

Small-for-Gestational-Age Newborns Exhibit Altered Placental and Cord Blood Methylation of Genes Involved in Energy Homeostasis: Association with Fetal Growth and Postnatal Body Composition

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The authors have nothing to disclose

BACKGROUND

Fetal growth is partly regulated by epigenetic factors, such as DNA methylation.

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- Altered methylation status in placental genes has been related to gestational diabetes, preeclampsia and prematurity.
- However, the epigenetic mechanisms underlying fetal growth restraint in uncomplicated pregnancies remain unknown.

AIM

To identify new candidate genes related to fetal growth, by assessing DNA methylation profiling in placenta and cord blood -as well as expression levels of differentially methylated genes- in newborns born appropriate- (AGA) or small-for-gestational-age (SGA).

SUBJECTS AND METHODS

- Placentas and cord blood samples were collected from uncomplicated pregnancies delivering term AGA [birthweight, between -1.1 and 1.1 SD; N=30) or SGA [birthweight, <-2 SD; N=21) newborns.
- Placental methylation profiling was performed using Agilent DNA Methylation array design to detect CpG sites located within promoter regions of 14.475 genes. A first analysis of the array revealed that 39 genes were differentially methylated (all, p<0.009). Among those genes, we selected those related to energy homeostasis (n=8) which were validated by bisulfite pyrosequencing (BSP) and also analyzed in cord blood by BSP.
- A differential methylation pattern was confirmed in four of the eight genes; of those, three were hypermethylated (*GPR120, ATG2B*, and *NKX6.1*) and one was hypomethylated (*SLC13A5*). Placental and cord blood expression of the confirmed genes was performed by real-time PCR.
- Glucose, insulin and IGF-I were measured in cord blood; body composition was assessed by DXA at age 15 days.

RESULTS

Table 1. Bivariate correlations between placental and cord blood gene

 methylation and expression and selected parameters in all subjects.

Figure 1. Methylation (top) and expression levels (bottom) of validated genes in placentas from infants born appropriate (AGA, n=30) or small-for-gestational age (SGA, n=21).

