Placental and Cord Blood Methylation of Genes Involved in Energy Homeostasis: Association with Fetal Growth and Neonatal Body Composition

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MD, CG, GS, FdZ, ALB and LI have nothing to declare.

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Abstract

Low weight at birth associates with subsequent susceptibility to diabetes. Epigenetic modulation is among the mechanisms potentially mediating this association. We performed a genome-wide DNA methylation analysis in placentas from term infants born appropriate-for-gestational-age (AGA) or small-for-gestational-age (SGA), to identify new genes related to fetal growth and neonatal body composition. Candidate genes were validated by bisulfite pyrosequencing (30 AGA, 21 SGA) and also analyzed in cord blood. Gene expression analyses were performed by RT-PCR. Neonatal body composition was assessed by dual X-ray absorptiometry at age 2 weeks. The *ATG2B*, *NKX6.1* and *SLC13A5* genes (respectively related to autophagy, beta-cell development and function, and lipid metabolism) were hypermethylated in placenta and cord blood from SGA newborns, whereas *GPR120* (related to free fatty acid regulation) was hypomethylated in placenta and hypermethylated in cord blood. Gene expression levels were opposite to methylation status, and both correlated with birth weight, with circulating IGF-I, and with total and abdominal fat at age 2 weeks. In conclusion, alterations in methylation and expression of genes involved in the regulation of energy homeostasis were found to relate to fetal growth and neonatal body composition, and may thus be among the early mechanisms modulating later susceptibility to diabetes.

Individuals born small-for-gestational age (SGA) are at increased risk for developing insulin resistance, obesity, metabolic syndrome and, subsequently, cardiovascular disease and type 2 diabetes in adulthood (1,2). Growth restraint before birth is thought to confer such risk, particularly when followed by excessive weight gain after birth (3). By the postnatal age of 4 months, SGA infants already have an altered endocrine-metabolic profile with elevated concentrations of circulating IGF-I and high-molecular-weight (HMW) adiponectin (4). These abnormalities are influenced by early nutrition and evolve towards a profile with more hepatic and visceral adiposity in childhood (5). The mechanisms underpinning these abnormalities remain poorly understood.

Epigenetic regulation of the placenta evolves during pre-implantation and progresses further during gestation. Strictly regulated DNA methylation has a key role in tissue-specific gene regulation and transcription; DNA methylation in CpG islands has been so far the most investigated target (6). Although DNA methylation has traditionally been viewed as an epigenetic "silencing" mark, recent advances suggest that the impact of DNA methylation on gene expression depends more on the genomic location of the CpG site (7). An adverse intrauterine environment can modify placental epigenetic marks and gene expression profiles, resulting in fetal growth restraint (8-10). Differential patterns of placental and cord blood methylation and/or expression have been described in selected imprinted and non-imprinted genes (11,12) in heterogeneous populations, including preterm infants (11) and growth-restricted fetuses from pregnancies complicated by hypertension or pre-eclampsia (12). Genome-wide methylation abnormalities in adipose-derived stem cells from SGA individuals have also been reported (13).

Here, we tested whether placental and cord blood methylation and/or expression of genes involved in the regulation of energy homeostasis (which may influence both early growth and later diabetes risk) differ between offspring born appropriate-for-gestational-age (AGA) or born SGA after an uncomplicated, term, singleton pregnancy.

Research Design and Methods

Study population

The study cohort consisted of 51 mother-placenta-newborn trios, with postnatal follow-up (Supplemental Figure 1). Thirty infants were born AGA (17 girls, 13 boys) and 21 SGA (9 girls, 12 boys). The inclusion and exclusion criteria are detailed as Online Supplemental Material.

Maternal age at conception, parity and height, as well as pre-gestational weight and body mass index [BMI, weight (Kg)/height (m^2)], were retrieved from clinical records. Gestational age was calculated by last menses and confirmed by first-trimester ultrasound (~10 wk).

The infant's weight and length were measured by the same investigator (GS) at birth and at the postnatal age of approximately 2 weeks. Weight was measured with a beam balance (Seca, Hamburg, Germany) and length with a length board, using the mean of three measurements.

DNA methylation microarray in placenta

DNA methylation profiling was performed in 8 AGA and 8 SGA samples with the Agilent DNA Methylation array (ID 049738, Agilent Technologies), which examines 27,800 highly informative CpG sites located within the proximal promoter regions of 14,475 genes. Nearly 100% of these CpG sites were localized within CpG islands. The process for isolating methylated DNA from purified DNA samples, and labelling and hybridization to Human DNA Methylation Array was conducted following the manufacturer's protocol (Agilent Microarray Analysis of Methylated DNA Immunoprecipitation v 1.1, Agilent Technologies). Briefly, 5μg of purified DNA was sonicated in PBS; DNA fragments were run in a 1.5% agarose gel and ranged from 200 to 1000 bp. Enrichment of methylated regions was performed using an antibody against methylcytosine. Enriched and unenriched DNAs were labelled with Cy3 and Cy5, respectively, and hybridized in the microarray. The microarray

chip was then washed and immediately scanned using a Microarray Scanner (Model G2505C, Agilent Technologies).

Gene expression analysis in placenta and cord blood by quantitative real time in differentially methylated genes validated by pyrosequencing

Steady state mRNA levels of *GPR120, ATG2B, NKX6-1* and *SLC13A5*, and the housekeeping gene *GAPDH* were measured using gene-specific primers: *GPR120*: forward 5'-CTGCCTCTCTGCGTCTTCTT-3', reverse 5'-CTCTTGCATCCAGGAGGTGT-3'; *ATG2B*: forward 5'-ATCCCAGCCTTTTGAAGGAC-3', reverse 5'-AGCAAAAGCAGTGGGTTGAT-3'; *NKX6-1*: forward 5'- CTTCTGGCCCGGAGTGAT-3', reverse 5'-CCCGCCAAGTATTTTGTTTG-3'; *SLC13A5*: forward 5'-CCTGCTGGATTGGAA GGTAA-3', reverse 5'-CACTCAGTGAACACGGCAAC-3'; GAPDH: forward 5'-GTCAGTGGTGGACCTGACCT-3', reverse 5'-TGCTGTAGCCAAATTCGTTG-3) and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). PCR reactions were performed using a 7500 Real-Time PCR System (Applied Biosystems), by mixing 1 µl of cDNA (20 ng) with 24 µl of reaction mixture (14 µl 2X SYBR Green I Master Mix buffer, 2 µl forward primer (2.5 µM); 2 µl reverse primer (2.5 µM), and 6 µl of nuclease-free water. Relative expression was then calculated according to the $2^{-\Delta Ct}$ method (14).

The details involving cord blood and placenta collection, DNA extraction and modification, microarray data pre-processing, validation by bisulfite pyrosequencing, RNA extraction and retro-transcription, as well as the Assays & Neonatal Body Composition procedures are provided as Online Supplemental Material.

Statistics and ethics

Statistical analyses were performed using SPSS software 23.0 (Inc. Chicago, IL). Unpaired t-test was used to study differences between AGA and SGA subgroups. Correlation

and stepwise multiple regression analysis were used to study the associations between methylation status and expression levels in placenta and cord blood, and auxological and body composition parameters. Covariance analysis was used to adjust for sex, gestational age, type of delivery and pre-gestational BMI. The level of significance was set at $P<0.05$.

The study was approved by the Institutional Review Board of Hospital Sant Joan de Déu at Barcelona University; written informed consent was obtained before delivery.

Results

Maternal and offspring characteristics

Table 1 summarizes the anthropometric parameters in the mothers and newborns by birth weight subgroups. The delivery rate by caesarian section was not different between the AGA and SGA subgroups (13% *vs* 19%, respectively). SGA infants displayed lower circulating levels of insulin and IGF-I in cord blood, and less fat and lean mass at age 2 weeks, as expected, while their mothers were shorter, and had a lower pre-gestational weight and BMI (14).

