.85	12.54 ± 3.04	0.25
4-10 years	9.51 ± 2.71	11.33 ± 5.70	0.396
10-12.5 years	9.25 ± 1.91	8.08 ± 1.81	0.313
12.5-14.5 years	6.77 ± 0.56	8.92 ± 1.85	0.065
14.5-18 years	6.26 ± 0.62	8.11 ± 2.26	0.04*

*Statistically significant at $p < 0.05$

Table 2. Comparison of amino-terminal propeptide of C-type natriuretic peptide concentration by body weight classification within each age group of children

	Total (mean ± SD)			Females (mean ± SD)			Males (mean ± SD)		
	NW	OW/O	p	NW	OW/O	p	NW	OW/O	p
0-1 month*	-	-	-	62.93 ± 7.76	-	-	63.02 ± 11.32	-	-
1-12 months[a]	22.70 ± 5.98	21.15 ± 3.73	0.63	23.82 ± 5.67	21.76 ± 2.98	0.519	22.05 ± 6.17	20.40 ± 4.73	0.696
1-4 years[a]	14.56 ± 5.21	13.38 ± 1.84	0.550	15.57 ± 5.85	13.26 ± 1.21	0.328	12.54 ± 3.04	13.48 ± 2.35	0.699
4-10 years	10.26 ± 4.23	9.86 ± 2.38	0.597	9.51 ± 2.71	9.66 ± 2.39	0.519	11.33 ± 5.70	10.08 ± 2.39	0.846
10-12.5 years	8.81 ± 1.91	8.83 ± 2.11	0.805	9.25 ± 1.91	9.60 ± 2.68	0.731	8.08 ± 1.81	8.47 ± 1.74	0.556
12.5-14.5 years	7.85 ± 1.72	8.46 ± 3.37	0.909	6.77 ± 0.56	7.51 ± 3.35	0.971	8.92 ± 1.85	9.35 ± 3.23	0.812
14.5-18 years	7.12 ± 1.82	7.80 ± 2.15	0.176	6.26 ± 0.62	7.09 ± 2.04	0.214	8.11 ± 2.26	8.83 ± 1.95	0.479

*Newborns were not further divided into subgroups according to the body mass index percentiles

[a]Body mass index percentiles were used to identify overweight (≥95th percentile), not obesity, in groups younger than 2 years of age

SD: sandard deviation, NW: normal weight, OW/O: overweight/obesity

Statistical Analysis

Shapiro-Wilk test was used to analyze the distribution pattern of the continuous variables in this study. No assumptions of normal distribution of the data were made. Comparison of NT-proCNP concentration within each age group of NW-group based on gender was performed by either Mann-Whitney U test or t-test, based on the distribution of NT-proCNP concentration in age groups. For the correlations of NT-proCNP concentration of NW-group with age, weight, and height, Spearman rho correlation coefficients were calculated. Comparison of NT-proCNP concentration within each age group based on BMI, i.e., NW-group vs. OW/O-group, was performed by Mann-Whitney U test. All these analyses were carried out using IBM SPSS Statistics 21.0 software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Statistical significance level was taken as $p < 0.05$.

Results

Comparison of Amino-Terminal Propeptide of C-Type Natriuretic Peptide Concentration within Each Age Group of NW-Group Based on Gender

Analysis of plasma NT-proCNP concentrations in the different age groups of healthy girls and boys in the NW-group showed that gender was not a factor that alters the age-dependent plasma NT-proCNP concentration until puberty in this group (Figure 1; Table 1). In subjects older than 12.5 years, plasma NT-proCNP concentrations were higher in boys than in girls, and in the 14.5-18-year-old group, this difference was statistically significant ($p < 0.05$).

Correlations within the NW-Group of Children

Plasma NT-proCNP concentration was negatively correlated with age ($n = 146$; $r = -0.878$; $p < 0.001$), weight ($n = 146$; $r = -0.863$; $p < 0.001$), and height ($n = 146$; $r = -0.866$; $p < 0.001$) in the NW-group.

Comparison of Amino-Terminal Propeptide of C-Type Natriuretic Peptide Concentration within Each Age Group Based on Body Mass Index

Plasma NT-proCNP concentrations in the NW-group and the OW/O-group were compared in each age group. The results revealed that the plasma NT-proCNP concentrations of girls and boys in the OW/O-group did not differ from that of their peers in the NW-group at any age group studied (Table 2).

Since two previous studies analyzed plasma CNP concentration of adolescents with overweight/obesity at a very narrow age interval, i.e., 11.8 ± 0.4 years for the first study (18) and 12.8 ± 2.4 years for the second study (19), a similar analysis was also performed in this study in a group of 109 children [31 children with normal weight (19 girls and 12 boys) and 78 children with overweight/obesity (34 girls and 44 boys)] at age 12.64 ± 1.58 years. The results revealed once again that mean plasma NT-proCNP concentration in children of normal weight (8.22 ± 1.85 pmol/L) was comparable to that of children with overweight/obesity (8.76 ± 2.72 pmol/L).

Discussion

The primary goal of the present study was to analyze and compare, in a Turkish population of different age groups, plasma NT-proCNP concentrations in a NW-group and an OW/O-group and to evaluate NT-proCNP as a biomarker in childhood obesity.

To test the reliability of plasma NT-proCNP concentrations obtained in this study, a confirmatory first experiment was designed, during which the plasma NT-proCNP

concentration of healthy children of normal weight was analyzed through an age-dependent group model (Figure 1, and Table 1). The goal was to demonstrate, in a Turkish population, the previously published relationship between plasma CNP concentration and height velocity during child growth (5,12,13,28,29,42). In the second experiment, on the other hand, according to the above-mentioned primary goal of this study, an age- and gender-matched comparison was made between the NW-group and the OW/O-group in terms of plasma NT-proCNP concentrations.

The technique used to analyze plasma CNP and/or NT-proCNP concentration has been one of the most variable part of the studies published previously in this field. Two generally accepted applications include radioimmunoassay (RIA) (5,12,13) and enzyme-linked immunosorbent assay (ELISA) (12). A previously evaluated (12) commercially available ELISA kit was used in this present study for the analysis of plasma NT-proCNP concentration. It was reported that the correlation between the RIA for NT-proCNP and this commercially available ELISA kit for NT-proCNP was significant ($r = 0.748$, $p < 0.0005$) (12). However, "the commercial ELISA" revealed values that were, on the average, 21% of the RIA values (range, 11-52%) (12). When evaluated in this context, plasma NT-proCNP concentrations obtained in girls and boys in the NW-group at different age groups (Figure 1; Table 1) were comparable to data reported by Olney et al (12). In addition, as reported previously (5,12,13,28,29,42), our results also showed that in children, plasma NT-proCNP concentration was negatively correlated with age, weight, and height. Gender was not a factor that alters the age-dependent plasma NT-proCNP concentration until puberty in the NW-group. After 12.5 years of age, plasma NT-proCNP concentration was higher in boys than in girls. All these results were in agreement with the literature (5,12,13,28,29,42), a finding supporting the appropriate design and also the reliability of the measurement of plasma NT-proCNP concentrations in this study.

To evaluate NT-proCNP as a potential biomarker in early detection of obesity-related disease conditions during child growth, a sex- and age-matched comparison was performed between the plasma NT-proCNP concentration of the OW/O-group and that of the NW-group. Plasma NT-proCNP concentration of girls, boys, and of children overall (girls + boys) in the OW/O-group did not differ from that of NW-group at any age group studied.

Since two previous studies, which suggested a lower plasma CNP concentration in adolescents with overweight/obesity, analyzed plasma CNP concentration at a very narrow age interval, i.e., 11.8 ± 0.4 years for the first study (18) and 12.8 ± 2.4 years for the second study (19), a similar analysis was also performed in this study in a group of 109 children aged 12.64 ± 1.58 years. This age interval was important in terms of reflecting the period at which growth velocity peaks during puberty both in girls and boys. Results revealed once again that mean plasma NT-proCNP concentration in the NW-group (8.22 ± 1.85 pmol/L) was comparable to that of the OW/O-group (8.76 ± 2.72 pmol/L).

Based on these results, it was concluded that plasma NT-proCNP concentration may be an important growth marker during childhood as suggested in the literature (5,12,13,28,29,42). However, unlike previous statements (18,19), in our subjects, plasma NT-proCNP concentration of the NW-group and the OW/O-group did not differ from one another at any age group studied, including adolescents.

The literature is very limited in terms of CNP and its relation to obesity. Other than two studies that suggested lower plasma CNP levels in adolescents with overweight/obesity (18,19), there are some studies which suggest melanocortin receptors as targets in the treatment of obesity (16), and CNP as a melanocortin receptor analog in mice (17). Yamada-Goto et al (17) reported that intracerebroventricular administration of CNP suppresses food intake via activation of the melanocortin system in mice. There are also some studies that investigated the relation between CNP and hypercholesterolemia (43), for which obesity is considered as a major risk factor. However, it has been shown that systemic BNP and CNP levels are not altered in patients affected by hypercholesterolemia (43).

Study Limitations

In terms of study limitations, this study was performed only on Turkish children. Additionally, a previously evaluated (12) commercially available ELISA kit was used in this present study for the analysis of plasma NT-proCNP concentration. It has been reported that "the commercial ELISA" revealed values that were, on the average, 21% of the RIA values (range, 11-52%) (12).

On the other hand, the technique used to analyze plasma CNP and/or NT-proCNP concentration has been one of the most variable part of the studies published previously in this field. Two generally accepted applications include RIA (5,12,13) and ELISA (12). It was reported that the correlation between the RIA for NT-proCNP and the commercially available ELISA kit for NT-proCNP was significant ($r = 0.748$, $p < 0.0005$) (12).

Our findings show that unlike previous reports, plasma NT-proCNP concentration of overweight/obese children was not significantly lower than that of children with normal weight in age groups analyzed in a Turkish population. However, the conclusion established in our study should be confirmed in subsequent studies.

Conclusion

At this stage, it is clear that CNP signaling may somehow be related to obesity and/or its treatment strategies. However, it is too early to conclude that it is a potential biomarker in the early detection of obesity and/or obesity-related disease conditions during child growth. Future studies are necessary to address this question.

Ethics

Ethics Committee Approval: This study was approved by the Institutional Clinical Ethics Review Board of Pamukkale University Faculty of Medicine, Denizli, Turkey (decision dated 27.05.2014 and numbered 2014/08, approval number: 1).

Informed Consent: Written consent was taken from all participants and/or parents/legal guardians of the participating children.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N. Lale Şatıroğlu Tufan, A. Çevik Tufan, Seda Topçu, Design: N. Lale Şatıroğlu Tufan, A. Çevik Tufan, Seda Topçu, Data Collection and Processing: Seda Topçu, Bayram Özhan, Filiz Şimşek Orhon, Sevgi Başkan, Betül Ulukol, Merih Berberoğlu, Zeynep Şıklar, Analysis and Interpretation: Afra Alkan, Mesut Akyol, N. Lale Şatıroğlu Tufan, A. Çevik Tufan, Seda Topçu, Literature Research: N. Lale Şatıroğlu Tufan, A. Çevik Tufan, Seda Topçu, Writing: N. Lale Şatıroğlu Tufan, A. Çevik Tufan, Seda Topçu.

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References

1. Chusho H, Tamura N, Ogawa Y, Yasoda A, Suda M, Miyazawa T, Nakamura K, Nakao K, Kurihara T, Komatsu Y, Itoh H, Tanaka K, Saito Y, Katsuki M, Nakao K. Dwarfism and early death in mice lacking C-type natriuretic peptide. Proc Natl Acad Sci U S A 2001;98:4016-4021. Epub 2001 Mar 20
2. Tamura N, Doolittle LK, Hammer RE, Shelton JM, Richardson JA, Garbers DL. Critical roles of the guanylyl cyclase B receptor in endochondral ossification and development of female reproductive organs. Proc Natl Acad Sci U S A 2004;101:17300-17305. Epub 2004 Nov 30
3. Bartels CF, Bükülmez H, Padayatti P, Rhee DK, van Ravenswaaij-Arts C, Pauli RM, Mundlos S, Chitayat D, Shih LY, Al-Gazali LI, Kant S, Cole T, Morton J, Cormier-Daire V, Faivre L, Lees M, Kirk J, Mortier GR, Leroy J, Zabel B, Kim CA, Crow Y, Braverman NE, van den Akker F, Warman ML. Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. Am J Hum Genet 2004;75:27-34. Epub 2004 May 14
4. Yasoda A, Komatsu Y, Chusho H, Miyazawa T, Ozasa A, Miura M, Kurihara T, Rogi T, Tanaka S, Suda M, Tamura N, Ogawa Y, Nakao K. Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. Nat Med 2004;10:80-86. Epub 2003 Dec 14
5. Prickett TC, Lynn AM, Barrell GK, Darlow BA, Cameron VA, Espiner EA, Richards AM, Yandle TG. Amino-terminal proCNP: a putative marker of cartilage activity in postnatal growth. Pediatr Res 2005;58-334-340. Epub 2005 Jul 8
6. Olney RC. C-type natriurectic peptide in growth: a new paradigm. Growth Horm IGF Res 2006;16(Suppl A):S6-14. Epub 2006 May 22
7. Olney RC, Bükülmez H, Bartels CF, Prickett TC, Espiner EA, Potter LR, Warman ML. Heterozygous mutations in natriuretic peptide receptor-B (NPR2) are associated with short stature. J Clin Endocrinol Metab 2006;91:1229-1232. Epub 2005 Dec 29
8. Potter LR, Abbey-Hosch S, Dickey DM. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. Endocr Rev 2006;27:47-72. Epub 2005 Nov 16
9. Alan T, Tufan AC. C-type natriuretic peptide regulation of limb mesenchymal chondrogenesis is accompanied by altered N-cadherin and collagen type X-related functions. J Cell Biochem 2008;105:227-235.
10. Tezcan B, Serter S, Kiter E, Tufan AC. Dose dependent effect of C-type natriuretic peptide signaling in glycosaminoglycan synthesis during TGF-β1 induced chondrogenic differentiation of mesenchymal stem cells. J Mol Histol 2010;41:247-258. Epub 2010 Aug 19
11. Kocamaz E, Gok D, Cetinkaya A, Tufan AC. Implication of C-type natriuretic peptide-3 signaling in glycosaminoglycan synthesis and chondrocyte hypertrophy during TGF-β1 induced chondrogenic differentiation of chicken bone marrow-derived mesenchymal stem cells. J Mol Histol 2012;43:497-508. Epub 2012 Jun 20
12. Olney RC, Permuy JW, Prickett TC, Han JC, Espiner EA. Amino-terminal propeptide of C-type natriuretic peptide (NTproCNP) predicts height velocity in healthy children. Clin Endocrinol (Oxf) 2012;77:416-422.
13. Del Ry S, Cantinotti M, Cabiati M, Caselli C, Storti S, Prescimone T, Murzi B, Clerico A, Giannessi D. Plasma C-type natriuretic peptide levels in healthy children. Peptides 2012;33:83-86. Epub 2011 Nov 10
14. Babadagli ME, Tezcan B, Yilmaz ST, Tufan AC. Matrilin-3 as a putative effector of C-type natriuretic peptide signaling during TGF-β induced chondrogenic differentiation of mesenchymal stem cells. Mol Biol Rep 2014;41:5549-5555. Epub 2014 Jun 17
15. Serkant Unal M, Cevik Tufan A. Mesenchymal Stem Cells and C-type Natriuretic Peptide Signaling: A Proposal for a New Treatment Approach for Skeletal Dysplasias. Curr Stem Cell Res Ther 2016;11:513-521.
16. Lam DD, Farooqi IS, Heisler LK. Melanocortin receptors as targets in the treatment of obesity. Curr Top Med Chem 2007;7:1085-1097.
17. Yamada-Goto N, Katsuura G, Ebihara K, Inuzuka M, Ochi Y, Yamashita Y, Kusakabe T, Yasoda A, Satoh-Asahara N, Ariyasu H, Hosoda K, Nakao K. Intracerebroventricular administration of C-type natriuretic peptide suppresses food intake via activation of the melanocortin system in mice. Diabetes 2013;62:1500-1504. Epub 2012 Dec 28
18. Del Ry S, Cabiati M, Bianchi V, Storti S, Caselli C, Prescimone T, Clerico A, Saggese G, Giannessi D, Federico G. C-type natriuretic peptide plasma levels are reduced in obese adolescents. Peptides 2013;50:50-54. Epub 2013 Oct 8
19. Del Ry S, Cabiati M, Bianchi V, Caponi L, Maltinti M, Caselli C, Kozakova M, Palombo C, Morizzo C, Marchetti S, Randazzo E, Clerico A, Federico G. C-type natriuretic peptide is closely associated to obesity in Caucasian adolescents. Clin Chim Acta 2016;460:172-177. Epub 2016 Jul 2
20. Sudoh T, Minamino N, Kangawa K, Matsuo H. C-type natriuretic peptide (CNP): a new member of natriuretic peptide family identified in porcine brain. Biochem Biophys Res Commun 1990;168:863-870.
21. Lippert S, Goetze JP. C-type natriuretic-derived peptides as biomarkers in human disease. Biomark Med 2010;4:631-639.

22. Wiegand S, Dannemann A, Krude H, Grüters A. Impaired glucose tolerance and type 2 diabetes mellitus: a new field for pediatrics in Europe. Int J Obes (Lond) 2005;29(Suppl 2):S136-142.
23. Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. Pediatrics 2006;117:1560-1567.
24. Nathan BM, Moran A. Metabolic complications of obesity in childhood and adolescence: more than just diabetes. Curr Opin Endocrinol Diabetes Obes 2008;15:21-29.
25. Prickett TC, Yandle TG, Nicholls MG, Espiner EA, Richards AM. Identification of amino-terminal pro-C-type natriuretic peptide in human plasma. Biochem Biophys Res Commun 2001;286:513-517.
26. Wu C, Wu F, Pan J, Morser J, Wu Q. Furin-mediated processing of Pro-C-type natriuretic peptide. J Biol Chem 2003;278:25847-25852. Epub 2003 May 7
27. Kuehnl A, Pelisek J, Bruckmeier M, Safi W, Eckstein HH. Comparative measurement of CNP and NT-proCNP in human blood samples: a methodological evaluation. J Negat Results Biomed 2013;12:7.
28. Olney RC, Prickett TC, Yandle TG, Espiner EA, Han JC, Mauras N. Amino-terminal propeptide of C-type natriuretic peptide and linear growth in children: effects of puberty, testosterone, and growth hormone. J Clin Endocrinol Metab 2007;92:4294-4298. Epub 2007 Aug 7
29. Prickett TC, Dixon B, Frampton C, Yandle TG, Richards AM, Espiner EA, Darlow BA. Plasma amino-terminal pro C-type natriuretic Peptide in the neonate: relation to gestational age and postnatal linear growth. J Clin Endocrinol Metab 2008;93:225-232. Epub 2007 Oct 30
30. Bundak R, Furman A, Gunoz H, Darendeliler F, Bas F, Neyzi O. Body mass index references for Turkish children. Acta Paediatr 2006;95:194-198.
31. Neyzi O, Furman A, Bundak R, Gunoz H, Darendeliler F, Bas F. Growth references for Turkish children aged 6 to 18 years. Acta Paediatr 2006;95:1635-1641.
32. Neyzi O, Günöz H, Furman A, Bundak R, Gökçay G, Darendeliler F, Baş F. Weight, height, head circumference and body mass index references for Turkish children. Çocuk Sağlığı ve Hastalıkları Dergisi 2008;51:1-14.
33. Günöz H, Bundak R, Furman A, Darendeliler F, Saka N, Baş F, Neyzi O. Z-score reference values for height in Turkish children aged 6 to 18 years. J Clin Res Pediatr Endocrinol 2014;6:28-33.
34. Rogol AD, Clark PA, Roemmich JN. Growth and pubertal development in children and adolescents: effects of diet and physical activity. Am J Clin Nutr 2000;72(2 suppl):521S-528S.
35. Gelander L. Children's growth: a health indicator and a diagnostic tool. Acta Paediatr 2006;95:517-518.
36. Keane V. Assesment of growth. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. Nelson Textbook of Pediatrics 19th ed. Saunders Elseiver; 2011, p. 39-44.
37. Tanner JM. Fetus into man: physical growth from conception to maturity. Cambridge, MA: Harvard University Press; 1989.
38. Himes JH, Dietz WH. Guidelines for overweight in adolescent preventive services: recommendations from an expert committee. The Expert Committee on Clinical Guidelines for Overweight in Adolescent Preventive Services. Am J Clin Nutr 1994;59:307-316.
39. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320:1240-1243.
40. Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, Robinson TN, Scott BJ, St Jeor S, Williams CL. Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. Circulation 2005;111:1999-2012.
41. Obezite Tanı ve Tedavi Kılavuzu, Türkiye Endokrinoloji ve Metabolizma Derneği, 2015, p. 31. ISBN: 978-605-4011-19-3.
42. Prickett TC, Charles CJ, Yandle TG, Richards AM, Espiner EA. Skeletal contributions to plasma CNP forms: evidence from regional sampling in growing lambs. Peptides 2009;30:2343-2347. Epub 2009 Aug 5
43. Lupattelli G, Marchesi S, Siepi D, Bagaglia F, Palumbo B, Roscini AR, Schillaci G, Vaudo G, Sinzinger H, Mannarino E. Natriuretic peptides levels are related to HDL-cholesterol with no influence on endothelium dependent vasodilatation. Vasa 2006;35:215-220.

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Childhood Sustained Hypercalcemia: A Diagnostic Challenge

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What is already known on this topic?

Hypercalcemia is a rare finding in children which may be due to a wide variety of factors and possibly left undiagnosed in mild elevations, while carrying the potential to lead to serious complications if proper intervention is not achieved.

What this study adds?

Suspicion of hypercalcemia and measurement of serum calcium level in children with clinical findings not attributable to any other clinical condition is essential. In case of hypercalcemia, young infants are more vulnerable to development of nephrolithiasis and should be evaluated as soon as possible.

Abstract

Objective: This study aimed to call attention to hypercalcemia, a rare finding in children which carries the potential of leading to serious complications without proper intervention.

Methods: Diagnosis, treatment, and clinical course of children with sustained hypercalcemia admitted between the years 2006-2016 were reviewed. Group 1 [parathyroid hormone (PTH)-dependent] consisted of patients with high/unsuppressed PTH levels and group 2 (PTH-independent) included cases with normal/suppressed PTH levels.

Results: Twenty patients (11 male, 9 female) with a median age of 6.25 (0.03-17.88) years were evaluated. Symptoms were mostly related with the gastrointestinal system, while six patients (30%) were asymptomatic. Physical examination findings were diverse, non-specific, and normal in four patients (20%). Median time of diagnosis was 45 (2-720) days. Patients were divided into group 1 (n=7) and group 2 (n=13). Most frequent etiologies were primary hyperparathyroidism (n=5), idiopathic infantile hypercalcemia (IIH) (n=5), and malignancy (n=4). A moderate positive correlation was noted between serum calcium and creatinine levels ($r=0.53$, $p=0.02$). Nephrocalcinosis was the most common complication (n=9) (45%). Treatment was not implemented in 2 patients with mild hypercalcemia, while other patients received medical treatment ± surgery. Treatment-resistant patients were cases of malignancies and neonatal severe hyperparathyroidism. Long-term follow-up displayed resistant hypercalciuria in three infants diagnosed as IIH.

Conclusion: Many patients with childhood hypercalcemia are asymptomatic or exhibit a non-specific and heterogeneous clinical presentation, resulting in delayed diagnosis. Mild cases may not be recognized, while symptoms may be missed in the presence of accompanying illnesses. Nevertheless, serious complications may only be avoided with prompt diagnosis and intervention.

Keywords: Hypercalcemia, hyperparathyroidism, idiopathic infantile hypercalcemia, malignancy-related hypercalcemia, pediatrics

Introduction

Hypercalcemia is defined as a state of total serum calcium level consistently higher than 11 mg/dL (2.75 mmol/L) or an ionized calcium concentration exceeding 5.4 mg/dL (1.35 mmol/L) (1). Measurement of ionized calcium is recommended for correct interpretation of hypercalcemia as it represents the physiologically active fraction. If not available, total calcium levels may be taken into account, while "corrected calcium" levels should be calculated in cases of hypoalbuminemia (2).

Hypercalcemia is a commonly encountered problem in adults, with most cases due to hyperparathyroidism

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and malignancy. In children, although not frequent, hypercalcemia may be observed in a wide range of diseases. Its clinical features tend to be diverse, non-specific, and sometimes insignificant. Some patients may be asymptomatic, while hypercalcemia can cause serious end-organ damage such as neurological complications and kidney failure if not diagnosed and treated promptly (3,4,5).

Understanding the physiology of calcium balance is mandatory for correct diagnosis and treatment. Calcium balance is maintained by complex interplay between the parathyroid gland, bones, the intestine, and the kidneys (6). The principle regulators of this process are considered to be parathyroid hormone (PTH) and 1,25-dihyroxyvitamin D [1,25-$(OH)_2D_3$] (3). The calcium-sensing receptor (CaSR), which is expressed mainly in the parathyroid gland and kidneys, plays a critical role in the sustainment of plasma calcium levels. Increased ionized calcium levels activate CaSR which in turn acts to reduce PTH secretion (3,4). PTH is secreted at a rate inversely proportional to the circulating level of ionized calcium (7). It functions to mobilise calcium from bones through stimulation of osteoclastic activity. PTH also endorses reabsorption of calcium while inhibiting renal phosphate reabsorption at the distal renal tubule and enhances 1-α hydroxylation of 25-hydroxyvitamin D_3 (25-OH-D_3) to 1,25-$(OH)_2D_3$ (calcitriol). Calcitriol promotes calcium and phosphate absorption from the intestine, increases bone mineralization and renal calcium reabsorption.

Identification of the underlying pathology of hypercalcemia is essential as treatment modality should be targeted on the specific cause. It is practical to recognize circulating levels of PTH initially and decide whether the cause of hypercalcemia is PTH-dependent or PTH-independent (4).

This study aimed to call attention to hypercalcemia, a rare finding in children, which may be due to a wide variety of factors and possibly left undiagnosed in mild elevations, while carrying the potential to lead to serious complications if proper intervention is not achieved.

Methods

This retrospective cohort study was conducted in Ankara University Faculty of Medicine Department of Pediatric Endocrinology. The study protocol was approved by the Clinical Research Ethics Committee of our university (approval number: 10-428-16; May 2016).

We identified a total of 20 children who were diagnosed to have "sustained" hypercalcemia in the time period between 2006 and 2016. Sustained hypercalcemia is defined as persisting hypercalcemia detected in at least two consecutive days of measurement (8). Exclusion criteria were outlined as incomplete data and "transient" hypercalcemia, which is characterized as normal serum calcium measurement following a single high value.

Each patient's age, presenting symptoms, vital signs (with particular attention on pulse rate and blood pressure), and physical examination findings related with hypercalcemia were recorded.

Laboratory Evaluation of Patients

Laboratory evaluations performed to establish the underlying diagnosis were noted for each patient. Corrected total serum calcium, phosphate, alkaline phosphatase, PTH, electrolytes, renal function tests, 25-OHD, and urinary calcium/creatinine ratio results at the time of diagnosis and prior to treatment were recorded. Further investigations [parathormone-related peptide (PTHrP), genetic analysis, parathyroid gland ultrasonography, Tc-99m sestamibi scanning of the parathyroid gland, and examination of parents for abnormalities of calcium homeostasis] were likewise documented in selected patients.

Grouping of Patients

Definite diagnosis indicating the underlying etiology of hypercalcemia for each patient was listed. Finally, patients were categorized into two groups based on PTH level. Group 1, the PTH-dependent group, consisted of patients with high/unsuppressed PTH levels. Group 2 included cases with normal/suppressed PTH levels and was called the PTH-independent group. We defined PTH suppression as a PTH level of <14 pg/mL (normal range: 14-72 pg/mL) (9). Hypercalcemia was defined as mild, moderate, or severe according to corrected total serum calcium levels. We considered total serum calcium levels between 11-12 mg/dL as mild, >12 and ≤ 14 mg/dL as moderate, and levels higher than 14 mg/dL as severe hypercalcemia (10).

Evaluation of Complications

Urinary tract ultrasonography was performed to detect possible nephrolithiasis and/or nephrocalcinosis resulting from hypercalciuria. Skeletal survey findings were recorded in patients who had undergone the evaluation. Hypertension, band keratopathy, and acute pancreatitis were reported as well.

Treatment and Long-term Follow-up of Patients

Treatment modalities received by each patient for hypercalcemia, as well as the period of time required to normalize serum calcium levels and the course of the hypercalcemia in long-term monitoring were recorded.

Statistical Analysis

Statistical analysis was performed using the SPSS statistical package (v.21.0). Counts and percentages for categorical variables, median values and ranges for continuous variables were recorded. Count comparisons were analyzed using non-parametric methods due to the small size of the study group. Contingency tables (2x2) were analyzed using Fisher's exact test, while higher dimensional tables were analyzed using the chi-square test. Mann-Whitney U and Kruskal-Wallis tests were conducted for comparisons involving continuous variables. Degree of association between variables was evaluated by Spearman's correlation coefficient. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 20 patients (9 female, 11 male) with a median age of 6.25 (0.03-17.88) years were evaluated. The most frequent presenting symptoms were related with the gastrointestinal system (nausea, vomiting, anorexia, abdominal pain, constipation), while 30 % (n = 6) of patients did not show any symptoms related with hypercalcemia. Of these, 4 cases were asymptomatic and were diagnosed incidentally, while two patients had malignancy and displayed symptoms associated with the primary diagnosis. In addition, we observed a wide range of symptoms such as polyuria, polydipsia, change in behavior, weight loss, muscle weakness, bone pain, and restlessness (Table 1). The median time elapsed from the onset of symptoms until diagnosis was 45 (2-720) days in symptomatic patients. With exception of a history of nephrolithiasis in first-degree relatives in two patients, family histories did not reveal presence of hypercalcemia, parathyroid gland disease and/or surgery.

Table 1. Frequency of symptoms related with hypercalcemia on admission

Symptoms	Number of patients (n)	Percentage (%)
Anorexia	7	35
Weight loss	6	30
Nausea-vomiting	4	20
Restlessness	4	20
Abdominal pain	3	15
Polyuria	3	15
Polydipsia	2	10
Constipation	2	10
Proximal muscle weakness	2	10
Change in behavior	2	10
Bone pain	1	5
Asymptomatic	6	30

We encountered diverse and non-specific physical examination findings such as signs of dehydration, hypertension, syndromic features, short stature, proximal myopathy, acute pancreatitis, and findings suggestive of malignancy such as lymphadenopathy and hepatosplenomegaly. Physical examination was recorded as normal in four patients (20 %).

Median corrected total serum calcium level of the study group at the time of diagnosis was 12.6 (11-16.5) mg/dL. Excluding the patient with chronic renal insufficiency, a moderate positive correlation was noted between corrected total serum calcium and creatinine levels ($r = 0.53$, $p = 0.02$) although serum creatinine level was within the normal range in all patients.

Based on PTH level, group 1 (n = 7) consisted of patients with elevated or unexpectantly unsuppressed PTH measurement (Figure 1). Group 2 (n = 13) comprised patients with normal or suppressed PTH level. The most frequent etiologies were primary hyperparathyroidism (PHPT) (n = 5), idiopathic infantile hypercalcemia (IIH) (n = 5), and malignancy-related hypercalcemia (n = 4).

Median PTH level in group 1 patients was 282.6 (10-764) pg/mL. Median age was 16.32 (10.02-17.25) years. Six patients in this group had high PTH levels. One adolescent patient with bone cysts exhibited a normal PTH level. Parathyroid gland ultrasonography in this patient revealed a parathyroid adenoma. Parathyroid ultrasonography revealed hypoechoic,

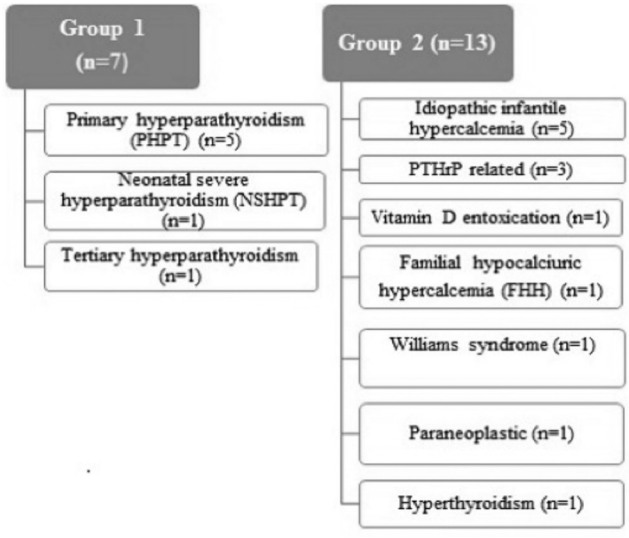

Figure 1. Etiology of hypercalcemia

PHPT: primary hyperparathyroidism, NSHPT: neonatal severe hyperparathyroidism, PTHrP: parathormone-related peptide, FHH: familial hypocalciuric hypercalcemia

lobulated extrathyroidal masses with well-defined margins in 6 patients. Possible diagnosis was parathyroid adenoma. Five of these patients were diagnosed as PHPT. One patient with chronic renal insufficiency was considered to develop tertiary hyperparathyroidism following renal transplantation. Only one patient, who was a neonate, displayed a high PTH level and normal ultrasonographic findings. Magnetic resonance imaging of the neck was likewise normal in this patient. This same patient was found to have a de novo homozygote *CaSR* gene mutation (PI1e81Lys point mutation) and was diagnosed as neonatal severe hyperparathyroidism (NSHPT) (11). His parents were heterozygote for the same mutation. Tc-99m sestamibi scanning of the parathyroid gland was conducted in five patients reporting focal increased activity compatible with parathyroid adenoma in three. Operative and pathologic findings revealed a single adenoma in six patients, while two parathyroid glands were reported to be hyperplastic in the patient with NSHPT.

In group 2, five infants of ages ranging between 15 days to 8 months had presented with mild hypercalcemia, low serum PTH, normal serum 25OHD, and increased urinary calcium/creatinine ratio. These patients were accepted as cases of IIH. Mutational screening of *CYP24A1* gene accomplished in one patient revealed a normal configuration.

Three patients with malignancies (chronic myeloid leukemia, hepatoblastoma, and rhabdomyosarcoma) in this group had elevated PTHrP (13.3-55.1 pg/mL) and low PTH levels. One patient with acute lymphoblastic leukemia displayed moderate hypercalcemia, low serum PTH (10 pg/mL), normal serum 25OHD, PTHrP, and renal function tests.

One female patient who exhibited mild hypercalcemia, low PTH (8.6 pg/mL), normal 25OHD, and hypocalciuria was found to carry a de novo heterozygote mutation of the *CaSR* gene (p.994delK (C.2981del AGA) and was diagnosed as a case of familial hypocalciuric hypercalcemia (FHH). Her mother was heterozygote for the same mutation, was normocalcemic, and had a normal urinary calcium/creatinine ratio.

An eighteen-month-old infant with moderate hypercalcemia and distinctive facial features together with supravalvular aortic stenosis was noted to have an elastin gene mutation on chromosome 7q.11.23 in fluorescent *in situ* hybridization analysis. This patient was diagnosed as Williams syndrome.

One child recently diagnosed as hyperthyroidism was found to have mild hypercalcemia which was detected incidentally. This four-month-old baby was referred because of restlessness, anorexia, and vomiting. She had severe hypercalcemia (16.4 mg/dL). She was admitted 10 days following the last dose of oral vitamin D (300,000 IU/dose) which she had received for 3 consecutive months. Serum 25OHD was high (291.4 mcg/L), serum PTH was low (5.2 pg/mL) in this patient and she was hypercalciuric. Vitamin D intoxication was the final diagnosis.

Bone mineral densitometry of two patients revealed results close to lower limits of normal (Z score: -1.75 and -1.8). One patient with parathyroid adenoma was found to have osteitis fibrosa cystica, detected by both skeletal survey and computed tomography. Band keratopathy was not recognized in any patient. All patients were evaluated with ultrasonography for renal stones. Medullary nephrocalcinosis and/or nephrolithiasis were identified at the time of diagnosis in seven patients, while two patients developed renal stones during follow-up, reaching a frequency of 45% in the total study group. The median age of patients with renal stones was younger compared to patients without renal stones ($p = 0.03$), while no relationship was detected between serum total calcium, serum PTH level, urinary calcium/creatinine level, and presence of nephrocalcinosis and/or nephrolithiasis (Table 2).

Hypertension was detected in two patients. Two patients (PHPT) were diagnosed as acute pancreatitis with hypercalcemia. One of these patients had been priorly diagnosed as Familial Mediterranean fever (FMF) with no response to colchicine treatment.

Therapeutic Interventions and Clinical Course

Two patients with PHPT and mild hypercalcemia did not receive medical therapy before surgery. Intravenous hydration

Table 2. Comparison of different parameters based on presence of nephrocalcinosis and/or nephrolithiasis

Parameters	Nephrocalcinosis and/or nephrolithiasis		
	Yes	No	p
Decimal age (years)	0.38 (0.03-17.25)	10.42 (0.04-17.88)	0.03
Serum Ca^{+2} (mg/dL)	12.0 (11.3-16.5)	12.76 (11-14.5)	0.97
Serum PTH (pg/mL)	10 (1-764)	11.9 (2.9-352)	0.70
Urinary calcium/creatinine ratio	1.04 (0.05-6.06)	0.52 (0.19-1.23)	0.12

Ca: calcium, PTH: parathormone

and bisphosphonates were applied to the remaining patients in group 1. Normocalcemia was achieved in two patients before surgery and on the postoperative day one in the remaining patients, with no recurrence. Although the neonate diagnosed as NSHPT with severe hyperalcemia received intensive treatment modalities including cinacalcet, only partial response was accomplished before surgery. When surgery took place at seven months, three parathyroid glands were resected and total thyroidectomy was performed because of inability to locate the fourth parathyroid gland. Normocalcemia was maintained on the first postoperative day, but serum calcium level reached 17.5 mg/dL in one week.

Two patients (IIH and FHH) resembling mild hypercalcemia were not given any treatment and spontaneous resolution was observed in both. The remaining patients with IIH were treated with glucocorticoids ± intravenous hydration. Resolution of hypercalcemia took 4-14 days. Repeated mild hypercalcemia was identified in 2 patients. Four infants with IIH were followed for 4-6.5 years and transient hypercalciuria was achieved in three of them.

Intravenous hydration and bisphosphonates were administered to patients with malignancy. Hypercalcemia resolved in 7-22 days but reappeared in all. Intravenous hydration, furosemide, glucocorticoids, and bisphosphonates were administered to the infant with vitamin D intoxication. Normocalcemia was achieved in 15 days with no recurrence. The patient with Williams syndrome was treated with intravenous hydration, furosemide, and glucocorticoids. Hypercalcemia ameliorated within two days. The patient diagnosed as Graves' disease was treated with antithyroid drugs with achievement of normocalcemia in four days. He was subsequently operated.

Discussion

Hypercalcemia is an infrequent finding in children but may be observed in a diverse range of clinical conditions with non-specific signs and symptoms in many cases. Moreover, determination of serum calcium is not included in many routine biochemistry profiles in children. Consequently, recognition of hypercalcemia can be a challenge in many occasions. Our study attempted to call an attention to this electrolyte imbalance.

Previous reviews mention diverse, sometimes non-specific, and occasionally absent clinical presentation in childhood hypercalcemia (3,5). We encountered similar results in our study, indicating the importance of suspecting hypercalcemia in patients with clinical findings non-attributable to other clinical conditions.

We found the median time of diagnosis as 45 days from the initiation of symptom. This period was even longer in some cases. For instance, two patients previously diagnosed as FMF and bone cysts were subsequently diagnosed as PHPT 36 and 72 months later, respectively. We suppose the main reason for delayed diagnosis was presence of non-specific and diverse clinical findings and symptoms. Moreover, a study of patients with PHPT reported the median time of diagnosis as 24 months (range: 1-60 months) which signified even later detection than in our cohort (12).

We noted a moderate positive correlation between total serum calcium and creatinine levels. We argue that normal but close to upper limits of serum creatinine levels might serve as an early indicator of renal damage in hypercalcemia.

A practical approach to hypercalcemia in children requires measurement of accompanying levels of PTH to determine whether the cause is PTH-dependent or PTH-independent (4). Unlike adults, PHPT accounts for only a small portion of childhood hypercalcemia and represents 1% of all cases (12,13). On the other hand, we defined 7 cases of PTH-dependent hypercalcemia, constituting 35% of our series. We think that this high ratio resulted from our institution serving as a reference tertiary center for complex cases necessitating multidisciplinary approach including surgery. Consistent with previously published reports, the majority of this group were adolescents (5,13). The hallmark laboratory findings of PHPT are hypercalcemia in the setting of elevated or inappropriately normal serum PTH level (14). One study including 52 children with PHPT reported that 85% of patients displayed elevated and 15% normal PTH (13). We had one patient with a normal PTH level. As in our case, we suggest further evaluation (e.g. parathyroid gland imaging) of children, especially adolescents, with unexplained hypercalcemia despite a normal PTH level.

Imaging to define the source of hyperparathyroidism comprise renal and neck ultrasonography and Tc-99m sestamibi scanning of the parathyroid gland. These imaging modalities achieve to localize adenomas in 80-90% of older children but their diagnostic success is lower in multigland hyperplasia (3). We observed positive findings in three of five patients who had undergone parathyroid gland scanning. Ultrasonographic evaluation indicated parathyroid adenoma in six patients, while it failed to detect any abnormality in one neonate. This is compatible with the literature informing the efficacy of ultrasonography in detection of parathyroid gland abnormality in only a third of cases with NSHPT (2).