$ \begin{array}{ c c c c c c } \hline Placenta & Cord Blood & Placenta & Cord Blood \\ \hline ATG2B & \beta & P & \beta & P & \beta & P & \beta & P \\ \hline Birth weight & -0.316 & 0.03 & -0.411 & 0.002 & 0.522 & <0.0001 & - & - \\ \hline Birth length & -0.358 & 0.013 & -0.435 & 0.002 & 0.495 & <0.0001 & - & - \\ \hline HOMA-IR & - & - & - & - & - & - & 0.442 & 0.001 & - & - \\ \hline IGF-I & -0.293 & 0.045 & - & - & 0.442 & 0.001 & - & - \\ \hline Abdominal fat & - & - & - & 0.416 & 0.003 & - & - \\ \hline Abdominal fat & - & - & - & 0.416 & 0.003 & - & - \\ \hline NKK6 & & & & & & & & \\ Birth weight & -0.358 & 0.025 & -0.509 & <0.0001 & 0.428 & 0.002 & - & - \\ \hline NKK6 & & & & & & & & \\ Birth weight & -0.358 & 0.025 & -0.593 & <0.0001 & 0.428 & 0.002 & - & - \\ \hline NK76 & & & & & & & & & & \\ Birth weight & -0.358 & 0.025 & -0.593 & <0.0001 & 0.473 & <0.001 & 0.399 & 0.027 \\ \hline Insulin & - & - & - & - & - & & - & - \\ IGF-I & -0.328 & 0.002 & -0.420 & 0.003 & 0.448 & 0.001 & 0.327 & 0.028 \\ \hline Fat Mass & -0.492 & 0.001 & - & - & 0.324 & 0.02 & - & - \\ IGF-I & -0.328 & 0.002 & -0.420 & 0.003 & 0.448 & 0.001 & 0.327 & 0.028 \\ \hline Fat Mass & -0.492 & 0.001 & - & - & 0.324 & 0.02 & - & - \\ \hline Abdominal fat & -0.417 & 0.008 & -0.313 & 0.031 & 0.325 & 0.02 & - & - \\ \hline IGF-I & -0.324 & 0.044 & -0.560 & <0.0001 & 0.476 & <0.0001 & - & - \\ \hline IGF-I & -0.323 & 0.001 & -0.575 & <0.0001 & 0.415 & 0.002 & 0.661 & <0.001 \\ \hline Insulin & - & - & -0.330 & 0.031 & - & - & 0.346 & 0.027 \\ \hline Insulin & - & - & -0.330 & 0.031 & - & - & 0.346 & 0.027 \\ \hline Insulin & - & - & -0.330 & 0.031 & - & - & 0.346 & 0.027 \\ \hline Insulin & - & - & -0.330 & 0.031 & - & - & 0.346 & 0.027 \\ \hline Insulin & - & - & - & - & - & - & - & - \\ IGF-I & -0.333 & 0.035 & - & - & - & - & - & - \\ IGF-I & -0.333 & 0.034 & -0.360 & 0.0011 & 0.386 & 0.005 & 0.629 & <0.0001 \\ \hline Insulin & - & - & 0.330 & 0.031 & - & & 0.346 & 0.027 \\ \hline Insulin & - & - & 0.330 & 0.031 & - & & 0.346 & 0.027 \\ \hline Insulin & - & - & 0.330 & 0.031 & - & & 0.346 & 0.027 \\ \hline Insulin & - & - & 0.333 & 0.035 & - & - & & - & & - \\ \hline IGF-I & -0.333 & 0.024 & -0.360 & 0.0011 & 0.386 & 0.005 $		Methylation				Expression			
ATG2BβPβPβPβPβPBirth weight-0.3160.03-0.4410.0020.522<0.0001Birth length-0.3580.013-0.4350.0020.495<0.0001HOMA-IRIGF-I-0.2930.0450.4420.001Abdominal fat0.4420.001Lean Mass-0.3340.022-0.509<0.00010.4280.002NKX6Birth weight-0.3580.025-0.593<0.0010.473<0.0010.3290.027InsulinHOMA-IRIGF-I-0.3280.002-0.4200.0030.4480.0010.3290.027InsulinHOMA-IRHOMA-IRIGF-I-0.3280.002-0.4200.0030.4480.0010.3270.028Fat Mass-0.4170.008-0.3130.0310.3250.02IG		Pla	centa	Cord Blood		Placenta		Cord Blood	
Birth weight -0.316 0.03 -0.441 0.002 0.522 <0.001	ATG2B	β	P	β	Р	β	Р	β	Р