Gene Ontology & Functional Pathway Analysis

We identified n=409 genes differentially methylated in SGA newborns (FDR < 0.1) (Supplemental Table 1). The GO analysis disclosed that most ~~of them~~ were involved in the regulation of metabolic processes, DNA binding and transcription, and biosynthesis of macromolecules (Supplemental Table 2). The KEGG software identified enriched numbers of epigenetic changes in n=11 canonical pathways, the most significant being those related to cell development and function, lipid metabolism, autophagy and signalling (Table 2). The top-ranked hyper- and hypomethylated genes in these pathways (n=39) are listed in Supplemental Tables 3 and 4. Since validation of all 39 genes by pyrosequencing was not feasible (costly and technically complex), we selected – in accordance with the study purpose– those genes (n=8) which were relevant to the regulation of glucose and lipid

metabolism; of those, three were hypomethylated (*MAPK8IP1, CAMK4* and *GPR120*) and five were hypermethylated (*ATG2B, KLF11, NKX6.1, NRIP1* and *SLC13A5*) in placentas of SGA newborns (Supplemental Tables 3 & 4).

Differentially methylated genes in placenta and cord blood

Validation of DNA methylation patterns by pyrosequencing demonstrated that *ATG2B, NKX6.1* and *SLC13A5* were hypermethylated in SGA placenta (respectively $P<0.02$, $P<0.008$ and $P<0.0001$ *vs* AGA) and SGA cord blood (all $P<0.0001$), while *GPR120* was hypomethylated in SGA placenta ($P=0.0006$) and hypermethylated in SGA cord blood ($P<0.0001$) (Figures 1 and 2). The differences in methylation were maintained after adjusting for sex, gestational age, type of delivery, and pre-gestational BMI. Pyrosequencing analysis failed to demonstrate differences in methylation status in *MAPK8IP1*, *CAMK4, KLF11* and *NRIP1* genes.

Expression of the validated genes in placenta and cord blood

Expression levels of *ATG2B, NKX6.1, SLC13A5* and *GPR120* in placenta and cord blood were opposite to their methylation status (all $P \leq 0.004$ in placenta; and all $P \leq 0.01$ in cord blood) (Figures 1 and 2). The differences in gene expression were maintained after adjusting for sex, gestational age, type of delivery, and pre-gestational BMI.

Correlation and multiple regression analyses between genetic and endocrine-metabolic data are provided as Online Supplemental Data.

Discussion

We identified differential methylation profiles in placenta and cord blood from SGA infants in 18 CpG sites along the *ATG2B, NKX6.1, SLC13A5* and *GPR120* genes – all non-imprinted – and report for the first time associations with birth weight, endocrine-metabolic markers and neonatal body composition. Neither differences in methylation and expression,

nor associations with growth and endocrine-metabolic markers were attributable to differences in gestational age or maternal BMI, or to sex or delivery route. The finding that the methylation and the expression of highlighted genes do mirror each other, as well in placenta as in cord blood, suggests that the expression of those genes is mainly under epigenetic control which, in turn, is presumably controlled by multiple maternal and environmental factors, most of which remain to be identified.

Hypermethylation and down-regulation of *ATG2B* (autophagy-related 2B) in placenta and cord blood was associated with smaller size at birth and lower fat mass. Autophagy is critical in the maintenance of maternal-fetal material exchange during placental development, in survival immediately after birth (15) and in cell differentiation, including in adipogenesis (16). In SGA infants, decreased autophagy resulting from down-regulation of *ATG2B* – and thus compromising nutrient supply – could be among the mechanisms accounting for prenatal growth restraint.

NKX6.1 (*NKX6* homeodomain 1) – a transcription factor known to be involved in pancreatic differentiation and β cell homeostasis – was hypermethylated and down-regulated in placenta and cord blood from SGA infants, and associated with birth weight and neonatal fat mass. In the pancreas, *NKX6.1* is exclusively expressed in β cells, and is required for prenatal β cell development and function and postnatal β cell expansion (17). In placenta, it remains to be studied whether the epigenetically controlled expression of *NKX6.1* is a modulator of nutrient transfer, and thus perhaps an indirect modulator of fetal β cell development, insulin secretion and fat mass.

Mammalian Indy homologue (*SLC13A5*, mIndy) encodes for a sodium-coupled citrate transporter (NaCT) that is mainly expressed in hepatocytes, and serves as a link between carbohydrate catabolism and lipogenesis. Loss of *SLC13A5* augments energy expenditure and hepatic fat oxidation, and attenuates hepatic lipogenesis, reducing overall growth (18). *SLC13A5* was hypermethylated and down-regulated in SGA placenta and cord blood. These findings align well with the knowledge that SGA infants are less adipose and more insulin

sensitive than AGA infants, not only in early infancy but also – if breastfed – in late infancy (19). Reduced expression of *SLC13A5* may conceivably be a programming mechanism attempting to protect those fetuses from excessive fat accumulation and impaired insulin action early during spontaneous catch-up.

GPR120 (G-protein coupled receptor 120), also known as free fatty acid receptor 4 (*FFAR4*), is a lipid sensor that regulates whole-body energy homeostasis in humans and rodents, mediating insulin sensitizing effects *in vivo* by repressing inflammation (20). *GPR120* activation indirectly stimulates pancreatic insulin secretion by inducing the release of glucagon-like-peptide-1 from the gut (21). Here, *GPR120* was found to be hypomethylated and hyperexpressed in SGA placenta. The human placenta expresses *GPR120* mainly in the microvillous membrane of the syncytiotrophoblast (22) where it presumably transfers unsaturated long-chain fatty acids from the maternal circulation into the syncytiotrophoblast, rather than into the fetal circulation. In contrast, *GPR120* was hypermethylated and hypoexpressed in cord blood of SGA infants, who have a reduced fat mass across early infancy (4). Interestingly, in the critical window of adipogenesis, *GPR120* is thought to be a key contributor to adipocyte differentiation (23).

This preliminary study is the first to associate epigenetic settings of non-imprinted genes to size at birth and to markers of endocrine-metabolic homeostasis and neonatal body composition in healthy term AGA and SGA infants. Study limitations include the small sample size, the lack of adjustment for cell composition in placenta/cord blood (24), the influence of maternal genetics on placental gene expression, the potential underrepresentation of differential methylation in CpG islands (25), and the inability to obtain - for ethical reasons- target tissues for assessing tissue-specific DNA methylation/expression. The strengths include the strict inclusion criteria (leading to distinct AGA and SGA populations, and avoiding perinatal confounders), and the parallel assessments of placenta/cord blood pairs.

In conclusion, in SGA placenta and cord blood, we identified differential methylation and expression patterns of genes involved in the regulation of glucose homeostasis and lipid metabolism. These epigenetic variations may influence fetal growth, early body composition, and lifelong diabetes risk.

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The authors have no conflicts of interest to disclose.

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L.I. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Figure legends

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). Values are mean ± SEM.

ATG2B, NKX6.1 and *SLC13A5* were hypermethylated, whereas *GPR120* was hypomethylated in SGA infants. Expression levels of *ATG2B, NKX6.1, SLC13A5* and *GPR120* were opposite to their methylation status.

*P<0.05; **P<0.01; ***P<0.0001 *vs* AGA.

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). Values are mean ± SEM.

ATG2B, NKX6.1, SLC13A5 and *GPR120* were hypermethylated in SGA infants. Expression levels of *ATG2B, NKX6.1, SLC13A5* and *GPR120* were opposite to their methylation status.

*P<0.05; **P<0.01; ***P<0.0001.

