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Lack of association of the *HSD11B1* gene polymorphisms with obesity and other traits of metabolic syndrome in children and adolescents

ABSTRACT

Introduction. Obesity and its related disorders, clustered into metabolic syndrome (MetS), are increasingly diagnosed in children and adolescents. Clinical features, which define MetS are also encountered in patients with glucocorticoid excess. Since no evident hypercortisolaemia was detected in obesity and MetS, investigations turned to the local modulators of cortisol action. 11β-hydroxysteroid dehydrogenase type 1, encoded by *HSD11B1* gene, controls tissue availability of cortisol by its regeneration from inert cortisone. Changes in *HSD11B1* expression and enzyme activity may be influenced by its sequence variants and seem implicated in MetS pathogenesis. Our study was designed to evaluate plausible association of the *HSD11B1* polymorphisms with early-onset obesity and features of MetS in Polish children and adolescents.

Material and methods. The study comprised of 258 obese children (136 females), aged 12.3 ± 3.6 years, with excessive body mass lasting 7.1 ± 3.8 years. Anthropometric and blood pressure measurements, baseline biochemical analyses and oral glucose tolerance test were performed in all participants. Genotyping of the *HSD11B1* variants rs12086634, rs846910, rs4844880, and rs3753519 was conducted in obese youth and compared with 568 lean blood donors.

Results. Mean relative body mass index in obese cohort was 164.7 ± 27.1%. Hypertension was detected in 12.4%, impaired fasting glucose in 8.9%, impaired glucose tolerance in 10.8%, diabetes in 2.7%, and dyslipidemia in 31.4% children and adolescents. None of the studied *HSD11B1* polymorphisms displayed significant difference in frequency between obese and lean individuals. MetS was diagnosed in 27.6% of 203 patients with obesity aged 10–18 years. Further genotype-stratified analyses of relationship between *HSD11B1* variants and particular features of MetS did not confirm increased susceptibility to develop early-onset hyperglycaemia, dyslipidaemia and hypertension in carriers of specific genotypes at rs4844880, rs846910, rs3753519, and rs12086634 ($p \geq 0.05$ in all tests).

Conclusion. Our study does not support the implication of the *HSD11B1* polymorphisms in early-onset obesity and other features of MetS. (Clin Diabetol 2016; 5, 6: 178–184)

Key words: childhood obesity, *HSD11B1* gene, polymorphism, cortisol, metabolic syndrome

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Introduction

Obesity is a soaring healthcare problem in modern societies. It increasingly affects the youngest populations and leads to a number of complications, which subsequently contribute to higher morbidity and mortality [1]. Metabolic syndrome (MetS) is a cluster of obesity-related traits associated with an elevated risk of type 2 diabetes (T2D) and cardiovascular disease [2]. Individuals who develop MetS in childhood have a doubled or tripled future risk of atherosclerosis and diabetes compared to those with no MetS at youth [1]. Of note, typical features, which define MetS are equally encountered in patients suffering from glucocorticoid excess, i.e. Cushing's syndrome, either of endogenous or exogenous origin [3, 4]. These clinical observations prompted detailed studies of the hypothalamo-pituitary-adrenal (HPA) axis in patients with obesity and MetS. However, despite some indices of the enhanced HPA reactivity to physiologic and pharmacologic stimuli, consistently elevated cortisol levels were not confirmed in obese individuals [5].

Identification of enzymes, which exert modulatory role on cortisol action in tissues, paved the way for new hypotheses on glucocorticoid role in cardiometabolic disorders. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) primarily acts as a ketoreductase and controls local availability of cortisol by its NADPH--dependent regeneration from the inert cortisone [6]. The enzyme, encoded by the *HSD11B1* gene, is widely expressed in the liver, adipose tissue, muscle and central nervous system, where it amplifies glucocorticoid action [7]. Variable degree of 11β-HSD1 activity may account for enhanced or attenuated local glucocorticoid effects. Transgenic mice with selective overexpression of 11β-HSD1 in their adipose tissue display features of MetS — visceral obesity, insulin resistance and hyperlipidaemia [8]. On the contrary, mice with targeted enzyme knockout or its selective inhibition seem protected from the adverse effects of the high-fat diet [9, 10]. In humans, increased expression of 11β--HSD1 was confirmed in visceral adipose tissue of obese, insulin resistant individuals [11]. A positive correlation between body mass index (BMI) and *HSD11B1* mRNA in adipose tissue was noted [11, 12]. Women with the highest enzyme activity in their visceral adipose tissue presented enhanced lipolysis and insulin resistance indexes, together with the lowest HDL cholesterol and adiponectin levels [13].

