

**Original Article**

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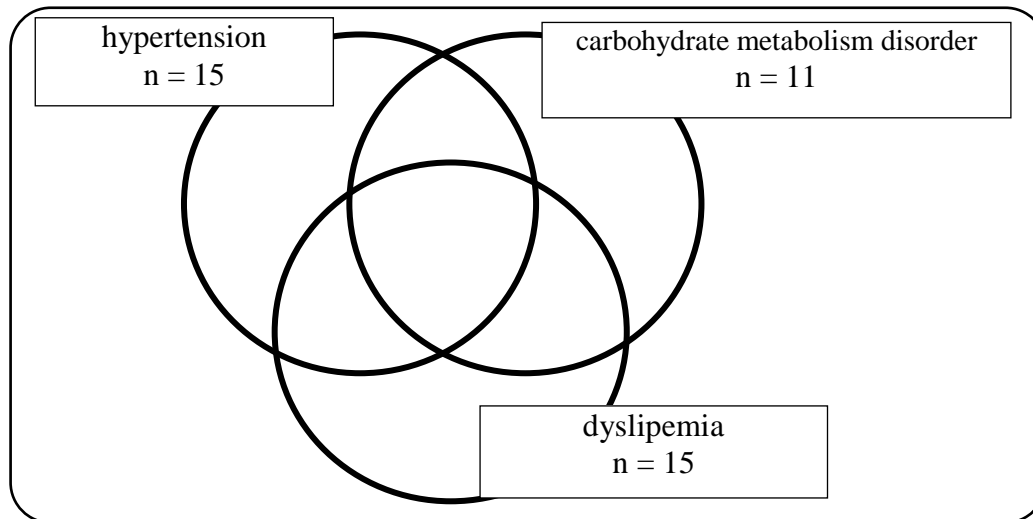
# Methylation Status of *CYP27B1* and *IGF2* Correlate to BMI SDS in Children with Obesity

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## Supplemental Material

**Supplementary figures**



**Supplementary fig. 1.** The prevalence of metabolic consequences in obese children (N = 82) 27 patients of 82 obese children had either hypertension (according to 24-hour monitoring), carbohydrate metabolism disorder (impaired glucose tolerance) and/or dyslipidemia (elevated cholesterol level). One patient had all three of the above mentioned comorbidities and also presented elevated uric acid level. According to our results hypertension and dyslipidemia individually was present in 18.29–18.29 % and carbohydrate metabolism disorder was present in 13.41% of the cases. 9 of 55 the patients without hypertension, impaired glucose tolerance and dyslipidemia presented isolated hyperuricemia (data not shown). 46 patients did not present any signs of comorbidities.

**Supplementary table 1.** Comparing some anthropometric and labor data of obese Hungarian girls and boys

	<b>Girls</b> (mean ± SD)	<b>Boys</b> (mean ± SD)	<b>p value</b>
<b>Puberty status (pub / all; %)</b>	33/42 (78.57 %)	27/40 (67.5 %)	
<b>Age (years)</b>	12.521 ± 3.096	12.935 ± 2.688	0.5819
<b>BMI SDS</b>	4.505 ± 0.288	3.964 ± 0.244	0.1571
<b>Waist circumference (cm)</b>	99.328 ± 17.688	104.222 ± 14.439	0.2547
<b>Mother's weight gain during pregnancy (kg)</b>	11.94 ± 1.471	13.67 ± 2.513	0.5488
<b>Cholesterol (mmol/L)</b>	4.588 ± 0.957	4.55 ± 0.897	0.2214
<b>HDL (mmol/L)</b>	1.146 ± 0.263	1.165 ± 0.333	0.7863
<b>Triglyceride (mmol/L)</b>	1.404 ± 0.561	1.459 ± 0.646	0.6837
<b>ALP (IU/L)</b>	184.2 ± 15.589	230.3 ± 16.41	<b>0.0452</b>
<b>SGOT (IU/L)</b>	20.29 ± 6.115	22.17 ± 7.114	0.3130
<b>SGPT (IU/L)</b>	18.23 ± 1.335	28.10 ± 3.813	<b>0.0228</b>
<b>Fasting glucose (mmol/L)</b>	4.974 ± 0.429	5.11 ± 0.486	0.1818
<b>Vitamin D (ng/mL)</b>	18.252 ± 6.414	19.26 ± 7.505	0.5146

*SD: standard deviation, BMI SDS: body mass index standard deviation score, HDL: High-density lipoprotein, ALP: Alkaline phosphatase, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate-pyruvate transaminase*

**Supplementary table 2.** Correlation of vitamin D level with anthropometric, metabolic parameters and methylation status