Table 1. Auxological and clinical parameters in the studied subjects and in their mothers

	AGA	**SGA**	***P* value**
Mothers	N=30	N=21	
Age (yr)	31.8 ± 1.1	31.8 ± 1.0	ns
Height (cm)	164 ± 0.01	159 ± 0.01	0.01
Weight (Kg)	66.0 ± 2.6	53.6 ± 1.6	0.0005
BMI (Kg/m^2)	24.6 ± 0.9	21.1 ± 0.6	0.003
Pregnancy weight gain (Kg)	13.4 ± 0.9	11.5 ± 0.7	ns
Primiparous (%)	57	76	ns
Caesarean section (n, %)	4 (13)	4 (19)	ns
Singleton Newborns	N=30	N=21	
Gender (% female)	57	43	ns
Gestational age (wk)	40.2 ± 0.2	38.7 ± 0.3	0.0008
Birth Weight (Kg)	3.3 ± 0.1	2.3 ± 0.1	<0.0001
Birth Weight Z-score	0.1 ± 0.1	-2.3 ± 0.1	<0.0001
Birth Length (cm)	50.6 ± 0.3	45.6 ± 0.5	<0.0001
Birth Length Z-score	0.2 ± 0.1	-1.9 ± 0.2	<0.0001
Placental weight (Kg)	0.60 ± 0.02	0.51 ± 0.03	0.022
Endocrine-metabolic variables at birth			
Glucose (mmol/L)	4.4 ± 0.2	4.5 ± 0.4	ns
Insulin (pmol/L)	27.8 ± 3.5	17.36 ± 2.1	0.009
IGF-I (nmol/L)	0.09 ± 0.01	0.06 ± 0.01	0.001
HMW adiponectin (mg/L)	43 ± 3	41 ± 3	ns
Body composition at 2 wk			
Weight Gain since Birth (g)	184 ± 53	310 ± 60	ns
Length Gain since Birth (cm)	1.7 ± 0.2	2.2 ± 0.6	ns
Bone Mineral Density (g/cm^2)	0.27 ± 0.01	0.24 ± 0.01	0.026
Bone Mineral Content (g)	94.4 ± 2.2	69.9 ± 2.8	<0.0001
Fat Mass (%)	18 ± 1	16 ± 1	0.04
Fat Mass (g)	696 ± 39	467 ± 38	0.0001
Abdominal Fat (%)	4.1 ± 0.2	3.3 ± 0.2	0.01
Abdominal Fat (g)	29 ± 2	16 ± 3	0.0007
Lean Mass (%)	82 ± 1	83 ± 1	ns
Lean Mass (g)	3110 ± 55	2279 ± 68	<0.0001

Data are mean ± SEM. BMI: body mass index; IGF-I: insulin-like growth factor-I; HMW adiponectin: high-molecular weight adiponectin.

Table 2. Differentially methylated genes involved in KEGG pathways. Pathways are arranged (top to bottom), according to *P* value.
The genes in bold (n=39) display the lowest (top-ranked) individual *P* values.

Canonical Pathway	*P*-value	Methylation status	Gene Symbol
Cell development and function	3.7E-04	Hyper	*PHOX2B, NDEL1,* ***GFOD1****, SMAD7,****KCNK13****, EFNA1,* ***CHRM2****, CNTN4,* ***NKX2-5, NKX3-2****,* ***NKX6-1****,* ***LMO2, PYGO2***
Lipid metabolism	4.0E-04	Hypo	*SOAT1, LYPLA2P1,* ***GPR120****, SORL1,* ***MAPK8IP1,*** *ECHDC2,* ***PEX1****, LYPLA2,* ***ATG2B****, HSD17B4*
Autophagy	5.4E-04	Hyper	*DRAM1,* ***ATG2B****, AMBRA1,* ***SOGA1***
Cellular signalling	6.6E-04	Hypo	*EIF2AK1, SLK,* ***CAMK4, MAPK13****, PAK4, CHKB,* ***PRKG1****, ALK,* ***STARD3NL****, PAPSS1, AKT3*
Cation channel activity	1.3E-03	Hyper	***CALHM2****,SCN3B, SLC24A2,* ***SLC13A5****,* ***KCNK13***
Regulation of immunity	1.7E-03	Hyper	*STAT5B,* ***ICOSLG****, PTPN22, RARA*
Endoplasmic reticulum metabolism	2.4E-03	Hyper	*SOAT1, DERL2, SPTLC1,* ***CLSTN2****, LOC729316, RTN2, SYNE1, NDEL1,* ***LRP10****, LARGE, C3ORF57, FAM156A, GNAS, FAM156B,* ***EIF2AK1,*** *POM121C,* ***KPNA4****, EDA, DLG1*
DNA-binding	2.4E-03	Hypo	*FLYWCH1,* ***HIST1H2B1****, HIST4H4, HIST1H4K, LOC100045887, NFKB2, SRF, GM11275, ARNT, HOXA3, LOC674678, HIST1H4A, HIST1H4B, NKX3-2, POU3F4, SRRM1, HIST1H4F, HIST1H4C, HIST1H4D, HIST1H4I, LBR, HIST1H4J, HIST1H4H, ZBTB48, CEBPD, NEUROG1, ZFP3, MECOM, HIST2H4,* ***UHRF2****,* ***SFPQ****, TOP3B*
Transcription regulation	3.2E-03	Hyper	*ZNF583, POU6F2, STAT5B, ZNF827, RHOQ, HSBP1, CNOT4, ZNF343,* ***ZGPAT****,* ***GTF2H2C****, BRPF1,* ***ZNF182****,* ***ZNF436****, GTF2H2C, ZNF680, ZFP90, PSMC3IP, RARA, SOX17, FOXB1, GATAD1, MBD3L1, PHOX2B, RCOR3*
Transcription regulation	3.2E-03	Hypo	*SCAI, JUNB, MCM5,* ***NRIP1****, GTF2H2B,* ***ZNF500****, ZNF234, TAF11, PRDM9, MED18, LIN54, EDA, HMX3, ZNF468, GPBP1L1, SCML1, NFYC, MYBL1, HOXA1, THAP1, NKX2-5,* ***MLLT3****, SMAD7, KLF11, SOX30, TFCP2,STAT1, FOXP4,* ***NKX6-1****, ZNF22, ZNF212, DR1, NARFL,* ***FMR1,*** *SFPQ,* ***TOP3B***
Apoptosis	3.7E-03	Hyper	*FAM176A, DOCK1, LTBR, AIMP2, STAG3L3, FAM82A2,* ***KLF11****, PAK1*
Protein-interaction	8.4E-03	Hyper	*DOCK1,* ***DOCK5****, ABI1,* ***SKAP2****, EPS8L1, RASA1, DLG1*