Therefore, early development of MetS might be connected with an altered *HSD11B1* gene expression and enzyme activity. As indicated *in vitro* and confirmed by *in vivo* studies, transcriptional activity and subsequent 11β-HSD1 expression may be affected by single nucleotide polymorphisms (SNPs) in the *HSD11B1* gene [14–17]. Rs846910 and rs12086634 appeared to be significantly associated with T2D in Pima Indians and with glucose uptake in the euglycaemic-hyperinsulinaemic clamp, although no direct association with BMI was found [18]. A Korean study revealed plausible association of the *HSD11B1* polymorphisms with MetS, fasting plasma glucose and BMI [19]. In European populations, a relationship of rs846910 and rs12086634 with the risk of MetS was described in Italian women [15]. Moreover, an association of the latter variant with an elevated *HSD11B1* mRNA in subcutaneous adipose tissue and enhanced conversion of cortisone to cortisol assessed in 24 h urine collections were reported [15]. In Spanish children rs3753519 appeared to be associated with obesity, although no effect on glucose balance was detected [20]. In a small American study an association between an adenine insertion in *HSD11B1* gene intron 3 (ins4436A, rs45487298) and increased BMI, waist-to-hip ratio and insulin resistance indices in children was found [21]. More recent analysis in young Mexican-Americans confirmed that rs846910 was associated with Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and circulating triglycerides but not with obesity [22]. Finally, a Chinese study linked several *HSD11B1* variants with childhood obesity and its cardiometabolic complications — hypertension, hypercholestarolaemia, and hyperglycaemia [23]. On the contrary, a study conducted among the elderly Dutch, revealed no association of *HSD11B1* gene with body composition and glucose metabolism [24]. However, plausible influence of genetic factors is rather expected to be more prominent in early-onset disorders. Baring this in mind, the current study was designed to evaluate plausible association of the *HSD11B1* polymorphisms with early-onset obesity and other features of the metabolic syndrome among Polish children and adolescents.

Material and methods

Two hundred fifty eight unrelated children (136 females, 122 males) referred to the Department of Paediatric Diabetes and Obesity at Poznan University of Medical Sciences for the reason of obesity, were enrolled. Their mean age ± standard deviation (± SD) was 12.3 ± 3.6 years and mean duration of excessive body mass was 7.1 ± 3.8 years. Their pubertal development evaluated according to the Tanner scale was stage 1 in 22.4%, stage 2 in 19.4%, stage 3 in 14.7%, stage 4 in 17.4% and stage 5 in 26.0% individuals. Children suffering from the endocrine-related, medication--induced or syndromic obesity were excluded from the study. Informed consent was obtained from parents of the minor patients and additionally from participants

above 16 years old. Local ethical committee at Poznan University of Medical Sciences has approved the study protocol (decision 233/13) and all procedures were in accordance with the Declaration of Helsinki.

Control samples for genotyping were obtained from 568 healthy lean blood donors recruited at the Regional Blood Transfusion Centre. Their mean age was 38.1 ± 10.2 years and mean BMI was 23.6 ± 1.3 kg/m^2.

Clinical evaluation of obese patients comprised their weight and height measurement with calibrated stable stadiometer and electronic scales. BMI was calculated as weight [kg]/height [m^2]. For the purpose of this study, obesity was defined as BMI-for-age value ≥ 95th percentile (i.e. BMI standard deviation score > 2) according to the Polish growth charts [25]. Relative body mass index (RBMI) was calculated as proportion of individual BMI vs. mean BMI at the 50th percentile for age and gender [26]. Additionally waist circumference (WC) was measured in all patients and referred to the Polish percentile charts for children and adolescents [27]. Blood pressure (BP) measurements were taken between 9 a.m. and 11 a.m. on three consecutive days, in a seated position after 10 min rest, using a validated oscillometric sphygmomanometer with a suitable cuff size. Hypertension was defined as mean value of three measurements exceeding 95th percentile for gender and age [28].

Biochemical analyses required morning blood sampling after overnight fast. Additionally, all patients underwent standard 2-hour oral glucose tolerance test (OGTT) with 1.75 g of glucose/kg (max. 75 g) and evaluation of glycaemia and insulinaemia. Biochemical analyses including glucose, total cholesterol, HDL cholesterol, and triglycerides were performed by standard enzymatic methods with Olympus AU680 automated chemistry analyzer (Beckman Coulter Inc, Miami, FL, USA). Serum insulin was determined by immunochemiluminescence (Architect System, Santa Clara, USA).