Birth length -0.358 0.013 -0.435 0.002 0.495 <0.001 - - HOMA-IR - </td <td>Birth weight</td> <td>-0.316</td> <td>0.03</td> <td>-0.441</td> <td>0.002</td> <td>0.522</td> <td><0.0001</td> <td>-</td> <td>-</td>	Birth weight	-0.316	0.03	-0.441	0.002	0.522	<0.0001	-	-
HOMA-IR <td>Birth length</td> <td>-0.358</td> <td>0.013</td> <td>-0.435</td> <td>0.002</td> <td>0.495</td> <td><0.0001</td> <td>-</td> <td>-</td>	Birth length	-0.358	0.013	-0.435	0.002	0.495	<0.0001	-	-
IGF-I-0.2930.0450.3380.023Fat Mass0.4420.001Abdominal fat0.4160.003Lean Mass-0.3340.022-0.509<0.0001	HOMA-IR	-	-	-	-	-	-	-	-
Fat Mass0.4420.001Abdominal fat0.4160.003Lean Mass-0.3340.022-0.509<0.0001	IGF-I	-0.293	0.045	-	-	-	-	0.338	0.023
Abdominal fat - - - 0.416 0.003 - - Lean Mass -0.334 0.022 -0.509 <0.0001 0.428 0.002 - - NKX6 - - - 0.001 0.553 <0.001 0.399 0.007 Birth weight -0.358 0.025 -0.593 <0.001 0.473 <0.001 0.399 0.007 Birth length -0.395 0.001 -0.581 <0.001 0.473 <0.001 0.329 0.027 Insulin -	Fat Mass	-	-	-	-	0.442	0.001	-	-
Lean Mass -0.334 0.022 -0.509 <0.0001 0.428 0.002 - - NKX6 Birth weight -0.358 0.025 -0.593 <0.001	Abdominal fat	-	-	-	-	0.416	0.003	-	-
NKX6 Birth weight -0.358 0.025 -0.593 <0.001	Lean Mass	-0.334	0.022	-0.509	<0.0001	0.428	0.002	-	-
Birth weight -0.358 0.025 -0.593 <0.001 0.553 <0.001 0.399 0.007 Birth length -0.395 0.001 -0.581 <0.001	NKX6								
Birth length-0.3950.001-0.581<0.0010.473<0.00010.3290.027Insulin0.3100.046HOMA-IRIGF-I-0.3280.002-0.4200.0030.4480.0010.3270.028Fat Mass-0.4920.0010.3240.02Abdominal fat-0.4170.008-0.3130.0310.3250.02Lean Mass-0.3240.044-0.560<0.0001	Birth weight	-0.358	0.025	-0.593	<0.0001	0.553	<0.0001	0.399	0.007
Insulin0.3100.046HOMA-IRIGF-I-0.3280.002-0.4200.0030.4480.0010.3270.028Fat Mass-0.4920.0010.3240.02Abdominal fat-0.4170.008-0.3130.0310.3250.02Lean Mass-0.3240.044-0.560<0.001	Birth length	-0.395	0.001	-0.581	<0.0001	0.473	<0.0001	0.329	0.027
HOMA-IR <td>Insulin</td> <td>-</td> <td>-</td> <td>-0.310</td> <td>0.046</td> <td>-</td> <td>-</td> <td>-</td> <td>-</td>	Insulin	-	-	-0.310	0.046	-	-	-	-
IGF-I-0.3280.002-0.4200.0030.4480.0010.3270.028Fat Mass-0.4920.0010.3240.02Abdominal fat-0.4170.008-0.3130.0310.3250.02Lean Mass-0.3240.044-0.560<0.0001	HOMA-IR	-	-	-	-	-	-	-	-
Fat Mass-0.4920.0010.3240.02Abdominal fat-0.4170.008-0.3130.0310.3250.02Lean Mass-0.3240.044-0.560<0.0001	IGF-I	-0.328	0.002	-0.420	0.003	0.448	0.001	0.327	0.028
Abdominal fat Lean Mass-0.4170.008-0.3130.0310.3250.02Lean Mass-0.3240.044-0.560<0.00010.476<0.001SLC13A5Birth weight-0.560<0.0001-0.505<0.00010.4150.0020.661<0.0001Birth length-0.4630.001-0.4770.0010.498<0.0010.597<0.0001Insulin0.3300.031IGF-I-0.3930.004-0.3600.00110.3860.0050.629<0.001Fat Mass-0.3130.0250.4580.0010.4350.003	Fat Mass	-0.492	0.001	-	-	0.324	0.02	-	-