Figure 1

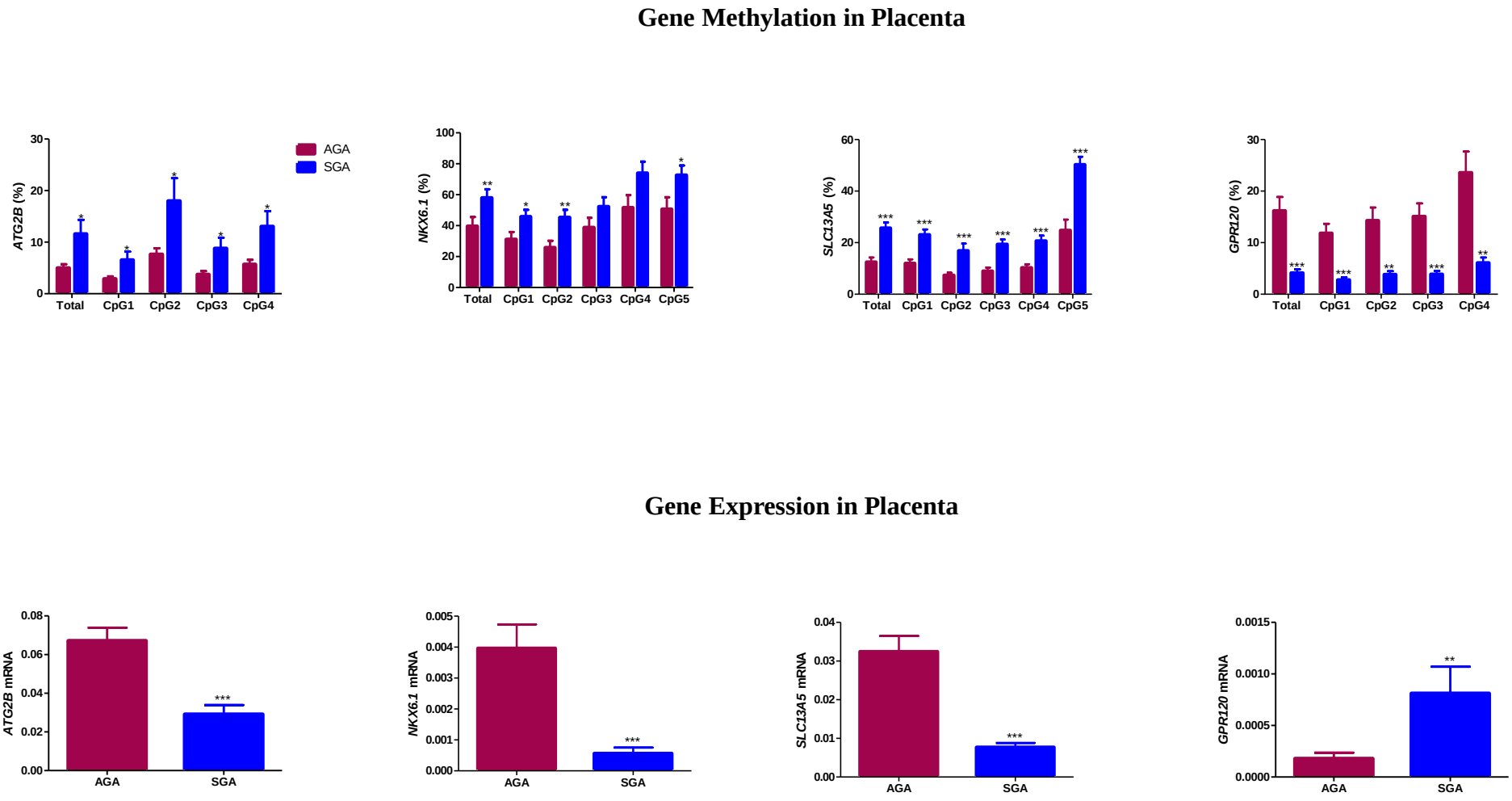
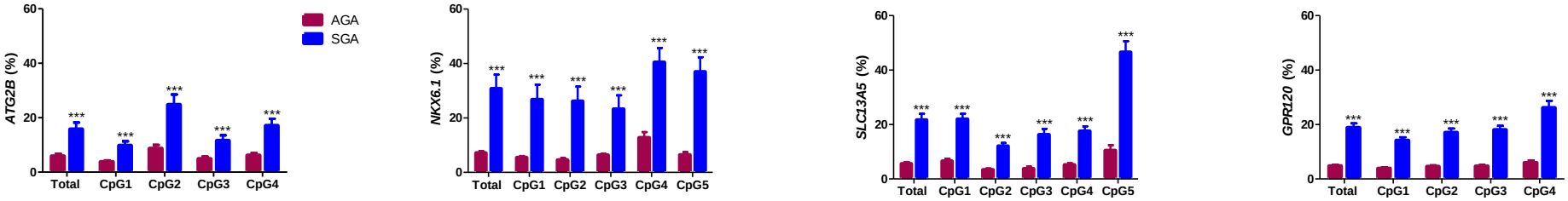
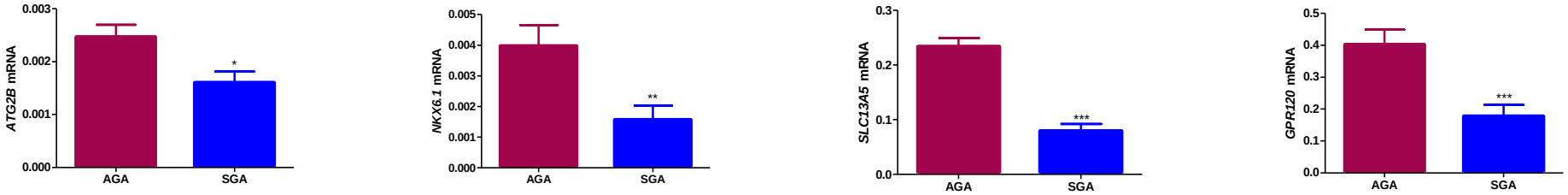

Gene Methylation in Placenta
AGA
SGA
ATG2B (%)
NKX6.1 (%)
SLC13A5 (%)
GPR120 (%)
Total
CpG1
CpG2
CpG3
CpG4
CpG5
Gene Expression in Placenta
ATG2B mRNA
NKX6.1 mRNA
SLC13A5 mRNA
GPR120 mRNA
AGA
SGA

Figure 2

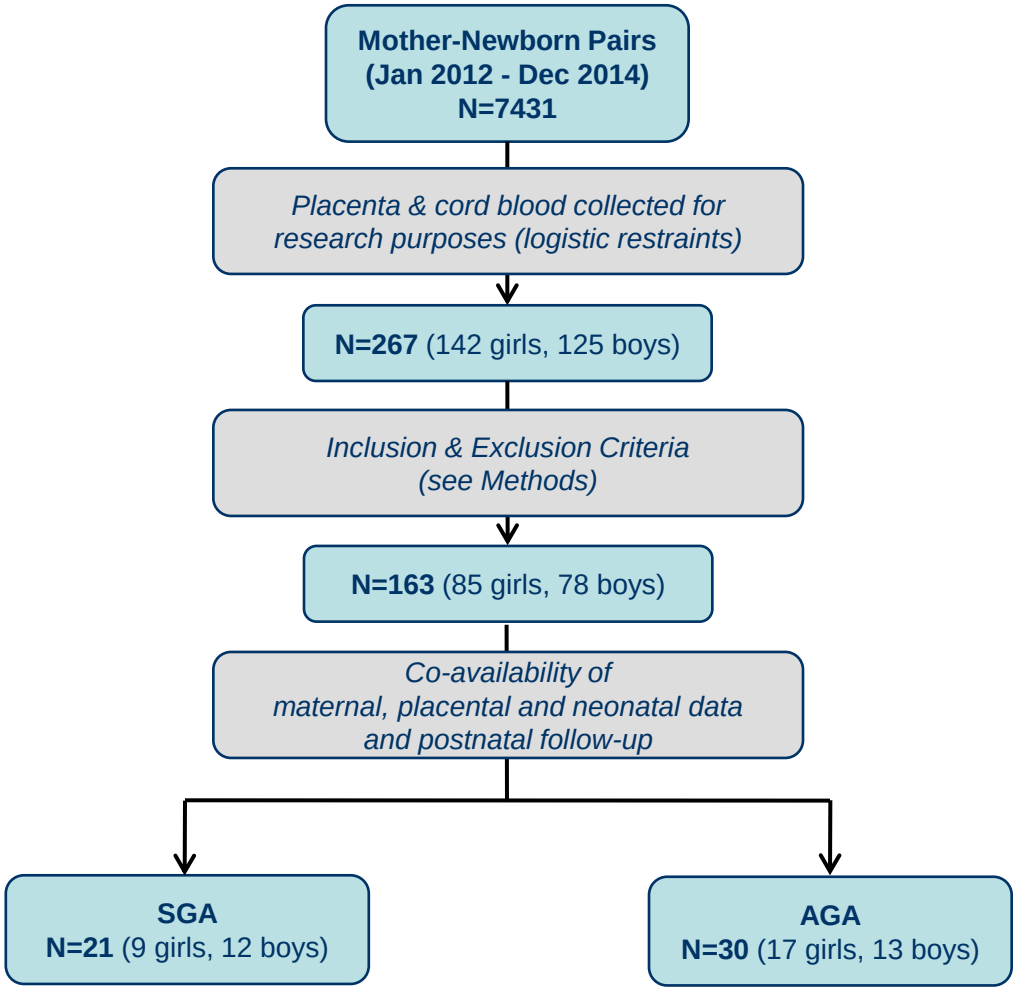
Gene Methylation in Cord Blood



Gene Expression in Cord Blood



Supplemental Figure 1.
Recruitment of the study population.
AGA: appropriate for gestational age
SGA: small for gestational age



Supplemental Table 1. Differentially methylated genes ranked by *P* value between AGA and SGA subgroups.
Q values, coefficients of regression (R^2) and the variation are also shown.

* Selected genes for validation by pyrosequencing.