Disorders of glucose tolerance were defined based upon guidelines of the International Society for Paediatric and Adolescent Diabetes (ISPAD) [29]. HOMA-IR was calculated as fasting serum insulin (mU/L) × fasting serum glucose (mmol/L) / 22.5. Low-density lipoprotein (LDL) cholesterol was determined by Friedewald's formula. MetS was defined according to the International Diabetes Federation (IDF) consensus criteria for children and adolescents in subjects aged 10–15 years old and standard criteria for adults in individuals aged 16 and more years [2, 30].

Genotyping

Genomic DNA was extracted from the peripheral blood using Gentra Puregene Blood Kit (Qiagen, Hilden, Germany). Genotyping of the *HSD11B1* gene SNPs: rs4844880, rs846910, rs3753519, and rs12086634, was carried out by allelic discrimination analysis using the 7900HT Real-Time PCR System and validated commercial TaqMan SNP Genotyping assays (C_2502442_10, C_8887157_10, C_27474627_10, and C_22275467_10, respectively) following the conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA). Data acquisition and analysis were performed using the allelic discrimination analysis module in SDS v. 2.3 software (Applied Biosystems). The genotypes were confirmed in 8% samples by direct DNA sequencing with BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI Prism 3730 Genetic Analyzer, Foster City, CA). The samples of confirmed genotypes were run as controls in all reactions and 10% samples were re-genotyped blind to ensure accuracy.

Statistical analysis

Statistical analyses were performed by means of GraphPad Prism 6.0c (GraphPad Software, La Jolla, CA). Quantitative data are presented as mean ± SD, median and interquartile range, while categorical data — as percentage. Hardy-Weinberg equilibrium of the genotyped SNPs was tested using online calculator devised at Tufts University (Medford, MA). Data normality was checked with Shapiro-Wilk test. Considering the low frequency of homozygotes for the minor alleles of the studied SNPs, these subjects were merged for statistical purposes with the heterozygotes and this group was compared with the homozygotes for the common wild-type allele. Normally distributed genotype-stratified data were subsequently compared using t-student test for unpaired samples, whereas those with non-normal distribution were analysed by nonparametric Mann-Whitney test. Two-tailed p-values < 0.05 were considered statistically significant. Assuming odds ratio (OR) of 1.5 at a 0.05 level of significance, the power of this study to detect an effect was calculated based on PS Power and Sample Size calculator v. 2.1.30 (Vanderbilt University, Nashville, TN).

Results

Mean RBMI in the studied cohort was equal to 164.7 ± 27.1%. Hypertension was detected in 32 (12.4%) obese individuals. Based upon the OGTT results, normal glucose tolerance was found in 200 (77.5%) subjects, impaired fasting glucose — in 23 (8.9%), impaired glucose tolerance — in 28 (10.8%) and diabetes — in 7 (2.7%) children and adolescents. Mean HOMA-IR value exceeded 3.0, indicating widespread insulin resistance in this cohort. Dyslipidemia defined according to the IDF criteria of MetS was detected in

Table 1. Clinical and biochemical characteristics of the studied cohort of obese children and adolescents

	Mean	SD	Median	IQR
RBMI (%)	164.7	27.1	160.0	144.4–179.3
SBP [mm Hg]	114	3	108	105–117
DBP [mm Hg]	71	2	67	64–76
Fasting glucose [mmol/L]	4.95	0.52	4.89	4.67–5.18
Fasting insulin [mU/L]	16.0	9.3	13.9	9.7–20.3
Glucose 2 h OGTT [mmol/L]	6.54	1.40	6.39	5.72–7.22
Insulin 2 h OGTT [mU/L]	77.7	61.8	57.8	36.6–101.5
HOMA-IR [mmol/L × mU/L]	3.56	2.16	3.06	2.00–4.34
Triglycerides [mmol/L]	1.39	0.77	1.16	0.92–1.64
Total cholesterol [mmol/L]	4.65	0.84	4.69	4.09–5.21
HDL cholesterol [mmol/L]	1.15	0.26	1.14	0.96–1.32
LDL cholesterol [mmol/L]	2.84	0.69	2.77	2.38–3.29