IIH is a rare cause of childhood hypercalcemia, mostly appearing in the first year of life (15,16). Serum PTH level is suppressed with normal or elevated 25OHD and calcitriol

levels. It was first identified in the 1950s in the UK when nearly 200 infants with hypercalcemia of unknown origin were reported in a short period of time (17). Excluding infants carrying dismorphic features and supravalvular aortic stenosis who were soon diagnosed as Williams-Beuren syndrome, most infants were regarded as "idiopathic". Henceforth, IIH was considered as a diagnosis of exclusion in infants with hypercalcemia (1). In 2011, Schlingmann et al (18) were the first researchers who defined loss-of-function mutations in the cytochrome *P450 24A1* gene (*CYP24A1*), which encodes vitamin D hydroxylase in patients with IIH. Mutations of this metabolising enzyme of calcitriol causes increased sensitivity to vitamin D. Another gene defect involving *SLC34A1* gene which encodes renal sodium-phosphate co-transporter was identified later in patients with IIH (19). Five infants of our study group without dismorphic features displayed mild hypercalcemia, hypercalciuria, low serum PTH, normal serum 25OHD, and were diagnosed as IIH. We performed mutational screening of *CYP24A1* gene in only one of our patients which reflected a negative result. The rest of our patients were diagnosed before description of this gene.

Unlike adults, hypercalcemia of malignancy is rare in children with an overall incidence of 0.4-1.3% (20). We had four patients with malignancy. Three of them were considered to develop humoral hypercalcemia (high PTHrP level), whereas one patient resembling normal PTHrP was believed to have hypercalcemia related with tumor synthesis of osteoclast-activating factors [interleukin (IL)-1, IL-6, tumor necrosis factor-α, and prostaglandins] (3,21).

Vitamin D intoxication is another cause of childhood hypercalcemia. Although the specific vitamin D intake that causes excess or intoxication is not clearly defined in pediatrics, current recommendations address 4000 IU as the upper tolerable daily intake (22). Furthermore, polymorphisms in genes that function in regulation of vitamin D synthesis have been implicated to affect circulating vitamin D levels (23). The Endocrine Society defines serum concentrations of 25OHD exceeding 150 ng/dL as intoxication for both children and adults and this cut-off is accepted by the Pediatric Endocrinology Society (24,25). One patient of our study group presenting with severe hypercalcemia was diagnosed as vitamin D intoxication.

FHH is an autosomal dominant disorder and typically carries good prognosis not necessitating treatment and close monitoring (2). Most patients carry a heterozygous inactivating mutation in the *CASR* gene, leading to an elevation of the set point for maintaining normal plasma calcium levels (26). Although it is characterised by a positive family history, hypercalcemia and hypocalciuria accompanied by normal or marginally elevated PTH, some patients may not demonstrate all these features (27). In such instances, biochemical profiles may hardly be distinguished from that of PHTP (3). Thus, molecular screening for CASR mutations should be performed when FHH is considered in the differential diagnosis of hypercalcemia, especially in the absence of one or more cardinal features (9). One of our patients demonstrated the aforementioned characteristics with the exception of a normal PTH level and a family history of hypercalcemia. Finally, both the patient and her mother were found to carry the same heterozygote CASR mutation.

Williams syndrome (Online Mendelian Inheritance in Man 194050) is caused by a deletion in chromosome 7 presenting with typical facial features and congenital heart disease, most commonly supravalvular aortic stenosis and peripheral pulmonary stenosis. Approximately 15% of cases are associated with hypercalcemia during infancy which is believed to result from increased sensitivity to vitamin D (7,28). One of our patients exhibited typical features of Williams syndrome along with severe hypercalcemia and this diagnosis was verified by genetic analysis.

It is known that mild hypercalcemia resulting from stimulation of osteoclastic bone resorption by high plasma levels of T_3 may occur in hyperthyroidism (3). We had a similar patient who showed resolution of hypercalcemia after restoring normal thyroid gland functions.

We observed that nephrocalcinosis and/or nephrolithiasis was mostly present at younger ages, especially occurring in the first year of life. We believe this finding may indicate that younger children with hypercalcemia are more vulnerable to occurrence of nephrolithiasis and should be evaluated for this complication as early as possible.

In our series, resolution of hypercalcemia was achieved in all patients with PHPT and one patient with tertiary HPT following surgery without recurrence. A study consisting of 52 study subjects reported repeated operations in 17% (n = 9) of patients with PHPT (12). Although our data indicate better surgical success and less recurrence of adenomas, we recognize that the follow-up period of our patients [median 11 (6-54) months vs. 13 (3-23] years) was relatively short as compared to the study cited above.

We had one neonate who presented with a severe course of hypercalcemia consistent with previous reports of NSHPT and repeated hypercalcemia occurring shortly after surgery, suggesting an ectopic fifth parathyroid gland (29,30).

One out of five patients diagnosed as IIH did not require treatment, while repeated hypercalcemia was

observed in two cases. Studies suggest that although hypercalcemia usually resolves spontaneously in months in IIH, hypercalciuria may persist for years. A clinical study investigating long-term follow-up of infants diagnosed as IIH reported 63 % resolution of hypercalcemia by 2 years of age, while persistent or recurrent hypercalciuria was identified in 5 out of 11 patients (15). Our experience reflected even higher rates of persistent hypercalciuria (3 of 4 patients with long-term follow-up), suggesting the need of ongoing monitoring in IIH.

Our patients with malignancy-related hypercalcemia (n = 4) demonstrated a treatment-resistant and repeated course. This was an expected finding as primary disease control was not achieved in these patients. Mild hypercalcemia not necessitating treatment with spontaneous resolution in the patient with FHH was compatible with the classically known clinical course of FHH (2).

Study Limitations

This study has a few limitations. The most noteworthy limitation seems to be the small size of the study group, creating difficulty in comparisons between groups. Secondly, molecular analysis of CYP24A1 mutations, if performed, would have reinforced diagnosis of IIH. Thirdly, a longer follow-up period would have provided more detailed information about natural course of both hypercalcemia and hypercalciuria, recurrences, and complications such as nephrolithiasis.

Conclusion

In conclusion, this study shows once again that childhood hypercalcemia may be due to a variety of factors. A considerable fraction of the patients remain asymptomatic, while many cases exhibit a non-specific and heterogeneous clinical presentation, resulting in delayed diagnosis. Mild cases may not be recognized. Moreover, symptoms may be missed in the cases with coexistence of a serious illness such as malignancy. On the other hand, serious complications may only be avoided with correct diagnosis and prompt intervention. Suspicion of hypercalcemia and measurement of serum calcium level in children with clinical findings non-attributable to any other clinical condition is essential.

Ethics

Ethics Committee Approval: This study was approved by Clinical Research Ethical Committee of Ankara University (approval number: 10-428-16; May 2016).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Merih Berberoğlu, Zeynep Şıklar, Nisa Eda Çullas İlarslan, Concept: Merih Berberoğlu, Zeynep Şıklar, Design: Nisa Eda Çullas İlarslan, Merih Berberoğlu, Zeynep Şıklar, Data Collection or Processing: Nisa Eda Çullas İlarslan, Analysis or Interpretation: Zeynep Şıklar, Nisa Eda Çullas İlarslan, Literature Search: Nisa Eda Çullas İlarslan, Zeynep Şıklar, Writing: Nisa Eda Çullas İlarslan.

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References

1. Rodd C, Goodyer P. Hypercalcemia of the newborn: etiology, evaluation, and management. Pediatr Nephrol 1999;13:542-547.
2. Phillips P, Pain R. Correcting the calcium. Br Med J 1977;1:1473.
3. Davies JH. Approach to the Child with Hypercalcaemia. Endocr Dev 2015;28:101-118. Epub 2015 Jun 12
4. Davies JH, Shaw NJ. Investigation and management of hypercalcaemia in children. Arch Dis Child 2012;97:533-538. Epub 2012 Mar 23
5. Mallet E; Working Group on Calcium Metabolism. Primary hyperparathyroidism in neonates and childhood. The French experience (1984-2004). Horm Res 2008;69:180-188. Epub 2008 Jan 8
6. Hoorn EJ, Zietse R. Disorders of calcium and magnesium balance: a physiology-based approach. Pediatr Nephrol 2013;28:1195-1206. Epub 2012 Nov 10
7. Lietman SA, Germain-Lee EL, Levine MA. Hypercalcemia in children and adolescents. Curr Opin Pediatr 2010;22:508-515.
8. McNeilly JD, Boal R, Shaikh MG, Ahmed SF. Frequency and aetiology of hypercalcemia. Arch Dis Child 2016;101:344-347. Epub 2016 Feb 22
9. Koltin D, Racmiel M, Wong BY, Cole DE, Harvey E, Sochett E. Mild infantile hypercalcemia: diagnostic tests and outcomes. J Pediatr 2011;159:215-221. Epub 2011 Mar 17
10. Shane E. Diagnostic approach to hypercalcemia. In: UpToDate, Post TW (Ed), UpToDate, Waltham MA, 2014 (Accessed on Nov 08, 2016).
11. Kahvecioglu D, Atasay B, Berberoglu M, Yildiz D, Cakir U, Akduman H, Erdeve O, Siklar Z, Magdelaine C, Lienhardt-Roussie A, Akar M, Ozbek MN, Arsan S. A novel mutation in the calcium sensing receptor gene in a neonate with severe hyperparathyroidism. Genet Couns 2014;25:331-335.
12. Kollars J, Zarroug AE, van Heerden J, Lteif A, Stavlo P, Suarez L, Moir C, Ishitani M, Rodeberg D. Primary hyperparathyroidism in pediatric patients. Pediatrics 2005;115:974-980.
13. Roizen J, Levine MA. Primary hyperparathyroidism in children and adolescents. J Chin Med Assocc 2012;75:425-434.
14. Markowitz ME, Underland L, Gensure R. Parathyroid Disorders. Pediatr Rev 2016;37:524-535.
15. Huang J, Coman D, McTaggart SJ, Burke JR. Long-term follow-up of patients with idiopathic infantile hypercalcaemia. Pediatr Nephrol 2006; 21:1676-1680. Epub 2006 Aug 24
16. Dinour D, Davidovits M, Aviner S, Ganon L, Michael L, Modan-Moses D, Vered I, Bibi H, Frishberg Y, Holtzman EJ. Maternal and infantile hypercalcemia caused by vitamin-D hydroxylase muatations and vitamin D intake. Pediatr Nephrol 2015;30:145-152. Epub 2014 Sep 7

17. Lightwood R, Stapleton T. Idiopathic hypercalcemia in infants. Lancet 1953;265:255-256.

18. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, Misselwitz J, Klaus G, Kuwertz-Bröking E, Fehrenbach H, Wingen AM, Güran T, Hoenderop JG, Bindels RJ, Prosser DE, Jones G, Konrad M. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. N Engl J Med 2011;365:410-421. Epub 2011 Jun 15

19. Schlingmann KP, Ruminska J, Kaufmann M, Dursun I, Patti M, Kranz B, Pronicka E, Ciara E, Akcay T, Bulus D, Cornelissen EA, Gawlik A, Sikora P, Patzer L, Galiano M, Boyadzhiev V, Dumic M, Vivante A, Kleta R, Dekel B, Levtchenko E, Bindels RJ, Rust S, Forster IC, Hernando N, Jones G, Wagner CA, Konrad M. Autosomal-Recessive Mutations in SLC34A1 Encoding Sodium-Phosphate Cotransporter 2A Cause Idiopathic Infantile Hypercalcemia. J Am Soc Neprol 2016;27:604-614. Epub 2015 Jun 5

20. Micheal JF, Susan RR. Oncologic emergencies. In: Pizzo P, Poplack DG, (eds). Principles and practice of pediatric oncology. 6th ed. Philalephia, Lipincott Williams and Wilkins, 2011:1145-1146.

21. Niizuma H, Fujii K, Sato A, Fujiwara I, Takeyama J, Imaizumi M. PTHrP-independent hypercalcemia with increased proinflammatory cytokines and bone resorption in two children with CD19-negative precursor B acute lymphoblastic leukemia. Pediatr Blood Cancer 2007;49:990-993.

22. Vogiatzi MG, Jacobson-Dickman E, DeBoer MD; Drugs, and Therapeutics Committee of The Pediatric Endocrine Society.Vitamin D supplementation and risk of toxicity in pediatrics: a review of current literature. J Clin Endocrinol Metab 2014;99:1132-1141. Epub 2014 Jan 23

23. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180-188. Epub 2010 Jun 10

24. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-1930. Epub 2011 Jun 6

25. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M; Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics 2008;122:398-417.

26. Taki K, Kogai T, Sakumoto J, Namatame T, Hishinuma A. Familial hypocalciuric hypercalcemia with a de novo heterozygous mutation of calcium-sensing receptor. Endocrinol Diabetes Metab Case Rep 2015;2015:150016. Epub 2015 May 1

27. Guarnieri V, Canaff L, Yun FH, Scillitani A, Battista C, Muscarella LA, Wong BY, Notarangelo A, D'Agruma L, Sacco M, Cole DE, Hendy GN. Calcium-sensing receptor (CASR) mutations in hypercalcemic states: studies from a single endocrine clinic over three years. J Clin Endocrinol Metab 2010;95:1819-1829. Epub 2010 Feb 17

28. Cagle AP, Waguespack SG, Buckingham BA, Shankar RR, Dimeglio LA. Severe infantile hypercalcemia associated with Williams syndrome successfully treated with intravenously administered pamidronate. Pediatrics 2004;114:1091-1095.

29. Murphy H, Patrick J, Báez-Irizarry E, Lacassie Y, Gómez R, Vargas A, Barkemeyer B, Kanotra S, Zambrano RM. Neonata severe hyperparathyroidism caused by homozygous mutation in CASR: A rare cause of life-threatening hypercalcemia. Eur J Med Genet 2016;59:227-231. Epub 2016 Feb 6

30. Savas-Erdeve S, Sagsak E, Keskin M, Magdelaine C, Lienhardt-Roussie A, Kurnaz E, Cetinkaya S, Aycan Z. Treatment experience and long-term follow-up data in two severe neonatal hyperparathyroidism cases. J Pediatr Endocrinol Metab 2016;29:1103-1110.

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Reliability and Validity of the Diabetes Eating Problem Survey in Turkish Children and Adolescents with Type 1 Diabetes Mellitus

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What is already known on this topic?

Noticing and treating eating disorders in children and adolescents with type 1 diabetes is important because of their potentially severe consequences. Using a screening tool designed specifically for individuals with type 1 diabetes when assessing disturbed eating behaviors in this population is important.

What this study adds?

No validated disease-specific short screening tool for children and adolescents with type 1 diabetes in Turkey has so far been established. The Turkish version of Diabetes Eating Problem Survey-Revised can be used as a valid screening tool for disordered eating behaviors in type 1 diabetes. This short, self-administered diabetes-specific screening tool for disordered eating behavior can be used in the clinical care of children and adolescents with type 1 diabetes.

Abstract

Objective: The aim of this study was to show the reliability and validity of a Turkish version of Diabetes Eating Problem Survey-Revised (DEPS-R) in children and adolescents with type 1 diabetes mellitus.

Methods: A total of 200 children and adolescents with type 1 diabetes, ages 9-18 years, completed the DEPS-R Turkish version. In addition to tests of validity, confirmatory factor analysis was conducted to investigate the factor structure of the 16-item Turkish version of DEPS-R.

Results: The Turkish version of DEPS-R demonstrated satisfactory Cronbach's $\propto$ (0.847) and was significantly correlated with age ($r = 0.194$; $p < 0.01$), hemoglobin A_{1c} levels ($r = 0.303$; $p < 0.01$), and body mass index-standard deviation score ($r = 0.412$; $p < 0.01$) indicating criterion validity. Median DEPS-R scores of Turkish version for the total samples, females, and males were 11.0, 11.5, and 10.5, respectively.

Conclusion: Disturbed eating behaviors and insulin restriction were associated with poor metabolic control. A short, self-administered diabetes-specific screening tool for disordered eating behavior can be used routinely in the clinical care of adolescents with type 1 diabetes. The Turkish version of DEPS-R is a valid screening tool for disordered eating behaviors in type 1 diabetes and it is potentially important to early detect disordered eating behaviors.

Keywords: Diabetes eating problem survey-revised, distributed eating behaviors, type 1 diabetes mellitus, children and adolescent

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Introduction

Adolescence is a developmental period with characteristic properties such as physiological changes (including weight gain, increase of adipose tissue) and psychological changes. These changes may lead to body image dissatisfaction and to an increase in prevalence of eating disorders, especially in females (1). The term "disturbed eating behaviors (DEB)", encompasses mild as well as more extreme dieting behavior, binge eating attacks, and compensatory behavior for weight control. Studies indicate that type 1 diabetes mellitus (T1D) is a risk factor for the development of DEB (2,3). Although most of the studies focus on females, some researchers suggest that adolescent males with T1D also may have an increased risk of development of DEB (4).

Etiology of DEB is complex and multifactorial (5,6). Individual, familial, and sociocultural factors can contribute to the development of DEB (5). A lot of mechanisms have been proposed to explain the relationship between DEB and T1D. The mechanisms proossed are the effects of a chronic medical condition on body image satisfaction and low self-esteem as well as the effects of treatment of hypoglycemia and insulin use, given the focus on diet and carbohydrate intake and weight gain associated with these treatment modules (7,8). Additionally, omitting or giving less insulin than required (i.e. insulin purging) is a unique tool to reduce weight in T1D.

Early detection and treatment of eating disorders in children and adolescents with T1D is important because of their potentially severe consequences. Early detection is critical in this population in order to maintain optimum health status and decrease the chances of complications such as retinopathy, neuropathy, and diabetic ketoacidosis. The complexity of diabetes management in combination with eating disorder treatment, it is necessary to detect those most at risk as early as possible. A number of screening questionnaires and structured clinical interviews help to identify and diagnose eating disorders in children and young people with diabetes (9). It is important to use a screening measure designed specifically for individuals with T1D when establishing DEB in this population. To date, no validated disease-specific short screening tool for children and adolescents with T1D is in use in Turkey.

The Diabetes Eating Problem Survey-Revised (DEPS-R) developed by Markowitz et al (10) is a diabetes-specific self-report instrument to screen eating disorders for individuals with T1D. This present study aims to establish the reliability and validity of a Turkish version of DEPS-R in a representative sample of Turkish children and adolescents with T1D.

Methods

In this cross-sectional study, the children and adolescents were asked to answer a questionnaire during a regularly scheduled medical visit after written informed consent had been obtained from the subjects and their parents. All subjects were T1D patients who were being treated with multiple daily insulin injections or with insulin pump therapy. Patient records were reviewed for the following eligibility criteria:

- Duration of type 1 diabetes ≥1 year,

- A regular follow-up for at least 1 year,

- No major medical problems (celiac disease, cystic fibrosis, psychiatric disorders, or communication difficulties).

Two hundred adolescents with T1D-90 (45%) males, 110 (55%) females-aged 9–18 years were included in the study.

DEPS-R is a 16-item diabetes-specific self-report questionnaire to test for diabetes-specific eating disorders. Answers are scored on a six-point Likert scale, with higher scores indicating more DEB and a total score of ≥20 indicating a high risk for eating disorders (range 0-80). The original DEPS-R has been shown to have a good internal consistency (Cronbach's alpha = 0.86) and construct validity in a sample of pediatric population with T1D (10). Back-translation techniques were employed to develop Turkish versions of the DEPS-R. The translation techniques followed standardized procedure suggested by Brislin (11) in which the inventory items and scale were translated from English into Turkish by a professional translator.

This study was approved by the Ege University Faculty of Medicine Clinical Research Ethics Committee (approval number: 14-12.1/9). The purpose of the study was explained to each participant and written informed consent was obtained. The study procedures were in accordance with the Declaration of Helsinki.

All subjects were asked to complete the DEPS-R.

In all subjects, height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured unclothed to the nearest 0.1 kg using a calibrated balance scale. Body mass index (BMI) was calculated by the weight (kg)/height (m^2) equation. Standard deviation scores (SDS) for weight, height, and BMI were calculated according to age and gender using reference values for Turkish children and the participants were categorized into four different groups as underweight (BMI-SDS <-1), of normal weight (BMI-SDS ≥ -1 - $< +1$), overweight (BMI-SDS $\geq +1$ - $+2 <$ BMI-SDS), and obese (BMI-SDS $\geq +2$) (12).

Hemoglobin A_{1c} (HbA_{1c}) measurements were performed with capillary method using the NycoCard II Reader (Axis-Shield Diagnostics Ltd, Dundee, UK) device.

Statistical Analysis

All data are presented as median for skewed data (age, HbA_{1c} (%), duration of T1D, and DEPS-R score), mean ± SD for BMI-SDS, or percent as indicated. Normal distribution was tested for continuous data. Group differences were investigated using the t-test for normally distributed data and the Mann-Whitney U test for data not showing a normal distribution. Correlations using Pearson's and Spearman's correlation coefficients were calculated to explore relationships of DEPS-R score with age, HbA_{1c} (%), duration of T1D, and BMI-SDS. Correlation values of 0.10-0.29 were interpreted as small, 0.30-0.49 as medium, and 0.50-1.0 as large (13). The factorial structure of the Turkish version of DEPS-R was examined by confirmatory factor analysis and the internal consistency was tested using Cronbach's coefficient. Chi square tests were used for categorical variables. Level of significance was defined as $p < 0.05$. Statistical analyses were conducted using Statistical Package for the Social Sciences (IBM SPSS Statistics) version 22.0 (SPSS Inc., Chicago, Illinois, USA) and SPSS Amos.

Results

Table 1 shows the sample characteristics. Median age of the 200 patients was 14.0 years (9.0-18.0). Median diabetes duration, HbA_{1c}, and mean BMI-SDS values were 64.5 months (12-210), 8.05% (5.5-15.0), and 0.64 ± 1.24 SD, respectively. There were no significant differences in age, diabetes duration, HbA_{1c} (%) levels, and BMI-SDS values between females and males (Table 1). Seventy-one percent of the patients were on multiple daily injections (MDI) (≥4 daily injections), while 28.5 % were on insulin pump therapy. The median total daily insulin dose was 0.83 (0.46-2.09) U/for patients on MDI and 0.93 (0.23-1.53) U/kg for patients on insulin pump therapy. There was no significant difference in total daily insulin dose between MDI and insulin pump therapy groups.

Internal consistency: The Cronbach coefficients for the DEPS-R Turkish version were 0.847, 0.857, and 0.830 for the entire sample, females, and males, respectively.

Confirmatory factor analysis: After the suitability of data for factor analysis was assessed, confirmatory factor analysis was performed on the 16 items of the DEPS-R Turkish version. Among the main factors and sub-factors, the established model was statistically significant (Table 2).

Prevalence of disturbed eating behaviors risk: The median scores obtained with the Turkish version of DEPS-R for the total sample, for females, and males were 11.0 (0-55), 11.5 (0-55), and 10.5 (0-55), respectively. There was no significant difference between females and males ($p = 0.122$). The median scores of the DEPS-R for the MDI group was 11.0 (0-47) and 11.0 (0-55) for the insulin pump group. There was no significant difference between MDI and insulin pump therapy groups ($p = 0.813$). A recommended cut-off score of ≥20 has been empirically established as a threshold indicating the need for further clinical assessment of eating pathology (10). A total of 29.1% of the females and 17.8% of the males scored above this cut-off value on the DEPS-R Turkish version and there was no significant difference between females and males.

Among females, the prevalence of DEPS-R scores increased steadily and significantly from 7 among underweight patients to 10 for normal weight, 21 for overweight, and

Table 1. Characteristics of the study participant

	All	Females	Males	p
n	200	110	90	
Age (years)	14.0 (9.0-18.0)	14.0 (9.0-18.0)	14.0 (9.0-18.0)	ǂNS
HbA_{1c} (%)	8.05 (5.5-15.0)	8.10 (5.5-15.0)	7.75 (5.9-13.3)	ǂNS
BMI-SDS, mean ± SD	0.64 ± 1.24	0.16 ± 1.25	-0.57 ± 1.23	ǂǂNS
Diabetes duration (months)	64.5 (12-210)	67.5 (12-188)	60 (12-210)	ǂNS
Insulin pump therapy, %	28.5	26.4	31.1	*NS
Multiple daily injections ≥4, %	71.5	73.6	68.9	*NS

Data are medians (minimum-maximum), unless otherwise indicated

The p-values refer to the significance of the difference between females and males

ǂMann-Whitney U test, ǂǂIndependent-sample t-test, *Chi-square test

BMI: body mass index, SDS: standard deviation score, HbA_{1c}: hemoglobin A_{1c}

Table 2. Results of confirmatory factor analysis

Goodness of fit index	Acceptable fit	Model
X^2/df (CMIN/df)	< 3 good, < 5 sometimes permissible	1.824
RMSEA	≤0.05 good, 0.05-0.10 moderate, > 0.10 bad	0.064
PCLOSE	≤0.05 good fit	0.057
CFI	≥0.95 great, ≥0.90 traditional, ≥0.80 sometimes permissible	0.907
GFI	≥0.90 good fit	0.90
AGFI	≥0.80 good fit	0.855
NFI	≥0.90 good fit	0.819

Df: degrees of freedom, CMIN: minimum discrepancy function, RMSEA: root mean square error of approximation, CFI: comparative fit index, AGFI: adjusted goodness of fit index, GFI: goodness of fit index, NFI: normed fit index

Table 3. Median scores of Diabetes Eating Problem Survey-Revised according to categories of body mass index-standard deviation score

	All	Female	Male	p
Underweight	7.0 (0-43)	7.0 (0-43)	6.0 (1-25)	NS
Normal weight	10.5 (0-46)	10.0 (0-46)	12 (0-45)	NS
Overweight	19.0 (4-55)	21.0 (6-55)	16 (4-55)	NS
Obese	22.0 (7-47)	26.5 (7-41)	15 (10-47)	NS

The data reflect the median values of the Diabetes Eating Problem Survey-Revised Turkish version total score for participants categorized in four different groups of body mass index-standard deviation score (BMI-SDS) (adjusted for age and sex): underweight (BMI-SDS < -1), normal weight (BMI-SDS ≥ -1 - < + 1), overweight (BMI-SDS ≥ + 1 - < + 2 < BMI-SDS), and obese (BMI-SDS ≥ + 2). The p-values refer to the significance of the difference between males and females

Table 4. Correlations of Diabetes Eating Problem Survey-Revised Turkish version scores with age, body mass index-standard deviation score, hemoglobin A_{1c}, and diabetes duration

	All	Female	Male
Age	0.194**	0.322**	0.054
Onset of diabetes	0.131	0.121	0.144
Diabetes duration	0.055	0.146	-0.074
HbA_{1C} (%)	0.303**	0.343**	0.258*
BMI-SDS	0.412**	0.455**	0.351**

Spearman correlation

*Differences were significant at $p < 0.05$, differences were significant at **$p < 0.01$

BMI-SDS: body mass index-standard deviation score, HbA_{1c}: hemoglobin A_{1c}

26.5 for the obese patients. Among males, the median score was 6 for underweight, 12 for normal weight and 16/15 for overweight/obese patients. There was no significant difference between overweight and obese groups in both females and males (Table 3).

Correlations with Diabetes Eating Problem Survey-Revised Turkish Version

There were significant positive correlations of DEPS-R Turkish version scores with age ($r = 0.194$; $p < 0.01$), HbA_{1c} levels ($r = 0.343$; $p < 0.01$), and BMI-SDS ($r = 0.455$; $p < 0.01$) among females. There was a positive small correlation between DEPS-R Turkish version scores and HbA_{1c} levels ($r = 0.258$; $p < 0.05$) and there was positive medium correlation with BMI-SDS ($r = 0.351$; $p < 0.01$) among males (Table 4).

Discussion

This study examined the psychometric properties of the Turkish version of DEPS-R and its clinical utility. The DEPS-R is a 16-item instrument designed to screen for DEB in T1D. Markowitz et al (10) first used the English version of DEPS-R and showed that this screening tool had good internal consistency (Cronbach's $\propto = 0.86$) and validity in a representative sample of young people with T1D. As the original version, Norwegian (Cronbach's $\propto = 0.89$) and German versions (Cronbach's $\propto = 0.84$) of DEPS-R also showed that DEPS-R has a good internal consistency and validity (14,15). The Turkish version of DEPS-R showed a very good internal consistency in children and adolescents with T1D with a Cronbach's $\propto$ of 0.847 (in females Cronbach's $\propto = 0.85$, in males Cronbach's $\propto = 0.83$) consistent with the previous validation studies.

The confirmatory factor analysis is a kind of structural equation model which is used to determine the relationship between observable and latent variables and has a significant value in scale adaptation studies. After the suitability of data for factor analysis was assessed, confirmatory factor analysis was performed and confirmatory factor loadings of the subscales were shown to be at an acceptable level

[goodness of fit index (GFI) 0.90, adjusted GFI 0.855, comparative fit index 0.91, κ^2/degrees of freedom 1.82, and root mean square error of approximation 0.064].

In this sample, DEPS-R scores were similar to those reported in previous studies (9.8, 11.0, 12.0, 8.0, vs. 11.0) (10,14,15,16). In addition, despite the fact that there were no significant differences in median DEPS-R scores 11.0 (0-55) between females and males in these studies, in our sample, DEPS-R scores of males were slightly higher than in previous studies (9.3, 7.7, 9.4, 5.0, vs. 10.5).

Previous studies defined a score of DEPS-R ≥20 as an indicator of high risk for DEB (10,15,16). Twenty-five percent of our participants scored DEPS-R ≥20. Of these 67% were females and 33% were males. Thirty-one percent of the patients who scored ≥20 were not receiving enough insulin to cover the food when they overate, and 23% skipped the following insulin dose after overeating. While a greater proportion of the females scored above cut-off, surprisingly, more males reported insulin restriction and insulin omission in this group. Among females, insulin restriction and insulin omission rates were 28 and 22 % and among males these rates were 37.5 and 25 %, respectively. When insulin is omitted, with the catabolism of lipids andthe induced glycosuria results in excretion of calories with urine and contributes to weight loss (17,18). Insulin omission is associated with frequent events of diabetic ketoacidosis, and DEB is linked with recurrent episodes of severe hyperglycemia and about one-third of individuals with T1D intentionally omit insulin (8,19). Considering the social pressure about thinness and/or to be fit, young people with T1D are particularly at risk of weight loss practices such as insulin restriction or omission. Most studies focus on females but some researchers suggest that adolescent males with T1D also may have an increased risk of development of DEB (4). In our sample, as stated above, especially males appeared to be at risk for omission of insulin and insulin restriction. The use of a diabetes-specific screening instrument such as DEPS-R may be important for detection of the risk in both genders with T1D. Weight issue and external appearance may be the main problems in females but depressed mood, low self-esteem maybe a problem in boys. In order to further understand the increase in omission and restriction rate of insulin as well as other problems encountered in boys, these participants are under further psychiatric evaluation.

Consistent with the literature, in our series also, patients with scores above the cut-off on DEPS-R had significantly higher HbA_{1c} levels ($p = 0.002$) in both genders (10,15,20). No significant differences between females and males in relation to HbA_{1c} was found. Participants with higher scores on the DEPS-R had a higher BMI-SDS, a finding which is in accordance with previous studies (10,14,15,20). In our study, median score of DEPS-R increased significantly from 7 in the underweight group to 10 in the normal weight group and to 22 in the obese group ($p = 0.012$). Participants who were overweight ($p \leq 0.0001$) or obese ($p \leq 0.0001$) had higher scores on DEPS-R than those who were of normal weight. Females scored higher scores in all groups, but there was no significant difference between females and males. Olmsted et al (21) performed a longitudinal study on a sample of 126 T1D females age ranging from 9-13 years and reported that a higher BMI value predicted the onset of DEB. Wisting et al (14) in 770 children and adolescent with T1D (9-11 years old) reported that the prevalence of DEB increased considerably with increasing weight, especially for females. Markowitz et al (16), in their study on a sample of 43 patients of ages 10-17 years who were on insulin pump therapy, reported that participants who were overweight or obese scored higher on DEPS-R than those who were of normal weight. Thus, more weight and shape concerns, more negative feelings about one's physical appearance, and more unhealthy weight control behaviors like insulin omission and insulin restricting can explain the relationship between BMI and DEB. As an exploratory analysis, we examined the stability of the DEPS-R scores by age groups. We found that median DEPS-R scores were 1.5 times and significantly higher in 90 adolescents aged 14-18 years than in 110 adolescents aged 9-13 years ($p \leq 0.0001$). Our results are similar to those reported in a previous DEPS-R validation study and a Norwegian sample study (10,14).

First DEPS-R validation study showed positive correlations of DEPS-R scores with age, age- and gender-adjusted BMI and HbA_{1c} levels (10).

Criterion validity of the Turkish version of the DEPS-R was shown through significant positive medium correlations with BMI-SDS ($r = 0.412$, $p < 0.01$) and HbA_{1c} levels ($r = 0.303$, $p < 0.01$) and a small but positive correlation with age ($r = 0.194$, $p < 0.01$) that may have been influenced by the presence of DEB in children and adolescents with T1D. Therefore, in clinical settings, we recommend assessment of DEPS-R score in relation to BMI-SDS and age in T1D patients. Higher BMI-SDS, higher HbA_{1c}, and older age appear to be risk factors for the development of DEBs among children and adolescents with T1D.

Study Limitations

A major limitation of this study is its inability to validate the Turkish version of DEPS-R with a structured clinical diagnostic interview by a pediatric psychiatrist. This may have led to overestimation of DEB.

Conclusion

Our data has shown that children and adolescents with T1D who have higher BMI values and higher HbA_{1c} levels appear to exhibit more DEBs. DEB and insulin restriction were associated with poor metabolic control. Diabetes health care professionals should be aware of comorbid eating disorders in children and adolescent with T1D. A short, self-administered diabetes-specific screening tool for DEB can be used routinely in the clinical care of children and adolescents with T1D. The Turkish version of DEPS-R is a valid screening tool for DEB in T1D and it is potentially important to detect DEB at an early stage. We propose that future research should focus on the validity of the DEPS-R as compared with a structured clinical diagnostic interview.

Ethics

Ethics Committee Approval: The study was approved by Ege University Faculty of Medicine Clinical Research Ethics Committee (approval number: 14-12.1/9).

Informed Consent: Written informed consent had been obtained from the subjects and their parents.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Damla Gökşen, Samim Özen, Şükran Darcan, Concept: Yasemin Atik Altınok, Damla Gökşen, Design: Yasemin Atik Altınok, Data Collection or Processing: Yasemin Atik Altınok, Analysis or Interpretation: Suriye Özgür, Reci Meseri, Literature Search: Yasemin Atik Altınok, Writing: Yasemin Atik Altınok.

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References

1. Meltzer LJ, Johnson SB, Prine JM, Banks RA, Desrosiers PM, Silverstein JH. Disordered eating, body mass, and glycemic control in adolescents with type 1 diabetes. Diabetes Care 2001;24:678-682.
2. Mannucci E, Rotella F, Ricca V, Moretti S, Placidi GF, Rotella CM. Eating disorders in patients with type 1 diabetes: a meta-analysis. J Endocrinol Invest 2005;28:417-419.
3. Jones JM, Lawson ML, Daneman D, Olmsted MP, Rodin G. Eating disorders in adolescent females with and without type 1 diabetes: cross sectional study. BMJ 2000;320:1563-1566.
4. Svensson M, Engström I, Aman J. Higher drive for thinness in adolescent males with insulin-dependent diabetes mellitus compared with healthy controls. Acta Paediatr 2003;92:114-117.
5. Dalle Grave R. Eating disorders: progress and challenges. Eur J Intern Med 2011;22:153-160. Epub 2011 Jan 14
6. Colton P, Olmsted M, Daneman D, Rydall A, Rodin G. Disturbed eating behavior and eating disorders in preteen and early teenage girls with type 1 diabetes: a case-controlled study. Diabetes Care 2004;27:1654-1659.
7. Young V, Eiser C, Johnson B, Brierley S, Epton T, Elliott J, Heller S . Eating problems in adolescents with Type 1 diabetes: a systematic review with meta-analysis. Diabet Med 2013;30:189-198.
8. Pinhas-Hamiel O, Levy-Shraga Y. Eating disorders in adolescents with type 2 and type 1 diabetes. Curr Diab Rep 2013;13:289-297.
9. Smart CE, Annan F, Bruno LP, Higgins LA, Acerini CL; International Society for Pediatric and Adolescent Diabetes. ISPAD Clinical Practice Consensus Guidelines 2014. Nutritional management in children and adolescents with diabetes. Pediatr Diabetes 2014;15(Suppl 20):135-153.
10. Markowitz JT, Butler DA, Volkening LK, Antisdel JE, Anderson BJ, Laffel LM. Brief screening tool for disordered eating in diabetes: internal consistency and external validity in a contemporary sample of pediatric patients with type 1 diabetes. Diabetes Care 2010;33:495-500. Epub 2009 Dec 23
11. Brislin RW. The wording and translation of research instruments. F Methods Cross-Cultural Res 1986:137-164.
12. Neyzi O, Günöz H, Furman A, Bundak R, Gökçay G, Darendeliler F, Baş F. Weight, height, head circumference and body mass index references for Turkish children. J Child Heal Dis 2008;51:1-14.
13. Cohen J. Statistical power analysis for the behavioral sciences. Stat Power Anal Behav Sci 1988:567.
14. Wisting L, Frøisland DH, Skrivarhaug T, Dahl-Jørgensen K, Rø Ø. Psychometric properties, norms, and factor structure of the diabetes eating problem survey-revised in a large sample of children and adolescents with type 1 diabetes. Diabetes Care 2013;36:2198-2202. Epub 2013 Mar 27
15. Saßmann H, Albrecht C, Busse-Widmann P, Hevelke LK, Kranz J, Markowitz JT, Marshall LF, Meurs S, de Soye IH, Lange K . Psychometric properties of the German version of the Diabetes Eating Problem Survey-Revised: additional benefit of disease-specific screening in adolescents with Type 1 diabetes. Diabet Med 2015;32:1641-1647. Epub 2015 May 15
16. Markowitz JT, Alleyn CA, Phillips R, Muir A, Young-Hyman D, Laffel LM. Disordered eating behaviors in youth with type 1 diabetes: prospective pilot assessment following initiation of insulin pump therapy. Diabetes Technol Ther 2013;15:428-433. Epub 2013 Apr 3
17. Colton P, Rodin G, Bergenstal R, Parkin C. Eating Disorders and Diabetes: Introduction and Overview. Diabetes Spectr 2009;22:138-142.
18. Scheuing N, Bartus B, Berger G, Haberland H, Icks A, Knauth B, Nellen-Hellmuth N, Rosenbauer J, Teufel M, Holl RW; DPV Initiative; German BMBF Competence Network Diabetes Mellitus. Clinical characteristics and outcome of 467 patients with a clinically recognized eating disorder identified among 52,215 patients with type 1 diabetes: A multicenter german/austrian study. Diabetes Care 2014;37:1581-1589. Epub 2014 Mar 12
19. Weinert LS, Scheffel RS, Severo MD, Cioffi AP, Teló GH, Boschi A, Schaan BD. Precipitating factors of diabetic ketoacidosis at a public hospital in a middle-income country. Diabetes Res Clin Pract 2012;96:29-34. Epub 2011 Dec 5
20. Wisting L, Frøisland DH, Skrivarhaug T, Dahl-Jørgensen K, Rø Ø. Disturbed eating behavior and omission of insulin in adolescents receiving intensified insulin treatment: a nationwide population-based study. Diabetes Care 2013;36:3382-3387. Epub 2013 Aug 20
21. Olmsted MP, Colton PA, Daneman D, Rydall AC, Rodin GM. Prediction of the onset of disturbed eating behavior in adolescent girls with type 1 diabetes. Diabetes Care 2008;31:1978-1982. Epub 2008 Jul 15

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Wrist Circumference and Frame Size Percentiles in 6-17-Year-Old Turkish Children and Adolescents in Kayseri

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What is already known on this topic?

Studies on reference values for wrist circumference and their use in assessing cardio-metabolic risks are limited.

What this study adds?

This is the first study on wrist circumference in Turkish children and adolescents and was conducted on a quite large sample. The study also included estimation of frame size based on wrist circumference.

Abstract

Objective: The aim of the current study was to provide wrist circumference (WrC) and body frame size (height/WrC) percentile values in Turkish children and adolescents aged 6-17 years.

Methods: In this cross-sectional study, the data of "Determination of Anthropometric Measures of Turkish Children and Adolescents" (DAMTCA II) study in Kayseri/Turkey were used. A total of 4330 observations were recorded (1931 boys, 2399 girls). The WrC and frame size reference values were produced with generalized additive models for location, scale and shape.

Results: The WrC percentiles (3rd-97th) were calculated. The frame size (height/WrC) was estimated as small, medium, and large (< 15th, 15-85th, and ≥85th percentiles, respectively). For both genders, WrC linearly increased with age (13.0-16.8 cm for boys and 12.5-15.5 cm for girls). In boys and girls, the mean ± standard deviation of WrC is 13.00 ± 0.89 cm and 12.48 ± 0.93 cm (6 years) and increases to 16.83 ± 1.16 and 15.58 ± 0.86 cm (17 years), respectively. The WrC values in all age groups were higher in boys compared with girls. The increment in frame size from 6 to 17 years were 1.25 cm in boys and 0.85 cm in girls.

Conclusion: WrC is a simple, easy-to-detect anthropometric index which is not subject to measurement errors. Additionally, WrC can be used both to decide about frame size and to determine metabolic risks related to obesity. We consider that this easy-to-get anthropometric index can be used both in screening procedures and clinical assessment procedure for obesity-related metabolic consequences.