Lean Mass-0.3240.044-0.560<0.00010.476<0.0001SLC13A5Birth weight-0.560<0.0001	Abdominal fat	-0.417	0.008	-0.313	0.031	0.325	0.02	-	-
SLC13A5 Birth weight -0.560 <0.0001	Lean Mass	-0.324	0.044	-0.560	<0.0001	0.476	<0.0001	-	-
Birth weight-0.560<0.0001-0.505<0.00010.4150.0020.661<0.0001Birth length-0.4630.001-0.4770.0010.498<0.0001	SLC13A5								
Birth length-0.4630.001-0.4770.0010.498<0.00010.597<0.0001Insulin0.3300.0310.3460.027HOMA-IR-0.3230.035IGF-I-0.3930.004-0.3600.00110.3860.0050.629<0.0001	Birth weight	-0.560	<0.0001	-0.505	<0.0001	0.415	0.002	0.661	<0.0001
Insulin0.3300.0310.3460.027HOMA-IR-0.3230.035IGF-I-0.3930.004-0.3600.00110.3860.0050.629<0.0001	Birth length	-0.463	0.001	-0.477	0.001	0.498	<0.0001	0.597	<0.0001
HOMA-IR-0.3230.035IGF-I-0.3930.004-0.3600.00110.3860.0050.629<0.0001	Insulin	-	-	-0.330	0.031	-	-	0.346	0.027
IGF-I-0.3930.004-0.3600.00110.3860.0050.629<0.0001Fat Mass-0.3130.0250.4580.0010.4350.003	HOMA-IR	-0.323	0.035	-	-	-	-	-	-
Fat Mass -0.313 0.025 0.458 0.001 0.435 0.003	IGF-I	-0.393	0.004	-0.360	0.0011	0.386	0.005	0.629	<0.0001
	Fat Mass	-0.313	0.025	-	-	0.458	0.001	0.435	0.003
Abdominal fat -0.358 0.01 -0.305 0.033 0.488 <0.0001 0.410 0.005	Abdominal fat	-0.358	0.01	-0.305	0.033	0.488	<0.0001	0.410	0.005
Lean Mass -0.557 <0.0001 -0.512 <0.0001 0.430 0.002 0.647 <0.0001	Lean Mass	-0.557	<0.0001	-0.512	<0.0001	0.430	0.002	0.647	<0.0001
GPR120	GPR120								
Birth weight 0.642 <0.0001 -0.621 <0.0001 -0.421 0.002 0.378 0.012	Birth weight	0.642	<0.0001	-0.621	<0.0001	-0.421	0.002	0.378	0.012
Birth length 0.592 <0.0001 -0.621 <0.0001 -0.407 0.003	Birth length	0.592	<0.0001	-0.621	<0.0001	-0.407	0.003	-	-
Insulin 0.349 0.019 -0.325 0.036	Insulin	0.349	0.019	-0.325	0.036	_	_	-	-
HOMA-IR 0.331 0.030.353 0.022 0.417 0.01	HOMA-IR	0.331	0.03	-	_	-0.353	0.022	0.417	0.01
IGF-I 0.475 <0.0001 -0.414 0.004 - 0.445 0.004	IGF-I	0.475	<0.0001	-0.414	0.004	-		0.445	0.004
Fat Mass 0.615 <0.00010.295 0.038 0.514 <0.0001	Fat Mass	0.615	< 0.0001	-	-	-0.295	0.038	0.514	< 0.0001
Abdominal fat 0.588 <0.0001 -0.369 0.011 -0.421 0.002 0.431 0.003	Abdominal fat	0.588	< 0.0001	-0.369	0.011	-0.421	0.002	0.431	0.003
Lean Mass 0.617 <0.0001 -0.615 <0.0001 -0.309 0.029 0.320 0.034	Lean Mass	0.617	<0.0001	-0.615	<0.0001	-0.309	0.029	0.320	0.034



Figure 2. Methylation (top) and expression levels (bottom) of validated genes in cord blood from infants born appropriate (AGA, n=30) or small-for-gestational age (SGA, n=21).



HOMA-IR: Homeostasis model assessment-insulin resistance; IGF-I: insulinlike growth factor-I



CONCLUSION

Epigenetic modifications of placenta and cord blood-expressed genes related to energy homeostasis contribute to explain fetal growth restraint and postnatal fat mass gain in term SGA newborns from uncomplicated pregnancies.

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