<b>Vitamin D (ng/mL)</b>	<b>correlation with</b>	<b>r value</b>	<b>p value</b>
	BMI SDS	-0.1191	0.2866
	Waist circumference (cm)	-0.1193	0.3682
	Mother's weight gain during pregnancy (kg)	0.06752	0.6021
	Cholesterol(mmol/L)	-0.01635	0.8848
	HDL(mmol/L)	-0.1465	0.1946
	Triglyceride (mmol/L)	-0.0764	0.5061
	ALP (U/L)	0.03449	0.7705
	SGOT (U/L)	0.1546	0.2786
	SGPT (U/L)	0.04095	0.787
	Fasting glucose (mmol/L)	0.002146	0.9847
	Fasting insulin (mIU/L)	-0.02664	0.8145
	HOMA index	-0.04825	0.6669
	Uric acid (umol/L)	0.05179	0.6482
	<i>VDR_2</i> methylation %	0.2053	0.066
	<i>VDR_3</i> methylation %	0.1649	0.1388
	<i>CYP27B1_1</i> methylation %	0.1245	0.2652
	<i>CYP27B1_2</i> methylation %	0.1033	0.3618
	<i>IGF2</i> methylation %	-0.05146	0.6503
	<i>POMC</i> methylation %	0.1664	0.1352

*BMI SDS: body mass index standard deviation score, HDL: High-density lipoprotein, ALP: Alkaline phosphatase, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate-pyruvate transaminase, VDR: vitamin D receptor gene, CYP27B1: 1-alfa-hydroxylase gene, IGF2: insulin like growth factor gene, POMC: proopiomelanocortin gene*

**Supplementary table 3.** Correlation of BMI SDS with anthropometric, metabolic parameters and methylation status

<b>BMI SDS</b>	<b>correlation with</b>	<b>r value</b>	<b>p value</b>
	Waist circumference (cm)	0.5655	< <b>0.0001</b>
	Mother's weight gain during pregnancy (kg)	0.06616	0.6094
	Cholesterol (mmol/L)	0.1545	0.1684
	HDL(mmol/L)	-0.07089	0.532
	Triglyceride (mmol/L)	0.02641	0.8185
	ALP (IU/L)	-0.1698	0.1481
	SGOT (IU/L)	-0.0574	0.6891
	SGPT (IU/L)	0.01273	0.9331
	Fasting glucose (mmol/L)	-0.1765	0.1127
	Fasting insulin (mIU/L)	0.3539	<b>0.0013</b>
	HOMA index	0.2757	<b>0.0122</b>
	Uric acid (umol/L)	0.2333	<b>0.0373</b>
	<i>VDR_2</i> methylation %	-0.06579	0.5595
	<i>VDR_3</i> methylation %	-0.1531	0.1697
	<i>CYP27B1_1</i> methylation %	0.1816	0.1025
	<i>CYP27B1_2</i> methylation %	0.2371	<b>0.0342</b>
	<i>IGF2</i> methylation %	-0.305	<b>0.0059</b>
	<i>POMC</i> methylation %	0.1334	0.2321

*BMI SDS: body mass index standard deviation score, HDL: High-density lipoprotein, ALP: Alkaline phosphatase, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamate-pyruvate transaminase, VDR: vitamin D receptor gene, CYP27B1: 1-alfa-hydroxylase gene, IGF2: insulin like growth factor gene, POMC: proopiomelanocortin gene*

**Supplementary table 4.** Linear regression models including IGF2 and CYP27B1 methylation status, vitamin D as predictive variables and BMI SDS as dependent variable

Model	Unstandardized Coefficients		Standardized Coefficients Beta	t	Sig.
	B	Std. Error			
1*	(Constant)	5.094	1.863	2.735	0.008
	IGF2 met%	-0.083	0.034	-0.264	<b>0.018</b>
	CYP27B1 met %	0.197	0.104	0.207	<b>0.061</b>
	vitamin D (ng/mL)	0.028	0.031	0.100	<b>0.365</b>
2	(Constant)	5,339	1.841	2.899	0.005
	IGF2 met%	-0.079	0.034	-0.251	<b>0.023</b>
	CYP27B1 met%	0.209	0.103	0.219	<b>0.046</b>

\*Model 1 included CYP27B1, IGF2 methylation status and Vitamin D level as variables. CYP27B1 (p= 0.061) and IGF2 (p= 0.018) methylation status were significantly associated with BMI SDS while vitamin D level did not show significant association (p= 0.365).

*BMI SDS: body mass index standard deviaton score, CYP27B1: 1-alfa-hydroxylase gene, IGF2: insulin like growth factor gene*

**Supplementary table 5.** Model fit for Model 1 and Model 2 (final model) (final model)

Model		Sum of Squares	df	Mean Square	F	Sig.p
1	Regression	31.175	3	10.392	3.767	0.014 <sup>b</sup>
	Residual	206.917	75	2.759		
	Total	238.092	78			
2	Regression	28.882	2	14.441	5.246*	<b>0.007<sup>c</sup></b>
	Residual	209.210	76	2.753		
	Total	238.092	78			

\*Model 2 as statistically significant final model predicting BMI SDS accounted for 9.8 % of the variance (  $F(2,78) = 5.246$   $p=0.007$ ).

<sup>a</sup> Dependent Variable: BMI SDS <sup>b</sup> Predictors: (Constant), D vitamin (ng/mL), *CYP27B1* met % , *IGF2* met % <sup>c</sup> Predictors: (Constant), *CYP27B1* met% , *IGF2* met %

*BMI SDS*: body mass index standard deviation score, *CYP27B1*: 1- $\alpha$ -hydroxylase gene, *IGF2*: insulin like growth factor gene