Keywords: Adolescents, anthropometry, children, frame size, growth percentiles, wrist circumferences

Introduction

Health professionals frequently use direct anthropometric measurements and indices derived from these measurements as tools to determine cardiometabolic risks and obesity. Definition of nutritional status, assessment of growth and development status, evaluation of differences in body proportions between populations, as well as contribution to diagnosis and treatment are some of these uses (1,2). Although waist circumference (WC) is the most frequently used index to assess nutritional, metabolic, and cardiovascular disorders, mid-upper arm, wrist, and neck circumferences may also be used (3,4).

Wrist circumference (WrC) is a new and possibly promising measurement to assess body frame size. A recent cross-

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sectional study suggests that WrC may also be associated with insulin resistance in obese children and adolescents (5). Most recently, in an 8.8-year follow-up study in a sample of 9330 adult Iranians, WrC was reported to be a significant predictor of diabetes in both genders (6). Although there is no established consensus on the routine use of WrC for clinical purposes as yet, there are studies suggesting that WrC may be a promising measurement.

The aim of this current study was to provide reference values for WrC and height/wrist ratio to assess body frame size in Turkish children and adolescents. To our knowledge, this will be the first study in the Turkish population on this issue. Additionally, producing frame size percentiles in this study would provide the assessment of WrC adjusted for height.

Methods

Study Design and Sampling

We used the data of the "Determination of Anthropometric Measures of Turkish Children and Adolescents (DAMTCA II)" study, which was conducted during October 2007 and April 2008. The DAMTCA II study was performed in Kayseri province which has more than 1,200,000 inhabitants and which is a leading industrial and trade center in Turkey. A total of 4330 observations were recorded (1931 boys, 2399 girls). The sampling design of the study was cross-sectional in which multi-stage probability sampling was performed (4).

Of the 708 schools in Kayseri, 17 primary, secondary, and high schools were randomly selected to recruit children and adolescents aged 6-17 years. Chronological age was calculated as the decimal age by subtracting the observation date from the birth date. Each year elapsed from their birthdates was noted as one age (e.g. 7.00-7.99 is accepted as 7 years old). The study protocol was approved by the Ethics Committee of Erciyes University and by the administration of the local educational authority (approval number: 2008/28, date: 08.01.2008). Parents' written consent was obtained prior to the study, and the procedures were in accordance with those outlined by the Declaration of Helsinki.

Data Collection and Questionnaire

Anthropometric indices: All measurements were performed twice by well-trained health professionals and the arithmetic mean was recorded for evaluation.

Wrist circumference: WrC was measured with children/adolescents in a seated position using a tension-gated tape measure positioned over the Lister tubercle of the distal radius and over the distal ulna. The Lister tubercle, a dorsal tubercle of the radius, can be easily palpated at the dorsal aspect of the radius around the level of the ulna head, about 1 cm proximal to the radiocarpal joint space. A tension-gated tape measure was used to ensure equivalent tape pressure between subjects (5).

Height: Height was measured with a portable Seca stadiometer sensitive to changes up to 1 cm. Daily calibration was performed to the portable devices. Measurements were done with subjects barefoot, the heels, hips, and shoulders touching the stadiometer, and the head in neutral position with eyes gazing forward. **Grant index:** Height (cm)/WrC (cm) (skeletal value) (1,7).

Questionnaire: The survey was based on a questionnaire sent home prior to evaluation and collection of anthropometric data from participating children/parents and adolescents.

Statistical Analysis

Outliers were examined first by checking the discontinuities in age-related WrC z scores plot, also $z < -10$ standard deviation scores (SDS), and $z > 6$ SDS liberal cut-off values were applied for automatic outlier detection (8). The remaining 4330 observations (1931 boys, 2399 girls) were randomly split into training and test sets (70%, 30%). The training set was used to fit the models (for each distribution and each gender), and the test set was applied to avoid overfitting, validate models, and to choose the convenient distribution.

Generalized additive models for location scale and shape (GAMLSS) were used to fit the age-related WrC model (9). Maximum penalized likelihood estimation was used to fit the model using Rigby and Stasinopoulos algorithm and Fisher scoring procedure. Box-Cox power exponential (BCPE), Box-Cox t (BCT), and Box-Cox Cole and Green (BCCG) distributions (distributions of LMS, LMST, and LMSP methods, respectively) were used to fit models, and cubic splines were used as smoothing functions. Each gender was modelled separately. The GAMLSS package (version 4.1-1) of R 2.14.0 program (www.r-project.org) was employed to fit our data.

For BCPE distribution modeling of boys, we followed the three-step optimization procedure of Rigby and Stasinopoulos (10) and the Generalized Akaike Information Criterion (GAIC#3) for model selection. In the first step, identity link functions for μ and ν, log link functions for σ and τ were chosen. An initial age transformation was chosen as $x = age^{\lambda=2.5}$ after a grid search of λ between -3 to 3 in steps of 0.5. In the second step, initial degrees of freedom

of all four parameters were set to 1, and values of df (μ), λ, and df (σ) were optimized, respectively. For df (μ) and df (σ), we made a search between 1 to 20 in steps of 1 and for λ between 2 to 3 in steps of 0.01. Starting model led to BCPE [3,1, df (ν), df (τ), 2.53] and fitted to all combinations of df (ν) and df (τ) ranging between 0 to 9 and 0 to 4 in steps of 1, respectively. In the last step of the procedure, fine tuning was used for the model BCPE (3, 1, 1, 0, 2.53) with changing values of df (σ), df (μ), df (ν), df (τ), and λ. We obtained BCPE (2.9, 1.0, 0.9, 0.2, 2.51) as the final model.

The same procedure was performed for girls and for BCT distribution models. For BCCG distribution, similar but a little different procedure was performed because of the absence of τ parameter. The difference is that values of df (μ) and λ were optimized first and combinations of df (σ) and df (ν) were searched next in a second step for same link functions (for μ, σ and ν). Finally, BCPE (2.9, 1.0, 0.9, 0.2, 2.51), BCT (2.8, 1.0, 0.9, 0.0, 2.20), and BCCG (2.9, 1.0, 1.0, 2.30) models for boys and BCPE (3.6, 1.1, 1.0, 0.6, 1.55), BCT (8.4, 1.0, 1.0, 0.1, 0.80), and BCCG (9.1, 0.8, 1.8, 1.20) for girls were obtained as optimum models for LMSP, LMST, and LMS methods, respectively. Based on the minimum GAIC#3 values, we decided to fit the LMSP method to construct percentile curves for both boys and girls.

A similar procedure was applied to model age-related frame size percentiles. Optimum models were obtained as BCPE (1.40, 4, 1, 1) for boys and BCCG (-0.75, 7, 1, 1) for girls. Differences between both genders in each age were assessed by independent samples t-test. Two-tailed p-values of < 0.05 were considered statistically significant.

Results

Table 1 shows the mean and percentile values for WrC in 6-17-year-old Turkish boys and girls. For both genders, WrC linearly increases with age (13.0 to 16.8 cm for boys and 12.5 to15.5 cm for girls, respectively for 6 and 17 years). In boys and girls, the mean ± standard deviation of WrC is 13.00 ± 0.89 cm and 12.48 ± 0.93 cm (6 years) and increases to 16.83 ± 1.16 and 15.58 ± 0.86 cm (17 years), respectively. The WrC values in all age groups were higher in boys when compared with girls. In boys, the 50th percentile ranged from 12.92 cm (at age 6) to 16.85 cm (at age 17) and in girls, the 50th percentile ranged from 12.41 cm (at age 6) to 15.54 cm at age 17.

Figure 1 shows the fitted percentile curves of frame size (height/WrC) and WrC (3rd, 5th, 10th, 15th, 25th, 50th, 75th, 85th, 90th, 95th, 97th) of related models for 6-17-year-old boys and girls. WrC steadily increases until 17 years in boys, while it makes plateau after age 14 years in girls.

Table 2 shows the percentile values for frame size (height/WrC) of 6-17-year-old Turkish boys and girls. In Table 3, the age- and gender-related frame size is shown according to its small, medium, and large percentiles. The three-frame size

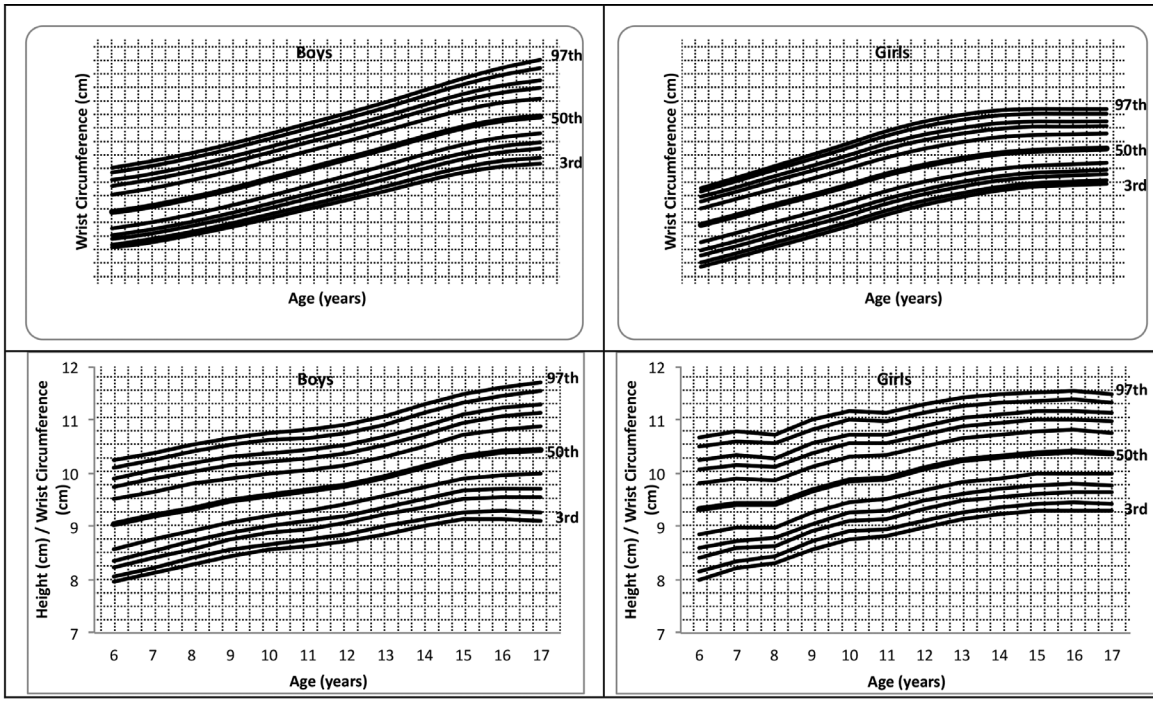

Figure 1. The distribution of wrist circumference and frame size in boys and girls aged 6-17 years with fitted percentile curves

Table 1. Age-related wrist circumference (cm) percentiles of 6-17-year-old Turkish boys and girls

Age (years)		Percentiles										
		3rd	5th	10th	15th	25th	50th	75th	85th	90th	95th	97th
Boys	**n**											
6	127	11.47	11.60	11.82	11.99	12.27	12.92	13.65	14.01	14.23	14.56	14.75
7	174	11.70	11.84	12.06	12.23	12.52	13.18	13.91	14.27	14.50	14.82	15.02
8		11.99	12.12	12.36	12.53	12.83	13.50	14.23	14.59	14.82	15.14	15.34
185												
9	156	12.31	12.46	12.70	12.88	13.19	13.86	14.59	14.95	15.18	15.51	15.71
10	179	12.67	12.83	13.08	13.27	13.58	14.27	15.00	15.36	15.59	15.92	16.12
11	168	13.05	13.21	13.48	13.68	14.00	14.69	15.42	15.78	16.01	16.34	16.55
12	126	13.42	13.59	13.88	14.08	14.41	15.11	15.83	16.19	16.43	16.76	16.97
13	139	13.79	13.98	14.28	14.50	14.84	15.54	16.26	16.62	16.86	17.20	17.42
14	153	14.19	14.39	14.71	14.94	15.29	15.99	16.70	17.08	17.32	17.68	17.91
15	223	14.55	14.76	15.10	15.34	15.69	16.39	17.11	17.50	17.76	18.15	18.40
16	217	14.80	15.03	15.38	15.63	15.99	16.69	17.41	17.82	18.11	18.54	18.83
17	84	14.92	15.16	15.54	15.79	16.16	16.85	17.58	18.02	18.33	18.83	19.17
Girls	**n**											
6	135	10.70	10.87	11.16	11.37	11.70	12.41	13.08	13.37	13.55	13.78	13.91
7	175	11.09	11.26	11.55	11.76	12.09	12.80	13.48	13.78	13.96	14.21	14.35
8	191	11.51	11.68	11.97	12.17	12.51	13.21	13.89	14.21	14.40	14.66	14.81
9	162	11.92	12.09	12.38	12.58	12.91	13.60	14.29	14.62	14.82	15.10	15.27
10	193	12.35	12.52	12.80	13.01	13.33	14.02	14.72	15.05	15.26	15.56	15.74
11	136	12.81	12.98	13.26	13.46	13.78	14.46	15.15	15.50	15.72	16.04	16.23
12	165	13.21	13.38	13.65	13.84	14.16	14.82	15.51	15.86	16.09	16.42	16.62
13	167	13.53	13.69	13.96	14.15	14.45	15.10	15.78	16.13	16.36	16.69	16.90
14	150	13.80	13.95	14.21	14.39	14.69	15.32	15.99	16.33	16.56	16.88	17.09
15	379	13.97	14.12	14.36	14.54	14.83	15.44	16.09	16.43	16.64	16.96	17.15
16	413	14.04	14.18	14.43	14.60	14.89	15.49	16.12	16.43	16.64	16.96	17.15
17	133	14.09	14.24	14.49	14.67	14.95	15.54	16.15	16.46	16.65	16.96	17.15

Age indicates whole age group (e.g. 7.00-7.99 years, etc.)

categories are determined according to certain percentiles: small ≤15th percentile, medium >15th percentile and <85th percentile, and large ≥85th percentile) (1). The increment in mean frame size from 6 years to 17 years was 1.26 cm in boys and 0.85 cm in girls. The change in WrC distribution through 6 to 17 years for each gender is shown in Figure 2 to demonstrate the difference between each gender and age-related increment. In Figures 3A and 3B, we compared the 50th WrC percentiles in the available three different studies to reveal geographic differences (2,11).

Discussion

Body circumferences are recommended for use in the clinical evaluation of nutritional and cardiometabolic disorders as a measure of compartmental body fat content or as an index of body fat distribution. As a novel clinical measure, we aimed to design a frame to calculate percentiles of WrC and also indices in which WrC is an important determinant to assess body frame. We found that WrC was higher in boys than in girls aged 6-17 years. The gender difference is observed to be about 0.5-1.0 cm from 6 to 12 years. Later, through 12 to 18 years, the gender difference gradually increases to 1.0-2.0 cm in favor of boys. (Table 1, Figure 2). Collinearity of WrC and WC is the most promising character of WrC, since it can be measured very easily. Additionally, WrC measurement is free from distracting factors, such as respiration and abdominal distension that can alter the reproducibility of WC.

In obese Italian children, hyperinsulinemia-related increase in free insulin-like growth factor-1 (IGF-1) levels was considered to enhance bone development and consequently lead to increase in WrC (5). Thus, WrC measurements were suggested to contribute to the assessment of

Table 2. Age-related frame size [height (cm)/wrist circumference (cm)] percentiles of 6-17-year-old Turkish boys and girls

Age (years)	Percentiles										
	3rd	5th	10th	15th	25th	50th	75th	85th	90th	95th	97th
Boys											
6	7.95	8.05	8.23	8.35	8.57	9.03	9.51	9.74	9.89	10.10	10.23
7	8.11	8.22	8.40	8.53	8.74	9.19	9.65	9.89	10.04	10.25	10.38
8	8.28	8.40	8.58	8.71	8.91	9.34	9.79	10.02	10.17	10.40	10.54
9	8.44	8.55	8.74	8.87	9.08	9.49	9.91	10.14	10.30	10.52	10.67
10	8.55	8.67	8.87	9.00	9.20	9.59	10.00	10.22	10.38	10.61	10.76
11	8.63	8.76	8.96	9.09	9.29	9.67	10.06	10.28	10.43	10.66	10.82
12	8.73	8.86	9.07	9.21	9.41	9.78	10.16	10.38	10.53	10.76	10.92
13	8.86	9.00	9.22	9.36	9.57	9.94	10.32	10.54	10.69	10.92	11.08
14	9.00	9.15	9.37	9.52	9.73	10.12	10.51	10.73	10.89	11.12	11.28
15	9.12	9.27	9.51	9.67	9.89	10.30	10.71	10.94	11.09	11.33	11.49
16	9.14	9.30	9.55	9.72	9.96	10.39	10.82	11.06	11.22	11.45	11.61
17	9.10	9.27	9.54	9.72	9.98	10.43	10.88	11.12	11.29	11.53	11.69
Girls											
6	7.99	8.15	8.41	8.59	8.84	9.32	9.81	10.07	10.24	10.50	10.67
7	8.22	8.36	8.59	8.74	8.98	9.43	9.89	10.15	10.33	10.60	10.78
8	8.30	8.43	8.63	8.78	8.99	9.42	9.86	10.12	10.29	10.56	10.73
9	8.58	8.71	8.91	9.05	9.27	9.69	10.13	10.38	10.55	10.82	10.99
10	8.77	8.90	9.11	9.25	9.46	9.88	10.31	10.56	10.73	10.99	11.16
11	8.82	8.95	9.15	9.29	9.51	9.91	10.34	10.57	10.73	10.98	11.14
12	8.99	9.12	9.33	9.47	9.68	10.08	10.50	10.73	10.89	11.13	11.29
13	9.14	9.27	9.48	9.62	9.83	10.23	10.65	10.87	11.03	11.26	11.42
14	9.22	9.35	9.56	9.70	9.91	10.31	10.72	10.94	11.09	11.32	11.47
15	9.28	9.42	9.62	9.77	9.98	10.37	10.78	11.00	11.15	11.37	11.52
16	9.31	9.45	9.65	9.79	10.00	10.40	10.80	11.01	11.16	11.38	11.53
17	9.30	9.43	9.63	9.77	9.98	10.37	10.76	10.97	11.12	11.33	11.47

hyperinsulinemic obesity, in addition to calculating frame size (frame size = height/WrC). The initial recommendation for WrC as a risk factor for metabolic disorders in adult population depends on a recent prospective study (6), although the first study in adolescents for WrC references was conducted in Bolivian 12-18-year-old adolescents (2). This present study is the third study on WrC and has been conducted on a large sample size with a broad age spectrum.

According to an adult study in the USA and Canada, there is a statistically significant positive association between WrC and diabetes (odds ratio = 1.3, $p = 0.006$). In this study, diabetic women also had larger neck, bust, and wrist circumferences, when adjusted for age and body mass index (12). In another pilot study, non-diabetic Italian adolescent athletes with a positive family history demonstrated statistically significant higher WrC values when compared to athletes with a negative family history and the difference was more significant in males (13). We consider that our WrC data can be used to investigate the possibility of a similar association of cardiometabolic risk in our children and adolescents.

As mentioned above, WrC measurement can be applied easily and has relatively low measurement errors. It is a simple, easy-to-detect anthropometric index, and it is not subject to measurement problems due to estimation of precise anatomic definition or to effects such as respiratory movements in WC measurements (14).

Another contribution of this present study is producing frame size as well as WrC references. Frame size classifies the skeletal structure as small, medium, and large, a characteristic that provides information in assessment of body composition. Fat mass, fat-free mass, and bone mass compositions are used in assessment of cardiometabolic risk. Frame size may contribute significantly to assess the

Table 3. The frame size categories [height (cm)/wrist circumference (cm)] for each gender

Age (years)	Boys						Girls					
	Mean (SD)	Small	n	Medium	n	Large	Mean (SD)	Small	n	Medium	n	Large	n
6	9.18 (0.63)*	≤8.41	19	8.41 > and < 9.85	89	≥9.85	19	9.37 (0.70)	≤8.65	12	8.65 > and < 10.15	60	≥10.15	12
7	9.28 (0.65)*	≤8.52	26	8.52 > and < 10.04	121	≥10.04	27	9.49 (0.67)	≤8.77	26	8.77 > and < 10.17	123	≥10.17	26
8	9.42 (0.63)	≤8.74	27	8.74 > and < 10.16	131	≥10.16	27	9.50 (0.62)	≤8.91	28	8.91 > and < 10.15	134	≥10.15	29
9	9.56 (0.59)*	≤8.94	21	8.94 > and < 10.23	112	≥10.23	23	9.91 (0.69)	≤9.14	24	9.14 > and < 10.67	113	≥10.67	25
10	9.69 (0.59)*	≤9.05	27	9.05 > and < 10.30	126	≥10.30	26	9.86 (0.65)	≤9.19	29	9.19 > and < 10.60	135	≥10.60	29
11	9.65 (0.54)*	≤9.09	25	9.09 > and < 10.21	118	≥10.21	25	10.02 (0.65)	≤9.34	21	9.34 > and < 10.71	95	≥10.71	20
12	9.91 (0.59)*	≤9.29	19	9.29 > and < 10.49	88	≥10.49	19	10.23 (0.55)	≤9.60	24	9.60 > and < 10.83	117	≥10.83	24
13	9.98 (0.54)*	≤9.45	22	9.45 > and < 10.54	96	≥10.54	21	10.27 (0.56)	≤9.67	25	9.67 > and < 10.86	117	≥10.86	25
14	10.28 (0.60)	≤9.65	23	9.65 > and < 10.92	107	≥10.92	23	10.30 (0.65)	≤9.63	22	9.63 > and < 11.03	106	≥11.03	22
15	10.37 (0.64)	≤9.70	33	9.70 > and < 11.03	157	≥11.03	33	10.42 (0.59)	≤9.76	58	9.76 > and < 11.03	262	≥11.03	59
16	10.37 (0.69)	≤9.66	32	9.66 > and < 11.07	153	≥11.07	32	10.40 (0.57)	≤9.82	62	9.82 > and < 11.03	289	≥11.03	62
17	10.43 (0.73)	≤9.67	12	9.67 > and < 11.19	60	≥11.19	12	10.32 (0.58)	≤9.65	20	9.65 > and < 10.94	289	≥10.94	62

Frame size categories: Small ≤15th percentile, medium > 15th percentile and < 85th percentile, and large ≥85th percentile

*In comparison between genders statistically significant mean frame sizes, p < 0.05

SD: standard deviation

Figure 2. The change in wrist circumference between each gender through 6-17 years (mean ± 2 standard deviation)

Figure 3A. Comparison of 50th percentiles of boys Wrist circumferences in Determination of Anthropometric Measures of Turkish Children and Adolescents-II, childhood and adolescence surveillance and prevention of adult non-communicable disease-IV, and Multi-Ethnic Study of Atherosclerosis studies

DAMTCAII: Determination of Anthropometric Measures of Turkish Children and Adolescents, CASPIAN-IV: childhood and adolescence surveillance and prevention of adult non-communicable disease, MESA: Multi-Ethnic Study of Atherosclerosi

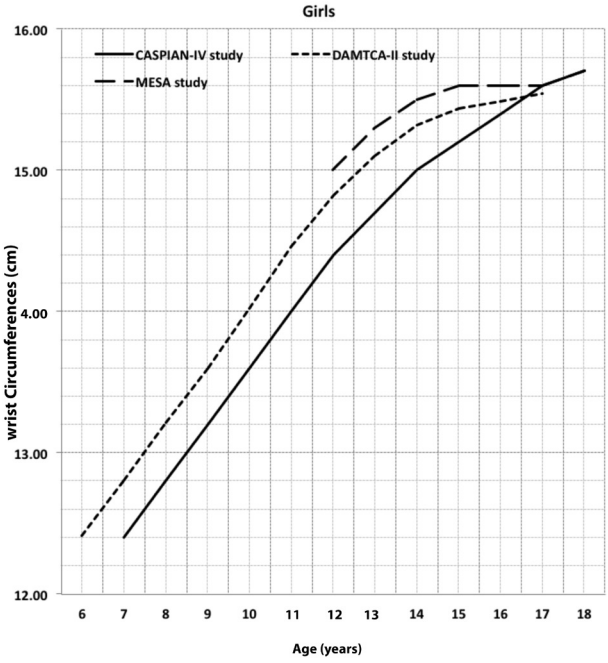

Figure 3B. Comparison of 50th percentiles of girls Wrist circumferences in Determination of Anthropometric Measures of Turkish Children and Adolescents-II, childhood and adolescence surveillance and prevention of adult non-communicable disease-IV, and Multi-Ethnic Study of Atherosclerosis studies

DAMTCAII: Determination of Anthropometric Measures of Turkish Children and Adolescents, CASPIAN-IV: childhood and adolescence surveillance and prevention of adult non-communicable disease, MESA: Multi-Ethnic Study of Atherosclerosis

level cardiometabolic risk since individuals with similar body fat content or body fat distribution may have different risk levels according to their frame size (15). The calculated cut-offs for frame size in the current study may then be used in assessment of cardiometabolic risk or obesity for both genders in children and adolescents. WrC is defined as an inexpensive, non-invasive, safe, inner-, inter-observable valid measure (16). Additionally, WrC is a measurement to predict insulin resistance (16). Finally, to the best of our knowledge, our study has been conducted on the largest sample size to date.

Study Limitations

On the other hand, the cross sectional study design and relatively underrepresenting total country population may be the limitations of this present study.

Conclusion

In conclusion, we may consider that WrC is a well-known anthropometric, however a novel clinical measure which can also be used to assess body frame size that may reflect cardiometabolic risk factors. This is the first comprehensive study, providing both WrC and frame size percentiles in Turkish children and adolescents. In short, WrC measurement is an easy to apply method with a relatively low risk of faulty measurement. However, studies on its use and applicability as a reference or risk factor are limited. Finally, WrC measurements and estimation of frame size have also the potential to be used in ergonomics for product design, human-machine harmony, and for analysis of physical environment for health.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Erciyes University and by the administration of the local educational authority (approval number: 2008/28, date: 08.01.2008).

Informed Consent: Parents' written consent was obtained prior to the study, and the procedures were in accordance with those outlined by the Declaration of Helsinki.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Selim Kurtoğlu, Concept: Selim Kurtoğlu, Ahmet Öztürk, Gökmen Zararsız, Design: Ahmet Öztürk, M. Mümtaz Mazıcıoğlu, Data Collection or Processing: Betül Çiçek, Ahmet Öztürk, Gökmen Zararsız, Analysis or Interpretation: Ahmet Öztürk, Gökmen Zararsız, Literature Search: Selim Kurtoğlu, Ahmet Öztürk, M. Mümtaz Mazıcıoğlu, Writing: Ahmet Öztürk, M. Mümtaz Mazıcıoğlu, Gökmen Zararsız.

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References

1. Bowers LJ. Assessment of nutritional status. In: Mootz RD, Vernon HT, (eds). Best Practices in Clinical Chiropractic. ASPEN Publication, USA, 1999:70.
2. Baya Botti A, Pėrez-Cueto FJ, Vasquez Monllor PA, Kolsteren PW. Anthropometry of height, weight, arm, wrist, abdominal circumference and body mass index, for Bolivian adolescents 12 to 18 years-Bolivian adolescent percentile values from the MESA study. Nutr Hosp 2009;24:304-311.
3. Mazıcıoğlu MM, Hatipoğlu N, Oztürk A, Çiçek B, Ustünbaş HB, Kurtoğlu S. Waist circumference and mid-upper arm circumference in evaluation of obesity in children aged between 6 and 17 years. J Clin Res Pediatr Endocrinol 2010;2:144-150. Epub 2010 Dec 10
4. Mazicioglu MM, Kurtoglu S, Ozturk A, Hatipoglu N, Cicek B, Ustunbas HB. Percentiles and mean values for neck circumference in Turkish children aged 6-18 years. Acta Paediatrica 2010;99:1847-1853.
5. Capizzi M, Leto G, Petrone A, Zampetti S, Papa RE, Osimani M, Spoletini M, Lenzi A, Osborn J, Mastantuono M, Vania A, Buzzetti R. Wrist circumference is a clinical marker of insulin resistance in overweight and obese children and adolescents. Circulation 2011;123:1757-1762. Epub 2011 Apr 11

6. Jahangiri Noudeh Y, Hadaegh F, Vatankhah N, Momenan AA, Saadat N, Khalili D, Azizi F. Wrist circumference as a novel predictor of diabetes and prediabetes: results of cross-sectional and 8.8-year follow-up studies. J Clin Endocrinol Metab 2013;98:777-784. Epub 2013 Jan 22
7. Lagua RT, Claudio VS. Nutrition and Diet Therapy Reference Dictionary. 4th ed. New-York, Chapman & Hall, USA, Appendix, 1996;21:415.
8. van Buuren S, Hayes DJ, Stasinopoulos DM, Rigby RA, ter Kuile FO, Terlouw DJ. Estimating regional centile curves from mixed data sources and countries. Stat Med 2009;28:2891-2911.
9. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape. Appl Statis 2005;54:507-554.
10. Rigby RA, Stasinopoulos DM. Smooth centile curves for skew and kurtotic data modelled using the Box-Cox power exponential distribution. Stat Med 2004;23:3053-3076.
11. Kelishadi R, Qorbani M, Hosseini M, Bahreynian M, Djalalinia S, Motlagh ME, Ardalan G, Khoramdad M, Mansourian M, Asayesh H, Heshmat R. Percentiles for anthropometric measures in Iranian children and adolescents: the CASPIAN-IV study. J Pediatr Endocrinol Metab 2016;29:1069-1076.
12. Freedman DS, Rimm AA. The relation of body fat distribution, as assessed by six girth measurements, to diabetes mellitus in women. Am J Public Health 1989;79:715-720.
13. Pomara F, Grosso F, Basile D, Polizzi V, Marcianó C, Adamo V, De Vita A, Petrucci M. A pilot study on adolescents of both sexes. Correlation between phenotype, athletic performances and family history to type 2 diabetes. Minerva Pediatr 2010;62:425-430.
14. Wills SD, Bhopal RS. The challenges of accurate waist and hip measurement over clothing: Pilot data. Obes Res Clin Pract 2010;4:163-246.
15. Chumlea WC, Wisemandle W, Guo SS, Siervogel RM. Relations between frame size and body composition and bone mineral status. Am J Clin Nutr 2002;75:1012-1016.
16. Campagna G, Zampetti S, Gallozzi A, Giansanti S, Chiesa C, Pacifico L, Buzzetti R. Excellent Intra and Inter-Observer Reproducibility of Wrist Circumference Measurements in Obese Children and Adolescents. PLoS One 2016;11:e0156646.

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Serum Thyroid-Stimulating Hormone Levels and Body Mass Index Percentiles in Children with Primary Hypothyroidism on Levothyroxine Replacement

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What is already known on this topic?

There have been studies in adults that show that higher thyroid-stimulating hormone (TSH) levels are associated with higher body mass index (BMIs). No studies done in children.

What this study adds?

It raises the question on whether we should treat pediatric patients with hypothyroidism aiming to keep TSH in the lower end of normal in an effort to improve their BMI.

Abstract

Objective: To determine the association, if any, between thyroid-stimulating hormone (TSH) levels and body mass index (BMI) percentiles in children with primary hypothyroidism who are chemically euthyroid and on treatment with levothyroxine.
Methods: This retrospective cross-sectional study consisted of a review of medical records from RUSH Medical Center and Stroger Hospital, Chicago, USA of children with primary hypothyroidism who were seen in the clinic from 2008 to 2014 and who were chemically euthyroid and on treatment with levothyroxine for at least 6 months. The patients were divided into two groups based on their TSH levels (0.34-<2.5 mIU/L and ≥2.5-5.6 mIU/L). The data were analyzed by Spearman rank correlation, linear regression, cross tabulation and chi-square, Mann-Whitney U test, and Kruskal-Wallis test.
Results: One hundred and forty-six children were included, of which 26% were obese (BMI ≥95%), 21.9% overweight (BMI ≥85-<95%), and 52.1% of a healthy weight (BMI ≥5-<85%). There was a significant positive correlation between TSH and BMI percentiles ($r = 0.274$, $p = 0.001$) and a significant negative correlation between TSH and serum free T4 ($r = -0.259$, $p = 0.002$). In the lower TSH group, 68.4% of the children had a healthy weight, while the percentage of obese children was 60.5% in the upper TSH group ($p = 0.012$).
Conclusion: In children diagnosed with primary hypothyroidism who are chemically euthyroid on treatment with levothyroxine, there is a positive association between higher TSH levels and higher BMI percentiles. However, it is difficult to establish if the higher TSH levels are a direct cause or a consequence of the obesity. Further studies are needed to establish causation beyond significant association.
Keywords: Thyroid-stimulating hormone, hypothyroidism, pediatric, children, obesity, body mass index, euthyroid

Introduction

Overt hypothyroidism may be associated with changes in energy expenditure and gain in body weight. Most patients stop gaining or lose weight after beginning treatment with levothyroxine and restoration of a biochemically euthyroid state. Weight gain in hypothyroid patients is related partly to myxedema, but this fluid frequently disappears after one to four weeks of treatment with levothyroxine (1,2).

On the other hand, serum thyroid-stimulating hormone

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(TSH) levels are often elevated in obese children and adults (3,4,5,6,7,8,9,10,11). The mechanism underlying this finding is not yet fully understood. The hyperthyrotropinemia appears to be a result rather than the root of the obesity, but the question as to whether high TSH levels contribute to difficulties with weight loss in obese patients remains unanswered.

TSH has wide normal ranges, but most laboratories use values of about 4.5 to 5.0 mIU/L as the upper limit of what is considered normal range. Approximately 95% of healthy Americans have serum TSH levels between 0.45 and 4.12 mIU/L. According to the Third National Health and Nutrition Examination Survey, for individuals who do not suffer from thyroid disease, the upper normal limit of serum TSH levels is 4.5 mIU/L (12).

Recent attention has focused on the potential relationship between minor abnormalities of thyroid function and changes in body weight in euthyroid subjects. To investigate whether there is a relationship between serum TSH levels and body mass index (BMI) percentiles in children with primary hypothyroidism who are chemically euthyroid and on treatment with levothyroxine, we retrospectively studied the charts of 146 children.

Methods

We conducted a retrospective, cross-sectional study that consisted of chart reviews of all cases from 2008 to 2014 of children aged between 6 months and 21 years with a diagnosis of primary hypothyroidism who were on treatment with levothyroxine for at least 6 months and chemically euthyroid. The study population consisted of patients of RUSH University Medical Center (RUMC) and Stroger Hospital (SH) Pediatric Endocrinology Outpatient Clinics (Chicago, IL, USA). One patient who was on nature-thyroid therapy (animal extract thyroid preparation) was also included. Subjects were identified using International Classification of Disease-9 codes. We analyzed the correlation between serum TSH levels and BMI percentiles.

Children with other endocrinopathies or other medical conditions that may affect weight, such as diabetes mellitus, celiac disease and pregnancy, those with abnormal TSH levels, and those who were on any medication that may alter serum TSH levels were excluded. We also excluded eight patients whose BMI and weight for length (W/L) percentiles were less than the 5%.

We enrolled 146 patients with primary hypothyroidism who were chemically euthyroid and met the inclusion criteria. Our study was approved by the Institutional Review Board of the RUMC and SH (approval number: 14062603-IRB01).

Anthropometric Measurements

Growth and thyroid function were assessed at each clinic visit every three months or bi-annually. Weight was measured with the subject wearing light clothing with his or her shoes off. Height was measured using a wall-mounted, fixed Harpender stadiometer. Recumbent length was measured using an infantometer in children who were two years of age or younger. BMI was calculated by dividing weight (kg) by height square (meter square) and plotted on the age- and gender-specific Centers for Disease Control (CDC) and National Center for Health Statistics BMI growth charts to obtain a percentile ranking. Weight, height, BMI, and W/L percentiles were calculated using CDC charts for girls and boys aged 2 to 20 years and <36 months. In children aged 2 to 18 years, obesity was defined as a BMI ≥95th percentile for age and gender. For this age group, overweight was defined as a BMI percentile of ≥85%-<95%, and healthy weight as a BMI percentile of ≥5%-<85% (13,14). In children aged less than 2 years, it is appropriate to use weight for recumbent length (W/L) percentile to evaluate their weight relative to linear growth (15,16). The term "overweight", rather than "obese" is used to describe these young children. For this population, overweight is defined as a W/L percentile of ≥95th.

Euthyroid status was defined as having a TSH level of 0.35-4.94 mIU/L at RUMC and 0.34-5.60 mIU/L at SH regardless of free T4 levels. The normal range of free T4 is 0.7-1.5 ng/dL at RUMC and 0.58-1.64 ng/dL at SH. Serum TSH and free T4 at RUMC were determined by two-site chemiluminescent enzyme micro particle immune assay (Architect, Abbott Diagnostics, U.S.). They were also determined at SH by two-site chemiluminescent enzyme microparticle immune assay (DXI, Beckman Coulter, U.S.).

To estimate the proportions of healthy weight, overweight, and obese patients within each TSH group, subjects were divided into two groups: those with a lower TSH (<2.5 mIU/L) and those with a higher TSH group (≥2.5 mIU/L). In addition, the subjects were divided according to category of BMI or W/L into healthy weight, overweight, and obese groups. Measurement obtained at the most recent visit were used in these calculations. Demographic (age, gender, and ethnicity) and clinical details were collected during routine, out-patient care.

Statistical Analysis

Data were recorded in Microsoft Excel 2010 and analyzed using SPSS version 18.0 software (SPSS Inc., U.S.). The correlations between various variables were assessed by the nonparametric Spearman rank correlation. The Mann-Whitney test was used for comparison between two groups, and the Kruskal-Wallis test was used for comparison among

larger groups. All reported p-values are two sided. A p-value ≤0.05 was considered as statistically significant.

Results

Anthropometric characteristics of the subjects are shown in Table 1. After exclusion of subjects who did not meet the inclusion criteria, a total of 327 participants were investigated, and 146 were eligible for these analyses. Forty-six boys (32%) and 100 girls (68%) with primary hypothyroidism who were chemically euthyroid were enrolled. Patients were divided according to their BMI or W/L percentiles (%). Thus 26% of the study group consisted of obese patients (≥95%), 21.9% of overweight patients (BMI ≥85 but less than 95%) and 52.1% of healthy weight patients (BMI ≥5% - < 85%).

The median [interquartile range (IQR)] of BMI and W/L percentiles were 82.25% (41.45%), while the median (IQR) of TSH levels was 2.11 mIU/L (2.15 mIU/L) (Table 1). The median TSH was 1.73 mIU/L for boys, while it was 2.35 mIU/L for girls, and the median BMI and W/L percentile values were 71.06 % in boys and 87.08 % in girls (Table 2). The correlation between serum TSH and BMI percentiles was strongly significant in girls ($r = 0.330$, $p = 0.001$), while in boys, this correlation was only marginally significant ($r = 0.250$, $p = 0.094$).

There was a significant positive correlation between serum TSH and BMI or W/L percentiles ($r = 0.274$, $p = 0.001$) and a significant negative correlation between BMI or W/L percentiles and free T4 ($r = -0.259$, $p = 0.002$).

A multivariate linear regression analysis for the association between serum TSH levels and different variables is shown in Table 3. In this analysis, we used BMI or W/L expressed in standard deviation scores (SDS) instead of percentiles. This analysis revealed that only BMI contributed significantly to the variance of TSH. This association was slightly attenuated after additional adjustments for age, gender, dose of levothyroxine, ethnicity, and body surface area (BSA) [$p = 0.013$, 95% confidence interval (CI) = 0.079-0.642]. A multivariate linear regression analysis for the association between serum free T4 and variables revealed that BMI, age, and ethnicity contributed significantly to the variance of serum free T4. In addition, the association between serum free T4 and BMI was slightly attenuated after adjustments for age, gender, dose of levothyroxine, ethnicity, and BSA ($p = 0.005$, 95% CI = -0.0.122- -0.022).

When subjects were divided according to category of TSH into two TSH groups (< than 2.5 mIU/L and ≥ than 2.5 mIU/L), the percentage of healthy weight subjects in the lower TSH group was 68.4%, while the percentage of obese subjects in the upper TSH group was 60.5% ($p = 0.012$) (Figure 1).