RBMI — relative body mass index; SBP — systolic blood pressure; DPB — diastolic blood pressure; OGTT — oral glucose tolerance test; HOMA-IR — Homeostatic Model Assessment of Insulin Resistance; HDL — high-density lipoprotein; LDL — low-density lipoprotein; SD — standard deviation; IQR — interquartile range

Table 2. Distribution of alleles and genotypes of the *HSD11B1* gene single nucleotide polymorphisms (SNPs) in 258 children and adolescents with obesity (OB) compared to 568 lean controls (CON)

SNP	Cohort	Genotypes (%)			Alleles (%)	
rs4844880		**TT**	**TA**	**AA**	**T**	**A**
	OB	166 (64.3)	83 (32.2)	9 (3.5)	415 (80.4)	101 (19.6)
	CON	385 (67.8)	157 (27.6)	26 (4.6)	927 (81.6)	209 (18.4)
	p		0.356		0.571	
rs846910		**GG**	**GA**	**AA**	**G**	**A**
	OB	222 (86.0)	34 (13.2)	2 (0.8)	478 (92.6)	38 (7.4)
	CON	508 (89.4)	59 (10.4)	1 (0.2)	1075 (94.6)	61 (5.4)
	p		0.201		0.113	
rs3753519		**GG**	**GA**	**AA**	**G**	**A**
	OB	191 (74.0)	61 (23.7)	6 (2.3)	443 (85.9)	73 (14.1)
	CON	438 (77.1)	124 (21.8)	6 (1.1)	1000 (88.0)	136 (12.0)
	p		0.294		0.218	
rs12086634		**GG**	**GT**	**TT**	**G**	**T**
	OB	163 (63.2)	85 (32.9)	10 (3.9)	411 (79.7)	105 (20.3)
	CON	317 (55.8)	222 (39.1)	29 (5.1)	856 (75.4)	280 (24.6)
	p		0.134		0.055	

81 (31.4%) individuals. Detailed biochemical analyses of the studied cohort are displayed in Table 1.

The frequencies of all investigated polymorphisms remained in Hardy-Weinberg equilibrium in patients and controls (p-values ≥ 0.058). Unfortunately, none of the four studied variants of the *HSD11B1* gene displayed significant difference in genotype and allele distribution between obese and lean individuals (Tab. 2).

Since diagnosing the metabolic syndrome is only recommended in children aged at least 10 years old, we reviewed the results of this subgroup of patients with regard to the IDF consensus criteria. The diagnosis of MetS was established in 56 (27.6%) out of 203 individuals aged 10–18 years old. Only this cohort was further studied in genotype-stratified analyses aiming to detect a relationship between *HSD11B1* gene polymorphisms and features of MetS. However, as presented in Table 3, these calculations did not confirm that carriers of specific genotypes of rs4844880, rs846910, rs3753519, and rs12086634 might be more susceptible to early-onset hyperglycaemia, dyslipidaemia and hypertension (p-values ≥ 0.05 in all tests).

Table 3. Association between features of the metabolic syndrome and polymorphic variants of the *HSD11B1* gene analysed in 203 obese children and adolescents aged 10–18 years old

SNP		rs4844880			rs846910			rs3753519			rs12086634		
Genotype		TT	TA+AA	p	GG	GA+AA	p	GG	GA+AA	p	TT	TG+GG	p
	n	120	83		171	32		144	59		114	89	
WC < 90th pc	54	33	21	0.727	45	9	0.832	37	17	0.648	26	28	0.166
WC ≥ 90th pc	149	87	62		126	23		107	42		88	61	
FPG < 5.6 mmol/L	166	99	67	0.747	138	28	0.460	118	48	0.921	97	69	0.241
FPG ≥ 5.6 mol/L	37	21	16		33	4		26	11		17	20	
HDL ≥ 1.03 mmol/L*	128	78	50	0.490	109	19	0.639	93	35	0.481	77	51	0.134
HDL < 1.03 mmol/L	75	42	33		62	13		51	24		37	38	
TG < 1.7 mmol/L	152	94	58	0.172	131	21	0.187	113	39	0.065	90	62	0.130
TG ≥ 1.7 mmol/L	51	26	25		40	11		31	20		24	27	
BP < 130/85 mm Hg	158	91	67	0.410	136	22	0.178	114	44	0.475	88	70	0.804
BP ≥ 130/85 mm Hg	45	29	16		35	10		30	15		26	19	

*In patients aged > 16 years old gender-related differences are considered, with cut-off value for males equal to 1.03 and for females 1.29 mmol/L. SNP — single nucleotide polymorphism; WC — waist circumference; pc — percentile; FPG — fasting plasma glucose; HDL — high-density lipoprotein cholesterol; TG — triglyceride; BP — blood pressure