Gene Symbol		Gene Denomination	*P value*	*Q value*	R^2	*Variation*
*NKX6-1**	1	NK6 homeobox 1	0.0002	0.012	0.68	12.73
HIST1H2BI	2	Histone cluster 1, H2bi	0.0002	0.012	0.88	12.70
UHRF2	3	Ubiquitin-like with PHD and ring finger domains 2, E3 ubiquitin protein ligase	0.0003	0.012	0.87	33.37
*MAPK8IP1**	4	Mitogen-activated protein kinase 8 interacting protein 1	0.0004	0.012	0.87	18.74
*CAMK4**	5	Calcium/calmodulin-dependent protein kinase IV	0.0004	0.012	0.83	15.36
CHRM2	6	Cholinergic receptor, muscarinic 2	0.0008	0.012	0.84	25.24
TOP3B	7	Topoisomerase (DNA) III beta	0.001	0.012	0.80	15.55
STARD3NL	8	STARD3 N-terminal like	0.001	0.012	0.79	32.32
LMO2	9	LIM domain only 2 (rhombotin-like 1)	0.001	0.012	0.82	24.88
*ATG2B**	10	Autophagy related 2B	0.002	0.012	0.78	28.79
MLLT3	11	Myeloid/lymphoid or mixed-lineage leukemia: translocated to, 3	0.002	0.012	0.89	11.96
CLSTN2	12	Calsyntenin 2	0.002	0.012	0.79	28.42
EIF2AK1	13	Eukaryotic translation initiation factor 2-alpha kinase 1	0.002	0.012	0.78	15.72
SFPQ	14	Splicing factor proline/glutamine-rich	0.002	0.012	0.78	18.80
*KLF11**	15	Kruppel-like factor 11	0.003	0.012	0.75	24.82
*SLC13A5**	16	Sodium-dependent citrate transporter), member 5	0.003	0.012	0.75	24.62
*NRIP1**	17	Nuclear receptor interacting protein 1	0.003	0.012	0.74	15.46
ZNF500	18	Zinc finger protein 500	0.003	0.012	0.76	15.58
DOCK5	19	Dedicator of cytokinesis 5	0.003	0.012	0.75	26.43
*GPR120**	20	Free fatty acid receptor 4	0.003	0.012	0.75	29.63
GFOD1	21	Glucose-fructose oxidoreductase domain containing 1	0.004	0.012	0.73	21.74
LRP10	23	Low density lipoprotein receptor-related protein 10	0.004	0.012	0.73	26.22
GTF2H2C	24	General transcription factor IIH, polypeptide 2C	0.004	0.012	0.73	42.19
PYGO2	25	Pygopus family PHD finger 2	0.004	0.012	0.73	17.30
PEX1	26	Peroxisomal biogenesis factor 1	0.004	0.012	0.74	12.73
PRKG1	27	Protein kinase, cGMP-dependent, type I	0.004	0.012	0.74	50.29
NKX3-2	28	NKX3 homeobox 2	0.004	0.012	0.72	24.89
ZGPAT	29	Zinc finger, CCCH-type with G patch domain	0.005	0.012	0.72	26.03
KCNK13	30	Potassium channel, subfamily K, member 13	0.005	0.012	0.71	20.44
SOGA1	31	Suppressor of glucose, autophagy associated 1	0.005	0.012	0.71	11.73
MAPK13	32	Mitogen-activated protein kinase 13	0.005	0.012	0.72	18.27
CALHM2	33	Calcium homeostasis modulator 2	0.006	0.012	0.85	9.22

SKAP2	34	Src kinase associated phosphoprotein 2	0.006	0.012	0.70	27.46
KPNA4	35	Karyopherin alpha 4 (importin alpha 3)	0.006	0.012	0.69	16.52
FMR1	36	Fragile X mental retardation 1	0.007	0.012	0.68	16.67
NKX2-5	37	NKX2 homeobox 5	0.008	0.012	0.85	23.80
ZNF182	38	Zinc finger protein 182	0.008	0.012	0.68	16.27
ZNF436	39	Zinc finger protein 436	0.008	0.012	0.65	49.76
ICOSLG	40	Inducible T-cell co-stimulator ligand	0.008	0.012	0.66	16.62
RASL12	41	RAS like family 12	0.01	0.012	0.76	23.22
ATP6V1F	42	ATPase H+ Transporting V1 Subunit F	0.01	0.012	0.69	20.60
RNF38	43	Ring Finger Protein 38	0.01	0.012	0.68	17.65
ZNF789	44	Zinc Finger Protein 789	0.01	0.012	0.68	40.11
HCG11	45	HLA Complex Group 11 (Non-Protein Coding)	0.01	0.012	0.68	21.58
LBR	46	Lamin B receptor	0.01	0.012	0.80	14.86
EMID1	47	EMI domain containing 1	0.01	0.012	0.76	23.34
BAMBI	48	BMP and activin membrane-bound inhibitor	0.01	0.012	0.74	49.69
RUSC2	49	Run and SH3 domain containing 2	0.01	0.012	0.74	28.64
MARC1	50	Mithocondrial amidoxime reducing component 1	0.01	0.012	0.73	21.71
PDZRN4	51	PDZ domain containing ring finger 4	0.01	0.012	0.72	16.24
TMCO1	52	Transmembrane and coiled-coil domains	0.01	0.012	0.71	16.94
AKR1E2	53	Aldo-Keto reductase family 1, member E2	0.01	0.012	0.71	20.85
SMAD7	54	SMAD Family Member 7	0.01	0.012	0.54	24.32
NDEL1	55	NudE Neurodevelopment Protein 1 Like 1	0.01	0.012	0.51	24.17
HSD17B7P2	56	Hydroxysteroid (17-beta) dehydrogenase 7 pseudogene 2	0.01	0.012	0.71	17.59
WIPF2	57	WAS/WASL interacting protein family member 2	0.01	0.012	0.71	33.71
PRDM9	58	PR domain 9	0.01	0.012	0.71	10.96
WIPI2	59	WD repeat domain, phosphoinositide interacting 2	0.01	0.012	0.70	26.85
ATP1B1	60	ATPase Na+/K+ Transporting Subunit Beta 1	0.01	0.012	0.70	20.72
DNLZ	61	DNL-Type Zinc Finger	0.01	0.012	0.70	15.78
FLRT2	62	Fibronectin Leucine Rich Transmembrane Protein 2	0.01	0.012	0.69	24.44
NRSN1	63	Neurensin 1	0.01	0.012	0.69	26.33
SERBP1	64	SERPINE1 mRNA Binding Protein 1	0.01	0.012	0.68	18.85
LIPT2	65	Lipoyl(Octanoyl) Transferase 2 (Putative)	0.01	0.012	0.68	30.22
ZDHHC17	66	Zinc Finger DHHC-Type Containing 17	0.01	0.012	0.68	27.09
ZBTB48	67	Zinc Finger And BTB Domain Containing 48	0.01	0.012	0.68	17.20
MPND	68	MPN Domain Containing	0.01	0.012	0.67	11.79
NOVA1	69	Neuro-Oncological Ventral Antigen 1	0.01	0.012	0.67	14.76
PSME1	70	Proteasome Activator Subunit 1	0.01	0.012	0.67	41.36
SNX12	71	Sorting Nexin 12	0.01	0.012	0.66	28.08
AKT3	72	V-Akt Murine Thymoma Viral Oncogene Homolog 3	0.01	0.012	0.66	24.74
STX18	73	Syntaxin 18	0.01	0.012	0.66	36.29