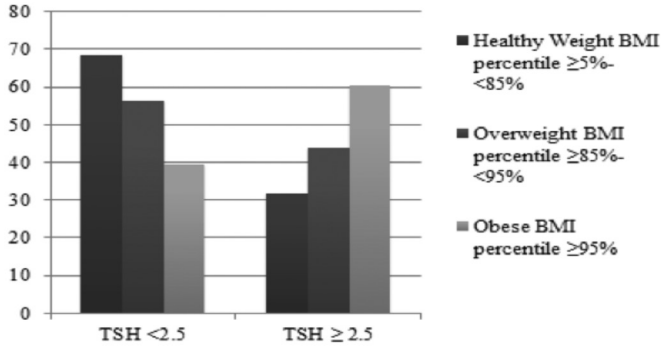

Figure 1. Body mass index percentiles according to thyroid-stimulating hormone (TSH) levels, TSH ≥ than 2.5 mIU/L and < than 2.5 mIU/L

BMI: body mass index, TSH: thyroid-stimulating hormone

Table 1. Anthropometric and hormonal parameters in the study subjects

	Mean ± SD	Median (IQR)	Minimum	Maximum
Age (years)	13.09 ± 6.64	15.27 (11.17)	0.56	20.89
Weight (kg)	55.80 ± 33.24	56.10 (48.31)	5.53	175.45
Weight percentile (%)	65.60 ± 33.10	75.49 (56.28)	0.14	100
Height or length (cm)	141.63 ± 33.88	156.25 (46.47)	61.50	193.00
Height percentile (%)	48.36 ± 33.58	45.82 (60.93)	0.10	100.00
BMI or weight/length percentiles (%)	71.28 ± 28.62	82.25 (41.45)	6.18	99.90
Serum free T4 (ng/dL)	1.09 ± 0.26	1.10 (0.30)	0.54	1.79
TSH (mIU/L)	2.39 ± 1.37	2.11 (2.15)	0.36	5.59
Dose of levothyroxine (mcg/kg/day)	1.82 ± 1.52	1.4040 (1.40)	0.19	15.09
Duration of therapy with LT4 (years)	3.89 ± 4.52	2.00 (3.88)	0.50	18.55

SD: standard deviation, IQR: interquartile range, BMI: body mass index, TSH: thyroid-stimulating hormone, T4: thyroxine, LT4: levothyroxine

Table 2. Comparison of median age, serum thyroid-stimulating hormone, serum fT4 levels, body mass index percentiles, and dose of LT4 between boys and girls

Median values	Boys	Girls	p
Age (years)	13.28	16.67	0.009
TSH (mIU/L)	1.73	2.35	0.090
Serum fT4 (ng/dL)	1.16	1.06	0.076
BMI or weight/length percentile %	71.06	87.08	0.464
LT4 dose (mcg/kg/day)	1.56	1.37	0.295

TSH: thyroid-stimulating hormone, BMI: body mass index, fT4: free thyroxine

Furthermore, when the subjects were divided according to category of BMI or W/L percentiles into healthy weight, overweight, and obese groups, there was a significant difference in the median serum TSH levels between these groups, which were 1.8 mIU/L in the healthy weight group, 2.3 mIU/L in the overweight group, and 3.2 mIU/L in the obese group (p = 0.006) (Figure 2). We also found a significant statistical difference in the median serum free T4 in these groups. It was 1.10 ng/dL in the heathy weight group, 1.10 ng/dL in the overweight group, and 0.96 ng/dL in the obese group (p = 0.027) (Figure 3).

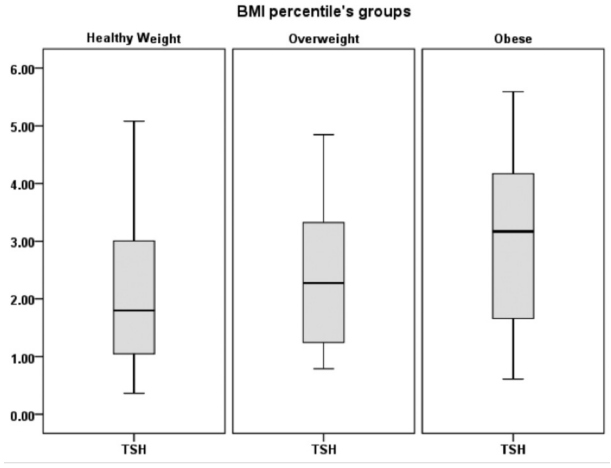

Figure 2. Serum thyroid-stimulating hormone (illustrated as median ± interquartile range) in 146 hypothyroid children divided into healthy weight body mass index (BMI) percentile ≥5- < 85 %, overweight BMI percentile ≥85- < 95 %, and obese BMI percentile ≥95 %

BMI: body mass index, TSH: thyroid-stimulating hormone

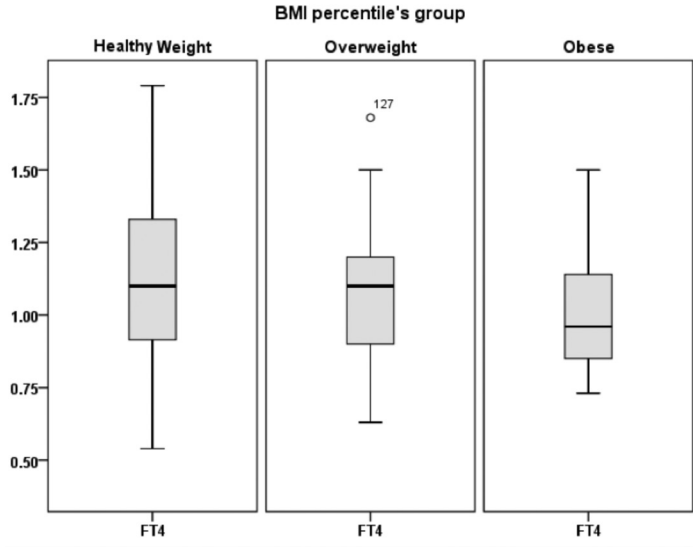

Figure 3. Serum free T4 (illustrated as median ± interquartile range) in 146 hypothyroid children divided into healthy weight body mass index (BMI) percentile ≥5- < 85 %, overweight BMI percentile ≥85- < 95 %, and obese BMI percentile ≥95

BMI: body mass index, fT4: free thyroxine

Table 3. Simple and multivariate linear regression analysis with serum thyroid-stimulating hormone as dependent variable

	Independent variable	Beta	p	95% CI
Module 1 (F, p) = (11.255, 0.001)	BMI or W/L SDS	0.269	0.001	(0.135-0.520)
Module 2 (F, p) = (5.059, 0.002)	BMI or W/L SDS	0.254	0.003	(0.110-0.507)
	Age (years)	0.042	0.616	(-0.026-0.043)
	Gender	0.124	0.082	(-0.053-0.891)
Module 3 (F, p) = (2.563, 0.022)	BMI or W/L SDS	0.295	0.013	(0.079-0.642)
	Age (years)	0.146	0.474	(-0.053-0.113)
	Gender	0.129	0.138	(-0.123-0.882)
	Dose of LT4 (mcg/kg/day)	-0.030	0.753	(-0.200-0.145)
	Ethnicity	0.015	0.855	(-0.518-0.624)
	BSA	-0.150	0.507	(-1.39-0.690)

CI: confidence interval, BMI: body mass index, W/L: weight/length, SDS: standard deviation score, BSA: body surface area, fT4: free thyroxine, LT4: levothyroxine

Discussion

In the present study of 146 children with primary hypothyroidism who were chemically euthyroid and on treatment with levothyroxine, we found an association between TSH, free T4, and BMI percentiles when we considered them as continuous variables. This association was also significant when we divided TSH into 2 categories. There was a significant difference in the proportion of healthy weight (BMI percentile ≥5- < 85 %) and obese subjects (BMI percentile ≥95 %) within the two TSH groups (p = 0.012). Linear regression revealed that BMI (expressed as SDS) contributed significantly to the variance of TSH. The high proportion of healthy weight patients within the lower TSH group as well as the patients with low serum TSH levels had low BMI values. Thus we venture to suggest that adjusting TSH to the lower end of normal range may be of benefit for weight reduction.

The association between serum TSH and BMI has been addressed by some researchers in both pediatric and adult patients, but the results are controversial. Most studies have been conducted in populations without thyroid disease, but several studies of these studies have reported a positive association between TSH and BMI, (3,4,5,6,7,8,9,10,11) and showed that even small changes in serum TSH levels can affect BMI. Some studies have shown a positive association between higher BMI and serum TSH level in euthyroid adults (17,18,19). Higher TSH levels may contribute to the difficulty of weight loss (5,20). Our patients with higher BMIs had higher TSH levels, although these were within the euthyroid range.

The mechanisms of the association between serum TSH and BMI have been proposed in an effort to explain the processes leading to high TSH levels in individuals with high BMI, including variation in the activity of peripheral deiodinases leading to possible changes in thyroid hormone action at the cellular level (21). Some studies have shown that thyroid function may vary during overfeeding or starvation. This may represent a physiologic adaptation, causing changes in metabolism and energy expenditure that may affect the ability to control weight gain during overfeeding and weight loss during starvation (1,13,20,22,23,24,25,26). Leptin has been proposed as a possible factor that causes higher TSH levels. Leptin regulates both prothyrotropin-releasing hormone (pro-TRH) synthesis and TRH expression (27). TSH can directly stimulate leptin secretion by adipocytes (28). Furthermore, the presence of TSH receptors in adipose tissue (29,30) causes proliferation of adipose tissue and differentiation of preadipocytes into adipocytes (31,32). Higher levels of leptin are proportional to overall fat percentage (33), and some studies have shown a positive correlation with TSH levels (34,35,36).

The high serum TSH levels in individuals with high BMI could be due to the decreased expression and down regulation of the TSH receptor gene, which is less expressed in obesity. Consequently, the plasma TSH increases to deal with peripheral hormone resistance (37). Furthermore, thyroxine controls the metabolic rate, and small changes in the LT4 dose in patients with long-term LT4 treatment have been shown to modify resting energy expenditure and weight changes (38). Our patients had been on levothyroxine replacement for at least 6 months, and this could also explain the association between TSH levels and BMI.

Study Limitations

A limitation of our study is that it was retrospective. The association between TSH and BMI and W/L percentiles could be affected by the presence of other modifying factors, such as different weight scales, diet, activity, emotional state, duration of treatment with levothyroxine, and compliance with medication.

Conclusion

A significant positive association was observed between the serum TSH concentration and BMI percentiles in hypothyroid children who are chemically euthyroid while on thyroid hormone replacement. In this population, whether variations in TSH and free T4 levels within the normal range can influence body weight or whether obesity per se can alter thyroid function cannot be stated at this time. We could speculate that in hypothyroid children who are on treatment with levothyroxine, aiming to keep the TSH in the lower half of the normal range will help them lose or maintain their weight. However, it is impossible to determine whether the higher TSH levels are the cause of the increased BMI or vice versa. Further studies are needed to assess the link between thyroid function and body weight.

We could associate the BMI and TSH levels with other parameters such as abdominal fat deposit measured by ultrasound scan, skin fold thickness, and waist and hip circumference, but since this study was retrospective, we did not have the data available.

Ethics

Ethics Committee Approval: The study was approved by RUSH University Medical Center and Stroger Hospital (approval number: 14062603-IRB01).

Informed Consent: Not needed as it was a retrospective study and no identifying information for patients was collected.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Carla Minutti, Design: Asma Shaoba, Carla Minutti, Data Collection or Processing: Asma Shaoba, Carla Minutti, Analysis or Interpretation: Asma Shaoba, Sanjib Basu, Literature Search: Asma Shaoba, Carla Minutti, Writing: Asma Shaoba, Carla Minutti, Sanjib Basu, Stelios Mantis.

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References

1. Kyle LH, Ball MF, Doolan PD. Effect of thyroid hormone on body composition in myxedema and obesity. N Engl J Med 1966;275:12-17.
2. Baron DN. Hypothyroidism; its aetiology and relation to hypometabolism, hypercholesterolaemia, and increase in body-weight. Lancet 1956;271:277-281.
3. Ghergherehchi R, Hazhir N. Thyroid hormonal status among children with obesity. Ther Adv Endocrinol Metab 2015;6:51-55.
4. Grandone A, Santoro N, Coppola F, Calabro P, Perrone L, Del Giudice EM. Thyroid function derangement and childhood obesity: an Italian experience. BMC Endocr Disord 2010;10:8.
5. Reinehr T. Thyroid function in the nutritionally obese child and adolescent. Curr Opin Pediatr 2011;23:415-420.
6. Stichel H, l'Allemand D, Grüters A. Thyroid function and obesity in children and adolescents. Horm Res 2000;54:14-19.
7. Bastemir M, Akin F, Alkis E, Kaptanoglu B. Obesity is associated with increased serum TSH level, independent of thyroid function. Swiss Med Wkly 2007;137:431-434.
8. Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, Jørgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab 2005;90:4019-4024. Epub 2005 May 3
9. Reinehr T, Isa A, de Sousa G, Dieffenbach R, Andler W. Thyroid hormones and their relation to weight status. Horm Res 2008;70:51-57. Epub 2008 May 21
10. Emokpae MA, Adeleke SI, Uwumarongie HO. Subclinical hypothyroidism in childhood obesity and its correlation with lipoproteins. Afr J Med Med Sci 2011;40:361-365.
11. Marras V, Casini MR, Pilia S, Carta D, Civolani P, Porcu M, Uccheddu AP, Loche S. Thyroid function in obese children and adolescents. Horm Res Paediatr 2010;73:193-197. Epub 2010 Mar 3
12. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87:489-499.
13. Krebs NF, Himes JH, Jacobson D, Nicklas TA, Guilday P, Styne D. Assessment of child and adolescent overweight and obesity. Pediatrics 2007;120(Suppl 4):193-228.
14. Koplan JP, Liverman CT, Kraak VI; Committee on Prevention of Obesity in Children and Youth. Preventing childhood obesity: health in the balance: executive summary. J Am Diet Assoc 2005;105:131-138.
15. Daniels SR, Hassink SG; Committee on Nutrition. The Role of the Pediatrician in Primary Prevention of Obesity. Pediatrics 2015;136:e275-292.
16. de Onis M, Onyango A, Borghi E, Siyam A, Blössner M, Lutter C; WHO Multicentre Growth Reference Study Group. Worldwide implementation of the WHOChild Growth Standards. Public Health Nutr 2012;15:1603-1610.
17. Bastemir M, Akin F, Alkis E, Kaptanoglu B. Obesity is associated with increased serum TSH level, independent of thyroid function. Swiss Med Wkly 2007;137:431-434.
18. Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, Jorgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab 2005;90:4019-4024. Epub 2005 May 3
19. Ambrosi B, Masserini B, Iorio L, Delnevo A, Malavazos AE, Morricone L, Sburlati LF, Orsi E. Relationship of thyroid function with body mass index and insulin-resistance in euthyroid obese subjects. J Endocrinol Invest 2010;33:640-643. Epub 2010 Mar 25
20. Reinehr T, de Sousa G, Andler W. Hyperthyrotropinemia in obese children is reversible after weight loss and is not related to lipids. J Clin Endocrinol Metab 2006;91:3088-3091. Epub 2006 May 9
21. Araujo RL, Carvalho DP. Bioenergetic impact of tissue-specific regulation of iodothyronine deiodinases during nutritional imbalance. J Bioenerg Biomembr 2011;43:59-65.
22. Danforth E Jr, Horton ES, O'Connell M, Sims EA, Burger AG, Ingbar SH, Braverman L, Vegenakis AG. Dietary-induced alterations in thyroid hormone metabolism during overnutrition. J Clin Invest 1979;64:1336-1347.
23. Portnay GI, O'Brian JT, Bush J, Vagenakis AG, Azizi F, Arky RA, Ingbar SH, BravermaN LE. The effect of starvation on the concentration and binding of thyroxine and triiodothyronine in serum and on the response to TRH. J Clin Endocrinol Metab 1974;39:191-194.
24. Onur S, Haas V, Bosy-Westphal A, Hauer M, Paul T, Nutzinger D, Klein H, Müller MJ. L-tri-iodothyronine is a major determinant of resting energy expenditure in underweight patients with anorexia nervosa and during weight gain. Eur J Endocrinol 2005;152:179-184.
25. Lawson EA, Klibanski A. Endocrine abnormalities in anorexia nervosa. Nat Clin Pract Endocrinol Metab 2008;4:407-414. Epub 2008 Jun 10
26. Reinehr T, Andler W. Thyroid hormones before and after weight loss in obesity. Arch Dis Child 2002;87:320-323.
27. Nillni EA, Vaslet C, Harris M, Hollenberg A, Bjorbak C, Flier JS. Leptin regulates prothyrotropin-releasing hormone biosynthesis. Evidence for direct and indirect pathways. J Biol Chem 2000;275:36124-36133.
28. Menendez C, Baldelli R, Camina JP, Escudero B, Peino R, Dieguez C, Casanueva FF. TSH stimulates leptin secretion by a direct effect on adipocytes. J Endocrinol 2003;176:7-12.
29. Crisp MS, Lane C, Halliwell M, Wynford-Thomas D, Ludgate M. Thyrotropin receptor transcripts in human adipose tissue. J Clin Endocrinol Metab 1997;82:2003-2005.
30. Bell A, Gagnon A, Grunder L, Parikh SJ, Smith TJ, Sorisky A. Functional TSH receptor in human abdominal preadipocytes and orbital fibroblasts. Am J Physiol Cell Physiol 2000;279:335-340.
31. Sorisky A, Bell A, Gagnon A. TSH receptor in adipose cells. Horm Metab Res 2000;32:468-474.
32. Valyasevi RW, Harteneck DA, Dutton CM, Bahn RS. Stimulation of adipogenesis, peroxisome proliferator-activated receptor-gamma (PPARgamma), and thyrotropin receptor by PPARgamma agonist in human orbital preadipocyte fibroblasts. J Clin Endocrinol Metab 2002;87:2352-2358.
33. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR,Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292-295.

34. Betry C, Challan-Belval MA, Bernard A, Charrie A, Drai J, Laville M, Thivolet C, Disse E. Increased TSH in obesity: Evidence for a BMI-independent association with leptin. Diabetes Metab 2015;41:248-251. Epub 2014 Dec 22

35. Iacobellis G, Ribaudo MC, Zappaterreno A, Iannucci CV, Leonetti F. Relationship of thyroid function with body mass index, leptin, insulin sensitivity and adiponectin in euthyroid obese women. Clin Endocrinol (Oxf) 2005;62:487-491.

36. Pinkney JH, Goodrick SJ, Katz J, Johnson AB, Lightman SL, Coppack SW, Mohamed-Ali V. Leptin and the pituitary-thyroid axis: a comparative study in lean, obese, hypothyroid and hyperthyroid subjects. Clin Endocrinol (Oxf) 1998;49:583-588.

37. Nannipieri M, Cecchetti F, Anselmino M, Camastra S, Niccolini P, Lamacchia M, Rossi M, Iervas, G, Ferrannini E. Expression of thyrotropin and thyroid hormone receptors in adipose tissue of patients with morbid obesity and/or type 2 diabetes: effects of weight loss. Int J Obes (Lond) 2009;33:1001-1006. Epub 2009 Jul 28

38. al-Adsani H, Hoffer LJ, Silva JE. Resting energy expenditure is sensitive to small dose changes in patients on chronic thyroid hormone replacement. J Clin Endocrinol Metab 1997;82:1118-1125.

ORIGINAL ARTICLE

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Is There an Association Between Cortisol and Hypertension in Overweight or Obese Children?

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What is already known on this topic?

It is known that obesity can lead to hypertension. However, the precise mechanisms behind the development of hypertension in overweight or obese children are not yet completely understood.

What this study adds?

There is evidence for an increased cortisol production rate with decreased renal 11β-hydroxysteroid dehydrogenase 2 activity and flattening of early-morning peak cortisol and cortisone in overweight or obese children. There is no evidence for a role for cortisol in hypertension-induced obesity.

Abstract

Objective: The precise mechanisms behind the development of hypertension in overweight or obese children are not yet completely understood. Alterations in hypothalamic-pituitary-adrenal axis activity may play a role. We aimed to investigate the association between cortisol parameters and hypertension in overweight or obese children.

Methods: Random urine (n = 180) and early-morning saliva samples (n = 126) for assessment of cortisol and cortisone were collected from 1) hypertensive overweight children (n = 50), 2) normotensive overweight children (n = 145), and 3) normotensive non-overweight children (n = 75).

Results: The age of participants was 10.4 ± 3.3 years and 53% were boys. The urinary cortisol-to-cortisone ratio [β 1.11, 95% confidence interval (CI) 1.05-1.19] as well as urinary cortisol/creatinine (β 1.38, 95% CI 1.09-1.54), and cortisone/creatinine ratios (β 1.26, 95% CI 1.17-1.36) were significantly higher in overweight or obese than in non-overweight children. After adjusting for body mass index-standard deviation score and urinary cortisone/creatinine ratio, but not cortisol/creatinine ratio, was significantly associated with presence of hypertension (β 1.12, 95% CI 1.02-1.23). Salivary cortisol and cortisone levels were significantly lower in overweight or obese than in non-overweight children (β -4.67, 95% CI -8.19- -1.15, and β 0.89, 95% CI 0.80-0.97 respectively). There were no significant differences in cortisol parameters between hypertensive and normotensive overweight or obese children.

Conclusion: This study provided further evidence for an increased cortisol production rate with decreased renal 11β-hydroxysteroid dehydrogenase 2 activity and flattening of early-morning peak cortisol and cortisone in overweight or obese children. However, there were no significant differences in cortisol parameters between hypertensive and normotensive overweight and obese children.

Keywords: Hypertension, obesity, children, cortisol, pathophysiology

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Introduction

As a result of the growing overweight and obesity epidemic, hypertension is increasingly common, even in childhood; 4–14% of overweight children and 11–33% of obese children are diagnosed with hypertension (1,2,3). Since both overweight and hypertension have a tendency to track from childhood into adulthood, these findings are of great concern (4,5).

Establishing the cause of hypertension in obese children is of utmost importance for the development of therapeutic strategies. However, the pathophysiology of hypertension in obesity is complex and not fully understood (6). Several studies suggest that alterations in the production and/or metabolism of glucocorticoids could play a role in the pathophysiology of the metabolic syndrome (7,8), given its phenotypic similarities with Cushing's syndrome (9,10). Glucocorticoids stimulate hepatic glucose production, lipolysis, vascular reactivity, and sodium reabsorption (11).

The tissue effects of glucocorticoids are for an important part regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes, which interconvert cortisol with its inert metabolite cortisone. There are two isozymes. Type 1 generates cortisol from cortisone and is expressed mainly in liver and adipose tissue, and type 2 catalyses the reverse reaction primarily in the kidney. Pharmacological inhibition of renal 11β-HSD2 activity, e.g. by heavy use of liquorice, leads to hypertension by exposure of renal mineralocorticoid receptors to excess cortisol concentrations. The role of 11β-HSD type 1 in blood pressure regulation and hypertension is less well understood (7,8,12).

There are few studies in children on 11β-HSD activity in obesity-induced hypertension (12,13,14). One case study of four 10-to-15-year-old hypertensive obese boys found excess urinary adrenal androgen and cortisol metabolites (13). Another study in children aged 14 to 15 years, that made comparisons between obese children with (n = 15) and without hypertension (n = 11), and normotensive normal-weight children (n = 15), found that the cortisol-to-cortisone ratio was higher in the hypertensive obese group than in the other two groups. Systolic blood pressure was positively associated with urinary tetrahydrocortisol (THF) + 5α-THF/THE (allo-THF/tetrahydrocortisone) ratio, indicative of a cortisol/cortisone shuttle that favours cortisol (12). Another study found that serum adrenocorticotropic hormone (ACTH) and cortisol levels were positively associated with blood pressure in obese children aged 4 to 18 years, yet there was no control group. These results suggest that the hypothalamic-pituitary-adrenal (HPA) axis is involved in the development of obesity-induced hypertension in children (14).

With the present study, we aimed to investigate if there is an association between cortisol parameters, including cortisol and cortisone in early-morning saliva and in random urine collections, and hypertension in overweight and obese children. To disentangle the contributions of hypertension and being overweight, we included two control groups, namely normotensive overweight and obese children and normotensive non-overweight children.

Methods

Population and Design

Non-fasting urine and early-morning fasting saliva samples were collected from a convenience sample of Dutch children aged 5 to 17 years, in the period between September 2013 and June 2015, consisting of: 1) n = 50 overweight and obese children with hypertension, 2) n = 145 overweight and obese children without hypertension, and 3) n = 75 non-overweight children without hypertension. In total, 36 children provided both urine and saliva samples, 141 children provided only urine samples, and 90 children only saliva samples.

Overweight and obese children with and without hypertension were recruited at a pediatric outpatient obesity clinic and through their participation in our study on the prevalence of hypertension in overweight and obese children (15). The control group of healthy non-overweight children was recruited at a general pediatric outpatient clinic, which they visited for various reasons, and at two schools. Children with conditions that might affect blood pressure, for example a history of urinary tract infections, were not eligible for inclusion.

The study protocol has been approved by the VU University Medical Center Ethics Committee (approval nuber: A2015,121). Informed consent was obtained from at least one of their parents and from all children above the age of 12 years.

Anthropometry and Blood Pressure Measurements

Height was measured to the nearest 0.1 centimetre using a stadiometer. Body weight was measured to the nearest 0.1 kilogram using a digital balance scale with children barefooted and wearing light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by the square of body height in meters and categorized according to the International Obesity Task Force (16).

Blood pressure was measured three consecutive times at the right arm after 5 minutes of rest in sitting position using an electronic oscillometric blood pressure device. An appropriate-sized cuff was used according to the guidelines

of the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents (17). Based on the lowest of three consecutive blood pressure measurements, hypertension was defined as ≥95th percentile for age, gender, and height (17).

Urine and Saliva Sample Analyses

Urine samples were collected on site. Early morning saliva samples were obtained using a Salivette® (Sarstedt AG & Co. Nümbrecht, Germany) swap which was provided during the visit together with a return envelope. Participants were requested to obtain saliva immediately after awakening, between 06.00 and 09.00 a.m. and prior to having breakfast and to return the sample by postal mailing.

Urine and saliva were stored at -80 °C. Both samples were analysed for cortisol and cortisone. 0.1 mL of urine or 0.1 mL of saliva was used to assess cortisol and cortisone concentrations, using an isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Internal standards ($^{13}C_3$ labeled cortisol and cortisone) were added to the samples. Samples were extracted using supported liquid extraction (Isolute, Biotage, Uppsala, Sweden) and analysed by LC-MS/MS [Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, Massachusetts, USA)]. Lower limit of quantitation was 1.0 nmol/L for cortisol and 0.5 nmol/L for cortisone. The intra-coefficients of variation (CV%) for cortisol were 7 and 4% at a level of 3 and >5 nmol/L, respectively, and for cortisone <5% at all levels >2.8 nmol/L. The inter-CV% was <11% for both cortisol and cortisone.

Outcome Measures

Cortisol/creatinine ratio in spot urine is a measure of cortisol production (18). The urinary cortisol-to-cortisone ratio reflects renal 11β-HSD2 activity (12,19). Cortisol and cortisone in early-morning saliva are indicators of the morning peak in HPA axis activity (20). The salivary cortisol-to-cortisone ratio is only a rough estimate of the systemic interconversion between 11β-HSDs.

Statistical Analysis

BMI and height standard deviation scores (SDSs) were calculated using the LMS method (16), based on reference values from the World Health Organization (21) and Centers for Disease Control, respectively (22). Blood pressure SDSs were calculated using the equations provided by the NHBPEP Working Group (17). Differences in characteristics between hypertensive overweight and obese children, normotensive overweight and obese children, and normotensive non-overweight children were tested with ANOVA and post-hoc t-tests. Linear regression analysis was used to test associations with cortisol parameters between children with and without hypertension, adjusted for BMI-SDS, and between overweight and non-overweight children, adjusted for blood pressure SDS. Results are expressed as beta coefficients with 95% confidence intervals (95% CI). A p-value <0.05 was considered statistically significant. The statistical analyses were performed with SPSS software version 22.0 (SPSS Inc., Chicago, Illinois).

Results

A total of 270 children and adolescents were included in the study. Urine samples were collected from 180 children (38 hypertensive overweight and obese children, 86 normotensive overweight and obese children, and 56 normotensive non-overweight children), and saliva samples from 126 children (17 hypertensive overweight and obese children, 64 normotensive overweight and obese children, and 45 normotensive non-overweight children). Demographic, anthropometric, and blood pressure data are presented in Table 1.

Salivary and urinary cortisol and cortisone, and cortisol-to-cortisone ratios are displayed in Table 2.

Salivary cortisol and cortisone levels, but not the salivary cortisol-to-cortisone ratio, were significantly lower in overweight or obese children than in non-overweight children (β 0.89, 95% CI 0.80-0.97, and β -4.67, 95% CI -8.19- -1.15, respectively). There were no significant differences in these parameters between hypertensive and normotensive children. Salivary cortisol levels were not significantly associated with systolic (β 0.61, 95% CI 0.31-1.21) or diastolic (β 1.20, 95% CI 0.74-1.97) blood pressure SDS.

Urinary cortisol/creatinine (β 1.38, 95% CI 1.09-1.54) and cortisone/creatinine ratios (β 1.26, 95% CI 1.17-1.36), and the cortisol-to-cortisone ratio (β 1.11, 95% CI 1.05-1.19) were significantly higher in overweight or obese than in non-overweight children. Urinary cortisol/creatinine (β 1.20, 95% CI 1.06-1.36) and cortisone/creatinine ratios (β 1.19, 95% CI 1.08-1.30), but not the urinary cortisol-to-cortisone ratio (β 1.02, 95% CI 0.95-1.09), were higher in hypertensive children than in normotensive children. After adjustment for BMI-SDS, the association between urinary cortisol/creatinine ratio and hypertension was no longer significant (β 1.11, 95% CI 0.97-1.25), but the association between cortisone/creatinine ratio and hypertension remained significant (β 1.12, 95% CI 1.02-1.23). After adjustment for BMI-SDS, urinary cortisol/creatinine ratio was not significantly associated with systolic (β 1.42, 95% CI 0.91-2.21) or diastolic (β 1.30, 95% CI 0.94-1.81) blood pressure SDS.

Table 1. Characteristics of the study sample divided into hypertensive overweight, normotensiveoverweight, and normotensive non-overweight categories

	Hypertensive overweight children (n = 50)	Normotensive overweight children (n = 145)	Normotensive non-overweight children (n = 75)
Boys (%)	24 (48)	73 (50)	47 (63)
Age (yrs)	10.0 ± 3.5	10.6 ± 3.3	10.4 ± 3.0
Weight			
BMI (kg/m²)	27.1 ± 7.0[a]	26.6 ± 5.2[b]	17.0 ± 2.2
BMI (SDS)	2.7 ± 0.7[a]	2.6 ± 0.8[b]	-0.3 ± 0.9
Overweight n (%)	14 (28)	50 (35)	-
Obese n (%)	18 (36)	53 (37)	-
Morbidly obese n (%)	18 (36)	42 (29)	-
Blood pressure			
Mean systolic BP (SDS)	1.98 ± 0.44[a,c]	0.50 ± 0.84[b]	0.11 ± 0.89
Mean diastolic BP (SDS)	1.03 ± 0.85[a,c]	0.23 ± 0.62[b]	-0.09 ± 0.68

Values are shown as n (%) or as mean ± standard deviation

[a]A significant difference between hypertensive overweight and normotensive non-overweight children ($p < 0.001$)

[b]A significant difference between normotensive overweight and non-overweight children ($p < 0.001$)

[c]A significant difference between hypertensive overweight and normotensive overweight children ($p < 0.001$)

Weight is categorised according to the definition of the International Obesity Task Force (24)

BMI: body mass index, BP: blood pressure, SDS: standard deviation score

Table 2. Salivary and urinary levels of cortisol and cortisone and cortisol/cortisone ratios in the subjects classified as hypertensive overweight, normotensive overweight, and normotensive non-overweight

	Hypertensive overweight children	Normotensive overweight children	Normotensive non-overweight children
Saliva samples	n = 17	n = 64	n = 45
sCortisol (nmol/L)	5.0 (3.7-9.5)	5.9 (3.9-7.7)[a]	7.4 (5.0-10.7)
sCortisone (nmol/L)	22.0 (18.3-29.0)	23.5 (17.3-30.0)[a]	28.0 (23.0-34.0)
sCCR	0.24 (0.17-0.32)	0.26 (0.20-0.31)	0.27 (0.20-0.32)
Urine samples	n = 38	n = 84	n = 56
uCortisol/cr (nmol/mmol)	10.9 (6.7-31.7)[b]	11.0 (7.2-17.3)[c]	5.1 (3.4-9.3)
uCortisone/cr (nmol/mmol)	31.2 (22.5-43.0)[b]	27.7 (19.1-35.1)[c]	13.6 (11.6-20.6)
uCCR	0.40 (0.23-0.55)	0.42 (0.34-0.52)[c]	0.32 (0.25-0.51)

Values are shown as median (interquartile range)

[a]A significant difference between normotensive overweight and non-overweight children ($p < 0.05$)

[b]A significant difference between hypertensive overweight and normotensive non-overweight children ($p < 0.001$)

[c]A significant difference between normotensive overweight and non-overweight children ($p < 0.001$)

CCR: cortisol/cortisone ratio, cr = creatinine

Discussion

This study provided evidence for an increased renal excretion of free cortisol and cortisone, with higher excretion of cortisol relative to cortisone, in overweight or obese children. It also showed that early morning salivary levels of cortisol and cortisone were lower in overweight or obese children. However, there were no differences in cortisol parameters between hypertensive and normotensive overweight or obese children.

The findings from this study confirm previous observations (20,23) that childhood obesity is associated with an increased cortisol production rate, decreased renal 11β-HSD2 activity, and flattening of early-morning peak cortisol and cortisone. We found no significant differences in cortisol parameters between hypertensive and normotensive overweight or obese children. This is in contrast to previous studies in obese children which showed a positive association of systolic blood pressure, as part of metabolic syndrome, with increased serum levels of cortisol and ACTH (14,24), and in free cortisol in 24-hr urine (25). An explanation for the lack of an association with hypertension in our study is that the groups of hypertensive and normotensive overweight or obese children might have been too similar regarding presence of features of metabolic syndrome, as an index of glucose tolerance was not tested, to be able to detect differences in cortisol parameters.

In the total sample of overweight and non-overweight children, we found significant associations between ratios of urinary cortisol or cortisone to creatinine and the presence of hypertension. However, after adjustment for BMI-SDS, both associations became weaker, and only the association with urinary cortisone/creatinine ratio remained significant.

Future studies should elucidate whether hypertensive and normotensive overweight or obese children differ in the metabolism of cortisol. Cortisol is metabolized reversibly by 11β-HSDs and irreversibly by A-ring reductases and CYP3A4. In adults, impaired metabolic clearance of cortisol has been implicated to play a role in metabolic disease susceptibility (26).

A major strength of our study is inclusion of three study groups consisting of hypertensive overweight and obese children, normotensive overweight and obese children, and normotensive non-overweight children. This approach enabled us to study the relative contributions in overweight or obesity and hypertension. Another strength is the method we used to measure cortisol and cortisone concentrations. LC-MS/MS is known to be a very accurate, specific and sensitive method to measure steroid hormones (27,28).

Study Limitations

Our study has several limitations. First, hypertension was based upon blood pressure measurements obtained on only one occasion, although three times consecutively. No 24-hour ambulatory blood pressure monitoring was performed to confirm the diagnosis of hypertension. Second, for practical reasons, only random daytime urine samples (with unspecified sampling times) were collected instead of 24-hr urine, although cortisol/creatinine ratio in spot urine has proven to be a reliable tool for the assessment of cortisol production (18). Third, only early-morning saliva samples were collected, so that association with diurnal rhythmicity in HPA axis activity could not be tested. Cortisol in scalp hair - as an index of long-term glucocorticoid exposure - was not tested in our sample, although recent studies have shown strong associations with indices of obesity (29). Yet another limitation is the cross-sectional study design of our study. A longitudinal study is necessary to gain insight into temporal relations. Ideally, the role of cortisol in the development of obesity-induced hypertension should be studied in a prospective cohort study with participants being sampled prior to developing overweight.

Conclusion

We found that overweight and obese children had an increased cortisol production rate. Furthermore, overweight and obesity were associated with a higher urinary cortisol-to-cortisone ratio, reflecting decreased renal 11β-HSD2 activity, as well as with lower levels of early-morning cortisol and cortisone. However, there were no significant differences in cortisol parameters between hypertensive and normotensive overweight and obese children. More research is needed to elucidate whether cortisol metabolism is involved in the pathogenesis of obesity-induced hypertension in children.

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Ethics

Ethics Committee Approval: The study was approved by the VU University Medical Center Ethical Committee.

Informed Consent: Informed consent was obtained from

at least one parent, and from all children above the age of 12 years.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: Martijn JJ Finken, Jeroen Nauta, Jaap W Groothoff, Joana E Kist-van Holthe, Design: Joana E Kist-van Holthe, Martijn JJ Finken, Data Collection or Processing: Aleid JG Wirix, Ines A von Rosenstiel-Jadoul, Martijn JJ Finken, Laboratory Analysis: Annemieke C Heijboer, Analysis or Interpretation: Aleid JG Wirix, Mai JM Chinapaw, Joana E Kist-van Holthe, Martijn JJ Finken, Literature Search: Aleid JG Wirix, Writing: Aleid JG Wirix, Martijn JJ Finken, Ines A von Rosenstiel-Jadoul, Annemieke C Heijboer, Jeroen Nauta, Jaap W Groothoff, Mai JM Chinapaw, Joana E Kist-van Holthe.

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References

1. Sorof JM, Lai D, Turner J, Poffenbarger T, Portman RJ. Overweight, ethnicity, and the prevalence of hypertension in school-aged children. Pediatrics 2004;113:475-482.
2. Maldonado J, Pereira T, Fernandes R, Carvalho M. Blood pressure distribution of a sample of healthy Portuguese children and adolescents: the AVELEIRA registry. Rev Port Cardiol 2009;28:1233-1244.
3. Polat M, Yıkılkan H, Aypak C, Görpelioğlu S. The relationship between BMI and blood pressure in children aged 7-12 years in Ankara, Turkey. Public Health Nutr 2014;17:2419-2424. Epub 2014 May 22
4. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation 2008;117:3171-3180. Epub 2008 Jun 16
5. Singh AS, Mulder C, Twisk JW, van Mechelen W, Chinapaw MJ. Tracking of childhood overweight into adulthood: a systematic review of the literature. Obes Rev 2008;9:474-488. Epub 2008 Mar 5
6. Wirix AJ, Kaspers PJ, Nauta J, Chinapaw MJ, Kist-van Holthe JE. Pathophysiology of hypertension in obese children: a systematic review. Obes Rev 2015;16:831-842. Epub 2015 Jun 22
7. Chapman K, Holmes M, Seckl J. 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev 2013;93:1139-1206.
8. Schnackenberg CG, Costell MH, Krosky DJ, Cui J, Wu CW, Hong VS, Harpel MR, Willette RN, Yue TL. Chronic inhibition of 11 β -hydroxysteroid dehydrogenase type 1 activity decreases hypertension, insulin resistance, and hypertriglyceridemia in metabolic syndrome. Biomed Res Int 2013;2013:427640. Epub 2013 Mar 18
9. Walker BR. Cortisol--cause and cure for metabolic syndrome? Diabet Med 2006;23:1281-1288.
10. Morton NM. Obesity and corticosteroids: 11beta-hydroxysteroid type 1 as a cause and therapeutic target in metabolic disease. Mol Cell Endocrinol 2010;316:154-164. Epub 2009 Oct 3
11. Walker BR. Glucocorticoids and cardiovascular disease. Eur J Endocrinol 2007;157:545-559.
12. Csábi GY, Juricskay S, Molnár D. Urinary cortisol to cortisone metabolites in hypertensive obese children. J Endocrinol Invest 2000;23:435-439.
13. Paize F, Isherwood D, Honour J, Jones CA. Abnormal urinary steroid profiles in four hypertensive obese children. J Pediatr Endocrinol Metab 2010;23:237-244.
14. Prodam F, Ricotti R, Agarla V, Parlamento S, Genoni G, Balossini C, Walker GE, Aimaretti G, Bona G, Bellone S. High-end normal adrenocorticotropic hormone and cortisol levels are associated with specific cardiovascular risk factors in pediatric obesity: a cross-sectional study. BMC Med 2013;11:44.
15. Wirix AJ, Nauta J, Groothoff JW, Rabelink TJ HiraSing RA, Chinapaw MJ, Kist-van Holthe JE. Is the prevalence of hypertension in overweight children overestimated? Arch Dis Child 2016;101:998-1003. Epub 2016 Mar 31
16. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 2012;7:284-294. Epub 2012 Jun 19
17. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114(2 Suppl):555-576.
18. Kapoor N, Job V, Jayaseelan L, Rajaratnam S. Spot urine cortisol-creatinine ratio - A useful screening test in the diagnosis of Cushing's syndrome. Indian J Endocrinol Metab 2012;16 (Suppl 2):376-377.
19. Stewart PM, Whorwood CB, Walker BR. Steroid hormones and hypertension: the cortisol-cortisone shuttle. Steroids 1993;58:614-620.
20. Kjölhede EA, Gustafsson PE, Gustafsson PA, Nelson N. Overweight and obese children have lower cortisol levels than normal weight children. Acta Paediatr 2014;103:295-299. Epub 2013 Dec 30
21. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ 2007;85:660-667.
22. Centers for Disease Control and Prevention National Center for Health Statistics. CDC growth charts: United States 2000. Retrieved October 2014, from www.cdc.gov/growthcharts.
23. Björntorp P, Rosmond R. Hypothalamic origin of the metabolic syndrome X. Ann N Y Acad Sci 1999;892:297-307.
24. Sen Y, Aygun D, Yilmaz E, Ayar A. Children and adolescents with obesity and the metabolic syndrome have high circulating cortisol levels. Neuro Endocrinol Lett 2008;29:141-145.
25. Reinehr T, Kulle A, Wolters B, Knop C, Lass N, Welzel M, Holterhus PM. Relationships between 24-hour urinary free cortisol concentrations and metabolic syndrome in obese children. J Clin Endocrinol Metab 2014;99:2391-2399.
26. Walker BR, Andrew R. Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease. Ann N Y Acad Sci 2006;1083:165-184.
27. Ackermans MT, Endert E. LC-MS/MS in endocrinology: what is the profit of the last 5 years? Bioanalysis 2014;6:43-57.
28. Vogeser M, Parhofer KG. Liquid chromatography tandem-mass spectrometry (LC-MS/MS)--technique and applications in endocrinology. Exp Clin Endocrinol Diabetes 2007;115:559-570.
29. Veldhorst MA, Noppe G, Jongejan MH, Kok CB, Mekic S, Koper JW, van Rossum EF, van den Akker EL. Increased scalp hair cortisol concentrations in obese children. J Clin Endocrinol Metab 2014;99:285-290. Epub 2014 Jan 1

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Hyperprolactinemia in Children with Subclinical Hypothyroidism

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What is already known on this topic?