Discussion

Despite the former reports suggestive of an association between *HSD11B1* polymorphisms and obesity and its related metabolic disorders, we could not confirm these findings in our study. Association of the *HSD11B1* gene variants with MetS was reported in South Indians, whereas relationship with T2D was detected in Native Americans originating from Pima population [18, 31]. In Europeans, association with MetS was found in Italian women, while a small study in Bosnians revealed correlations with BP, HOMA-IR and LDL cholesterol values [15, 32]. Moreover, *HSD11B1* gene appeared to influence glucose and HDL cholesterol level in Brazilian females of European descent [33]. Although data from adults do not confirm direct effect of *HSD11B1* polymorphisms on body mass, an association with obesity and its measures (BMI, WC, waist-to-hip ratio) was reported in Spanish, American and Chinese children [20, 21, 23]. Results with regard to insulin and its action are contradictory in Spanish and Mexican-American youth [20, 22]. This latter cohort also revealed a relationship between *HSD11B1* polymorphism and circulating triglycerides [22].

Although equally conducted among children and adolescents, our investigation failed to detect similar associations with the *HSD11B1* gene variants. One possible explanation relies on population differences, which are commonly encountered in association studies, including those concerning obesity [34]. Lack of sufficient power is another reason that should be considered, given a limited number of participants and low frequency of the minor alleles in our population, ranging from 5.4% for A at rs846910 to 24.6% for T at rs12086634. Therefore, assuming an OR of 1.5, the current analysis presented 49.5 to 93.2% power to detect an effect of the investigated polymorphisms. Finally, the choice of the studied SNPs might also play a role. Their selection was based upon former data indicating plausible association with obesity, but we also paid attention to experimental evidence of their functionality. The minor G allele of rs12086634 alone and in combination with rs846910 was found to affect *HSD11B1* gene transcriptional activity, whereas *in vitro* studies evaluating solely the functional aspect of rs846910 did not reveal differences in *HSD11B1* expression and enzyme activity [14–16, 18]. Moreover, an inhibitory effect on gene transcription was detected for the minor A allele at rs4844880 [17]. However, *in vitro* assays usually focus on specific gene variants, which are analysed separately, hence their results may not be relevant for the situation *in vivo*, when effects of several polymorphism cumulate and overlap. Therefore, we cannot exclude that the *HSD11B1* variants investigated in our study are of no functional significance. As a matter of fact, lack of association between *HSD11B1* gene polymorphisms and metabolic complications of obesity was reported in adult populations of French Canadians, Japanese and elderly Dutch [24, 35, 36]. Still, other gene variants might be implicated in development of MetS traits. A recent Polish study demonstrated an association between *HSD11B1* rs45487298 and essential hypertension in adults [37]. Moreover, within the hypertensive cohort this polymorphism appeared strong predictor of T2D and lower circulating HDL cholesterol,

although these observations were not confirmed in the control group [37].

On the other hand, our previous analysis conducted in patients with primary adrenal failure who require lifelong glucocorticoid substitution, revealed that *HSD11B1* rs3753519 was associated with BMI and fasting plasma glucose, whereas rs12086634 appeared associated with circulating cholesterol [38]. Analogous findings with regard to rs4844880, BMI and weight gain during glucocorticoid replacement were reported in a smaller Hungarian cohort of subjects with adrenocortical insufficiency [39]. These patients are usually treated with hydrocortisone, identical with the natural cortisol molecule, but its proper dosage, mimicking natural diurnal rhythm of cortisol secretion, remains a challenging issue. Differences in 11β-HSD1 activity may therefore enhance individual susceptibility to the adverse effects of inadvertent excess glucocorticoid supply. However, observations in this specific population constantly receiving exogenous steroids may not be relevant for obese patients, who rely on endogenous regulation of the HPA axis.

To summarize, our study does not corroborate the role of polymorphic variants of the *HSD11B1* gene in obesity and other features of the metabolic syndrome. Nevertheless, there are consistent experimental and clinical data to support the implication of 11β-hydroxysteroid dehydrogenase type 1 in pathogenesis of obesity-related metabolic disease. However, enzyme activity may be influenced by factors other than genetic polymorphisms. Investigation of modulators of the glucocorticoid action in tissues seems to be justified and promising area to explore in obesity and obesity related metabolic disorders.

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Conflict of interests

The authors declare no conflict of interests.

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