SHANK2	74	SH3 And Multiple Ankyrin Repeat Domains 2	0.01	0.012	0.66	34.90
EFNA1	75	Ephrin A1	0.01	0.012	0.66	30.52
C3ORF55	76	PQ Loop Repeat Containing 2-Like	0.01	0.012	0.66	12.22
CNTN4	77	Contactin 4	0.01	0.012	0.51	43.43
HOXA1	78	Homeobox A1	0.01	0.012	0.48	23.60
CA10	79	Carbonic Anhydrase 10	0.01	0.012	0.65	24.60
CDC14A	80	Cell Division Cycle 14A	0.01	0.012	0.65	33.53
IDS	81	Iduronate 2-Sulfatase	0.01	0.012	0.65	11.50
KIAA1274	82	Phosphatase Domain Containing, Paladin 1	0.01	0.012	0.59	25.16
KLHL5	83	Kelch Like Family Member 5	0.01	0.012	0.65	28.40
SOAT1	84	Sterol O-Acyltransferase 1	0.01	0.012	0.57	34.02
REXO1L2P	85	RNA exonuclease 1 Homolog-like 2	0.01	0.012	0.79	38.17
PSMC3IP	86	PSMC3 interacting protein	0.01	0.012	0.70	16.88
JUNB	87	Jun B Proto-Oncogene	0.01	0.012	0.65	26.19
CBLN3	88	Cerebellin 3 Precursor	0.01	0.012	0.65	48.79
WDR82	89	WD Repeat Domain 82	0.01	0.012	0.64	37.32
SIKE1	90	Suppressor Of IKBKE 1	0.01	0.012	0.64	13.49
RCOR3	91	REST Corepressor 3	0.01	0.012	0.64	42.14
FAM98A	92	Family With Sequence Similarity 98 Member A	0.01	0.012	0.64	12.44
ZNF451	93	Zinc Finger Protein 451	0.01	0.012	0.64	51.69
MAGED2	94	MAGE Family Member D2	0.01	0.012	0.63	56.81
SPTLC1	95	Serine Palmitoyltransferase Long Chain Base Subunit 1	0.01	0.012	0.63	11.35
EIF2S3	96	Eukaryotic Translation Initiation Factor 2 Subunit Gamma	0.01	0.012	0.63	34.49
RNF111	97	Ring Finger Protein 111	0.01	0.012	0.63	18.66
PITPNC1	98	Phosphatidylinositol Transfer Protein, Cytoplasmic 1	0.01	0.012	0.63	19.85
CNOT4	99	CCR4-NOT Transcription Complex Subunit 4	0.01	0.012	0.62	16.38
LARGE	100	Like-Glycosyltransferase	0.01	0.012	0.62	20.47
SCML1	101	Sex Comb On Midleg-Like 1 (Drosophila)	0.01	0.012	0.62	21.93
SLED1	102	Proteoglycan 3 Pseudogene	0.01	0.012	0.62	32.95
TSN	103	Translin	0.01	0.012	0.62	30.26
STAT1	104	Signal Transducer And Activator Of Transcription 1	0.01	0.012	0.62	35.80
STAT5B	105	Signal Transducer And Activator Of Transcription 5B	0.01	0.012	0.62	13.00
GBAP1	106	Glucosylceramidase Beta Pseudogene 1	0.01	0.012	0.62	10.89
SYK	107	Spleen Tyrosine Kinase	0.01	0.012	0.61	37.05
SRF	108	Serum Response Factor	0.01	0.012	0.61	33.12
ZNF766	109	Zinc Finger Protein 766	0.01	0.012	0.61	31.60
SNORD24	110	Small Nucleolar RNA, C/D Box 24	0.01	0.012	0.61	25.53
DUOX2	111	Dual Oxidase 2	0.01	0.012	0.61	13.24
RRP12	112	Ribosomal RNA Processing 12 Homolog	0.01	0.012	0.61	46.87
NTAN1	113	N-Terminal Asparagine Amidase	0.01	0.012	0.61	27.15

PAK4	114	P21 Protein (Cdc42/Rac)-Activated Kinase 4	0.01	0.012	0.60	45.41
POLQ	115	Polymerase (DNA) Theta	0.01	0.012	0.60	53.11
SNORA16A	116	Small Nucleolar RNA, H/ACA Box 16A	0.01	0.012	0.60	43.91
GNPDA2	117	Glucosamine-6-Phosphate Deaminase 2	0.01	0.012	0.60	13.90
MGAT5B	118	Mannosyl (Alpha-1,6-)-Glycoprotein Beta-1,6-N-Acetyl-glucosaminyltransferase, Isozyme B	0.01	0.012	0.60	37.02
LANCL3	119	LanC Like 3	0.01	0.012	0.60	11.23
CPOX	120	Coproporphyrinogen Oxidase	0.01	0.012	0.60	16.32
SLC35B2	121	Solute Carrier Family 35 Member B2	0.01	0.012	0.60	15.40
RUNDC3A	122	RUN Domain Containing 3[a]	0.01	0.012	0.60	20.99
TMEM9B	123	TMEM9 Domain Family Member B	0.01	0.012	0.60	34.17
ZFP90	124	ZFP90 Zinc Finger Protein	0.01	0.012	0.60	16.93
ATP5E	125	ATP Synthase, H+ Transporting, Mitochondrial F1 Complex, Epsilon Subunit	0.01	0.012	0.59	28.49
SDR39U1	126	Short Chain Dehydrogenase/Reductase Family 39U Member 1	0.01	0.012	0.59	49.63
FBXO46	127	F-Box Protein 46	0.01	0.012	0.59	18.44
ATP7B	128	ATPase Copper Transporting Beta	0.01	0.012	0.59	17.02
CRCP	129	CGRP Receptor Component	0.01	0.012	0.59	34.37
ASB7	130	Ankyrin Repeat And SOCS Box Containing 7	0.01	0.012	0.59	25.81
UBA1	131	Ubiquitin Like Modifier Activating Enzyme	0.01	0.012	0.59	51.16
RNF180	132	Ring Finger Protein 180	0.01	0.012	0.59	49.32
C22ORF40	133	Cysteine Rich DPF Motif Domain Containing 1	0.01	0.012	0.59	23.01
RSPO3	134	R-Spondin 3	0.01	0.012	0.59	10.53
C1ORF174	135	Chromosome 1 Open Reading Frame 174	0.01	0.012	0.59	16.15
ATG16L1	136	Autophagy Related 16 Like 1	0.01	0.012	0.59	43.09
ZKSCAN2	137	Zinc Finger With KRAB And SCAN Domains 2	0.01	0.012	0.59	23.54
BRPF1	138	Bromodomain And PHD Finger Containing 1	0.01	0.012	0.59	49.90
PLEKHG6	139	Pleckstrin Homology And RhoGEF Domain Containing G6	0.01	0.012	0.59	37.44
EIF3B	140	Eukaryotic Translation Initiation Factor 3 Subunit B	0.01	0.012	0.58	30.11
BTBD9	141	BTB Domain Containing 9	0.01	0.012	0.58	27.97
GPBP1L1	142	GC-Rich Promoter Binding Protein 1 Like 1	0.01	0.012	0.51	19.05
SCN3B	143	Sodium Voltage-Gated Channel Beta Subunit 3	0.01	0.012	0.50	16.18
PHOX2B	144	Paired Like Homeobox 2b	0.01	0.012	0.49	33.87
TAF11	145	TATA-Box Binding Protein Associated Factor 11	0.01	0.012	0.49	15.13
LYPLA2P1	146	Lysophospholipase II Pseudogene 1	0.01	0.013	0.68	18.79
DRAM1	147	DNA Damage Regulated Autophagy Modulator 1	0.01	0.013	0.64	16.38
AMBRA1	148	Autophagy And Beclin 1 Regulator 1	0.01	0.013	0.56	26.99
PAPSS1	149	3'-Phosphoadenosine 5'-Phosphosulfate Synthase 1	0.01	0.012	0.47	30.81
PTPN22	150	Protein Tyrosine Phosphatase, Non-Receptor Type 22	0.01	0.012	0.47	25.48
LOC739216	151	POM121 Membrane Glycoprotein Pseudogene	0.01	0.013	0.61	33.16
C3ORF57	152	Serine Palmitoyltransferase Small Subunit B	0.01	0.012	0.48	16.35
FAM156B	153	Family With Sequence Similarity 156 Member B	0.01	0.013	0.50	19.01