Hyperprolactinemia is common in adults with subclinical and overt primary hypothyroidism. Hyperprolactinemia in children has been linked to adverse metabolic outcomes. Prevalence of hyperprolactinemia in children with thyroid dysfunction, especially subclinical hypothyroidism, is not known.

What this study adds?

Hyperprolactinemia is common in children with hypothyroidism, observed in a third of children with subclinical hypothyroidism and more than half of children with overt hypothyroidism. Receiver operating characteristics analysis showed that thyroid-stimulating hormone ≥4.00 mIU/L has a good sensitivity and specificity in predicting hyperprolactinemia in children. Hyperprolactinemia may be an indication for treating subclinical hypothyroidism in children.

Abstract

Prevalence of hyperprolactinemia in children with subclinical hypothyroidism (ScH) is not known. This study aimed to determine the occurrence and predictors of hyperprolactinemia in euthyroid children and in children with ScH and overt primary hypothyroidism (OPH). Serum prolactin levels were estimated in consecutive children < 18 years of age undergoing thyroid function evaluation and diagnosed to have normal thyroid function, ScH, or OPH. Children with pituitary adenomas, secondary hypothyroidism, multiple pituitary hormone deficiency, comorbid states, and drug-induced hyperprolactinemia were excluded. From the initially screened 791 children, hormonal data from 602 children who fulfilled all criteria were analyzed. Seventy-one (11.79%) of these had ScH, and 33 (5.48%) had OPH. Occurrence of hyperprolactinemia was highest in the OPH group (51.51%), followed by ScH (30.98%) and euthyroid children (4.41%) ($p < 0.001$). Median (25^{th}–75^{th} percentiles) levels for prolactin in euthyroid, ScH, and OPH children were 13.3 (9.4-17.95), 19.15 (15.97-30.12), and 28.86 (17.05-51.9) ng/mL, respectively ($p < 0.001$). In children, prolactin levels were comparable in males and females. An age-related increase in serum prolactin was noted in euthyroid children, which was statistically significant in post-pubertal (16-18 years) children. Area under the curve for thyroid stimulating hormone (TSH) in predicting hyperprolactinemia in children was 0.758 (95% confidence interval: 0.673–0.829; $p < 0.001$). TSH ≥4.00 mIU/L had a sensitivity of 69.4% and specificity of 77.6% in detecting hyperprolactinemia. Hyperprolactinemia is common in children with ScH and OPH. TSH ≥4.00 mIU/L has a good sensitivity and specificity in predicting hyperprolactinemia in children. More studies are needed to establish if hyperprolactinemia should be an indication for treating ScH in children.

Keywords: Subclinical hypothyroidism, hyperprolactinemia, prolactin, thyroid stimulating hormone

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Introduction

Hyperprolactinemia is a common endocrinopathy encountered in clinical practice with a prevalence ranging from 0.4% to 5% (1). We recently reported that in a cohort of 2848 individuals, hyperprolactinemia was common in patients with subclinical hypothyroidism (ScH), especially in those with thyroid stimulating hormone (TSH) >7.5 mIU/L (2). TSH ≥7.51 mIU/L (females) and ≥8.33 mIU/L (males) had a sensitivity of ≈50% with a high specificity of >90% in detecting hyperprolactinemia (2). Overt hypothyroidism in children has been linked with pituitary enlargement, pituitary hyperplasia, and in rare cases, even with feedback pituitary adenoma and multiple pituitary hormone deficiency (3,4). This enlargement of the pituitary is primarily believed to be due to increase in the number and size of thyrotrophs and lactotrophs in the pituitary gland (3). Primary hypothyroidism leads to increased thyrotropin-releasing hormone (TRH) release from hypothalamus, which has a physiologic trophic effect on thyrotrophs and lactotrophs resulting in increased TSH and prolactin levels, respectively (3,5). Reduced prolactin clearance as well as reduced sensitivity to the inhibitory actions of dopamine and its agonists on prolactin release also contribute to hyperprolactinemia (6). However, prolactin levels have rarely been investigated in children with hypothyroidism, especially in those with ScH. Also, the prevalence of hyperprolactinemia in children with ScH is not known. Hence, the aim of this study was to determine the occurrence and predictors of hyperprolactinemia in children with a spectrum of thyroid dysfunction ranging from euthyroidism to ScH and overt primary hypothyroidism.

Methods

Consecutive children younger than 18 years undergoing thyroid function evaluation at the department of biochemistry were selected for investigation. Children diagnosed to have pituitary adenomas, secondary hypothyroidism, multiple pituitary hormone deficiency, subclinical hyperthyroidism, and overt hyperthyroidism were excluded. Also, children with associated comorbidities such as chronic liver disease, renal disease, syndromes, and those taking medications such as anti-epileptics, neuroleptics, and anti-psychotics were excluded. The study protocol was explained, and only those children or their parents/guardians who gave informed written consent were included in the study. Serum prolactin was assayed from samples of children undergoing thyroid function evaluation who fulfilled the above criteria and who were diagnosed to have either ScH, overt primary hypothyroidism, or normal thyroid function. The reference range of free tri-iodothyronine (FT3), free tetra-iodothyronine (FT4), and TSH in our laboratory is 2–4.4 pg/mL, 0.6–2.2 ng/dL, and 0.5–5 mIU/L, respectively. ScH was defined as normal FT4 levels with TSH levels above the normal range (7). Overt primary hypothyroidism was defined as TSH levels above the normal range accompanied by low FT4 levels (7). Serum was separated from samples collected and stored at −80 °C. Patients with drug-induced hyperprolactinemia were excluded. The study duration was from August 2014 to December 2016. The Institutional Ethics Committee of Dr. Ram Manohar Lohia Hospital and Post Graduate Institute of Medical Education and Research approved the study [approval number: 89 (13/2014/IEC/PGIMER/RML/1645) dated 11th June 2014]. Serum prolactin was measured using chemiluminescence microparticle immunoassay (CLIA) (VITROS® ECiQ Immunodiagnostic System, Johnson & Johnson, USA), a methodology which has been elaborated elsewhere (2). The normal ranges of serum prolactin in adults are 2.8–27 ng/mL in females and 2.1–17 ng/mL in males (2). We used the age- and sex-specific normal ranges of serum prolactin in children developed by Aitkenhead and Heales (8) for defining hyperprolactinemia in our study. There is no sex difference in serum prolactin among children till 1 year of age. All children 16 years old or younger, with serum prolactin levels higher than the upper limit (97th percentile) for their age and sex were defined to have hyperprolactinemia (Table 1). For children between 16 and 18 years of age, the adult reference range was used in the analysis. Hence, hyperprolactinemia among children in the 16-18 years old group was defined as a serum prolactin level >27 ng/mL in females and >17 ng/mL in males (2).

Table 1. Normal levels (ranging from 2.5th and 97.5th percentiles) for serum prolactin in children of different age groups

Age group	Serum prolactin levels (ng/mL) (mean values and ranges)	
	Males	Females
0-30 days	31.35–236.50	31.35–236.50
31-60 days	24.0–147.40	24.0–147.40
61-90 days	5.08–98.70	5.08–98.70
3-5 months	3.76–98.50	3.76–98.50
6-8 month	4.04–77.41	4.04–77.41
9-12 months	4.98–38.54	4.98–38.54
1-2 years	3.05–37.08	3.15–40.65
2-4 years	2.67–33.69	2.63–30.08
5-8 years	2.20–20.58	2.11–21.90
9-11 years	1.88–26.08	2.06–25.75
12-16 years	2.06–22.51	2.72–28.30

The VITROS® ECiQ Immunodiagnostic System, Johnson & Johnson, USA was also used for estimation of FT3, FT4, and TSH. The methodology has been elaborated elsewhere (9). Samples from children having elevated prolactin levels with normal thyroid function were evaluated for macroprolactinemia using polyethylene glycol (PEG) precipitation test (treatment of equal parts of serum with PEG followed by centrifugation) to remove macroprolactin. Macroprolactinemia was diagnosed if post-PEG prolactin level was < 40 % of pre-PEG levels (10).

Statistical Analysis

Normality of the distribution of variables was assessed using the Kolmogorov-Smirnov test, and accordingly parametric or non-parametric tests were used for statistical analysis. The receiver operating characteristics (ROC) curves were plotted, and areas under the curves (AUCs) with 95 % confidence interval (CI) were calculated to explore the diagnostic efficacy and determine cut-offs of serum TSH in predicting hyperprolactinemia in children. The Youden index, defined as (sensitivity + specificity) − 1 was used to determine the optimal cut-off points. A p-value < 0.05 was considered statistically significant. SPSS version 20 was used for the analyses.

Results

Of a total of 791 children younger than 18 years of age who underwent thyroid function evaluation, 649 (males:females = 200:402) who fulfilled all inclusion and exclusion criteria had their serum prolactin levels determined. Drug-induced hyperprolactinemia was diagnosed in 47 children who were excluded. Other reasons for exclusion were secondary hypothyroidism (n = 23), multiple pituitary hormone deficiency (n = 16), associated pituitary adenoma (n = 12), comorbid disease states (n = 85), and refusal to consent (n = 6). Hence, 602 children were evaluated in the study. Of these, 71 children (11.79 %) were diagnosed to have ScH and 33 (5.48 %) had overt primary hypothyroidism. The remaining 498 children were euthyroid. The occurrence of hyperprolactinemia in subclinically hypothyroid and overt hypothyroid children was 22 (30.98 %) and 17 (51.51 %), respectively, figures which were significantly higher as compared to euthyroid children (4.41 %) (p < 0.001). Among the 71 children with ScH, the occurrence of hyperprolactinemia in children with TSH levels of 5-7.5, 7.5-10, and > 10 mIU/L was 23.80 % (15/63), 80 % (4/5), and 100 % (3/3), respectively (p < 0.001). Median (25^{th}–75^{th} percentile) values for serum prolactin levels in euthyroid children, children with ScH, and those with overt primary hypothyroidism were 13.3 (9.4-17.95) ng/mL, 19.15 (15.97-30.12) ng/mL, and 28.86 (17.05-51.9) ng/mL, respectively (p < 0.001).

Among the 602 children evaluated in this study, 33 were in age group 0-5 years, 54 in age group 6-10 years, 211 in age group 11-15 years, and 304 in age group 16-18 years (Table 2). Median prolactin levels among children in the different age groups (euthyroid vs. hypothyroidism) are shown in Table 3. As shown in Table 3, serum prolactin levels increased with age in euthyroid children, an increase which was significantly higher in the post-pubertal (16-18 years) age group (p < 0.001). A similar trend was not seen in children with hypothyroidism (Table 3). Median prolactin levels of children in the different age groups were not significantly different based on the sex of the child (Table 4). The areas under the ROC curves were constructed to evaluate the predictive values of serum TSH in predicting hyperprolactinemia in children. The AUC for TSH in predicting hyperprolactinemia in children was 0.758 (95 % CI: 0.673–0.829; p < 0.001). A serum TSH ≥4.00 mIU/L had a sensitivity of 69.4 % and specificity of 77.6 % in detecting hyperprolactinemia (Figure 1).

Discussion

Undiagnosed and untreated hyperprolactinemia has been linked with poor bone mineral health, osteomalacia, hypogonadotropic hypogonadism, menstrual abnormalities, and ovulatory dysfunction in adults (11,12).

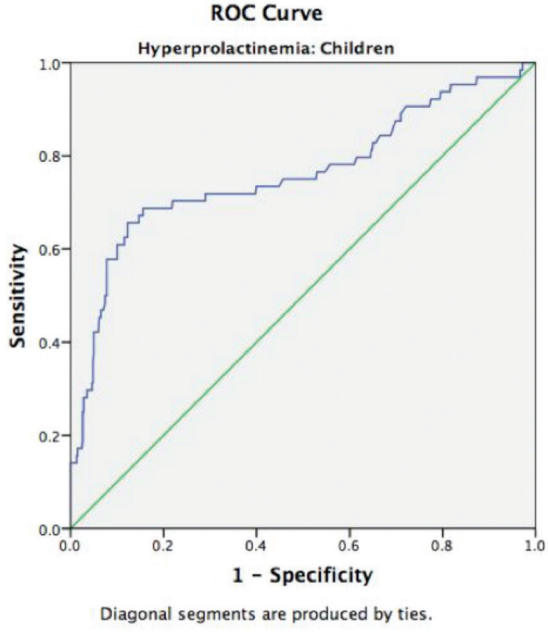

Figure 1. Receiver operating characteristics curve (blue line) showing the sensitivity and specificity of serum thyroid stimulating hormone in predicting hyperprolactinemia in children

ROC: receiver operating characteristics

Hyperprolactinemia is believed to be rare in children (13). Drug-induced hyperprolactinemia secondary to anti-epileptics, antipsychotics, and hyperprolactinemia secondary to pituitary tumors are believed to be the most common causes of hyperprolactinemia in children (14,15). Hyperprolactinemia in children has also been linked with impaired bone health, puberty problems, galactorrhea in females, and gynecomastia in males (16,17).

Our study highlighted for the first time that hyperprolactinemia is common in children with ScH (30.98%). The occurrence of hyperprolactinemia in children with overt hypothyroidism in this study was 51.51% and its occurrence in euthyroid children was 4.41%. These trends are similar to those observed in adults. We have previously reported the occurrence of hyperprolactinemia in adults with euthyroidism, ScH, and overt hypothyroidism to be 2.02-2.32%, 31.61-35.65%, and 39.53-42.95%, respectively (2).

An important observation of this study was that in contrast to adults (females have higher prolactin levels than males), no difference in prolactin levels was noted between male and female children. Prolactin levels in post-pubertal children (16-18 years of age) were significantly higher compared to prepubertal (0-5 years, 6-10 years) and peri-pubertal children (11-15 years). Increased sex steroid levels in the post-pubertal state, especially estrogen, may explain this finding. The trophic effect of estrogen on TRH-mediated prolactin secretion from lactotrophs has been reported in various studies (18).

A serum TSH level ≥4.00 mIU/L had a sensitivity and specificity of 69.4% and 77.6%, respectively in detecting hyperprolactinemia in children. We have previously demonstrated that TSH levels of ≥7.51 mIU/L in females and ≥8.33 mIU/L in males can be used as cut-off levels in detecting hyperprolactinemia in adults (2). There is often a lack of clarity among doctors with regard to indications for levothyroxine therapy in ScH (19). Hyperprolactinemia may be accepted as one of the indications for levothyroxine therapy in adults with ScH (2).

This study highlighted that the TSH thresholds for detecting hyperprolactinemia is much lower in children as compared to adults (4 mIU/L vs. 7.5 mIU/L). This statement is also supported by the observation that in our study, all children with ScH who had TSH levels ≥7.51 mIU/L had hyperprolactinemia as compared to only 49-61% of adults reported in a previous study (2). The limitations of this study include the lack of

Table 2. Occurrence of subclinical and overt primary hypothyroidism in children of different age groups in this study

Age group	Euthyroid	Subclinical hypothyroidism	Overt primary hypothyroidism
0-5 years (n = 33)	21	6	6
6-10 years (n = 54)	46	6	2
11-15 years (n = 211)	184	17	10
16-18 years (n = 304)	247	42	15

Table 3. Serum prolactin levels in euthyroid children as compared to those with subclinical or overt hypothyroidism in different age groups (median and 25th–75th percentile values)

Age group	Serum prolactin levels (ng/mL)		p
	Euthyroid	Hypothyroid (subclinical or overt)	
0-5 years (n = 33)	10.05 [5.87-13.98] (n = 21)	26.5 [19.17- 43.30] (n = 12)	< 0.001
6-10 years (n = 54)	10.45 [8.22-14.95] (n = 46)	18.35 [15.98- 33.75] (n = 8)	< 0.001
11-15 years (n = 211)	10.75 [7.45- 16.25] (n = 184)	17.5 [15.1-43.0] (n = 27)	< 0.001
16-18 years (n = 304)	15.0 [11.1-20.2] (n = 247)	23 [16-30] (n = 57)	< 0.001

p < 0.05 considered statistically significant

Table 4. Median prolactin levels in male and female children of different age groups (median and 25th–75th percentile values)

Age group	Serum prolactin levels (ng/mL)		p
	Males	Females	
0-5 years (n = 33)	16.33 [14.17-36.75]	14.4 [9.6-19.5]	0.995
6-10 years (n = 54)	12.0 [8.37-15.99]	15.1 [10.4-19.50]	0.273
11-15 years (n = 211)	9.5 [6.82-14.17]	15.1 [10.4-19.5]	0.134
16-18 years (n = 304)	13.4 [9.9-16.2]	16.7 [12.5-23.9]	0.286

p < 0.05 considered statistically significant

assessment of gonadotropins and sex steroids in children in different age groups and their relationship with prolactin and thyroid hormone levels. Obesity was also not assessed among children in our study cohort and serum prolactin levels were not adjusted for childhood obesity. There is a report suggesting that serum prolactin may be lower in children with obesity (20). However, it must be highlighted that our institute is a government medical college and hospital, providing free medical treatment to the population. The majority of our patients are the off spring of parents of low socio-economic status, and obesity is rare in these children. Being a cross-sectional study, the impact of levothyroxine supplementation on prolactin levels in ScH and overt primary hypothyroidism was not evaluated, and is a limitation.

To conclude, it may be said that hyperprolactinemia is common in children with hypothyroidism, observed in a third of children with ScH and in more than half of children with overt hypothyroidism. ROC analysis confirmed that a TSH ≥4.00 mIU/L has a good sensitivity and specificity in predicting hyperprolactinemia in children. Further longitudinal studies are warranted to evaluate the impact of this hyperprolactinemia on clinical outcomes in children and to establish if hyperprolactinemia should be considered as an indication for treating ScH in children.

Ethics

Ethics Committee Approval: The study was approved by the Institutional Ethics Committee of Dr. Ram Manohar Lohia Hospital and Post Graduate Institute of Medical Education and Research, New Delhi [approval number: 89 (13/2014/IEC/PGIMER/RML/1645) dated 11th June 2014].

Informed Consent: Consent was filled out by children and their parents/guardians.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Deep Dutta, Lokesh Kumar Sharma, Concept: Deep Dutta, Design: Deep Dutta, Neera Sharma, Data Collection or Processing: Lokesh Kumar Sharma, Neera Sharma, Analysis or Interpretation: Deep Dutta, Lokesh Kumar Sharma, Literature Search: Deep Dutta, Neera Sharma, Writing: Deep Dutta, Lokesh Kumar Sharma, Neera Sharma.

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References

1. Mancini T, Casanueva FF, Giustina A. Hyperprolactinemia and prolactinomas. Endocrinol Metab Clin North Am 2008;37:67-99.
2. Sharma LK, Sharma N, Gadpayle AK, Dutta D. Prevalence and predictors of hyperprolactinemia in subclinical hypothyroidism. Eur J Intern Med 2016;35:106-110.
3. Dutta D, Maisnam I, Ghosh S, Mukhopadhyay P, Mukhopadhyay S, Chowdhury S. Panhypopituitarism with empty sella a sequel of pituitary hyperplasia due to chronic primary hypothyroidism. Indian J Endocrinol Metab 2012;16:282-284.
4. Khawaja NM, Taher BM, Barham ME, Naser AA, Hadidy AM, Ahmad AT, Hamamy HA, Yaghi NA, Ajlouni KM. Pituitary enlargement in patients with primary hypothyroidism. Endocr Pract 2006;12:29-34.
5. Asa SL, Ezzat S. The pathogenesis of pituitary tumours. Nat Rev Cancer 2002;2:836-849.
6. Serri O, Chik CL, Ur E, Ezzat S. Diagnosis and management of hyperprolactinemia. CMAJ 2003;169:575-581.
7. Raber W, Gessl A, Nowotny P, Vierhapper H. Hyperprolactinaemia in hypothyroidism: clinical significance and impact of TSH normalization. Clin Endocrinol (Oxf) 2003;58:185-191.
8. Aitkenhead H, Heales SJ. Establishment of paediatric age-related reference intervals for serum prolactin to aid in the diagnosis of neurometabolic conditions affecting dopamine metabolism. Ann Clin Biochem 2013;50:156-158.
9. Sharma N, Sharma LK, Dutta D, Gadpayle AK, Anand A, Gaurav K, Mukherjee S, Bansal R. Prevalence and Predictors of Thyroid Dysfunction in Patients with HIV Infection and Acquired Immunodeficiency Syndrome: An Indian Perspective. J Thyroid Res 2015;2015:517173. Epub 2015 Dec 22
10. Samson SL, Hamrahian AH, Ezzat S; AACE Neuroendocrine and Pituitary Scientific Committee; American College of Endocrinology (ACE). American Association Of Clinical Endocrinologists, American College Of Endocrinology Disease State Clinical Review: Clinical Relevance Of Macroprolactin In The Absence Or Presence Of True Hyperprolactinemia. Endocr Pract 2015;21:1427-1435.
11. Poppe K, Velkeniers B, Glinoer D. Thyroid disease and female reproduction Clin Endocrinol (Oxf) 2007;66:309-321.
12. Lu KH, Chen HI, Grandison L, Huang HH, Meites J. Reduced luteinizing hormone release by synthetic luteinizing hormone-releasing hormone (LHRH) in postpartum lactating rats. Endocrinology 1976;98:1235-1240.
13. Catli G, Abaci A, Altincik A, Demir K, Can S, Buyukgebiz A, Bober E. Hyperprolactinemia in children: clinical features and long-term results. J Pediatr Endocrinol Metab 2012;25:1123-1128.
14. Saito E, Correll CU, Gallelli K, McMeniman M, Parikh UH, Malhotra AK, Kafantaris V. A prospective study of hyperprolactinemia in children and adolescents treated with atypical antipsychotic agents. J Child Adolesc Psychopharmacol 2004;14:350-358.
15. Catli G, Abaci A, Bober E, Büyükgebiz A. Clinical and diagnostic characteristics of hyperprolactinemia in childhood and adolescence. J Pediatr Endocrinol Metab 2013;26:1-11.
16. Galli-Tsinopoulou A, Nousia-Arvanitakis S, Mitsiakos G, Karamouzis M, Dimitriadis A. Osteopenia in children and adolescents with hyperprolactinemia. J Pediatr Endocrinol Metab 2000;13:439-441.
17. Selvan C, Dutta D, Ghosh S, Mukhopadhyay S, Chowdhury S. Growth hormone deficiency, secondary hypothyroidism, and empty sella following treatment of childhood macroprolactinoma. Indian J Endocrinol Metab 2013;17:132-134.
18. Carlson HE, Jacobs LS, Daughaday WH. Growth hormone, thyrotropin, and prolactin responses to thyrotropin-releasing hormone following diethylstilbestrol pretreatment. J Clin Endocrinol Metab 1973;37:488-490.
19. Surana V, Aggarwal S, Khandelwal D, Singla R, Bhattacharya S, Chittawar S, Kalra S, Dutta D; Society for Promotion of Education in Endocrinology and Diabetes Group, India. A 2016 clinical practice pattern in the management of primary hypothyroidism among doctors from different clinical specialties in New Delhi. Indian J Endocrinol Metab 2017;21:165-177.
20. Chirico V, Cannavò S, Lacquaniti A, Salpietro V, Mandolfino M, Romeo PD, Cotta O, Munafò C, Giorgianni G, Salpietro C, Arrigo T. Prolactin in obese children: a bridge between inflammation and metabolic-endocrine dysfunction. Clin Endocrinol (Oxf) 2013;79:537544.

CASE REPORT

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Dysmorphic Features, Frontal Cerebral Cavernoma, and Hyperglycemia in a Girl with a *De Novo* Deletion of 7.23 Mb in Region 7p13-p12.1

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What is already known on this topic?

Most glucokinase mutations are single nucleotide mutations. Glucokinase deletion mutations are very rare. Until now, a whole glucokinase deletion in non-syndromic patients has only been reported once. In syndromic patients, whole glucokinase gene deletions have been reported in combination with multiple gene deletions.

What this study adds?

Our patient with hyperglycemia and dysmorphic features had a deletion of 7.23 Mb comprising the region 7p13-p12.1, with involvement of 39 Online Mendelian Inheritance in Man genes, including glucokinase associated with maturity-onset diabetes of the young, CCM2 associated with type 2 cerebral cavernous malformations, insulin-like growth factors binding protein-3 associated with decreased postnatal growth, and oxoglutarate dehydrogenase associated with alpha-ketoglutarate dehydrogenase deficiency (short stature, hypotonia, cognitive impairment, and movement abnormalities). This previously unreported deletion explains the clinical picture of the patient and suggests that 7p13-p12.1 contains genes involved in intellectual disability and craniofacial development.

Abstract

We describe the case of a 7-year-old girl referred to our diabetes unit for hyperglycemia associated with facial dysmorphic features, intellectual disability, and cerebral cavernomas. Based on presence of anti islet antigen-2 (IA2) antibodies and a human leukocyte antigen of DR3/DR4/DQ2, the patient was initially diagnosed to be a case of type 1 diabetes mellitus. At follow-up, the very good metabolic control on a low insulin dose and negative IA2 antibodies led to a suspicion of glucokinase (*GCK*)-related maturity-onset diabetes of the young (MODY 2). This suspicion was substantiated in multiplex ligation-dependent probe amplification (MLPA) which showed a heterozygous *GCK* deletion (exons 1 to 12). However, the patient's parents did not have such a deletion and were clinically euglycemic. Given the clinical picture and the MLPA findings, array based comparative genomic hybridization was performed showing a monoallelic deletion of 7.23 Mb in the short arm of chromosome 7 (7p13-p12.1). The deleted intervals contain 39 genes listed in the Online Mendelian Inheritance in Man list, including *GCK* associated with MODY 2, *CCM2* associated with type 2 cerebral cavernous malformations, *IGFBP-3* associated with decrease in postnatal growth, and *OGD* associated with alpha-ketoglutarate dehydrogenase deficiency, with cognitive impairment and movement abnormalities. This previously unreported deletion was considered to explain the clinical picture of the patient. Also, the findings suggest that 7p13-p12.1 contains genes involved in intellectual disability and craniofacial development.

Keywords: Glucokinase, cerebral cavernous malformation, maturity-onset diabetes, oxoglutarate dehydrogenase, hyperglycemia, intellectual disability

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Introduction

Hyperglycemia is a frequent cause for presentation at pediatric endocrinology clinics. Autoimmune diabetes and insulin resistance associated with obesity or with monogenic diabetes (mainly maturity-onset diabetes of the young) are among the major causes of hyperglycemia. In Spain, mutations in *GCK* and *HNF-1α* explain most cases clinically diagnosed maturity-onset diabetes of the young (MODY) (1). Pedigrees with monogenic MODY-type diabetes typically show a dominant pattern of inheritance.

We describe the case of a 7-year-old girl referred to our diabetes unit for hyperglycemia associated with dysmorphic features, intellectual disability, frontal cerebral cavernoma, and no familial antecedents of diabetes. In the genetic study, we identified a deletion of 7.23 Mb in the 7p13-p12.1 region (genomic coordinates Chr7: 42807167 to 50040279).

Case Report

A 7-year-old girl presented to our diabetes unit with hyperglycemia. In the previous 4 years, her fasting blood glucose levels were reported to vary between 103-135 mg/dL. There was no history of polyuria, polydipsia, or polyphagia. Likewise, there was no history of weight loss or of any episode of ketosis.

Due to detection of bradycardia, the patient was delivered by caesarean section at a gestational age of 40 weeks. Her Apgar score was 6/9 and type 3 reanimation was needed. Birth weight was 2530 g [-2 standard deviation (SD)], birth length 47 cm (-1.7 SD), and head circumference was 35 cm (0.5 SD). No hypoglycemia or hyperbilirubinemia were noted in her neonatal life. However, she received early stimulation, starting at age 22 months, for language delay and hypotonia. She was reported to have three febrile seizure episodes and also tics. She showed poor school performance and poor social interaction. For these reasons, she was referred to the pediatric neurology clinic where she was diagnosed to have a frontal-cerebral cavernoma accompanied by intellectual disability and dysmorphic features (Figure 1).

Family history: She had healthy non-consanguineous parents [mother's height and father's height were 162 cm (0.13 SD) and 165.5 cm (-1.6 SD), respectively] and a healthy sister. There was no family history of diabetes, neurological or hereditary metabolic disorders.

Physical examination: Her weight and height were 20.5 kg (-0.96 SD) and 115.4 cm (-1.6 SD), respectively, with a body mass index of 15.4 kg/m^2 (-0.4 SD). Her target height was 157.3 cm (-1.1 SD). She had dysmorphic features with a triangular face, short forehead, depressed nasal bridge, low hair implantation, bushy eyebrows, synophridia, and microretrognathia (Figure 2). The rest of the examination was normal.

Initial genetic studies: In the initial study of the dysmorphic features and mental retardation, we determined the karyotype (46XX), investigated the subtelomeric regions and the *FMR1* gene, which were normal. Other laboratory tests were also ordered.

Biochemical analyses: Hemoglobin A1c (HbA1c) level was 6.0%. Oral glucose tolerance test (OGTT) (with 1.75 g/kg)

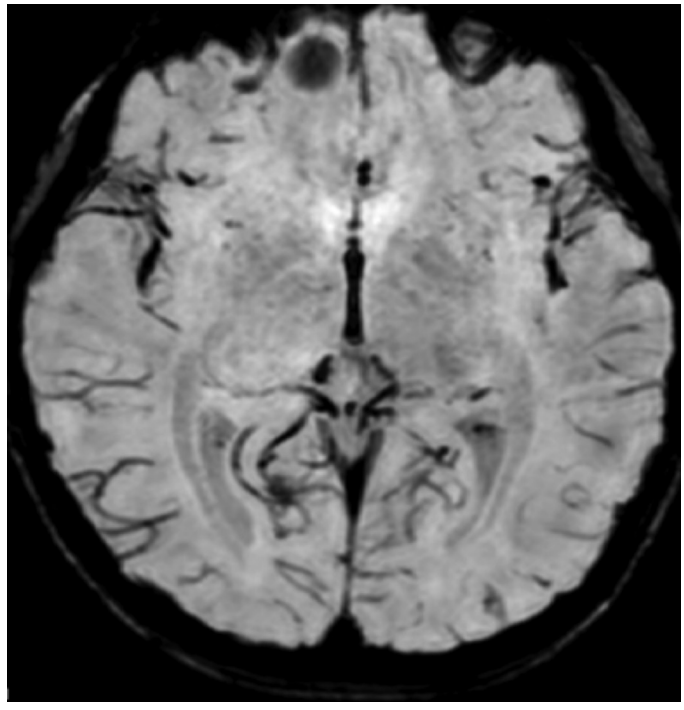

Figure 1. Cerebral magnetic resonance: Shows a frontal cerebral cavernoma (7x6 mm)

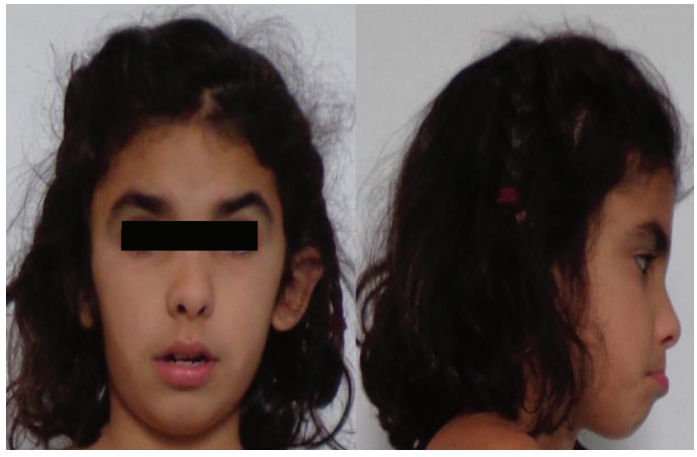

Figure 2. Facial dysmorphic features: Triangular face, short front, depressed nasal bridge, low hair implantation, bushy eyebrows, synophridia, and microretrognathia

revealed a basal glycemia of 129 mg/dL, a serum glucose level of 191 mg/dL after 1 hour and 150 mg/dL after 2 hours, a sign of impaired glucose tolerance. She had preserved her pancreatic reserves. In the course of the OGTT, c-peptide levels at 0, 60, 120 minutes were 0.8, 3.7 and 2.1 ng/mL, respectively. Thyroid, liver and kidney functions were normal.

Autoimmunity: The patient's anti-IA2 (anti-tyrosine phosphatase) level was 78.8 U/mL (positive > 7.5 U/ mL), and she did not have any anti-glutamic acid decarboxylase (GAD-0.0 U/mL). Human leukocyte antigen (HLA) determination revealed HLA-DR3/DR4 and HLA-DQ2.

Diagnosis: Given the presence of fasting hyperglycemia (129 mg/dL), the positivity for a pancreatic autoantibody (anti-IA2), and a HLA trait with increased risk for development of autoimmune diabetes (HLA DR3/DR4), a diagnosis of type 1 diabetes mellitus (T1DM) was made and treatment with insulin at 0.3 U/kg/day was started.

Follow-up: Nine months after T1DM diagnosis, the patient presented with a very good metabolic control while on a low insulin dose (0.15 U/kg/day). Her weight was 20.8 kg (-1.9 SD) and her height was 118.9 cm (-1.7 SD), with a linear growth velocity (LGV) of 4.38 cm/year (-1.5 SD). Her laboratory results were negative for anti-IA2 and anti-GAD antibodies (samples analyzed in two different laboratories). HbA1c was 5.8 %. Insulin-like growth factors (IGF)-1 and IGF binding protein-3 (*IGFBP-3*) levels, adjusted for age and sex, were 104.2 ng/mL (-0.6 SD) and 2947 ng/mL (-1 SD). The above findings suggested the need to further investigate an alternative diagnosis for her hyperglycemia.

GCK-related MODY 2 was suspected by persistent mild hyperglycemia in the fasting state. We requested a multiplex ligation-dependent probe amplification (MLPA) that showed a heterozygous *GCK* deletion (exons 1 to 12). However, the patient's parents did not have such a deletion and were clinically euglycemic. Previously, we had performed karyotype analysis to the parents of the patient and both were found to be normal, discarding chromosome translocations. These results increased the likelihood that the patient's deletion would be *de novo*.

Results of Array Based Comparative Genomic Hybridization (International System for Human Cytogenetic Nomenclature 2013): Given the clinical picture and the MLPA findings, an array based comparative genomic hybridization (CGH) (KaryoNIM® Postnatal 60k) was performed. This array-CGH platform is primarily used for intellectual disabilities and syndromes with multiple malformations. This method simultaneously detects the presence or absence of genetic and chromosomal variations (duplications or deletions) responsible for 160 genetic syndromes with a minimum resolution of approximately 275 kilobases between probes. The results revealed a pattern of arr[hg19] 7p13p12.1(42,807,167-50,040,279)x1 (female genomic pattern). This result shows a deletion of 7.23Mb in the 7p13-p12.1 region (genomic coordinates Chr7: 42807167 to 50040279). This region (Figure 3) contains 39 genes included in Online Mendelian Inheritance in Man (OMIM) (2) database. *GCK* associated with MODY 2, *CCM2* associated with type 2 cerebral cavernous malformations, *OGDH* associated with alpha-ketoglutarate dehydrogenase deficiency (short stature, cognitive impairment, and movement abnormalities), and *IGFBP-3* whose deletion has been associated with a 20 % decrease in postnatal growth are among these genes (3). Deletion of these genes may explain patient's clinical signs. With these findings, the decision was taken to discontinue insulin therapy.

At 8 years and 10 months old, the patient continued to have good metabolic control (HbA1c < 6.0 %) without insulin, although with a slowing in growth [height 122.2 cm (-1.9

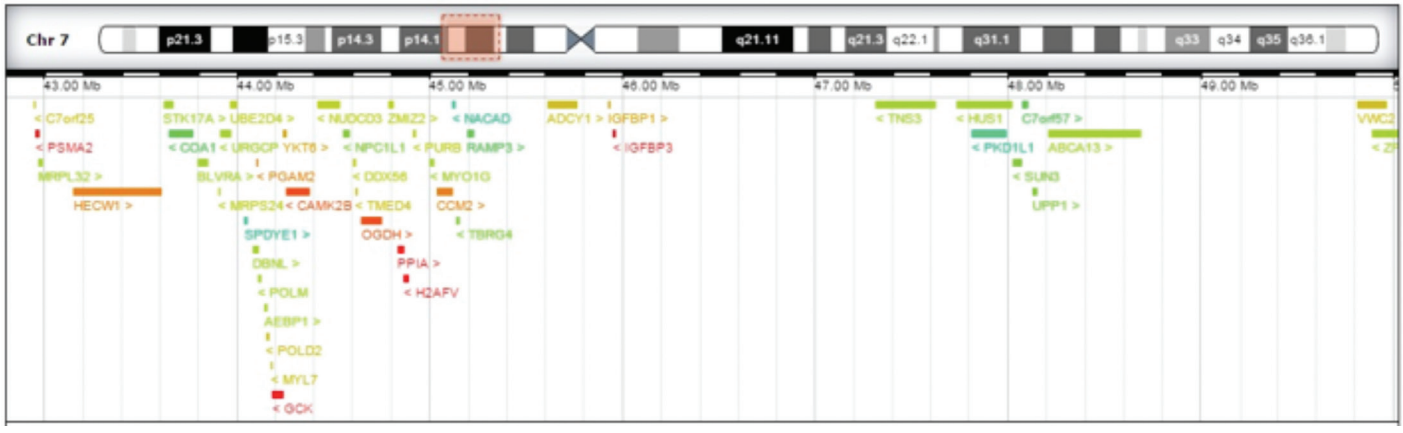

Figure 3. Region 7p13-p12.1 (genomic coordinates Chr7: 42807167 to 50040279): copy number variation 7.23 Mb contains 39 genes included in Online Mendelian Inheritance in Man list

SD) with a LGV of 3.5 cm/year (-2.3 SD)]. For this reason, IGFBP-3 deficit is being reevaluated again.

Discussion

Our patient had hyperglycemia with positive anti-IA2 antibodies. These findings and presence of HLA findings associated with risk for development of autoimmune diabetes (HLA DR3/DR4) were initially interpreted as signs of T1DM. At follow-up, and after nine months of insulin treatment, she presented in a state of very good metabolic control on a low insulin dose, with anti-IA2 antibodies which had become negative. These findings led us to further investigate for presence of other underlying causes of hyperglycemia.

Monogenic diabetes MODY 2 was suspected (mutations in *GCK* explains most cases of monogenic causes of diabetes in Spain) and MLPA was performed in the patient and her parents, showing an heterozygous *GCK* gene deletion and wild type results, respectively.

The presence of dysmorphic features and of a frontal cavernoma increased the suspicion of a genetic syndrome. Given the clinical picture and the finding in MLPA, array-CGH (KaryoNIM® Postnatal 60k) was performed, finding the real size of the deletion being 7.23 Mb long in the region 7p13-p12.1.

According to the "Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources" (DECIPHER) (4), the 7p13-p12.1 deleted interval contains five genes (*GCK, IGFBP-3, OGDH, PPIA*, and *PSMA2*) associated with low haploinsufficiency (HI) score (HI index <10%) (5) and/or high loss intolerance score (pLI ≥0.9) (6) and one intermediate (*CMM2*: HI 28.18% and pLI 0.48). Four of these genes described (Table 1) are consistently linked to clinical features observed in our patient, namely, mild hyperglycemia in the fasting state, cerebral cavernous malformations, decrease in postnatal growth, cognitive impairment, movement disorders (tics), and hypotonia. The clinical impact of deletion in this region is unknown, so close clinical monitoring is needed.

Most *GCK* mutations are single nucleotide mutations (7). *GCK* deletion mutations are very rare (8). Until now, a whole *GCK* deletion in non-syndromic patients have only been reported once (9). In syndromic patients, whole *GCK* gene deletions have been reported in combination with multiple gene deletions (10). The low birth weight (-2 SD) in this patient can be explained by the mother not having the *GCK* mutation and the fetus having the *GCK* mutation, which indirectly decreases fetal insulin secretion and thereby fetal growth (11,12).

At follow-up, the patient was found to have an altered postnatal growth (height -1.9 SD and LGV -2.3 SD) that could be explained in part by the HI of *IGFBP-3* and *OGHD*. *IGFBP-3* possesses both growth-inhibitory and potentiating effects on cells that are independent of IGF action and are mediated through specific *IGFBP-3*-binding proteins/receptors located at the cell membrane, cytosol, or nuclear compartments and in the extracellular matrix. *IGFBP-3* deletion has been associated with a 20% decrease in postnatal growth (3).

The patient received early stimulation, starting at age 22 months, for hypotonia and language delay. *OGDH* HI is associated, according to the Human Phenotype Ontology (13), with short stature, hypotonia, cognitive impairment, and movement abnormalities, among others features. For this reason, the patient was referred to the hereditary metabolic diseases unit for further assessment.

After a search in DECIPHER, we have not found another case with the same deletion. But we found a boy (DECIPHER ID 277032) with a more extensive deletion (9.26 Mb genomic coordinates Chr 7: 44085112-53341792) that includes the region 7p13-p12.1. This copy number variation contains 36 genes included in OMIM list. *PPIA, IGFBP-3, GCK, GRB10*, and *H2AFV* have HI <10% and that patient's phenotype includes global developmental delay, tall stature,

Table 1. Genes in deleted region (7p13-p12.1) and predicted phenotypic effects

Gene	OMIM number	Description	HI%	pLI	Phenotype
GCK	138079	Glucokinase	8.07	0.20	MODY type 2
CCM2	607929	CCM2 scaffolding protein	28.18	0.48	Cerebral cavernomatous malformations
IGFBP-3	146732	Insulin-like growth factor-binding protein 3	6.39	0.36	Decrease in postnatal growth
OGDH	613022	Oxoglutarate dehydrogenase	17.23	0.99	Short stature, hypotonia, cognitive impairment, and movement abnormalities

OMIM: Online Mendelian Inheritance in Man, MODY: maturity-onset diabetes of the young, HI: haploinsufficiency index - protein coding genes have been scored according to their predicted probability of exhibiting haploinsufficiency (5), pLI: lost intolerance score. Indicates probability that a gene is intolerant to a loss-of-function mutation (6)

macrocephaly, depressed nasal bridge, pectus excavatum, and hypospadias. Surprisingly, the patient had euglycemia and tall stature.

To summarize, our patient with hyperglycemia and dysmorphic features had a deletion of 7.23 Mb comprising the region 7p13-p12.1, with involvement of 39 OMIM genes, including: *GCK* associated with MODY 2, *CCM2* associated with type 2 cerebral cavernous malformations, *IGFBP-3* associated with decreased postnatal growth, and *OGDH* associated with alpha-ketoglutarate dehydrogenase deficiency. This previously unreported deletion is thought to explain the clinical picture of the patient and suggests that 7p13-p12.1 contains genes involved in intellectual disability and craniofacial development.

Ethics

Informed Consent: Written informed consent for publication of the data was given by the patient and his parents.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: Raquel Barrio, Design: Gilberto Pérez López, Beatriz Villafuerte Quispe, Data Collection and Processing: Gilberto Pérez López, Analysis and Interpretation: Luis Castaño, María José Cabrejas Núñez, Literature Research: Gilberto Pérez López, Writing: Gilberto Pérez López, Raquel Barrio.

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References

1. Barrio R, Bellanné-Chantelot C, Moreno JC, Morel V, Calle H, Alonso M, Mustieles C. Nine novel mutations in maturity-onset diabetes of the young (MODY) candidate genes in 22 Spanish families. J Clin Endocrinol Metab 2002;87:2532-2539.
2. Online Mendelian Inheritance in Man (OMIM). http://www.omim.org/. Last access: January 10, 2017.
3. Liao L, Chen X, Wang S, Parlow AF, Xu J. Steroid receptor coactivator 3 maintains circulating insulin-like growth factor I (IGF-I) by controlling IGF binding protein 3 expression. Mol Cell Biol 2008;28:2460-2469. Epub 2008 Jan 22
4. DatabasE of genomiC varIation and Phenotype in Humans using Ensembl Resources (DECIPHER). http://decipher.sanger.ac.uk/index Last access: January 14, 2017.
5. Huang N, Lee I, Marcotte EM, Hurles ME. Characterizing and predicting haploinsufficiency in the human genome. PLoS Genet 2010;6:e1001154.
6. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285-291.
7. Pruhova S, Dusatkova P, Sumnik Z, Kolouskova S, Pedersen O, Hansen T, Cinek O, Lebl J. Glucokinase diabetes in 103 families from a country-based study in the Czech Republic: geographically restricted distribution of two prevalent GCK mutations. Pediatr Diabetes 2010;11:529-535.
8. Garin I, Rica I, Estalella I, Oyarzabal M, Rodríguez-Rigual M, San Pedro JI, Pérez-Nanclares G, Fernández-Rebollo E, Busturia MA, Castaño L, Pérez de Nanclares G; Spanish MODY Group. Haploinsufficiency at GCK gene is not a frequent event in MODY2 patients. Clin Endocrinol (Oxf) 2008;68:873-878. Epub 2008 Feb 1
9. Ellard S, Thomas K, Edghill EL, Owens M, Ambye L, Cropper J, Little J, Strachan M, Stride A, Ersoy B, Eiberg H, Pedersen O, Shepherd MH, Hansen T, Harries LW, Hattersley AT. Partial and whole gene deletion mutations of the GCK and HNF1A genes in maturity onset diabetes of the young. Diabetologia 2007;50:2313-2317. Epub 2007 Sep 8
10. Schwarzbraun T, Windpassinger C, Ofner L, Vincent JB, Cheung J, Scherer SW, Wagner K, Kroisel PM, Petek E. Genomic analysis of five chromosome 7p deletion patients with Greig cephalopolysyndactyly syndrome (GCPS). Eur J Med Genet 2006;49:338-345. Epub 2005 Nov 28
11. Lachance CH. Practical Aspects of Monogenic Diabetes: A Clinical Point of View. Can J Diabetes 2016;40:368-375. Epub 2016 Feb 18
12. Bacon S, Schmid J, McCarthy A, Edwards J, Fleming A, Kinsley B, Firth R, Byrne B, Gavin C, Byrne MM. The clinical management of hyperglycemia in pregnancy complicated by maturity-onset diabetes of the young. Am J Obstet Gynecol 2015;213:236. Epub 2015 Apr 30
13. The Human Phenotype Ontology (HPO) database. http://human-phenotype-ontology.github.io/ Last access: January 15, 2017.

CASE REPORT

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Phenotypic Variability in a Family with Acrodysostosis Type 2 Caused by a Novel PDE4D Mutation Affecting the Serine Target of Protein Kinase-A Phosphorylation

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What is already known on this topic?

Acrodysostosis is a rare congenital multisystem condition characterized by skeletal dysplasia, varying degrees of intellectual disability, and possible resistance to multiple G protein-coupled receptor signalling hormones. Acrodysostosis type 2 is caused by mutations in cAMP-specific phosphodiesterase 4D (*PDE4D*). To date, 26 different *PDE4D* gene mutations have been reported in humans.

What this study adds?

Identification of a novel heterozygous missense mutation of the *PDE4D* gene c.569C > T (p.Ser190Phe) in a familial case of acrodysostosis type 2 affecting the serine target of protein kinase-A phosphorylation within the motif RRESF.
Evidence for a significant phenotypic variability of acrodysostosis in patients carrying the same mutation.

Abstract

Acrodysostosis is a very rare congenital multisystem condition characterized by skeletal dysplasia with severe brachydactyly, midfacial hypoplasia, and short stature, varying degrees of intellectual disability, and possible resistance to multiple G protein-coupled receptor signalling hormones. Two distinct subtypes are differentiated: acrodysostosis type 1 resulting from defects in protein kinase type 1-α regulatory subunit and acrodysostosis type 2 caused by mutations in phosphodiesterase 4D (*PDE4D*). Most cases are sporadic. We report on a rare multigenerational familial case of acrodysostosis type 2 due to a novel autosomal dominantly inherited *PDE4D* mutation. A 3.5-year-old boy presented with short stature, midfacial hypoplasia, severe brachydactyly, developmental delay, and behavioural problems. Laboratory investigations revealed mild thyrotropin resistance. His mother shared some characteristic features, such as midfacial hypoplasia and severe brachydactyly, but did not show short stature, intellectual disability or hormonal resistance. Genetic analysis identified the identical, novel heterozygous missense mutation of the *PDE4D* gene c.569C > T (p.Ser190Phe) in both patients. This case illustrates the significant phenotypic variability of acrodysostosis even within one family with identical mutations. Hence, a specific clinical diagnosis of acrodysostosis remains challenging because of great interindividual variability and a substantial overlap of the two subtypes as well as with other related Gsα-cAMP-signalling-linked disorders.

Keywords: Acrodysostosis, skeletal dysplasia, brachydactyly, inactivating parathyroid hormone/parathyroid hormone related protein signalling disorder, phosphodiesterase 4D

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Introduction

Acrodysostosis is a very rare congenital multisystem condition characterized by (1) skeletal dysplasia with severe brachydactyly, midfacial hypoplasia, and short stature, (2) varying degrees of intellectual disability, and (3) possible resistance to multiple G protein-coupled receptor (GPCR) signalling hormones, including parathyroid hormone (PTH) and thyrotropin (TSH) (1,2). The syndrome was first described by Robinow et al (3) in 1971. Recently, genetic defects in cAMP-dependent protein kinase type 1-α regulatory subunit (PRKAR1A) and cAMP-specific phosphodiesterase 4D (PDE4D) were identified in patients with acrodysostosis by candidate gene analysis (4) and exome sequencing (2,5) respectively.

Since then, two distinct genetic and phenotypic subtypes of acrodysostosis have been differentiated: acrodysostosis type 1 resulting from defects in *PRKAR1A*, and acrodysostosis type 2 caused by mutations in *PDE4D*. PRKAR1A as well as PDE4D play a crucial role in the GPCR-Gsα-cAMP-protein kinase A-signalling pathway (cAMP/PKA-pathway), which is present in almost all cell types and mediates a wide spectrum of biological functions, i.e. the physiological effects of several hormones (6).

In acrodysostosis type 1, the mutations in the regulatory subunit of *PRKAR1A* impair signalling through GPCR that use Gsα/adenyl cyclase/cAMP/PKA as a major signalling pathway, including PTH related protein (PTHrP), known to play an important role in endochondral bone development (7). In the case of acrodysostosis type 2, the presence of skeletal abnormalities whose characteristics and severity are indistinguishable from those seen in acrodysostosis type 1 supports the idea that during development, signalling by PTHrP through the PTHR-cAMP-PKA pathway is also attenuated and suggests that the mutations in *PDE4D* observed in this disease ultimately result in inappropriately increased PDE activity. The finding that injection into zebrafish of human *PDE4D* carrying mutations causing acrodysostosis type 2, but not wild-type *PDE4D*, produces developmental abnormalities is consistent with this idea (1). Many splice variants of *PDE4D* require PKA-induced phosphorylation to achieve maximal enzymatic activity (8,9,10). Based on the location of *PDE4D* mutations occurring in acrodysostosis type 2, it has been suggested that they may influence regulation by PKA (11). In contrast, mutations that would inactivate PDE4D (e.g. nonsense mutations, deletions, or mutations disrupting the catalytic site) cause a different phenotype, which is, in certain respects, a mirror-image of abnormalities seen in acrodysostosis type 2 (1). Another group, however, reported that *PDE4D* mutations causing acrodysostosis type 2 resulted in impaired enzyme activity, and that the observed phenotype resulted from an over-compensatory increased expression of other PDE4 isoforms (12,13). Thus, although the precise nature of the effect of PDE4 mutations in this disease remains controversial, all currently available evidence favours the conclusion that global PDE activity is inappropriately increased.

The vast majority of the cases of acrodysostosis occur sporadically, most probably due to *de novo* point mutations with evidence of a paternal age effect (5,14,15). Only very few families with an autosomal dominant inheritance of acrodysostosis type 2 have been reported so far (15,16).

Here, we report on a novel autosomal dominantly inherited heterozygous *PDE4D* mutation in a multigenerational family with acrodysostosis that illustrates the clinical variability of this syndrome even within one family. In doing so, we aim to raise awareness for this rare and clinically heterogeneous disease.

Case Reports

Patient 1

Patient 1 was born at 38 weeks' gestation by caesarean section because of oligohydramnion and pathological cardiotocography. At birth, he was small for gestational age with a weight of 2530 g [4.5th percentile, standard deviation score (SDS) -1.68] and a length of 48 cm (7th percentile, -1.47 SDS). During the neonatal period, the patient had feeding problems and several episodes of hypoglycemia occurred. Initial psychomotor milestones were reached rather late, but still within the normal range (walking at 1.6 years of age, speaking single words at 1.5 years of age). However, subsequently, he displayed a delay in motor development and regression of verbal development, and ergotherapy and speech therapy were started. Adenoidectomy and insertion of tympanic ventilation tubes were performed at the age of 3 years.

At the age of 3.5 years, he was referred to our clinic for further clinical evaluation. On examination, he had light-coloured blue eyes and red-coloured hair, a round face with widely spaced eyes and midfacial hypoplasia with flattening of the nasal bridge (Figure 1A). Moreover, he had small broad hands and feet with stubby digits with relative sparing of the first toe. Furthermore, pigmented skin lesions were noticed on the upper arms and legs. Neuropsychological examination showed behavioural problems and a delay of gross and fine motor skills and speech development with severely impaired speech comprehension and dyslalia. He had grown below the 3rd percentile since the age of 2 years with a height of 90.7 cm (0.9th percentile, SDS -2.38) at the

time of presentation. His weight was 12.8 kg (5.4th percentile, SDS -1.61) with a body mass index of 15.6 kg/m^2 (51.6th percentile, SDS +0.04). Radiographic examination showed severe brachydactyly with shortening of metacarpals, metatarsals and phalanges, except for the big toe, cone-shaped epiphyses, and a significantly advanced bone age (Figure 2A). Laboratory investigations showed normal results, except for a mildly elevated TSH of 7.98 mU/L, while thyroid hormone levels were normal.

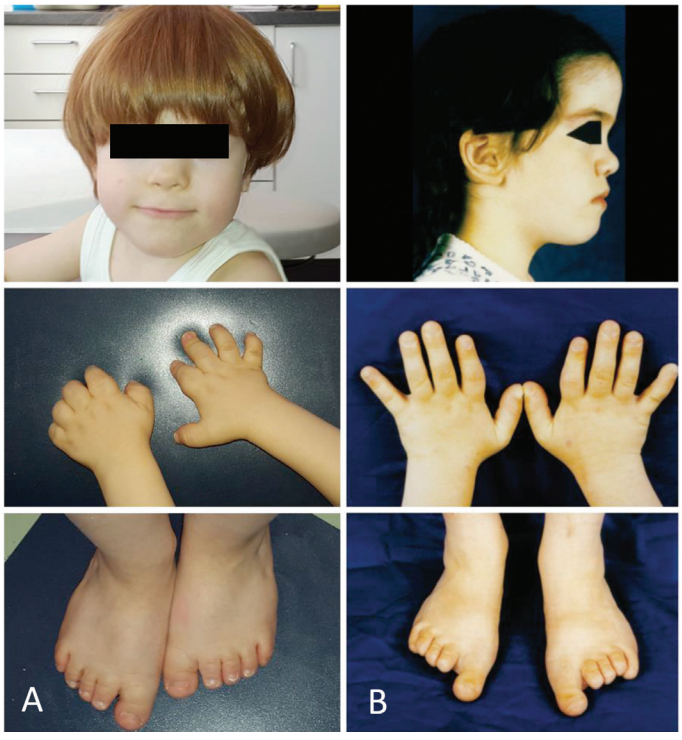

Figure 1. Photographs of the face, hands, and feet of patient 1 at the age of 3 years (A) and patient 2 at the age of 8 years (B). Note the facial dysostosis with flattening of the nasal bridge and the small broad hands and feet with relative sparing of the first toe [Figure 1B reproduced with permission of Springer (17)]

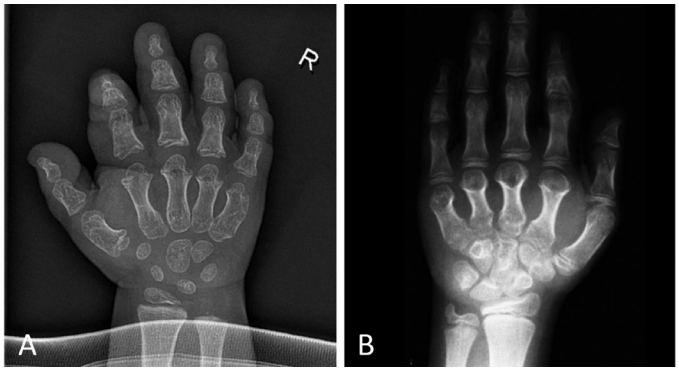

Figure 2. X-rays of the hands of patient 1 (A) and patient 2 (B). Note the severe brachydactyly with shortening of metacarpals and phalanges and cone-shaped epiphyses [Figure 2B reproduced with permission of Springer (17)]

Patient 2

Patient 2 is the mother of patient 1 (17). She presented at the age of 8.8 years. At presentation, she had macrocephaly with frontal bossing, midfacial hypoplasia, and small broad hands and feet with brittle nails (Figure 1B). The big toe of both feet appeared hyperplastic. The patient had a height between the 3rd and 10th percentiles, a body weight at the 50th percentile, and a head circumference above the 97th percentile. Radiographs showed severe brachydactyly with short metacarpals II-V, metatarsals II-IV and phalanges, first ray hyperplasia of the foot, cone-shaped epiphyses, and early epiphyseal fusion (Figure 2B). Bone age was accelerated by 3 years. Magnetic resonance imaging of the head showed a thickened calvarium, as well as a 2 cm supracerebellar arachnoid cyst and a lipoma at the corpus callosum as incidental findings. Furthermore, a heart defect with septal aneurysm and atrioventricular valve defect was diagnosed. At initial presentation, laboratory investigations showed normal thyroid function tests, a normal PTH, and adequate calcium and phosphate levels. In the further course, she developed autoimmune thyroiditis as well as vitamin D deficiency. According to the medical history, she had started speaking at the age of 4 years. However, her further mental development was normal. She graduated from high school and momentarily studies law at the university.

Clinical, radiological and laboratory characteristics of patient 1 and 2 are summarized in Table 1.

Similar dysmorphic clinical findings and radiological features of the hands and feet were reported in the mother of patient 2 (grandmother of patient 1). She had died from leukemia. Other family members have not been affected.

Molecular analysis in patient 1 and patient 2 identified a novel heterozygous missense mutation of the *PDE4D* gene (NM-001104631): c.569C>T (p.Ser190Phe) in exon 2 and thereby confirmed the clinical diagnosis of acrodysostosis type 2. Interestingly, this mutation affects the serine target of PKA phosphorylation within the motif RRESF.

Informed consent was obtained from the patient's family.

Discussion

In this article, we reported a very rare case of autosomal dominantly inherited acrodysostosis type 2 in a three-generational family caused by a novel mutation in the *PDE4D* gene illustrating the significant phenotypic variability of acrodysostosis and discuss differential diagnosis. Here by, we aim to increase the general awareness of this rare condition.

To our knowledge, this is the first report on a family with three generations to be affected by acrodysostosis type 2 due to a heterozygous missense mutation of the *PDE4D* gene c.569C>T (p.Ser190Phe) affecting the upstream conserved region 1 of the PDE4D. It is the second mutation affecting the serine target of PKA phosphorylation within the motif RRESF. To date, 26 missense mutations in *PDE4D* gene associated with acrodysostosis type 2 have been identified (including the novel mutation in the family discussed in this case report). All mutations described so far were sporadic, heterozygous de novo mutations, except for very few families with a genetically proven autosomal dominant inheritance of *PDE4D* mutations (15,16).

Second, in the present case, the phenotypic variability of affected family members sharing the identical mutation is striking. Interestingly, patient 1 was born small for gestational age and has short stature, whereas his mother (patient 2) who carried the same mutation has a normal height. Light-coloured blue eyes and red-coloured hair, originally described by Niikawa et al (18) in patients with acrodysostosis, were striking on clinical examination of patient 1 (15). In contrast, his mother presented with brown hair colour. Furthermore, patient 1 displayed behavioural problems and a delay of motor and speech development with severely impaired speech comprehension and dyslalia, whereas his mother graduated from high school and attends university. Patient 1 had mild TSH resistance, whereas his mother had normal thyroid functions tests at presentation. Clinical heterogeneity for acrodysostosis was also described by Lynch et al (15) in a family with three affected siblings whose father was found to have only subtle features of acrodysostosis but also carried the same mutation. Lynch et al (15) also found a high variability of intellectual abilities in this family. As possible explanations for the lack of full clinical expression, variable expressivity, mosaicism, and tissue specific imprinting of the gene are discussed (15).

Table 1. Clinical, radiological, and biochemical features in the patients with acrodysostosis

Features	Patient 1	Patient 2
Clinical features		
Facial dysostosis		
- Broad face with widely spaced eyes	Yes	Yes
- Maxillonasal hypoplasia	Yes	Yes
Peripheral dysostosis		
- Intrauterine growth retardation	Yes	-
- Short stature	Yes	No
- Small and broad hands and feet	Yes	Yes
- Enlargement of first toe	Yes	Yes
Intellectual disability	Yes	No
Radiological findings		
- Thickened calvarium	-	Yes
- Severe brachydactyly	Yes	Yes
- Cone-shaped epiphyses	Yes	Yes
- Advanced bone age	Yes	Yes
Laboratory findings		
- Hormonal resistance	Yes	No

Third, given the very low number of familial cases reported so far and the significant phenotypic variability of affected family members, it is most likely that some affected individuals are not considered for diagnosis because of subtle phenotypic features. Hence, familial cases might be underestimated. Therefore, careful clinical evaluation of the parents of affected patients for features of acrodysostosis is crucial to detect possible mutation carriers, to reveal new familial cases, and to gain information about the genotype-phenotype correlation in the future.

Fourth, considering the great clinical overlap between the subtypes of acrodysostosis and also with other Gsα-cAMP-signalling-linked disorders, a specific clinical diagnosis still remains difficult and challenging, if not even impossible.

In the past, the two subtypes of acrodysostosis were mainly differentiated by the presence or absence of hormonal resistance that was exclusively attributed to *PRKAR1A* mutations (19). However, Lindstrand et al (1) recently found that *PDE4D* mutations as well may lead to clinically significant endocrine abnormalities like PTH or TSH resistance in a small subset of patients (16). Moreover, the present case shows that even family members with the identical mutation in *PDE4D* might differ in the presence of hormonal resistance, illustrating the phenotypic variability. Therefore, endocrine follow-up remains important for all patients, regardless of the subtype of acrodysostosis. However, Elli et al (16) described different frequencies of phenotypic characteristics according to the mutated gene. Short stature and cone-shaped epiphyses were more often described in acrodysostosis type 1, whereas a more characteristic facial dysostosis, mental or behavioural defects, cryptorchidism and/or lack of pubertal spurt were found more often in acrodysostosis type 2 (16). Several non-mutually exclusive explanations for the phenotypic differences have been advanced, but further work is required to validate their importance (13,16,19,20). However, since the number of published cases of acrodysostosis, in particular type 2, is still low and single cases had a high impact on this comparative analysis, future alterations of observed clinical features are

likely. A single distinctive feature that allows clear clinical differentiation of both subtypes has not been revealed yet.

Differential diagnosis should not only include the different subtypes of acrodysostosis but also other entities of the "inactivating PTH/PTHrP signalling disorders" that clinically present with Albright hereditary osteodystrophy (AHO) (21,22). AHO was first described by Albright et al (23) in 1942 and comprises heterogeneous clinical features such as brachydactyly, rounded face, short stature, stocky build and subcutaneous ossifications. In pseudohypoparathyroidism type 1A and type 1C, AHO is associated with hormonal resistance as well as obesity and varying degrees of intellectual disability. They are caused by maternal loss-of-function mutations or imprinting defects in the GNAS gene encoding Gsα. AHO without any evidence of hormonal resistance, called pseudopseudohypoparathyroidism, is due to paternal loss-of-function mutations in GNAS. Because of substantial clinical overlap of acrodysostosis with other related Gsα-cAMP-signalling-linked disorders, a clear clinical diagnosis without genetic analysis remains difficult.

In summary, we identified a novel heterozygous *PDE4D* mutation in a family with acrodysostosis type 2. This case illustrates the phenotypic variability of acrodysostosis even within one family with identical mutations. We conclude that a specific clinical diagnosis of acrodysostosis remains challenging because of great interindividual variability and a substantial overlap of the two subtypes as well as with other related Gsα-cAMP-signalling-linked disorders.

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Ethics

Informed Consent: Informed consent was obtained from the patient's family.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Volker Schuster, Astrid Bertsche, Concept: Volker Schuster, Design: Julia Hoppmann, Volker Schuster, Data Collection or Processing: Caroline Silve, Chrystel Leroy, Franz Wolfgang Hirsch, Astrid Bertsche, Julia Hoppmann, Analysis or Interpretation: Caroline Silve, Chrystel Leroy, Literature Search: Julia Hoppmann, Julia Gesing, Volker Schuster, Franz Wolfgang Hirsch, Writing: Julia Hoppmann, Julia Gesing, Caroline Silve, Wieland Kiess, Roland Pfäffle, Astrid Bertsche.

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References

1. Lindstrand A, Grigelioniene G, Nilsson D, Pettersson M, Hofmeister W, Anderlid BM, Kant SG, Ruivenkamp CA, Gustavsson P, Valta H, Geiberger S, Topa A, Lagerstedt-Robinson K, Taylan F, Wincent J, Laurell T, Pekkinen M, Nordenskjöld M, Mäkitie O, Nordgren A. Different mutations in PDE4D associated with developmental disorders with mirror phenotypes. J Med Genet 2014;51:45-54. Epub 2013 Nov 7
2. Michot C, Le Goff C, Goldenberg A, Abhyankar A, Klein C, Kinning E, Guerrot AM, Flahaut P, Duncombe A, Baujat G, Lyonnet S, Thalassinos C, Nitschke P, Casanova JL, Le Merrer M, Munnich A, Cormier-Daire V. Exome sequencing identifies PDE4D mutations as another cause of acrodysostosis. Am J Hum Genet 2012;90:740-745. Epub 2012 Mar 29
3. Robinow M, Pfeiffer RA, Gorlin RJ, McKusick VA, Renuart AW, Johnson GF, Summitt RL. Acrodysostosis. A syndrome of peripheral dysostosis, nasal hypoplasia, and mental retardation. Am J Dis Child 1971;121:195-203.
4. Linglart A, Menguy C, Couvineau A, Auzan C, Gunes Y, Cancel M, Motte E, Pinto G, Chanson P, Bougnères P, Clauser E, Silve C. Recurrent PRKAR1A mutation in acrodysostosis with hormone resistance. N Engl J Med 2011;364:2218-2226.
5. Lee H, Graham JM Jr, Rimoin DL, Lachman RS, Krejci P, Tompson SW, Nelson SF, Krakow D, Cohn DH. Exome sequencing identifies PDE4D mutations in acrodysostosis. Am J Hum Genet 2012;90:746-751. Epub 2012 Mar 29
6. Assié G. One single signaling pathway for so many different biological functions: lessons from the cyclic adenosine monophosphate/protein kinase A pathway-related diseases. J Clin Endocrinol Metab 2012;97:4355-4357.
7. Karaplis AC, Goltzman D. PTH and PTHrP effects on the skeleton. Rev Endocr Metab Disord 2000;1:331-342.
8. Mika D, Conti M. PDE4D phosphorylation: a coincidence detector integrating multiple signaling pathways. Cell Signal 2016;28:719-724. Epub 2015 Nov 10
9. Sette C, Conti M. Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. J Biol Chem 1996;271:16526-16534.
10. Sheppard CL, Lee LC, Hill EV, Henderson DJ, Anthony DF, Houslay DM, Yalla KC, Cairns LS, Dunlop AJ, Baillie GS, Huston E, Houslay MD. Mitotic activation of the DISC1-inducible cyclic AMP phosphodiesterase-4D9 (PDE4D9), through multi-site phosphorylation, influences cell cycle progression. Cell Signal 2014;26:1958-1974. Epub 2014 May 9
11. Cedervall P, Aulabaugh A, Geoghegan KF, McLellan TJ, Pandit J. Engineered stabilization and structural analysis of the autoinhibited conformation of PDE4. Proc Natl Acad Sci USA 2015;112:1414-1422. Epub 2015 Mar 9
12. Houslay M. Hypertension linked to PDE3A activation. Nat Genet 2015;47:562-563.
13. Kaname T, Ki CS, Niikawa N, Baillie GS, Day JP, Yamamura K, Ohta T, Nishimura G, Mastuura N, Kim OH, Sohn YB, Kim HW, Cho SY, Ko AR, Lee JY, Kim HW, Ryu SH, Rhee H, Yang KS, Joo K, Lee J, Kim CH, Cho KH, Kim D, Yanagi K, Naritomi K, Yoshiura K, Kondoh T, Nii E, Tonoki H, Houslay MD, Jin DK. Heterozygous mutations in cyclic AMP phosphodiesterase-4D (PDE4D) and protein kinase A (PKA) provide new insights into the molecular pathology of acrodysostosis. Cell Signal 2014;26:2446-2459. Epub 2014 Jul 24
14. Jones KL, Smith DW, Harvey MA, Hall BD, Quan L. Older paternal age and fresh gene mutation: data on additional disorders. J Pediatr 1975;86:84-88.

15. Lynch DC, Dyment DA, Huang L, Nikkel SM, Lacombe D, Campeau PM, Lee B, Bacino CA, Michaud JL, Bernier FP; FORGE Canada Consortium, Parboosingh JS, Innes AM. Identification of novel mutations confirms PDE4D as a major gene causing acrodysostosis. Hum Mutat 2013;34:97-102.

16. Elli FM, Bordogna P, de Sanctis L, Giachero F, Verrua E, Segni M, Mazzanti L, Boldrin V, Toromanovic A, Spada A, Mantovani G. Screening of PRKAR1A and PDE4D in a large Italian series of patients clinically diagnosed with Albright hereditary osteodystrophy and/or pseudohypoparathyroidism. J Bone Miner Res 2016;31:1215-1224. Epub 2016 Feb 9

17. Schuster V, Kellner MW, Horwitz AE. Akrodysostose. Monatsschr Kinderheilkd 1999;147:936-939.

18. Niikawa N, Matsuda I, Ohsawa T, Kajii T. Familial occurrence of a syndrome with mental retardation, nasal hypoplasia, peripheral dysostosis, and blue eyes in Japanese siblings. Hum Genet 1978;42:227-232.

19. Linglart A, Fryssira H, Hiort O, Holterhus PM, Perez de Nanclares G, Argente J, Heinrichs C, Kuechler A, Mantovani G, Leheup B, Wicart P, Chassot V, Schmidt D, Rubio-Cabezas Ó, Richter-Unruh A, Berrade S, Pereda A, Boros E, Muñoz-Calvo MT, Castori M, Gunes Y, Bertrand G, Bougnères P, Clauser E, Silve C. PRKAR1A and PDE4D mutations cause acrodysostosis but two distinct syndrome with or without GPCR-signaling hormone resistance. J Clin Endocrinol Metab 2012;97:2328-2338. Epub 2012 Oct 5

20. Motte E, Le Stunff C, Briet C, Dumaz N, Silve C. Modulation of signaling through GPCR-cAMP-PKA pathways by PDE4 depends on stimulus intensity: Possible implications for the pathogenesis of acrodysostosis without hormone resistance. Mol Cell Endocrinol 2016;442:1-11. Epub 2016 Nov 28

21. Mantovani G, Spada A, Elli FM. Pseudohypoparathyroidism and Gsa-cAMP-linked disorders: current view and open issues. Nat Rev Endocrinol 2016;12:347-356. Epub 2016 Apr 22

22. Thiele S, Mantovani G, Barlier A, Boldrin V, Bordogna P, de Sanctis L, Elli F, Freson K, Garin I, Grybek V, Hanna P, Izzi B, Hiort O, Lecumberri B, Pereda A, Saraff V, Silve C, Turan S, Usardi A, Werner R, de Nanclares GP, Linglart A. From Pseudohypoparathyroidism to inactivating PTH/PTHrP Signalling Disorder (iPPSD), a novel classification proposed by the European EuroPHP network. Eur J Endocrinol 2016;175:1-17. Epub 2016 Jul 11

23. Albright F, Burnett CH, Smith PH, Parson W. Pseudohypoparathyroidism – an example of "Seabright-Bantam syndrome". Endocrinology 1942;30:922-932.

CASE REPORT

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Successful Growth Hormone Therapy in Cornelia de Lange Syndrome

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What is already known on this topic?

This manuscript describes a patient with Cornelia de Lange syndrome who was successfully treated with growth hormone because she was born small for gestational age and showed no catch up growth. The diagnosis of Cornelia de Lange syndrome was made years after the growth hormone treatment. To our knowledge, there is no published work about the effect of growth hormone therapy in Cornelia de Lange syndrome.

What this study adds?

This case report will contribute to more knowledge about treatment with growth hormone in Cornelia de Lange syndrome and also help fellow pediatricians and pediatric endocrinologists in their decision-making when considering the treatment in this patient group.

Abstract

Cornelia de Lange syndrome (CdLS) is a both clinically and genetically heterogeneous syndrome. In its classical form, it is characterised by distinctive facial features, intra-uterine growth retardation, short stature, developmental delay, and anomalies in multiple organ systems. *NIPBL, SMC1A, SMC3, RAD2*1 and *HDAC8*, all involved in the cohesin pathway, have been identified to cause CdLS. Growth hormone (GH) secretion has been reported as normal, and to our knowledge, there are no reports on the effect of recombinant human GH treatment in CdLS patients. We present a patient born small for gestational age with persistent severe growth retardation [height -3.4 standard deviation score (SDS)] and mild dysmorphic features, who was treated with GH from 4.3 years of age onward and was diagnosed 6 years later with CdLS using whole-exome sequencing. Treatment led to a height gain of 1.6 SDS over 8 years. Treatment was interrupted shortly due to high serum insulin-like growth factor-1 serum values. In conclusion, GH therapy may be effective and safe for short children with CdLS.

Keywords: Cornelia de Lange syndrome, growth hormone, small for gestational age, *NIPBL*, whole-exome sequencing

Introduction

The clinical features of Cornelia de Lange syndrome (CdLS) (synonym Brachmann-de Lange syndrome, de Lange syndrome; Online Mendelian Inheritance in Man #122470, #300590, #610759, #614701, #300882) were first described by de Lange (1) in 1933, although Brachmann (2) is believed to have reported a case in 1916. CdLS is known as a rare and genetically and clinically heterogeneous disorder. The reported prevalence of 0.5-10:100,000 may be an underestimation due to underdiagnosis of mild cases (3).

Mutations in the *NIPBL*, *SMC1A*, *SMC3*, *RAD21*, and *HDAC8* genes have been identified to cause CdLS (4). These genes code for subunits and regulatory proteins in the cohesin pathway. Most cases of CdLS are sporadic although autosomal and X-linked inheritance patterns have been described in some families (4).

CdLS is a disorder affecting multiple organ systems. Characteristic craniofacial features include well-defined arched eyebrows with synophrys, long and curly eyelashes, ptosis and low-set, posteriorly rotated ears. In addition, a

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variety of additional features such as craniofacial deviations and cardiovascular, gastrointestinal, genitourinary, and neurosensory abnormalities have been reported. Skeletal deformations usually affect the upper extremity. Linear growth is in general impaired, and affected patients show both pre- and postnatal growth retardation. CdLS specific growth charts are available showing a mean adult height of 155.8 cm for males and 131.1 cm for females (5). Cognitive and psychomotor abilities vary from mild learning disabilities, in which speech and language disorders are more distinct, to severe intellectual disability (4). With regard to the severity of clinical features, there is a trend of a genotype-phenotype association, depending on the causative gene and type of mutation. However, an identical mutation can be associated with quite different phenotypes, suggestive of a role of other modifying genetic or environmental factors (6).

Only few reports have commented on endocrine abnormalities in CdLS. Kline et al (7) reported a mildly delayed puberty in a group of 49 patients with CdLS, with a mean age of onset of 15 years for boys and 13 years for girls. In several reports, growth hormone (GH) secretion was assessed and found normal. We found no reports on the effect of recombinant human GH (r-hGH) treatment in children with CdLS.

We present a female patient born small for gestational age (SGA) with severe postnatal growth retardation and mild dysmorphic features. She was treated with GH from 4.3 years of age and was diagnosed 6 years later with CdLS using whole-exome sequencing (WES), thereby being the first reported CdLS patient to be treated with GH.

Case Report

This female patient is the third child of healthy non-consanguineous parents with normal heights (father 173 cm, mother 166 cm) and no known familiar diseases or genetic defects. Conditional target height (cTH) was 166 cm [-0.7 standard deviation score (SDS)] (8). Both her siblings had normal birth weight and postnatal growth. At the age of 10 months, she was referred to our centre for evaluation of short stature. After being carefully monitored in the prenatal period for intra-uterine growth retardation, she was born at a gestational age of 37 weeks and 5 days, after an uncomplicated delivery. At birth, her weight was 2340 grams (-1.7 SDS) (9), but subsequent measurements of weight in the first year of life were between -3.4 SDS and -4.0 SDS. Head circumference at birth was 32 cm (-2.4 SDS). Length was not measured at birth, but at 3 months of age length was 51.5 cm (-3.4 SDS), and all subsequent height measurements were below -3.0 SDS (Figure 1). Based on these data, we estimated the likelihood of a low birth length sufficient to diagnose the child as SGA with failure of catch-up growth (10).

At the age of 10 months, length was 63 cm (-3.7 SDS), weight 6.2 kg (-3.4 SDS), weight for length was normal (0.2 SDS), head circumference was 42.5 cm (-2.0 SD), and the ratio between crown-rump length and total length was 0.68 (2.1 SDS) (11). Arm span/height ratio was 0.92 (0.1 SDS) (12). Physical examination showed no abnormalities except for two dysmorphic features. First, the patient had well-defined and arched eyebrows with synophrys. Second, she had short digits of both hands, in particular the phalanges of both fifth digits (Figure 2).

Serum insulin-like growth factor-1 (IGF-1) was 8.0 nmol/L (-0.1 SDS), IGF-binding protein 3 1.8 mg/L (-1.1 SDS), and GH stimulation tests with clonidine and arginine showed serum GH of peaks of 4.4 µg/L and 12.1 µg/L, respectively. This made GH deficiency or insensitivity unlikely.

A skeletal survey performed in the first year of life and at the age of two years showed no signs of skeletal dysplasia. A 46,XX karyotype was determined at amniocentesis, and genetic evaluation of *SHOX*, *FGFR3*, *IGF-1,* and *IGF1R* revealed no mutations or copy number variants, and uniparental disomy of chromosome 7 and hypomethylation of the 11p15 imprinting region tested negative. At the age of 5 years, a single nucleotide polymorphism array (GeneChip Human Mapping 250K Nsp Array) was performed showing no abnormalities.

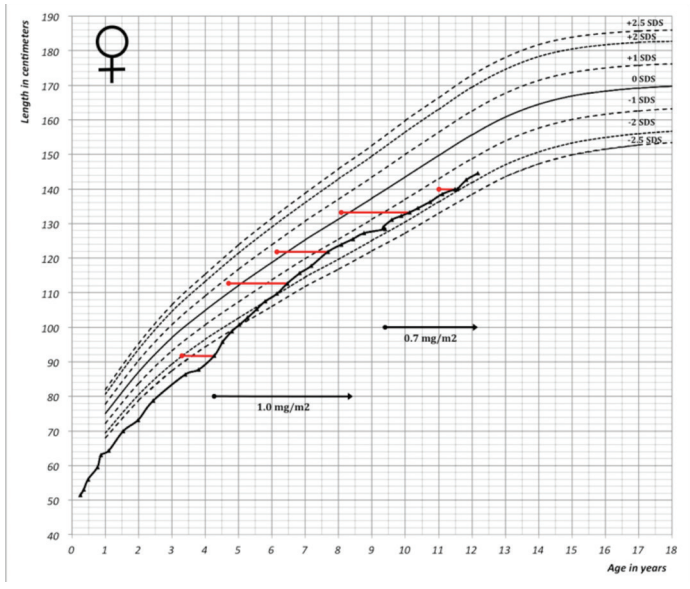

Figure 1. Growth curve of the patient plotted on the growth reference chart for female Dutch children (-2.5, -2.0, -1.0, 0, +1.0, +20 and +2.5 standard deviation lines are shown). Red lines indicate bone age, horizontal lines indicate recombinant human growth hormone treatment (8)

SDS: standard deviation score

At the age of 10 years, WES was performed in the index patient and her parents. Genomic DNA was isolated from peripheral blood samples using the Autopure LS Instrument (Gentra Systems). Cytogenetic microarray analysis was performed using the Affymetrix CytoScan HD Array according to the manufacturer's procedures. Copy number was assessed in the patient using ChAS software (Chromosome Analysis Suite). WES was performed as previously described by Hannema et al (13), using the Nimblegen SEqCap EZ V2 capture kit (Roche, Nimblegen, Inc, Madison, Wisconsin). Filtering for *de novo* variants in the index patient with a home-made variant filtering algorithm (Santen et al) (14) showed a heterozygous, *de novo* splice mutation (c.771 + 1G > A), chr5:36971139 build 37, in *NIPBL* gene. Although this mutation has not been described previously, it has been found in another patient with CdLS (15). The result was confirmed by Sanger sequencing. cDNA analysis was performed to investigate the effect of the splice mutation. An aberrant transcript besides the expected wild type PCR product was observed. Sequencing of the aberrantly spliced product revealed a loss of the last 21 bp of exon 7, which is expected to lead to an in-frame deletion of 7 amino acids in the protein, p.(Val251_Asp257del).