NFKB2	154	Nuclear Factor Kappa B Subunit 2	0.01	0.012	0.48	35.97
HIST1H4B	155	Histone Cluster 1, H4b	0.01	0.013	0.58	17.91
HIST1H4F	156	Histone Cluster 1, H4f	0.01	0.013	0.48	9.91
HIST1H4C	157	Histone Cluster 1, H4c	0.01	0.013	0.62	21.32
HIST1H4J	158	Histone Cluster 1, H4j	0.01	0.012	0.48	38.75
HIST1H4H	159	Histone Cluster 1, H4h	0.01	0.013	0.47	22.21
MCM5	160	Minichromosome Maintenance Complex Component 5	0.02	0.012	0.58	15.68
FZD7	161	Frizzled Class Receptor 7	0.02	0.012	0.58	20.01
TSTD1	162	Thiosulfate Sulfurtransferase Like Domain Containing 1	0.02	0.012	0.58	21.36
SLK	163	STE20 Like Kinase	0.02	0.012	0.58	35.08
SSR4	164	Signal Sequence Receptor, Delta	0.02	0.012	0.58	17.49
TMEM86A	165	Transmembrane Protein 86[a]	0.02	0.012	0.58	18.67
CPEB4	166	Cytoplasmic Polyadenylation Element Binding Protein 4	0.02	0.012	0.58	27.96
FAM125A	167	Multivesicular Body Subunit 12[a]	0.02	0.012	0.58	42.16
RTN2	168	Reticulon 2	0.02	0.012	0.58	25.53
SLC35B3	169	Solute Carrier Family 35 Member B3	0.02	0.012	0.57	16.83
RAB36	170	RAB36, Member RAS Oncogene Family	0.02	0.012	0.57	12.76
ZNF333	171	Zinc Finger Protein 333	0.02	0.012	0.57	25.95
PROCA1	172	Protein Interacting With Cyclin A1	0.02	0.012	0.57	13.60
FAM64A	173	Family With Sequence Similarity 64 Member A	0.02	0.012	0.57	29.55
GATAD1	174	GATA Zinc Finger Domain Containing 1	0.02	0.012	0.57	11.87
FAM105A	175	Family With Sequence Similarity 105 Member A	0.02	0.012	0.57	35.87
SNRPN	176	Small Nuclear Ribonucleoprotein Polypeptide N	0.02	0.012	0.57	24.56
ADAMTS19	177	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 19	0.02	0.012	0.57	16.11
GRIK5	178	Glutamate Ionotropic Receptor Kainate Type Subunit 5	0.02	0.012	0.57	10.91
ARNT	179	Aryl Hydrocarbon Receptor Nuclear Translocator	0.02	0.012	0.57	36.56
GRID1	180	Glutamate Ionotropic Receptor Delta Type Subunit 1	0.02	0.012	0.57	12.37
MB21D1	181	Mab-21 Domain Containing 1	0.02	0.012	0.57	39.38
TSLP	182	Thymic Stromal Lymphopoietin	0.02	0.012	0.57	46.08
USHBP1	183	USH1 Protein Network Component Harmonin Binding Protein 1	0.02	0.012	0.57	16.25
ZNF680	184	Zinc Finger Protein 680	0.02	0.012	0.57	23.15
POU3F4	185	POU Class 3 Homeobox 4	0.02	0.012	0.57	37.79
STXBP5	186	Syntaxin Binding Protein 5	0.02	0.012	0.57	31.65
FGF13	187	Fibroblast Growth Factor 13	0.02	0.012	0.57	11.40
ZNF468	188	Zinc Finger Protein 761	0.02	0.012	0.57	13.89
PALM2	189	PALM2-AKAP2 Readthrough	0.02	0.012	0.57	20.08
VBP1	190	Von Hippel-Lindau Binding Protein 1	0.02	0.012	0.57	12.10
RARA	191	Retinoic Acid Receptor Alpha	0.02	0.012	0.57	24.49
LYPLA2	192	Lysophospholipase II	0.02	0.012	0.57	10.39
PARVB	193	Parvin Beta	0.02	0.012	0.57	46.17

RPL29	194	Ribosomal Protein L29	0.02	0.012	0.56	17.97
RHOQ	195	Ras Homolog Family Member Q	0.02	0.012	0.56	43.80
SOX30	196	SRY-Box 30	0.02	0.012	0.56	18.34
CPNE1	197	Copine 1	0.02	0.012	0.56	11.73
SIRPA	198	Signal Regulatory Protein Alpha	0.02	0.012	0.56	15.11
IFT46	199	Intraflagellar Transport 46	0.02	0.012	0.56	22.43
SRRM1	200	Serine And Arginine Repetitive Matrix 1	0.02	0.012	0.56	23.81
SNTB1	201	Syntrophin Beta 1	0.02	0.012	0.56	10.84
SLC39A10	202	Solute Carrier Family 39 Member 10	0.02	0.012	0.56	41.97
GDI1	203	GDP Dissociation Inhibitor 1	0.02	0.012	0.56	12.34
L3MBTL1	204	L(3)Mbt-Like 1 (Drosophila)	0.02	0.012	0.56	37.32
TOMM34	205	Translocase Of Outer Mitochondrial Membrane 34	0.02	0.012	0.56	12.90
METTL10	206	Methyltransferase Like 10	0.02	0.012	0.56	39.27
ATF1	207	Activating Transcription Factor 1	0.02	0.012	0.56	38.04
ZNF37BP	208	Zinc Finger Protein 37B, Pseudogene	0.02	0.012	0.55	23.84
PIR	209	Pirin	0.02	0.012	0.55	45.71
KCNMB3	210	Potassium Calcium-Activated Channel Subfamily M Regulatory Beta Subunit 3	0.02	0.012	0.55	27.69
SLC41A3	211	Solute Carrier Family 41 Member 3	0.02	0.012	0.55	30.61
HMGCLL1	212	3-Hydroxymethyl-3-Methylglutaryl-CoA Lyase-Like 1	0.02	0.012	0.55	18.78
HMX3	213	H6 Family Homeobox 3	0.02	0.012	0.55	20.00
PSMG1	214	Proteasome Assembly Chaperone 1	0.02	0.012	0.54	22.20
PAK1	215	P21 Protein (Cdc42/Rac)-Activated Kinase 1	0.02	0.012	0.54	23.14
KARS	216	Lysyl-TRNA Synthetase	0.02	0.012	0.54	9.48
KRCC1	217	Lysine-Rich Coiled-Coil 1	0.02	0.012	0.54	29.83
MIF	218	Macrophage Migration Inhibitory Factor (Glycosylation-Inhibiting Factor)	0.02	0.012	0.54	21.57
RLTPR	219	Capping Protein Regulator And Myosin 1 Linker 2	0.02	0.012	0.54	28.96
POM121C	220	POM121 Transmembrane Nucleoporin C	0.02	0.012	0.54	29.62
HSD17B4	221	Hydroxysteroid (17-Beta) Dehydrogenase 4	0.02	0.012	0.54	48.97
DLG1	222	Discs Large Homolog 1, Scribble Cell Polarity Complex Component	0.02	0.012	0.54	15.34
CHKB	223	Choline Kinase Beta	0.02	0.012	0.54	48.49
TMEM14A	224	Transmembrane Protein 14A	0.02	0.012	0.54	27.31
RPL21	225	Ribosomal Protein L21	0.02	0.012	0.54	18.54
ZNF212	226	Zinc Finger Protein 212	0.02	0.012	0.54	34.36
MID1IP1	227	MID1 Interacting Protein 1	0.02	0.012	0.54	79.31
SCAI	228	Suppressor Of Cancer Cell Invasion	0.02	0.012	0.54	42.35
CTTNBP2	229	Cortactin Binding Protein 2	0.02	0.012	0.54	23.97
FAM197Y2P	230	Family With Sequence Similarity 197 Y-Linked Member 2, Pseudogene	0.02	0.012	0.54	25.33
GABRA1	231	Gamma-Aminobutyric Acid Type A Receptor Alpha1 Subunit	0.02	0.012	0.54	33.41
C2ORFf88	232	Chromosome 2 Open Reading Frame 88	0.02	0.013	0.54	24.80
KDM4C	233	Lysine Demethylase 4C	0.02	0.012	0.54	34.59