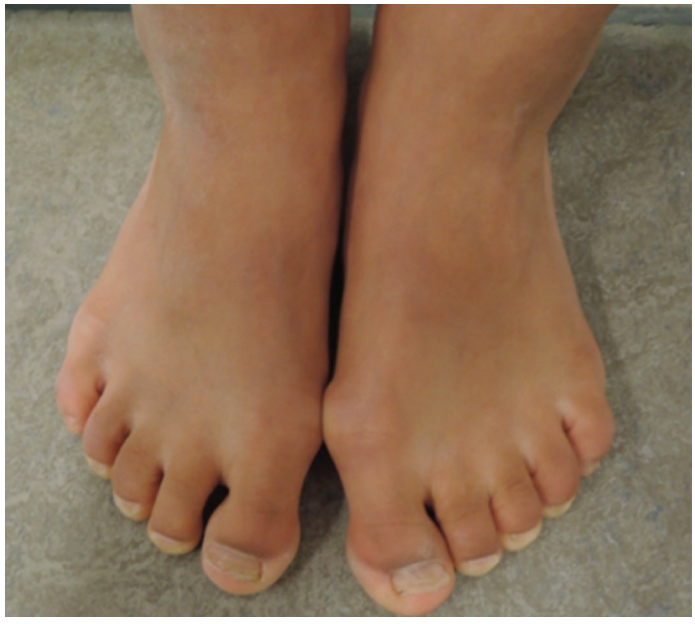

Figure 2a. Images of the hands a showing short digits. Published with permission of the patient's parents. No permission was given to publish photographs of facial features

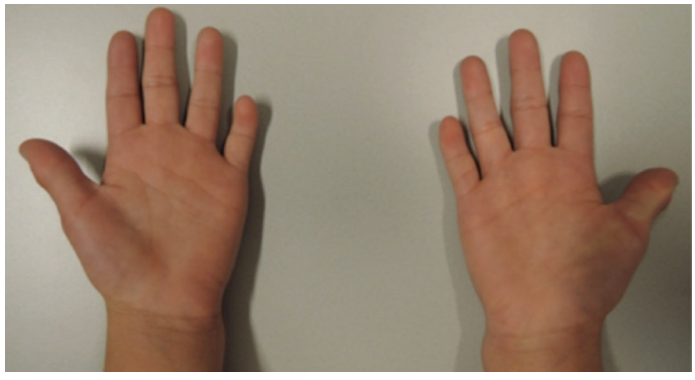

Figure 2b. Images of the hands showing short digits, especially of the thumbs and fifth digits. Published with permission of the patient's parents

Following the diagnosis of CdLS, an extensive medical screening was performed. Echocardiography revealed a structurally and functionally normal heart. An ultrasound of the urogenital tract showed normal structures. Otorhinolaryngologic and ophthalmologic evaluations showed no abnormalities except a mild nervus IV paresis of the left eye, but without resulting in a negative effect on the visual performance of the child.

Treatment with r-hGH in a dosage of 0.86 mg/m^2/day subcutaneously was started at 4.3 years of age at a height of 91.7 cm (-3.5 SDS) based on the indication of SGA with failure of catch-up growth. Bone age, assessed according to the Greulich and Pyle (16) method, was then 3.3 years. After the initiation of GH treatment regular outpatient clinic visits were scheduled. Every three months, anthropometric values were evaluated. Bone age and laboratory tests including IGF-1 were performed yearly. The growth response was appropriate (Figure 1) and at 8.5 years of age, height was 125.5 cm (-1.4 SDS). Following a series of increased serum IGF-1 values, as high as 5.3 SDS (Figure 3), therapy was interrupted for nine months, which led to deceleration of growth (height -1.7 SDS). At 9.3 years, r-hGH therapy was restarted at a lower dose of 0.7 mg/m^2. At present, at the age of 12.3 years, her height is 142.6 cm (-1.8 SDS) and IGF-1 is 2.2 SDS. The last measured bone age was 11.0 years at an age of 11.6 years. Predicted adult height at that point

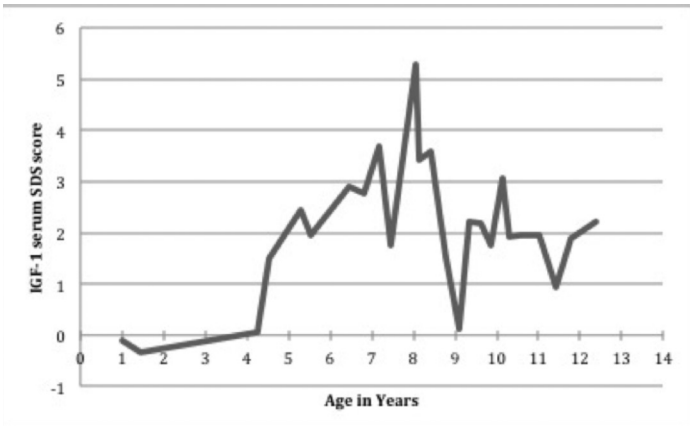

Figure 3. Patient's serum insulin-like growth factor-1 standard deviation score

IGF-1: insulin-like growth factor-1, SDS: standard deviation score

according to Bayley and Pinneau (17) was 154.4 cm, 1.8 SD below cTH-SDS. No side effects or health complaints occurred during therapy. Due to a speech and language disorder, she followed an adjusted educational programme. Her intellectual abilities were tested within the normal range with a nonverbal intelligence quotient of 98. Motor development was normal.

The patient's parents gave their consent for inclusion of the patient's pictures in this publication.

Discussion

In this case report, we present a female patient with extreme short stature and very mild clinical features of CdLS. She showed a substantial increase in height SDS of 1.8 SDS following treatment with r-hGH. To our knowledge, this is the first report on r-hGH treatment in a patient with CdLS. SGA without adequate catch-up growth is an established indication for GH therapy in most parts of the world including Europe and for this reason, GH was started in our patient. The described effect of r-hGH treatment in our patient resembles the increase in height previously reported in SGA patients treated with a similar GH dose (18,19). The initial extreme increase in IGF-1 SDS score on the 0.86 mg/m^2 GH dose is considerably higher than that reported for GH-treated children with SGA (18,20).

In approximately 70 % of CdLS patients, a genetic cause is detected (4). A *NIPBL* mutation is found in 80 % of these cases and results in more severe clinical problems in comparison to the other genes. However, missense mutations in this gene tend to give rise to a milder phenotype than splice site mutations, or nonsense and frameshift mutations (4). Our patient has a splice site mutation in the *NIPBL* gene, demonstrated by both DNA and RNA analysis, causing an in-frame deletion which resulted in mild clinical features. The most prominent clinical feature in this patient was growth retardation. Facial features of CdLS were not distinctive. Although she needed a special educational program for her speech language disorder, intelligence was within the normal range.

Although both prenatal and postnatal growth retardation is a hallmark of CdLS, little is known about CdLS and GH function. Schwartz et al (21), McArthur and Edwards (22), and Abraham and Russell (23) published data on GH concentration in 16 CdLS patients (see Table 1). Two patients reported by Schwartz et al (21) were diagnosed with classic GH deficiency. One of these well-nourished patient had low serum IGF-1 values and discordant results on stimulation tests, resulting in the conclusion that there was some degree of end-organ resistance to GH. McArthur and Edwards (22) and Abraham and Russell (23) measured GH concentration in 11 patients using multiple methods. GH concentrations of all patients were ≥14 µg/L.

In a more recent case series of 49 CdLS patients, 98 percent of the patients had heights below the 5th percentile compared to standard growth curves, but no tests for GH were conducted (7). Our patient had both normal GH concentrations after stimulation tests with arginine and clonidine as well as normal IGF-1 serum values, thereby excluding GH deficiency or insensitivity. The very high serum IGF-1 concentrations on a regular GH dosage suggest a form of IGF-1 insensitivity.

Although this case report suggests an appropriate growth response to r-hGH in this patient, we acknowledge that she has not achieved adult height yet. Still, our observation suggests that short children with CdLS (mildly affected) may be considered as candidates for r-hGH treatment.

Table 1. Overview of growth hormone test results in Cornelia de Lange syndrome patient

Author	Year	n	Study method	Results
McArthur and Edwards (22)	1967	2	Fasting GH concentration	GH concentration of 10 µg/L and 4.5 µg/L
Abraham and Russell (23)	1968	2	Insulin-induced hypoglycemia	All patient had GH concentration ≥14 µg/L
		7	Glucose tolerance test	
Schwartz et al (21)	1990	5	GH stimulation tests with insulin and clonidine	Two patients with classic GH deficiency, peak GH 2.4 and 4.5 µg/L One patient with both low IGF-1 and discordant stimulation results

GH: growth hormone, IGF-1: insulin-like growth factor-1

Ethics

Informed Consent: Informed consent was given by both parents of the presented case.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Wilma Oostdijk, Sarina G Kant, Monique Losekoot, Concept: Michael de Graaf, Wilma Oostdijk, Sarina G Kant, Data Collection or Processing: Michael de Graaf, Analysis or Interpretation: Egbert Johan Willem Redeker, Gijs Willem Eduard Santen, Annemieke Johanna Maria Henriëtta Verkerk, André Gerardus Uitterlinden, Literature Search: Michael de Graaf, Writing: Michael de Graaf, Wilma Oostdijk, Sarina G Kant, Jan Maarten Wit.

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References

1. de Lange C. Sur un type nouveau de degenerescence (typus Amstelodamensis). Arch Med Enfants 1933;36:713-719.
2. Brachmann W. Ein fall von symmetrischer monodaktylie durch Ulnadefekt, mit symmetrischer flughautbildung, in den ellebeugen, sowie anderen abnormitaten (zwerghaftogkeit, halsrippen, behaarung). Jarb Kinder Phys Erzie 1916;84:225-235.
3. Van Allen MI, Filippi G, Siegel-Bartelt J, Yong SL, McGillivray B, Zuker RM, Smith CR, Magee JF, Ritchie S, Toi A, et al. Clinical variability within Brachmann-de Lange syndrome: a proposed classification system. Am J Med Genet 1993;47:947-958.
4. Boyle MI, Jespersgaard C, Brondum-Nielsen K, Bisgaard AM, Tümer Z. Cornelia de Lange syndrome. Clin Genet 2015;88:1-12. Epub 2014 Oct 28
5. Kline AD, Barr M, Jackson LG. Growth Manifestations in the Brachmann-Delange Syndrome. Am J Med Genet 1993;47:1042-1049.
6. Mannini L, Cucco F, Quarantotti V, Krantz ID, Musio A. Mutation spectrum and genotype-phenotype correlation in Cornelia de Lange syndrome. Hum Mutat 2013;34:1589-1596. Epub 2013 Sep 16
7. Kline AD, Grados M, Sponseller P, Levy HP, Blagowidow N, Schoedel C, Rampolla J, Clemens DK, Krantz I, Kimball A, Pichard C, Tuchman D. Natural history of aging in Cornelia de Lange syndrome. Am J Med Genet C Semin Med Genet 2007;145:248-260.
8. van Dommelen P, Schönbeck Y, van Buuren S. A simple calculation of the target height. Arch Dis Child 2012;97:182. Epub 2011 Dec 18
9. Niklasson A, Albertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. BMC Pediatr 2008;8:8.
10. Wit JM, Ranke BM, Kelnar CJH. ESPE Classification of Paediatric Endocrine Diagnoses. Karger, 2007.
11. Fredriks AM, van Buuren S, van Heel WJ, Dijkman-Neerincx RH, Verloove-Vanhorick SP, Wit JM. Nationwide age references for sitting height, leg length, and sitting height/height ratio, and their diagnostic value for disproportionate growth disorders. Arch Dis Child 2005;90:807-812. Epub 2005 Apr 29
12. Gerver WJM, de Bruin R. Paediatric Morphometrics a reference manual. Utrecht Bunge, 1996.
13. Hannema SE, van Duyvenvoorde HA, Premsler T, Yang RB, Mueller TD, Gassner B, Oberwinkler H, Roelfsema F, Santen GW, Prickett T, Kant SG, Verkerk AJ, Uitterlinden AG, Espiner E, Ruivenkamp CA, Oostdijk W, Pereira AM, Losekoot M, Kuhn M, Wit JM. An activating mutation in the kinase homology domain of the natriuretic peptide receptor-2 causes extremely tall stature without skeletal deformities. J Clin Endocrinol Metab 2013;98:1988-1998. Epub 2013 Sep 20
14. Santen GW, Aten E, Sun Y, Almomani R, Gilissen C, Nielsen M, Kant SG, Snoeck IN, Peeters EA, Hilhorst-Hofstee Y, Wessels MW, den Hollander NS, Ruivenkamp CA, van Ommen GJ, Breuning MH, den Dunnen JT, van Haeringen A, Kriek M. Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome. Nat Genet 2012;44:379-380.
15. Yuan B, Pehlivan D, Karaca E, Patel N, Charng WL, Gambin T, Gonzaga-Jauregui C, Sutton VR, Yesil G, Bozdogan ST, Tos T, Koparir A, Koparir E, Beck CR, Gu S, Aslan H, Yuregir OO, Al Rubeaan K, Alnaqeb D, Alshammari MJ, Bayram Y, Atik MM, Aydin H, Geckinli BB, Seven M, Ulucan H, Fenercioglu E, Ozen M, Jhangiani S, Muzny DM, Boerwinkle E, Tuysuz B, Alkuraya FS, Gibbs RA, Lupski JR. Global transcriptional disturbances underlie Cornelia de Lange syndrome and related phenotypes. J Clin Invest 2015;125:636-651. Epub 2015 Jan 9
16. Greulich W, Pyle S. Radiograph Atlas of Skeletal Development of the Hand and Wrist. 2nd ed. California: Stanford University Press, 1959.
17. Bayley N, Pinneau SR. Tables for predicting adult height from skeletal age: revised for use with the Greulich-Pyle hand standards. J Pediatr 1952;4:423-441.
18. Hokken-Koelega A, van Pareren Y, Arends N, Boonstra V. Efficacy and Safety of Long-Term Continuous Growth Hormone Treatment of Children Born Small for Gestational Age. Horm Res 2004;62:149-154.
19. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. J Clin Endocrinol Metab 2003;88:3584-3590.
20. Tanaka T, Yokoya S, Seino Y, Togari H, Mishina J, Kappelgaard AM, Fujieda K. Long-term efficacy and safety of two doses of growth hormone in short japanese children born small for gestational age. Horm Res Paediatr 2011;76:411-418. Epub 2011 Nov 29
21. Schwartz ID, Schwartz KJ, Kousseff BG, Bercu BB, Root AW. Endocrinopathies in Cornelia de Lange syndrome. J Pediatr 1990;117:920-923.
22. McArthur R, Edwards JH. De Lange syndrome: report of 20 cases. Can Med Assoc J 1967;96:1185-1198.
23. Abraham JM, Russell A. De lange syndrome. A study of nine examples. Acta Paediat Scand 1968;57:339-353.

CASE REPORT

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A Mutation in *INSR* in a Child Presenting with Severe Acanthosis Nigricans

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What is already known on this topic?

Mutations in *INSR* lead to a wide spectrum of insulin resistance syndromes ranging from leprechaunism to type A insulin resistance. Rabson-Mendenhall syndrome (RMS) is an intermediate form of insulin resistance syndrome since the function of insulin receptor is only moderately reduced.

What this study adds?

To the best of our knowledge, we report the first case with RMS from Turkey diagnosed at molecular level. Sequencing of *INRS* revealed a novel homozygous mutation.

Abstract

Rabson-Mendenhall syndrome (RMS) is an autosomal recessive disorder due to mutations in the insulin receptor gene (*INSR*) which is mapped to 19p13.2. RMS is characterized by acanthosis nigricans, generalized lanugo, tooth and nail dysplasia, high nasal bridge, and growth retardation. A 5-year-old female patient was referred due to acanthosis nigricans and generalized lanugo. On her physical examination, severe acanthosis nigricans of the neck, axillae, the external genitalia and antecubital regions, generalized lanugo, mildly decreased subcutaneous fat, dysmorphic facial features, and polydactyly on her left hand were noted. Insulin resistance and impaired glucose tolerance were found. Sequence analysis of the *INSR* in the patient revealed c.3529 + 5G > A mutation in homozygous state. RMS should be suspected in a patient with characteristic physical features and insulin resistance.
Keywords: Rabson-Mendenhall syndrome, insulin resistance, *INSR*

Introduction

The human insulin receptor (IR) consists of two extracellular α subunits and two transmembrane intracellular β subunits. Insulin binds to α subunit and activates β subunit autophosphorylation and kinase activity, which is essential for transmembrane signaling of glucose transport. The α and β subunits of the IR are encoded by a single gene (*INSR*) which is mapped on the short arm of chromosome 19 (1).

Mutations of *INSR* lead to a wide spectrum of inherited insulin resistance syndromes ranging from leprechaunism (Donohue syndrome, autosomal recessive), which occurs in infancy and results in death, to type A insulin resistance (autosomal dominant), which leads to mild clinical symptoms after puberty. The severity of Rabson-Mendenhall syndrome (RMS) (autosomal recessive) is intermediate between the two aforementioned types (2). In RMS, the function of IR is less severely reduced, while little or no residual IR function is found in leprechaunism (3).

The loss of IR function results in various metabolic and growth defects. Metabolic defects in RMS are characterized by fasting hypoglycemia, postprandial hyperglycemia, later refractory hyperglycemia, extreme hyperinsulinemia, and late ketoacidosis. Affected patients also have postnatal

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growth restriction as well as impaired muscle and adipose tissue development due to the defective mitogenic action of insulin (3). Clinical findings of RMS include acanthosis nigricans, generalized lanugo, tooth and nail dysplasia, high nasal bridge, growth retardation, and hyperextensible joints (4). It is a rare genetic disorder, and in our country, no case with RMS diagnosed at molecular level has been reported to date.

Case Report

A 5-year-old female patient was referred due to acanthosis nigricans and generalized lanugo which developed in the last two years. According to the past medical history, she was born by cesarean section after an uneventful pregnancy with a birth weight of 3500 g and length of 49 cm. She was the first child of first-degree consanguineous parents. Her developmental milestones were normal. There is no family history of any remarkable medical problems. She had a healthy sibling who did not have any dysmorphic features.

Her weight was 18.7 kg [standard deviation (SD) score -0.08], height 98.7 cm (SD score -2.6), and body mass index 19.2 kg/m^2 (SD score 1.94). Severe acanthosis nigricans of the neck, axillae, the external genitalia and antecubital regions, generalized lanugo, mildly decreased subcutaneous fat, coarse face, large ears, high nasal bridge, upturned nose, abnormalities of the teeth, gingival hyperplasia, and polydactyly in her left hand were noted (Figure 1). Complete blood count, liver and renal function tests, electrolytes, lipid profile, fasting and postprandial glucose levels, glycosylated hemoglobin (HbA1c), thyroid function tests, insulin-like growth factor (IGF) 1, and serum IGF binding protein-3 levels were normal, while fasting insulin was extremely high (Table 1). After an overnight fast, an oral glucose tolerance test (OGTT) (1.75 g/kg) was performed and impaired glucose tolerance was detected. Bone age was consistent with 4 years according to the Greulich-Pyle atlas. Healthy eating and lifestyle changes were recommended to the patient for impaired glucose tolerance. Clinical features and metabolic status were found to be unchanged after one year of follow-up.

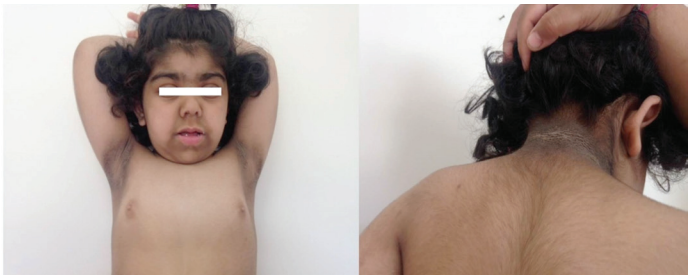

Figure 1. Dysmorphic features of the patient and acanthosis nigricans on her neck

Molecular Studies

After getting informed consent from the parents, DNA was extracted from peripheral leukocytes using standard methods. All exons and flanking intron regions of the *INSR* (NM_000208.3) were sequenced and a homozygous mutation was found: c.3529 + 5G > A (IVS19 + 5G > A) (Figure 2). This impact of variant was analyzed by using Human Splicing Finder V3 and Mutation Taster and both predicted this variant to be damaging. Our literature search yielded a heterozygote variant in a case with Donohue syndrome with compound heterozygote genotype (p.Arg1027* in exon 17 and c.3529 + 5G > A) that was recently reported in a congress session (5). There is no functional analysis data in the literature. Frequency of this variant was 4 in 121408 in EXAC database. This variant was also present in dbSNP database with rs764083259 code. DANN score is 0.8688.

Screening for the relevant mutation was performed in family members. The parents and the 3-year-old sibling were heterozygous for the same mutation (Figure 2).

Table 1. Laboratory findings of the patient

	Result	Normal range
Fasting glucose (mg/dL)	78	60-100
Post-prandial glucose (mg/dL)	102	100-140
Triglyceride (mg/dL)	59	< 150
Total cholesterol (mg/dL)	126	< 170
LDL-cholesterol (mg/dL)	54	< 130
HDL-cholesterol (mg/dL)	60	> 45
Fasting insulin (µIU/mL)	129	< 15
Free thyroxine (ng/dL)	0.82	0.7-1.56
TSH (µIU/mL)	1.98	0.56-5.4
HbA1c (%)	5.1	4-6
IGF-1 (ng/mL)	72	52-297
IGFBP-3 (ng/mL)	2440	1300-5600
OGTT		
Glucose level		
0 min (mg/dL)	77	60-100
120 min (mg/dL)	144	100-140
Insulin levels		
0 min (µIU/mL)	158	< 15
Insulin peak (µIU/mL)	> 300	< 100

LDL: low-density lipoprotein, HDL: high-density lipoprotein, TSH: thyroid-stimulating hormone, HbA1c: glycosylated hemoglobin, IGF-1: insulin-like growth factor-1, IGFBP-3: insulin-like growth factor binding protein-3, OGTT: oral glucose tolerance test

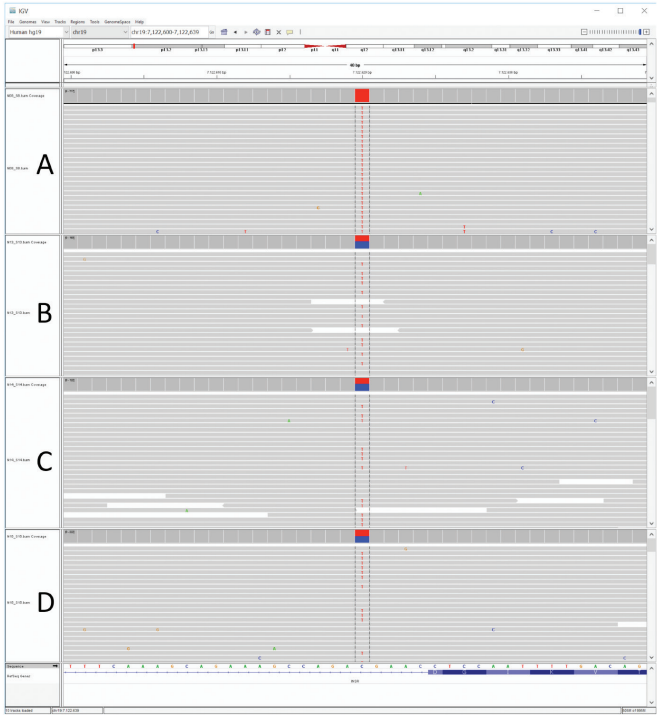

Figure 2. Homozygous (A, patient) and heterozygous (B, sibling; C, father; D, mother) *INSR* mutations identified in the family members

Discussion

RMS was first described in 1956 by Rabson and Mendenhall. Acanthosis nigricans, hypertrichosis, skin and dental abnormalities, phallic enlargement, prognathism, abdominal distension, pineal hyperplasia, coarse facies, insulin-resistant diabetes, growth retardation, lack of subcutaneous fat, lichenified skin, and fissuring of the tongue were reported to be the clinical symptoms of RMS (6,7). In our patient, since the age of 3 years, several symptoms of this condition (acanthosis nigricans, hypertrichosis, teeth abnormalities, gingival hyperplasia, and dysmorphic face) were present. Analysis of the *INSR* yielded a novel homozygous mutation.

Variant distributions given in UniProt database in both RMS and leprechaunism do not present a clear cut domain related distribution. Clinical variations seem mostly related with severity of functional effect of the mutation. The present mutation was first reported as a component of a compound heterozygous mutation found in a case with leprechaunism. The severe clinical picture was most probably due to the frame-shift mutation of the second allele of that patient (5). It is not possible to reach a similar conclusion for our patient without a functional analysis in such a splice site mutation at +5 position. However, *in silico* analyses indicate that the variant is damaging and that there was no other alteration in *INSR*.

Patients with RMS can develop fasting hypoglycemia, postprandial hyperglycemia, and ketoacidosis (8). In our patient, according to the OGTT, impaired glucose tolerance was detected. Since serum glucose level was not too high at 2-h OGTT, only diet and lifestyle changes were recommended as treatment.

Thakker (2) have reported 11 patients with RMS and type A insulin resistance who were diagnosed with diabetes at presentation and treated with high-dose insulin and insulin-sensitizing drugs. However, it has been also reported that, despite use of high doses of insulin or oral drugs like metformin and glitazones, poor glycemic control persisted in most patients with RMS (2). There is still no adequate treatment for RMS. In some studies, recombinant human IGF-1 has been shown to reduce fasting glucose, fasting insulin, C-peptide, and proinsulin levels and to improve glycemic control (9). However, these were short-term studies.

Another treatment approach for RMS is recombinant methionyl human leptin therapy (metreleptin). The beneficial effects of metreleptin on severe insulin resistance due to lipodystrophy syndromes and leptin deficiency are well known. Cochran et al (10) have demonstrated a significant reduction in blood glucose over 10 months in two patients with RMS. Brown et al (11) also demonstrated a 1.7% reduction in HbA1c with metreleptin treatment for 12 months in five patients with RMS. These authors stated that metreleptin could be an option for the treatment of RMS, but that additional therapies were needed to improve glycemic control (11). As another recent approach, vildagliptin, an oral anti-diabetic agent of the new dipeptidyl peptidase-4 inhibitor class of drugs, was reported by Moreira et al (12) to be an option for treatment of insulin resistance in cases with RMS. In their patient, vildagliptin was used as part of combination therapy which included metformin, pioglitazone, and acarbose (12). It is clear that long-term studies are needed to fully assess the effect of vildagliptin on insulin resistance syndromes. Since our patient did not need any medication in the follow-up, we have no experience with the above compounds.

In conclusion, this report describes a homozygous *INSR* mutation resulting in a clinical picture of insulin resistance and dysmorphic features of RMS and emphasizes the importance of screening the *INSR* in patients with insulin resistance and dysmorphic features. At the moment, there is no definitive treatment for RMS and new treatment approaches are needed.

Ethics

Informed Consent: Consent form was filled out by the patient's parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Hale Tuhan, Serdar Ceylaner, Özlem Nalbantoğlu, Korcan Demir, Concept: Hale Tuhan, Korcan Demir, Design: Hale Tuhan, Korcan Demir, Data Collection or Processing: Hale Tuhan, Serdar Ceylaner, Sezer Acar, Ayhan Abacı, Ece Böber, Korcan Demir, Analysis or Interpretation: Hale Tuhan, Serdar Ceylaner, Korcan Demir, Literature Search: Hale Tuhan, Korcan Demir, Writing: Hale Tuhan, Korcan Demir.

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References

1. Longo N, Wang Y, Smith SA, Langley SD, DiMeglio LA, Giannella-Neto D. Genotype-phenotype correlation in inherited severe insulin resistance. Hum Mol Genet 2002;11:1465-1475.
2. Thakker RV. 2010 Multiple Endocrine Neoplasia type 1. In: De Groot LJ, Jameson JL, eds. Endocrinology, 2- Volume Set, 6th ed. Saunders, Elsevier; 2719-2741.
3. Semple RK, Savage DB, Cochran EK, Gorden P, O'Rahilly S. Genetic syndromes of severe insulin resistance. Endocr Rev 2011;32:498-514. Epub 2011 May 2
4. Kim D, Cho SY, Yeau SH, Park SW, Sohn YB, Kwon MJ, Kim JY, Ki CS, Jin DK. Two novel insulin receptor gene mutations in a patient with Rabson-Mendenhall syndrome: the first Korean case confirmed by biochemical, and molecular evidence. J Korean Med Sci 2012;27:565-568. Epub 2012 Apr 25
5. Martínez R, Ortiz-Espejo M, Aguayo A, Velayos T, Urrutia I, Martínez de LaPiscina I, Barreiro J, Barrio R, Cardona-Hernández R, García-Puente I, Vela A, Rica I, Castaño L. Genotype-Phenotype Correlation in Severe Insulin Resistance Syndromes: 5 Cases with Recessive Mutations in INSR [abstract]. In: Proceedings of XXVI. Congreso de la Sociedad Latinoamericana de Endocrinologia Pediatrica; Buenos Aires, Oral Presentation Number 25, 2016.
6. Hardaway CA, Gibbs NF. What syndrome is this? Rabson-Mendenhall syndrome. Pediatr Dermatol 2002;19:267-270.
7. Rabson SM, Mendenhall EN. Familial hypertrophy of pineal body, hyperplasia of adrenal cortex and diabetes mellitus; report of 3 cases. Am J Clin Pathol 1956;26:283-290.
8. Desbois-Mouthon C, Magre J, Duprey J, Caron M, Blivet-Van Eggelpoel MJ, Daubas C, Gourmelen M, Chevallier B, Rizkalla S, Robert JJ, Capeau J. Major circadian variations of glucose homeostasis in a patient with Rabson-Mendenhall syndrome and primary insulin resistance due to a mutation (Cys284--> Tyr) in the insulin receptor alpha-subunit. Pediatr Res 1997;42:72-77.
9. McDonald A, Williams RM, Regan FM, Semple RK, Dunger DB. IGF-I treatment of insulin resistance. Eur J Endocrinol 2007;157(Suppl 1):51-56.
10. Cochran E, Young JR, Sebring N, DePaoli A, Oral EA, Gorden P. Efficacy of recombinant methionyl human leptin therapy for the extreme insulin resistance of the Rabson-Mendenhall syndrome. J Clin Endocrinol Metab 2004;89:1548-1554.
11. Brown RJ, Cochran E, Gorden P. Metreleptin improves blood glucose in patients with insulin receptor mutations. J Clin Endocrinol Metab 2013;98:1749-1756. Epub 2013 Aug 22
12. Moreira RO, Zagury RL, Nascimento TS, Zagury L. Multidrug therapy in a patient with Rabson-Mendenhall syndrome. Diabetologia 2010;53:2454-2455. Epub 2010 Aug 14

CASE REPORT

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Synchronous Solid Pseudopapillary Tumor and Insulinoma in an Adolescent MEN1 Patient Presenting with Diagnostic Dilemmas

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What is already known on this topic?

Multiple endocrine neoplasia (MEN1) is a rare autosomal dominant disorder characterized by primary hyperparathyroidism, enteropancreatic neuroendocrine tumors, and anterior pituitary adenomas. Insulinoma is a reported cause of hypoglycemia in an adolescent MEN1 patient.

What this study adds?

This is the first report of a case of MEN1 with a solid pseudopapillary tumor. This case report confirms the obligation to persistently question drug abuse in adolescents presenting with diagnostic challenges.

Abstract

Multiple endocrine neoplasia (MEN1) is a rare autosomal dominant disorder characterized by primary hyperparathyroidism, enteropancreatic neuroendocrine tumors, and anterior pituitary adenomas. A 16-year-old male presented to the emergency outpatient clinic with tonic convulsions. Physical examination in the postconvulsive period was unremarkable and revealed a muscular, postpubertal adolescent. Biochemical tests at admission were consistent with hyperinsulinemic hypoglycemia and remarkable for elevated levels of liver transaminases and creatine kinase. Work-up for a potential inborn error of metabolism and Doppler ultrasound for congenital portal-hepatic shunt were negative. When the patient was questioned, he reported using the anabolic steroid stanozolol to strengthen his muscles. His enzyme levels normalized after cessation of stanozolol. Hypoglycemia did not recur on diazoxide therapy. Magnetic resonance imaging showed two discrete lesions in the pancreas. Distal pancreatectomy revealed two masses 1.1 and 1.4 cm in diameter: a solid pseudopapillary tumor and an insulinoma. The patient also had asymptomatic primary hyperparathyroidism. DNA sequence analysis of the *MEN1* gene in the index patient and his father and brother revealed a previously reported "pW183S" heterozygous mutation. This case further adds to the "pancreatic tumor" phenotype of MEN1 with the presence of a solid pseudopapillary tumor. This case report also confirms the need to meticulously question drug abuse in adolescents presenting to clinics with diagnostic challenges.
Keywords: Hypoglycemia, hyperinsulinism, adolescent, multiple endocrine neoplasia 1, insulinoma, solid pseudopapillary tumor

Introduction

Multiple endocrine neoplasia (MEN1) is a rare autosomal dominant disorder characterized by primary hyperparathyroidism, enteropancreatic neuroendocrine tumors, and anterior pituitary adenomas (1). The *MEN1* gene is located on chromosome 11q13 and encodes the 610-amino acid menin protein that belongs to the class of tumor suppressors (1). Because of the high degree of penetrance of the *MEN1* gene, the majority of the patients develop

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clinical manifestations of the disorder by the fifth decade. Age-related penetrance has been studied, and the mutation seems to be non-penetrant in those younger than 5 years. It is more than 50% penetrant by 20 years of age and greater than 95% by 40 years (2). Primary hyperparathyroidism and insulinoma have been reported to occur as early as 8 and 5 years (3,4). Pancreatic involvement in asymptomatic individuals has been detected by abdominal imaging and by measurement of fasting concentrations of hormones and biomarkers such as gastrin, pancreatic polypeptide, and chromogranin A (1). Nonfunctioning pancreatic endocrine tumors have also been reported in children with MEN1 mutations (5). Herein, we describe an adolescent with MEN1 presenting with diagnostic dilemmas and the first case of a solid pseudopapillary tumor of the pancreas in a MEN1 patient.

Case Report

A 16-year-old male adolescent with a diagnosis of primary epilepsy was referred to our center due to tonic convulsions. Family history was significant for maternal death owing to metastatic lung adenocarcinoma and a paternal history of low-grade liposarcomas and recurrent nephrolithiasis. Physical examination was unremarkable and revealed a well-built, muscular adolescent. Biochemical tests at admission were consistent with hyperinsulinemic hypoglycemia as follows: venous blood glucose, 24 mg/dL; cortisol, 18 µg/dL; growth hormone, 13 ng/mL; insulin, 8 µU/mL; and C-peptide, 1.34 ng/mL. Anti-insulin antibodies were negative. Serum liver transaminase and creatine kinase levels were elevated. Alanine amino transferase was 349 U/L; aspartate aminotransferase, 158 U/L; and creatine kinase 834 U/L. The metabolic work-up for inborn errors of metabolism was negative; tandem mass spectrometry findings and urinary amino acid and organic acid levels were normal. Hepatic ultrasonography for a congenital portal-hepatic shunt was negative. Thin-slice pancreas computed tomography and pancreas magnetic resonance imaging (MRI) findings were normal. On further questioning, the patient admitted taking stanazolol (Winstrol) to strengthen his muscles. Liver transaminase and creatine kinase levels normalized within 3 weeks of stanozolol cessation. Hypoglycemia did not recur on diazoxide (200 mg/day) therapy. Endoscopic ultrasound to search for an insulinoma was scheduled, but the patient refused to undergo endoscopic ultrasound for 7 months. Biochemical evaluation also revealed asymptomatic primary hyperparathyroidism. Serum calcium level was 11.5 mg/dL; phosphorus, 2.6 mg/dL; alkaline phosphatase, 520 IU/L; parathyroid hormone, 320 pg/mL; 25-hydroxyvitamin D, 33 ng/mL; and 24-h urinary calcium, 4.2 mg/kg/day. Parathyroid scintigraphy revealed an adenoma in the inferior right parathyroid gland. The constellation of primary hyperparathyroidism and hyperinsulinemic hypoglycemia due to a possible insulinoma prompted us to search for a mutation in the menin gene. DNA sequence analysis of the menin gene revealed a previously reported heterozygous "pW183S" mutation at codon 183 on exon 3 of the menin gene located at chromosome 11 q13 (6). Plasma fasting gastrin and glucagon levels were within normal ranges. Screening of the father and the 14-year-old brother of the proband revealed the same mutation. The brother had asymptomatic hyperparathyroidism. Endoscopic ultrasound of the patient

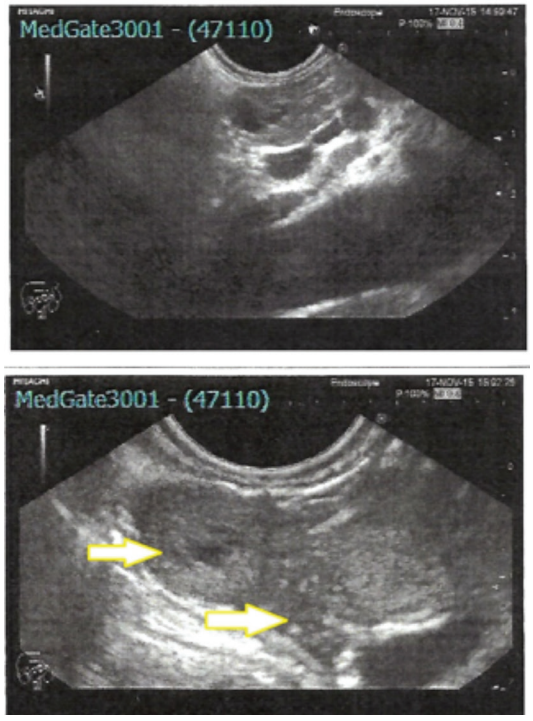


Figure 1. Endoscopic ultrasound images of the index patient showing the two pancreatic lesions (yellow arrows)

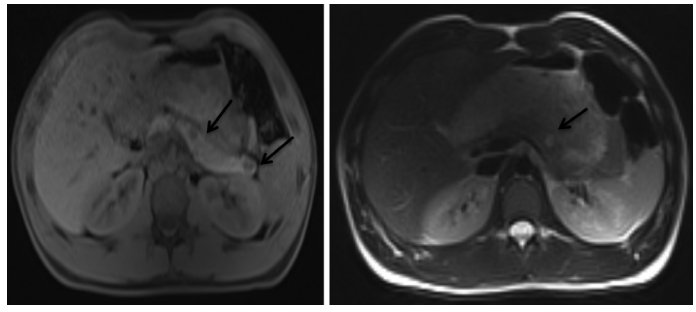


Figure 2. Non-contrast, T1-weighted axial pancreas magnetic resonance imaging (MRI) showing hypointense lesions (black arrows) in the tail and body of the pancreas (A). T2-weighted axial MRI revealing a hyperintense lesion (black arrow) in the body of the pancreas (B)

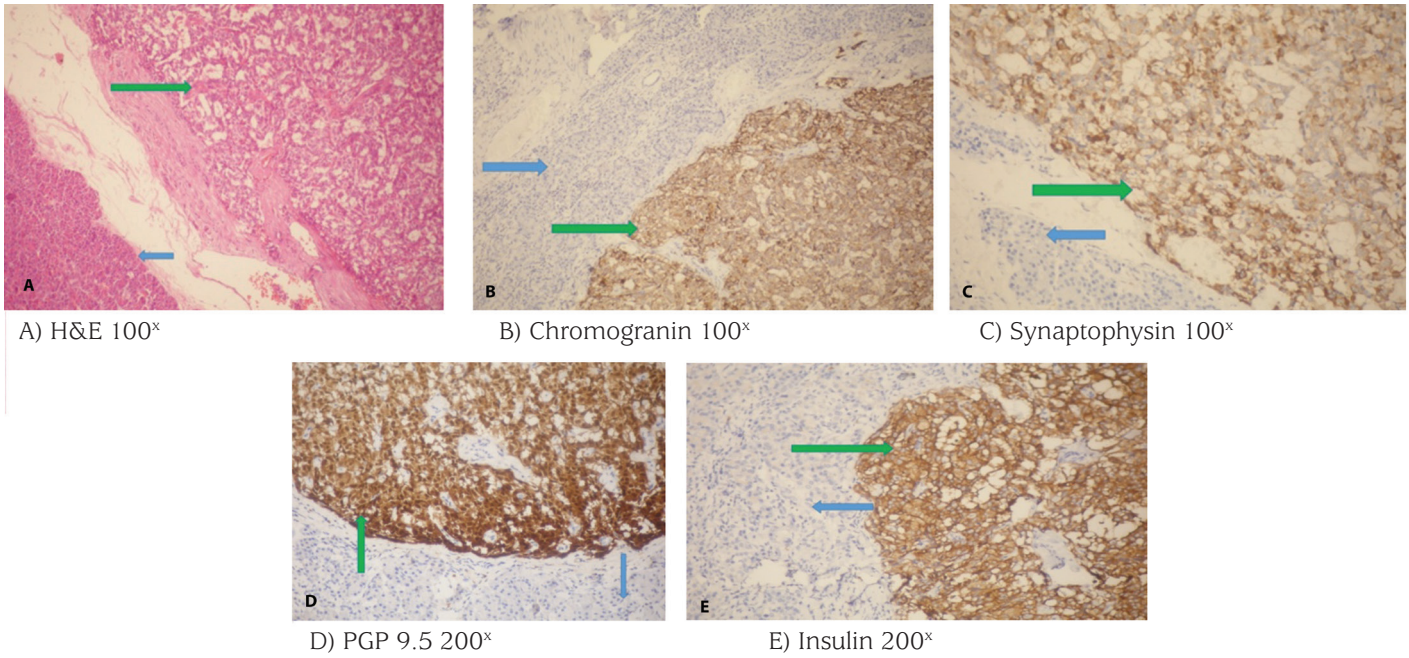
revealed two masses: one in the body (1.4 cm) and the other in the tail of the pancreas (1.1 cm) (Figure 1). Repeat pancreas MRI prior to surgery confirmed the presence of these masses (Figure 2). Histopathologic evaluation of the mass in the tail of the pancreas revealed tumor cells with round/oval nuclei and eosinophilic granular cytoplasm. The tumor nests were arranged in trabecular, insular, or sheet-like patterns (Figure 3A). Immunocytochemical evaluation of the mass in the tail of the pancreas was consistent with a grade 1 neuroendocrine tumor according to World Health Organization classification and stained positive for chromogranin, synaptophysin, protein gene product 9.5, and insulin (Figure 3B, 3C, 3D, 3E). Ki-67 index was less than 1 %. Thus, the distal mass was diagnosed as an insulinoma. The proximal region mass was composed of small- and medium-sized tumor cells with no apparent atypia. Pseudopapillary structures were observed in most of the areas (Figure 4A). The tumor was positive for β-catenin, CD56, progesterone receptor, chromogranin, and synaptophysin (Figure 4). The proximal mass was diagnosed as a solid pseudopapillary tumor. Ki-67 index was 6 % to 7 %. Staining of both tumors was negative for menin (images are unavailable since they were processed at another center which did not share the slides).

Postoperatively, hypoglycemia did not recur when diazoxide was discontinued. Conversely, the patient developed diabetes and normoglycemia was restored with insulin glargine at a mean dose of 0.45 U/kg/day. His requirement for insulin diminished 8 months postoperatively. Informed consent was taken from the father of the patient, and assent was also taken from the patient.

Discussion

The present case has unique aspects regarding the biochemical evaluation for hypoglycemia and the puzzling histopathologic findings of a solid pseudopapillary tumor and an insulinoma following distal pancreatectomy and splenectomy.

The etiology of hypoglycemia in the adolescent includes a variety of disorders reviewed elsewhere (7). Insulinoma is not an infrequent cause of hypoglycemia in adolescents and its presence should always prompt consideration of MEN1. The use of stanazolol in the current case further complicated the evaluation owing to elevated liver transaminase and creatine kinase levels, which prompted us to search for inborn errors of metabolism and congenital portosystemic shunts (Abernethy malformation), which have recently been reported as a cause of hyperinsulinemic hypoglycemia (8). In the current case, repeated interviews uncovered drug abuse as the cause of the discordant biochemical



A) H&E 100x B) Chromogranin 100x C) Synaptophysin 100x

D) PGP 9.5 200x E) Insulin 200x

Figure 3. Histopathology of the patient's distal pancreatic tumor. Hematoxylin and eosin (H&E) (A) and immunostaining (B-E) are shown. Green and blue arrows represent tumor and normal tissue, respectively. H&E revealed tumor cells with round or oval nuclei, "salt and pepper chromatin", and an eosinophilic granular cytoplasm. The tumor nests are arranged in trabecular, insular, or sheet-like patterns. Chromogranin A, synaptophysin, protein gene product 9.5, and insulin positivity confirm the diagnosis of insulinoma

H&E: hematoxylin and eosin, PGP: protein gene product

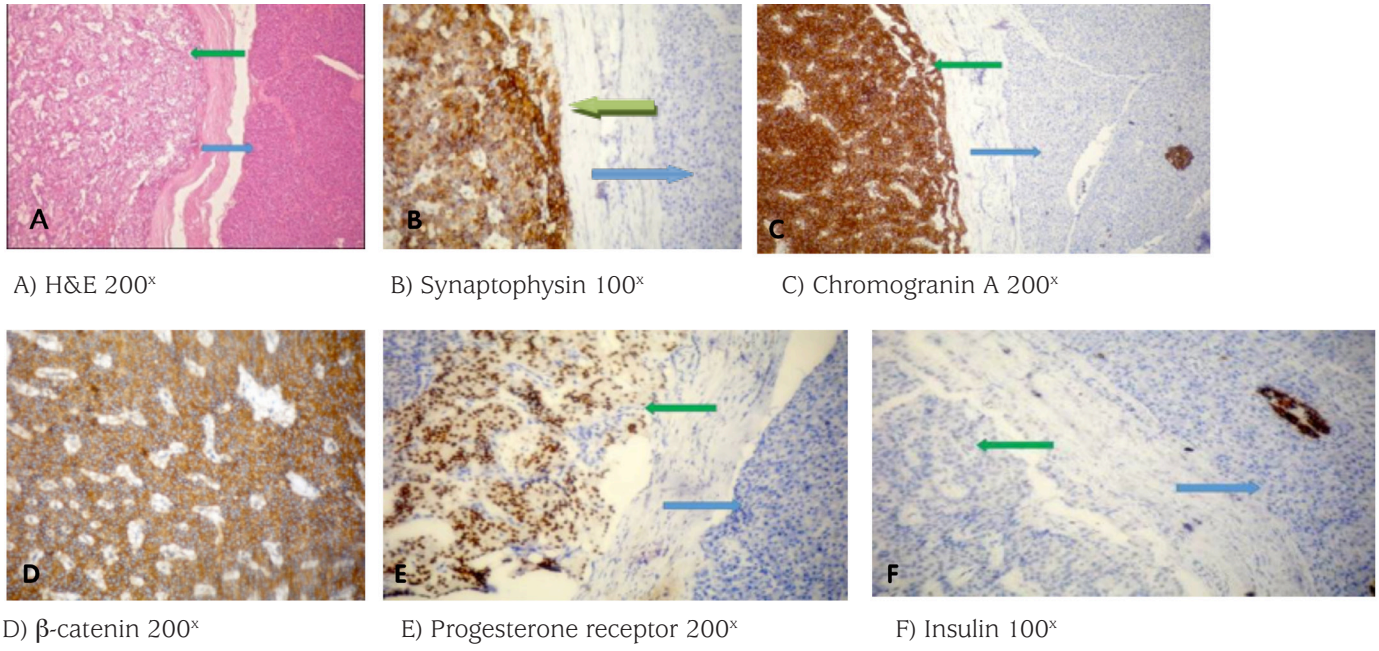


Figure 4. Histopathology of the patient's tumor in the body of the pancreas. Hematoxylin and eosin (H&E) (A) and immunostaining (B-F) are shown. Green and blue arrows represent tumor and normal tissue, respectively. H&E revealed small- and medium-sized tumor cells with no atypia. Pseudopapillary structures were observed in most areas. The tumor stained positive for synaptophysin (B), chromogranin A (C), β-catenin (D), and progesterone receptor (E) but was negative for insulin (F); these findings confirm a solid pseudopapillary tumor of the pancreas

H&E: hematoxylin and eosin

anomalies. Adolescence is a particularly vulnerable period for drug abuse that should always be considered as a reason for unusual clinical and/or biochemical findings that present diagnostic challenges.

To the best of our knowledge, this is the first report of a solid pseudopapillary tumor of the pancreas in a MEN1 patient. Moreover, this is also the first description of a synchronous nonfunctional tumor and insulinoma in a pediatric MEN1 patient. In the literature, there are two adult case reports of solid pseudopapillary tumor of the pancreas and functioning adenomas of the pancreas (9,10). However, these cases were not clinically or genetically diagnosed as having MEN1. Solid pseudopapillary tumor of the pancreas is very rare constituting less than 1% of all pancreatic masses (11). The cellular origin of the tumor is uncertain. Some theories suggest that these tumors originate from small duct epithelium, acinar or pluripotent stem cells capable of exocrine and endocrine differentiation (11). They are of benign nature or have a low-grade malignancy potential that typically occurs in young women. These tumors are very unusual in males. The tumor can be located throughout the pancreas. Although it is generally benign or has low-grade malignant potential, it may invade surrounding soft tissues and can even metastasize to the liver or peritoneum in rare cases. The tumor is a combination of solid growth pattern and cystic areas of loosely cohesive epithelioid cells that can become hemorrhagic. Microscopically, the tumors are positive for β-catenin, CD56, synaptophysin, and progesterone receptors (12). Nonfunctional pancreatic tumors have been associated with increased mortality in MEN1 patients.

Genetically confirming a clinical diagnosis of MEN1 in the proband is mandatory to assess other family members for the presence of the mutation so that they can be followed for potential endocrine problems and for avoiding unnecessary work-up due to "phenocopy." In the present case, the 14-year-old brother of the proband had asymptomatic hyperparathyroidism. The presence of the mutation indicates that he also needs lifetime surveillance for occurrence of MEN1-related tumors.

Drug abuse should be considered in adolescents presenting to clinics, particularly when the diagnosis is unclear. This case report adds to the "pancreatic tumor" phenotype of MEN1 with a solid pseudopapillary tumor.

Ethics

Informed Consent: Consent was taken from the father of the patient, and assent was taken from the patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Muharrem Battal, Ahmet Uçar, Concept: Ahmet Uçar, Design: Ahmet Uçar, Data Collection or Processing: Ahmet Uçar, Banu Özgüven, Muharrem Battal, Ferda Alparslan Pınarlı, Evrim Özmen, Analysis or Interpretation: Ahmet Uçar, Banu Özgüven, Muharrem Battal, Ferda Alparslan Pınarlı, Evrim Özmen, Literature Search: Ahmet Uçar, Ferda Alparslan Pınarlı, Aylin Yetim, Yasin Yılmaz, Evrim Özmen, Writing: Ahmet Uçar, Banu Özgüven, Aylin Yetim, Yasin Yılmaz.

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References

1. Thakker RV. Multiple endocrine neoplasia type 1 (MEN1). Best Pract Res Clin Endocrinol Metab 2010;24:355-370.
2. Thakker RV 2010 Multiple Endocrine Neoplasia type 1. In: De Groot LJ, Jameson JL, eds. Endocrinology, 2- Volume Set, 6th ed. Saunders, Elsevier; 2719-2741.
3. Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD, Edwards CR, Heath DA, Jackson CE, Jansen S, Lips K, Monson JP, O'Halloran D, Sampson J, Shalet SM, Wheeler MH, Zink A, Thakker RV. Clinical studies of multiple endocrine neoplasia type 1 (MEN1). QJM 1996;89:653-669.
4. Lévy-Bohbot N, Merle C, Goudet P, Delemer B, Calender A, Jolly D, Thiéfin G, Cadiot G; Groupe des Tumeurs Endocrines. Prevalence, characteristics and prognosis of MEN 1-associated glucagonomas, VIPomas, and somatostatinomas: study from the GTE (Groupe des Tumeurs Endocrines) registry. Gastroenterol Clin Biol 2004;28:1075-1081.
5. Newey PJ, Jeyabalan J, Walls GV, Christie PT, Gleeson FV, Gould S, Johnson PR, Phillips RR, Ryan FJ, Shine B, Bowl MR, Thakker RV. Asymptomatic children with multiple endocrine neoplasia type 1 mutations may harbor nonfunctioning pancreatic neuroendocrine tumors. J Clin Endocrinol Metab 2009;94:3640-3646.
6. Bassett JH, Forbes SA, Pannett AA, Lloyd SE, Christie PT, Wooding C, Harding B, Besser GM, Edwards CR, Monson JP, Sampson J, Wass JA, Wheeler MH, Thakker RV. Characterization of mutations in patients with multiple endocrine neoplasia type 1. Am J Hum Genet 1998;62:232-244.
7. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, Levitsky LL, Murad MH, Rozance PJ, Simmons RA, Sperling MA, Weinstein DA, White NH, Wolfsdorf JI; Pediatric Endocrine Society. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. J Pediatr 2015;167:238-245. Epub 2015 May 6
8. Bas S, Guran T, Atay Z, Haliloglu B, Abalı S, Turan S, Bereket A. Premature pubarche, hyperinsulinemia and hypothyroxinemia: novel manifestations of congenital portosystemic shunts (Abernethy malformation) in children. Horm Res Paediatr 2015;83:282-287.
9. Arafah M, Kfoury H, Naseem S. Hypoglycemia, a rare presentation of a solid pseudopapillary neoplasm of the pancreas: a case report. Turk J Gastroenterol 2011;22:544-547.
10. Bhanot P, Nealon WH, Walser EM, Bhutani MS, Tang WW, Logroño R. Clinical, imaging, and cytopathological features of solid pseudopapillary tumor of the pancreas: a clinicopathologic study of three cases and review of the literature. Diagn Cytopathol 2005;33:421-428.
11. Liu X, Rauch TM, Siegal GP, Jhala N. Solid-pseudopapillary neoplasm of the pancreas: Three cases with a literature review. Appl Immunohistochem Mol Morphol 2006;14:445-453.
12. Martin RC, Klimstra DS, Brennan MF, Conlon KC Solid-pseudopapillary tumor of the pancreas: a surgical enigma? Ann Surg Oncol 2002;9:35-40.

CASE REPORT

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Response to Growth Hormone Treatment in a Patient with Insulin-Like Growth Factor 1 Receptor Deletion

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What is already known on this topic?

Insulin-like growth factor receptor (IGF1R) mutations are suspected in patients born small for gestational age with normal endocrine and metabolic workup. Single nucleotide polymorphism microarray may help to identify those patients with deletions that include the IGF1R gene. Early initiation of growth hormone (GH) treatment with higher dosing than used in growth hormone deficiency may lead to significant improvement in growth parameters. Reports in the literature reveal variable rates of response to GH treatment.

What this study adds?

We report the dramatic response to growth hormone therapy in this patient which highlights the importance of identifying patients with IGF1R deletion and treating them early.

Abstract

We report a six-year-old boy who presented with short stature, microcephaly, dysmorphic features, and developmental delay and who was identified with a terminal deletion of 15q26.2q26.3 containing the insulin-like growth factor receptor (IGF1R) gene in addition to a terminal duplication of the 4q35.1q35.2 region. We compare our case with other reports of deletions and mutations affecting the IGF1R gene associated with pre-and postnatal growth restriction. We report the dramatic response to growth hormone therapy in this patient which highlights the importance of identifying patients with IGF1R deletion and treating them early.
Keywords: Growth hormone therapy, growth hormone receptor, short stature, 15q deletion, duplication 4q

Introduction

Fetal growth is dependent on maternal, placental, fetal, and environmental factors. The mechanisms of human fetal growth remain unknown in many cases. Insulin-like growth factor 1 (IGF-1) has a crucial role in the regulation of pre- and postnatal growth. It promotes growth during embryogenesis and postnatal life via DNA synthesis stimulation, cell proliferation, cellular differentiation, and also by increasing glucose uptake in adipose tissue and muscle cells (1). The role of IGF-1 is not limited to growth promotion and weight gain but is also important in promoting brain and inner ear development (2,3).

IGFs are produced primarily in the liver in response to growth hormone (GH), while their metabolic effects are mainly due to their binding of GH with its receptors on target cells.

The gene coding the IGF1 receptor (IGF1R), located on the long arm of chromosome 15, is involved in somatic development and glucose metabolism. Terminal microdeletion of the long arm of chromosome 15 is a rare cause of short stature. Most cases have been associated with pre- and postnatal growth restriction, microcephaly, and developmental delay (4,5).

Herein, we report a six-year-old male who presented with developmental delay and short stature with a terminal

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deletion of 15q26.2q26.3. This region includes the *IGF1R* gene and a terminal duplication of the 4q35.1q35.2 identified by single nucleotide polymorphism (SNP) microarray analysis. This report emphasizes the important role of SNP microarrays in investigating short stature where significant cognitive impairment or marked dysmorphism are not prominent features. It also elucidates the benefit of GH therapy in patients with IGF1R deletions and highlights the importance of early diagnosis and treatment of these patients.

Case Report

We report a male born at full term by cesarean section following a pregnancy complicated by oligohydramnios and severe intrauterine growth retardation (IUGR). At birth, his length was 43.18 cm (Z score -3.2) and weight was 2102 grams (Z score-3). His history was significant for gastroesophageal reflux during the first three months of life treated by ranitidine. On review of his milestones, he was smiling by six months, sitting at six months, standing at 20 months, and walking at 21 months. He had his first teeth at one year of age. He said his first words at 20 months. He initially presented to the genetics clinic at the age of 30 months for short stature. On physical examination, his height was 60.2 cm (Z score -9.3), weight was 8.49 kg (Z score -4.7), and head circumference was 45 cm (Z score -3). Dysmorphic features including mild frontal bossing, low-set ears, and marked clinodactyly of his fifth digits (Figure 1) were suggestive of Russell-Silver syndrome. However, his proportionate head size and developmental delay were inconsistent with this diagnosis. His chest, abdomen, genitourinary system examination, and neurologic findings were unremarkable. Initial laboratory evaluation showed normal thyroid function studies, IGF binding protein 3 (IGFBP3) was 2.4 mcg/mL (normal range 1.1-5.2), and IGF1 25 ng/mL (normal range 30-174). His biochemical workup results were within normal ranges. Bone age radiograph revealed the presence of normally shaped phalangeal epiphyses with a significantly delayed bone age 2 standard deviation below his chronological age. Abdominal ultrasound, echocardiography, and cerebral magnetic resonance imaging findings were normal. Developmental evaluation was done at the age of three years using the Wechsler Preschool and Primary Scales of Intelligence 4th edition and Vineland Adaptive Behavior Scales 2nd edition. His general cognitive ability was in the low range of intellectual functioning as measured by the Full Scale Intelligence Quotient = 72, 3rd percentile. His Verbal Comprehension Index was 84, in the below average range. His speech was normal for rate, rhythm, and prosody but with multiple articulation errors. Vineland Adaptive Behavior Composite Score was 61 (< 1 percentile). He was observed to be easily distracted and was slightly hyperactive.

SNP microarray analysis was performed using the Affymetrix Cytoscan HD platform. 250 ng of total genomic DNA extract was digested with NspI and then ligated to NspI adaptors, respectively, and amplified using Titanium Taq with a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California). Polymerase chain reaction products were purified using AM Pure beads (Agencourt Biosciences, Beverly, Massachusetts) and quantified using NanoDrop 8000 (Thermo Fisher, Wilmington, Delaware). Purified DNA was fragmented and biotin labeled and hybridized to the Affymetrix Cytoscan HD Gene Chip. The data were analyzed using Chromosome Analysis Suite. The analysis was based on the GRCh37/hg19 assembly. SNP microarray identified a 4.09 Mb terminal deletion of 15q26.2q26.3 [arr[hg19] 15q26.2q26.3(98,434,315-99,459,796)x1] and included the IGF1R gene. Microarray also showed a 6.41 Mb terminal duplication of the 4q35.1q35.2 [arr[hg19] 4q35.1q35.2(184,738,819-190,957,473)x3] (Figure 2). The duplicated region comprised numerous genes of uncertain clinical significance, this region being flanked by *ENPP6* and *DBET* genes.

Daily subcutaneous GH therapy was started at the age of 41 months with a mean dose of 35 ug/kg/day. His follow-up growth parameters 6 months later revealed improvement; his weight was 11.4 kg (Z score -3) and his height was 87.4 cm (Z score -3.7). The dose was increased to 40 ug/kg/day. His growth velocity just prior to GH treatment was 1.84 cm/

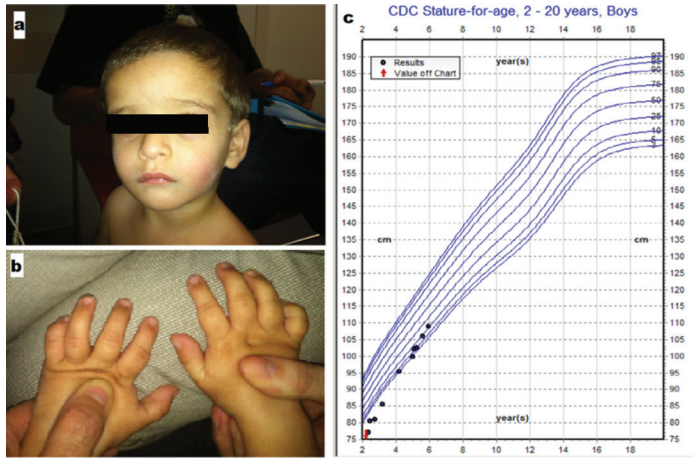

Figure 1. Photograph showing frontal bossing, low-set ears (a), and marked clinodactyly of both fifth digits (b). Height for age curve of the patient showing that the patient's height was below 3rd percentile for age until growth hormone (GH) treatment was started at the age of 41 months. Following institution of GH therapy, catch up growth was noted (c)

CDC: Center for Disease Control and Prevention

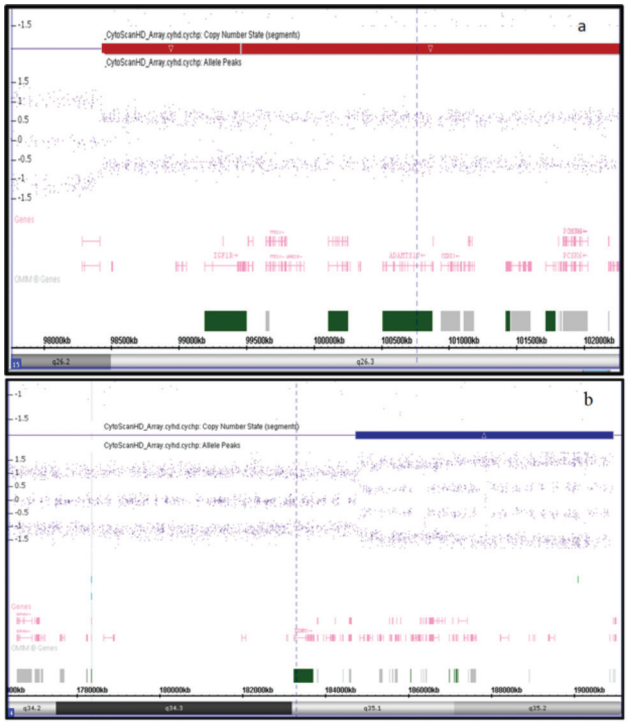

Figure 2. PSingle nucleotide polymorphism microarray showing the 4.09 Mb terminal deletion of 15q26.2- > 15qter arr [hg19] 15q26.2q26.3 (98,434,315-99,459,796) x1 (a) and the 6.41 Mb terminal duplication of 4q35.1- > 4qter arr[hg19] 4q35.1q35.2 (184,738,819-190,957,473) x3 (b)

year. His follow-up after one year shows improvement in his growth parameters. His weight was 14.9 kg (Z score -2), height was 99.8 cm (Z score -1.89), and head circumference was 46.5 cm (Z score -2). Over the past 15 months, he has received consistent treatment which is reflected in his post-treatment growth velocity of 8.47 cm/year. His height and weight is now at the 10th percentile for age. Additionally, there is an improved IGF1 level of 312 ng/mL and improved IGFBP3 level of 3.4 mg/L (normal range 1.5-3.4 mg/L).

The family history was significant for learning problems in the mother. Maternal height was 175 cm and paternal height was 180 cm, yielding a mid-parental target height of 184 cm. There was no consanguinity reported and no other significant medical history. Fluorescence *in situ* hybridization testing of the father was negative and permission for maternal testing could not be obtained. Informed consent was obtained from the family.

Discussion

In this report, we describe a patient with IUGR, short stature, microcephaly, developmental delay, and mild facial dysmorphism. SNP microarray demonstrated a terminal deletion of 15q26.2q26.3 containing the *IGF1R* gene and terminal duplication of the 4q35.1q35.2.

Although similar cases have been previously reported, clinical features in patients with IGF1R abnormalities have not been well-defined. Based on our report and previous studies, they all share the common features of growth retardation and microcephaly. However, mental performance is not essentially affected (6,7). Cardiac, lung, gastrointestinal, and renal anomalies have been reported in some patients (8,9). Choi et al (10) have reported a case with IGF1R haploinsufficiency due to deletion of the chromosome 15q26.2 in association with short stature, coarctation of the aorta, right cryptorchidism, left multicystic dysplastic kidney disease, and dysmorphic features including microcephaly, bilateral ptosis, strabismus, long palpebral fissure, and clinodactyly. These additional clinical features were mostly linked to other genes in the same region affected by the deletion (11,12).

As previously reported, multiple family members may show similar features of growth retardation and microcephaly, and molecular analysis of the affected family members has revealed similar IGF1R mutations (13,14,15). Several mutations have been so far described affecting 15q locus leading to IGF1R dysfunction. The first case of an interstitial deletion of chromosome 15 was reported by Fryns et al (16). Terminal deletions of 15q26 have been described by others (5,10). Clinical features and underlying mutations of reported cases are summarized in Table 1 (5,6,10,12,13,15,17,18,19).

In addition to 15q26.2q26.3 deletion, our patient was shown to have a terminal duplication of the 4q35.1q35.2. It is known that individuals with 4q duplication may exhibit variable phenotypes such as microcephaly, brachycephaly, slanting eyes, low-set ears, retromicrognathia, small or missing thumb, and congenital cardiac defects, the variability attributable to the specific band duplication (20). Our patient appears to have a milder phenotype than the reported case with a duplication of 4q24qter (21) and the siblings with duplication of 4q31-35 (22). It is possible that his phenotype is modified by the deletion of chromosomal 15q26.2q26.3 region or other unknown genetic/epigenetic factors.

Growth retardation caused by IGF1R haploinsufficiency was successfully treated with GH in some of the earlier reports (5,17). However, full catch-up was not achieved. Although the response to GH in patients with IGF1R mutations was inconsistent in some of the available reports (18,19), our patient has shown a promising initial response with an annual height velocity of 12.4 cm/year after 1.5 years of GH treatment. Similarly, Walenkamp et al (5) reported a 15-year-

Table 1. Clinical and other features of reported cases

Case	Age (y.)	Sex M = male F = female	Deletion/ mutation	Short stature	Microcephaly	Dysmorphic features	Other clinical findings	Developmental delay and/ or mental retardation	GH treatment	Reference
Our case	6	M	15q26.2q26.3 terminal deletion 4q35.1q35.2 duplication	+	+	Frontal bossing and low-set ears	Clinodactyly of both fifth digits	+	GH therapy started at 3.4 y. (0.7-0.8 mg) daily. His height and weight improved to the 10th percentile for age	
1	4.5	F	15q26.2 -> qter deletion	+	+	-	Delayed speech	-	GH therapy started at 5.3 y. (1mg/ m^2/day). Catch-up growth occurred, and reached her adult height at 15 years (1.6 SDS)	(5)
2	4.5	F	c.G1456A p.R108Q c.A1478C p.K115N in exon 2	+	-	-	Nonverbal learning disorder	+	GH therapy started at age of 4.5 y. with growth rate of 6.5 to 7.2 cm/ year	(6)
3	6.8	F	c.420del Cin exon 2 p.Ala110fsX0	+	NA	-	-	-	GH therapy started at 6y. (0.44 mg/ kg/wk). After 11 month of therapy her height SDS increased from -3.56 to -2.38	(10)
4	9.5	M	c.420del Cin exon 2 p.Ala110fsX0	+	NA	-	-	-	GH therapy started at 9.5y. (0.44 mg/ kg/wk). After 1 year of therapy, his height SDS increased from -3.42 to -2.78	(10)
5	4	F	15q26.2 deletion	+	-	Hypertelorism, broad nasal bridge, triangular face, large mouth, short pigmented upper lip, low-set and posterior rotated ears	Bilateral hearing loss, bilateral hip dysplasia, and club feet	+	GH therapy started at 4 years (1mg/ m^2/day). After 4 years of GH treatment, height SDS increased from -3.42 to -1.68	(12)

Table 1. Continue

6	7	M	15q26.3 deletion	+	-	Upward slanting, bilateral extra nipple, proximal implanted thumbs, and broad feet	-	+	GH therapy started at 7 years (1mg/m^2/day). After 4 years of GH treatment, her height SDS was increased from -3.57 by 1.69 SD	(12)
7	1.25	F	c.G3148A in exon16 p.Glu1050Lys	+	+	Triangular face, brachycephaly, mild hypertelorism, small mouth, and prominent ears	-	-	GH therapy started at 1.7 y. (1.4 mg/m^2/d). After 1.9 years of therapy, her height SDS increased from -2.3 to -1.2.	(13)
8	13.6	F	c.G1577A in exon 7 p. Arg481Glu	+	NA	Triangular face, small hands and feet	-	-	GH therapy started at 13.6 y. (0.07 mg/kg/day) for 6 months with no improvement in height	(15)
9	8.5	M	c.C265T in exon 2 p.Arg59Ter	+	+	Broad nasal bridge, long philtrum, thin upper lip, and inverted lower lip	Bilateral clinodactyly	+	GH therapy started at 6.5 y. (30 mcg/kg/d) for 24 months, his height SDS increased from -2.5 to -1.5	(17)
10	7.5	F	c. A1549T in exon 7 p.Tyr487Phe	+	+	-	-	-	GH therapy started at 3.4 y. (0.035mg/kg/d). After 4 years of therapy, height SDS increased from -3.19 to -2.39	(18)
11	9	F	c.T1886A in exon 2 p.Val599Glu	+	+	-	Attention deficit hyperactivity disorder	+	GH therapy started at age of 7.4 y. (0.031 - 0.036 mg/kg/day). Growth velocity was -1.81 SDS with marginal improvement after GH therapy	(19)

NA: not available, GH: growth hormone, SDS: standard deviation score

old girl with IGF1R deletion who responded dramatically to GH therapy started at the age of five years. A normal adult height was eventually achieved in this patient (5). In contrast, no height gain was observed in a 13.5-year-old girl reported by Inagaki et al (15) who received recombinant human GH for 6 months. Abuzzahab et al (6) reported a 4.5-year-old female whose growth rate increased to 7.2 cm per year during the first year of GH therapy and which slowed down after GH therapy discontinuation. Her height velocity increased to 6.5 cm per year when treatment was resumed (6).

The positive effect of GH treatment may be explained by its direct stimulatory effect or marked elevation of IGF1 levels that could stimulate partially insensitive receptors. It is recommended to start treatment with a small dose and titrate the dose up based on treatment response. Choi et al (10) proposed that higher doses of GH 0.217 mg to 0.7 mg/kg were needed to achieve optimal response. Variable response to GH may be explained by variable responsiveness of abnormal IGF1R resulting from the different underlying mutations. Variable mutation extent may also explain variability of the mutant receptor response to GH therapy.

Given the detrimental consequences of IGF1 unresponsiveness on both somatic growth and psychomotor development, the diagnosis of IGF1R mutation/deletion should be considered in a child with growth retardation and otherwise negative laboratory studies. Although rare, delay in treatment could seriously impact the growth and development pattern (15).

In conclusion, IGF1R mutations must be suspected in patients born small for gestational age with normal endocrine and metabolic workup results. SNP microarray may help to identify those patients with deletions that include the *IGF1R* gene. Early initiation of GH treatment with higher dosing than used in GH deficiency may lead to significant improvements in growth parameters and catch up growth.

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Ethics

Informed Consent: Consent form was filled out by parent who has provided full permission for the report.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Virginia Kimonis, Ajanta Naidu, Concept: Virginia Kimonis, Design: Virginia Kimonis, Data Collection or Processing: Ranim Mahmoud, Ajanta Naidu, Hiba Risheg, Virginia Kimonis, Analysis or Interpretation: Ranim Mahmoud, Ajanta Naidu, Hiba Risheg, Virginia Kimonis, Literature Search: Ranim Mahmoud, Virginia Kimonis, Writing: Ranim Mahmoud, Virginia Kimonis.

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References

1. Rudaks LI, Nicholl JK, Bratkovic D, Barnett CP. Short stature due to 15q26 microdeletion involving IGF1R: report of an additional case and review of the literature. Am J Med Genet A 2011;155:3139-3143. Epub 2011 Nov 7
2. Camarero G, Avendano C, Fernandez-Moreno C, Villar A, Contreras J, de Pablo F, Pichel JG, Varela-Nieto I. Delayed inner ear maturation and neuronal loss in postnatal Igf-1-deficient mice. J Neurosci 2001;21:7630-7641.
3. Carson MJ, Behringer RR, Brinster RL, McMorris FA. Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. Neuron 1993;10:729-740.
4. Veenma DC, Eussen HJ, Govaerts LC, de Kort SW, Odink RJ, Wouters CH, Hokken-Koelega AC, de Klein A. Phenotype-genotype correlation in a familial IGF1R microdeletion case. J Med Genet 2010;47:492-498. Epub 2009 Dec 2
5. Walenkamp MJ, de Muinck Keizer-Schrama SM, de Mos M, Kalf ME, van Duyvenvoorde HA, Boot AM, Kant SG, White SJ, Losekoot M, Den Dunnen JT, Karperien M, Wit JM. Successful long-term growth hormone therapy in a girl with haploinsufficiency of the insulin-like growth factor-I receptor due to a terminal 15q26.2->qter deletion detected by multiplex ligation probe amplification. J Clin Endocrinol Metab 2008;93:2421-2425. Epub 2008 Mar 18
6. Abuzzahab MJ, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfäffle R, Raile K, Seidel B, Smith RJ, Chernausek SD; Intrauterine Growth Retardation (IUGR) Study Group. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med 2003;349:2211-2222.
7. Kruis T, Klammt J, Galli-Tsinopoulou A, Wallborn T, Schlicke M, Müller E, Kratzsch J, Körner A, Odeh R, Kiess W, Pfäffle R. Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. J Clin Endocrinol Metab 2010;95:1137-1142. Epub 2010 Jan 26
8. Siebler T, Lopaczynski W, Terry CL, Casella SJ, Munson P, De Leon DD, Phang L, Blakemore KJ, McEvoy RC, Kelley RI, et al. Insulin-like growth factor I receptor expression and function in fibroblasts from two patients with deletion of the distal long arm of chromosome 15. J Clin Endocrinol Metab 1995;80:3447-3457.
9. Biggio JR Jr, Descartes MD, Carroll AJ, Holt RL. Congenital diaphragmatic hernia: is 15q26.1-26.2 a candidate locus? Am J Med Genet A 2004;126:183-185.
10. Choi JH, Kang M, Kim GH, Hong M, Jin HY, Lee BH, Park JY, Lee SM, Seo EJ, Yoo HW. Clinical and functional characteristics of a novel

heterozygous mutation of the IGF1R gene and IGF1R haploinsufficiency due to terminal 15q26.2->qter deletion in patients with intrauterine growth retardation and postnatal catch-up growth failure. J Clin Endocrinol Metab 2011;96:130-134. Epub 2010 Oct 20

11. Roback EW, Barakat AJ, Dev VG, Mbikay M, Chrétien M, Butler MG. An infant with deletion of the distal long arm of chromosome 15 (q26.1---qter) and loss of insulin-like growth factor 1 receptor gene. Am J Med Genet 1991;38:74-79.
12. Ester WA, van Duyvenvoorde HA, de Wit CC, Broekman AJ, Ruivenkamp CA, Govaerts LC, Wit JM, Hokken-Koelega AC, Losekoot M. Two short children born small for gestational age with insulin-like growth factor 1 receptor haploinsufficiency illustrate the heterogeneity of its phenotype. J Clin Endocrinol Metab 2009;94:4717-4727. Epub 2009 Oct 28
13. Walenkamp MJ, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruithof MF, Breuning MH, Romijn JA, Karperien M, Wit JM. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. J Clin Endocrinol Metab 2006;91:3062-3070. Epub 2006 Jun 6
14. Kawashima Y, Takahashi S, Kanzaki S. Familial short stature with IGF-I receptor gene anomaly. Endocr J 2012;59:179-185. Epub 2011 Oct 19
15. Inagaki K, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, Terekhov S, LeRoith D. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. J Clin Endocrinol Metab 2007;92:1542-1548. Epub 2007 Jan 30
16. Fryns JP, de Muelenaere A, van den Berghe H. Interstitial deletion of the long arm of chromosome 15. Ann Genet 1982;25:59-60.
17. Raile K, Klammt J, Schneider A, Keller A, Laue S, Smith R, Pfäffle R, Kratzsch J, Keller E, Kiess W. Clinical and functional characteristics of the human Arg59Ter insulin-like growth factor i receptor (IGF1R) mutation: implications for a gene dosage effect of the human IGF1R. J Clin Endocrinol Metab 2006;91:2264-2271. Epub 2006 Mar 28
18. Labarta JI, Barrio E, Audí L, Fernández-Cancio M, Andaluz P, de Arriba A, Puga B, Calvo MT, Mayayo E, Carrascosa A, Ferrández-Longás A. Familial short stature and intrauterine growth retardation associated with a novel mutation in the IGF-I receptor (IGF1R) gene. Clin Endocrinol (Oxf) 2013;78:255-262.
19. Wallborn T, Wüller S, Klammt J, Kruis T, Kratzsch J, Schmidt G, Schlicke M, Müller E, van de Leur HS, Kiess W, Pfäffle R. A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. J Clin Endocrinol Metab 2010;95:2316-2324. Epub 2010 Mar 31
20. Celle L, Lee L, Rintoul N, Savani RC, Long W, Mennuti MT, Krantz ID. Duplication of chromosome region 4q28.3-qter in monozygotic twins with discordant phenotypes. Am J Med Genet 2000;94:125-140.
21. Rinaldi R, De Bernardo C, Assumma M, Grammatico B, Buffone E, Poscente M, Grammatico P. Cytogenetic and molecular characterization of a de novo 4q24qter duplication and correlation to the associated phenotype. Am J Med Genet A 2003;118:122-126.
22. Otsuka T, Fujinaka H, Imamura M, Tanaka Y, Hayakawa H, Tomizawa S. Duplication of chromosome 4q: renal pathology of two siblings. Am J Med Genet A 2005;134:330-333.

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Autoimmune Limbic Encephalitis Associated with Type 1 Diabetes Mellitus

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To the Editor,

Limbic encephalitis (LE) is an autoimmune, neurological disorder characterized by confusion, memory disturbance, and seizures. An association between type 1 diabetes mellitus (T1D) and other autoimmune disorders is well-known. However, the co-occurrence of T1D and LE is very rare.

A 16-year-old boy was admitted to our emergency department with confusion and headache. Electroencephalography revealed temporal slowing, cerebral magnetic resonance imaging demonstrated hyperintense signal of the right mesiotemporal lobe, and positron emission tomography demonstrated increased activity in the right temporal lobe. Blood glutamic acid decarboxylase antibody (anti-GAD) level was 2114 IU/mL (0–10) and the cerebrospinal fluid anti-GAD level was 4.07 nmol/L (<0.02). These findings led to a consideration of autoimmune LE as a possible diagnosis. Pulse methylprednisolone was administered over five days. After steroid treatment, symptoms improved, but hyperglycemia occurred on the third day of treatment. Glycemia level reached 502 mg/dL. Concurrent insulin level was 42 μIU/mL. Hyperglycemia improved after cessation of steroid treatment. Glycated hemoglobin was 5.6%. The possibility of a steroid-induced hyperglycemia was considered. Six months later, the patient was readmitted with dyspnea and abdominal pain. The family reported occurrence of polyuria and polydipsia during the previous two months. Blood anti-GAD level was >2000 IU/mL. The patient was diagnosed to have T1D. With treatment, the ketoacidosis improved in 10 h. After being educated for diabetes, the patient was discharged. Two months later, he presented with a headache and confusion again. Intravenous immunoglobulin (IVIG) 1 g/kg/d for two days every month was administered. Neurological symptoms improved and the daily insulin dose was decreased.

GAD catalyzes the production of γ-aminobutyric acid which is the most important inhibitory neurotransmitter. Especially GAD65 is highly expressed in the central nervous system (1). It is also a target antigen in T1D (2). It was reported that the patients with high values of anti-GAD (>2000 IU/mL) encountered neurological disorders (3). A few cases have been reported in which T1D and LE were associated with a high titer of anti-GAD (4). In all these cases, the patients were diagnosed with T1D prior to development of encephalitis symptoms. In contrast, our patient was diagnosed with T1D six months after LE. The diabetes developed during steroid therapy for encephalitis and the patient was initially considered as having a steroid-induced diabetes. The effect of glucocorticoids on glucose metabolism is the result of both beta cell dysfunction and insulin resistance (5). The findings in our patient (42 μIU/mL insulin concurrent with 502 mg/dL glycemia) can be considered as a relative insulinopenia. It can be speculated that in this patient, the pathogenesis of the diabetic state could be a combination of steroid impact and impairment of beta cells due to anti-GAD antibodies during the beginning stages of T1D. We also observed that IVIG administration decreased the need for the average insulin dose. However, it is difficult to distinguish

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whether the decrease was due to the impact of IVIG or to a honeymoon phase.

In conclusion, there is a possible association between T1D and autoimmune neurologic disorders due to anti-GAD. Close follow-up is important for diabetic patients with anti-GAD to detect neurological deterioration. In addition, patients encountering GAD65-related neurological disorders should be followed carefully for T1D.

Keywords: Limbic encephalitis, diabetes, anti-glutamic acid decarboxylase

Ethics

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Onur Akın, Aylin Kılınç Uğurlu, Kıvılcım Gücüyener, Esra Döğer, Concept: Onur Akın, Aysun Bideci, Design: Onur Akın, Aysun Bideci, Data Collection or Processing: Yılmaz Akbaş, Neşe Karabacak, Emine Demet Akbaş, Analysis or Interpretation: Mahmut Orhun Çamurdan, Peyami Cinaz, Literature Search: Onur Akın, Özge Yüce, Writing: Onur Akın.

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References

1. Sloviter RS, Dichter MA, Rachinsky TL, Dean E, Goodman JH, Sollas AL, Martin DL. Basal expression and induction of glutamate decarboxylase and gaba in excitatory granule cells of the rat and monkey hippocampal dentate gyrus. J Comp Neurol 1996;373:593-618.
2. Kaufman DL, Clare-Salzler M, Tian J, Forsthuber T, Ting GS, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature 1993;366:69-72.
3. Saiz A, Blanco Y, Sabater L, Gonzalez F, Bataller L, Casamitjana R, Ramio-Torrenta L, Graus F. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: Diagnostic clues for this association. Brain 2008;131:2553-2563. Epub 2008 Aug 7
4. Korff CM, Parvex P, Cimasoni L, Wilhelm-Bals A, Hampe CS, Schwitzgebel VM, Michel M, Siegrist CA, Lalive PH, Seeck M. Encephalitis associated with glutamic acid decarboxylase autoantibodies in a child: a treatable condition? Arch Neurol 2011;68:1065-1068.
5. van Raalte DH, Nofrate V, Bunck MC, van Iersel T, Elassaiss Schaap J, Nassander UK, Heine RJ, Mari A, Dokter WH, Diamant M. Acute and 2-week exposure to prednisolone impair different aspects of beta-cell function in healthy men. Eur J Endocrinol 2010;162:729-735. Epub 2010 Feb 2

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