PEX3	234	Peroxisomal Biogenesis Factor	0.02	0.012	0.54	39.83
PRPF4	235	Pre-MRNA Processing Factor 4	0.02	0.012	0.53	11.70
CEBPD	236	CCAAT/Enhancer Binding Protein Delta	0.02	0.012	0.53	25.60
NMT2	237	N-Myristoyltransferase 2	0.02	0.012	0.53	15.51
FASTKD2	238	FAST Kinase Domains 2	0.02	0.012	0.53	25.78
SLC4A1AP	239	Solute Carrier Family 4 Member 1 Adaptor Protein	0.02	0.012	0.53	26.36
ENC1	240	Ectodermal-Neural Cortex 1	0.02	0.012	0.53	35.56
RBM4B	241	RNA Binding Motif Protein 4B	0.02	0.012	0.53	12.36
HLA-B	242	Major Histocompatibility Complex, Class I, B	0.02	0.012	0.53	49.40
FANCB	243	Fanconi Anemia Complementation Group B	0.02	0.012	0.53	31.92
MED18	244	Mediator Complex Subunit 18	0.02	0.012	0.53	13.64
CBLN2	245	Cerebellin 2 Precursor	0.02	0.012	0.53	17.47
NSUN4	246	NOP2/Sun RNA Methyltransferase Family Member 4	0.02	0.012	0.53	34.82
ALK	247	Anaplastic Lymphoma Receptor Tyrosine Kinase	0.02	0.012	0.53	33.33
HIST2H2BF	248	Histone Cluster 2, H2bf	0.02	0.012	0.53	21.72
PUM1	249	Pumilio RNA Binding Family Member 1	0.02	0.012	0.53	32.92
HIST4H4	250	Histone Cluster 4, H4	0.02	0.013	0.60	22.13
HIST1H4K	251	Histone Cluster 1, H4k	0.02	0.012	0.48	51.75
HIST1H4D	252	Histone Cluster 1, H4d	0.02	0.012	0.53	33.57
HIST1H4I	253	Histone Cluster 1, H4i	0.02	0.013	0.56	11.50
HIST2H4	254	Histone Cluster 4, H4	0.02	0.012	0.60	17.27
NFYC	255	Nuclear Transcription Factor Y Subunit Gamma	0.02	0.012	0.47	28.13
HOXA3	256	Homeobox A3	0.02	0.012	0.70	64.02
NARFL	257	Nuclear Prelamin A Recognition Factor Like	0.02	0.013	0.50	58.32
FAM176A	258	Eva-1 Homolog A, Regulator Of Programmed Cell Death	0.02	0.013	0.49	12.51
DOCK1	259	Dedicator of cytokinesis 1	0.02	0.012	0.47	24.22
LTBR	260	Lymphotoxin Beta Receptor	0.02	0.012	0.47	23.49
AIMP2	261	Aminoacyl TRNA Synthetase Complex-Interacting Multifunctional Protein 2	0.02	0.012	0.51	10.21
ABI1	262	Abl Interactor 1	0.02	0.012	0.47	63.72
EPS8L1	263	EPS8 Like 1	0.02	0.012	0.49	26.33
C14ORF145	264	Centrosomal Protein 128	0.03	0.012	0.49	29.98
ZNF827	265	Zinc Finger Protein 827	0.03	0.012	0.53	11.31
DNAJB3	266	DnaJ Heat Shock Protein Family (Hsp40) Member B3	0.03	0.012	0.53	28.51
SOX17	267	SRY-Box 17	0.03	0.012	0.53	47.09
CPNE8	268	Copine 8	0.03	0.012	0.53	13.06
ZNF22	269	Zinc Finger Protein 22	0.03	0.012	0.53	35.94
PEX5	270	Peroxisomal Biogenesis Factor 5	0.03	0.012	0.53	22.84
UTF1	271	Undifferentiated Embryonic Cell Transcription Factor 1	0.03	0.012	0.53	64.52
ADAMTS17	272	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 17	0.03	0.012	0.53	41.73
OAZ3	273	Ornithine Decarboxylase Antizyme 3	0.03	0.012	0.53	18.51

LDHB	274	Lactate Dehydrogenase B	0.03	0.012	0.53	20.18
MTMR1	275	Myotubularin Related Protein 1	0.03	0.012	0.52	29.15
DR1	276	Down-Regulator Of Transcription 1	0.03	0.012	0.52	10.32
POU6F2	277	POU Class 6 Homeobox 2	0.03	0.012	0.52	35.18
LIN54	278	Lin-54 DREAM MuvB Core Complex Component	0.03	0.012	0.52	13.60
RAD51L1	279	RAD51 Paralog B	0.03	0.012	0.52	30.22
SLC25A36	280	Solute Carrier Family 25 Member 36	0.03	0.012	0.52	37.55
RBBP5	281	Retinoblastoma Binding Protein 5	0.03	0.012	0.52	48.22
RAB33B	282	RAB33B, Member RAS Oncogene Family	0.03	0.012	0.52	36.33
NAA38	283	N(Alpha)-Acetyltransferase 38, NatC Auxiliary Subunit	0.03	0.012	0.52	22.43
STXBP3	284	Syntaxin Binding Protein 3	0.03	0.012	0.52	39.95
FAM82A2	285	Regulator Of Microtubule Dynamics 3	0.03	0.012	0.52	29.22
PTPRCAP	286	Protein Tyrosine Phosphatase, Receptor Type C Associated Protein	0.03	0.012	0.52	24.88
STAG3L3	287	Stromal Antigen 3-Like 3 (Pseudogene)	0.03	0.012	0.52	27.11
ADM	288	Adrenomedullin	0.03	0.012	0.52	19.47
CSRP2BP	289	Cysteine And Glycine Rich Protein 2	0.03	0.012	0.52	33.81
FOXP4	290	Forkhead Box P4	0.03	0.012	0.52	30.81
FLYWCH1	291	FLYWCH-Type Zinc Finger 1	0.03	0.012	0.52	55.51
DCTN6	292	Dynactin Subunit 6	0.03	0.012	0.52	39.82
ANPEP	293	Alanyl Aminopeptidase, Membrane	0.03	0.015	0.57	15.18
SORL1	294	Sortilin-Related Receptor, L(DLR Class) A Repeats Containing	0.03	0.012	0.51	14.34
MYBL1	295	MYB Proto-Oncogene Like 1	0.03	0.012	0.51	36.73
CHCHD3	296	Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 3	0.03	0.012	0.51	13.87
CASC5	297	Cancer Susceptibility Candidate 5	0.03	0.012	0.51	56.87
SLC24A2	298	Solute Carrier Family 24 Member 2	0.03	0.012	0.51	26.03
SMNDC1	299	Survival Motor Neuron Domain Containing 1	0.03	0.012	0.51	27.67
KCNQ3	300	Potassium Voltage-Gated Channel Subfamily Q Member 3	0.03	0.012	0.51	35.10
DLD	301	Dihydrolipoamide Dehydrogenase	0.03	0.012	0.51	21.66
ZFP3	302	ZFP3 Zinc Finger Protein	0.03	0.012	0.51	32.84
NDUFB7	303	NADH:Ubiquinone Oxidoreductase Subunit B7	0.03	0.012	0.51	39.76
FBXO34	304	F-Box Protein 34	0.03	0.012	0.51	31.20
ACTR10	305	Actin-Related Protein 10 Homolog	0.03	0.012	0.51	24.05
ZNF365	306	Zinc Finger Protein 365	0.03	0.012	0.51	44.13
TPTEP1	307	Transmembrane Phosphatase With Tensin Homology Pseudogene 1	0.03	0.015	0.51	27.69
VIPR1	308	Vasoactive Intestinal Peptide Receptor 1	0.03	0.012	0.51	18.63
COL12A1	309	Collagen Type XII Alpha 1	0.03	0.012	0.51	26.93
TMC5	310	Transmembrane Channel Like 5	0.03	0.012	0.51	26.85
TSSC1	311	Tumor Suppressing Subtransferable Candidate 1	0.03	0.012	0.51	15.76
EDA	312	Ectodysplasin A	0.03	0.012	0.51	44.32
ABCC5	313	ATP Binding Cassette Subfamily C Member 5	0.03	0.012	0.51	36.80

SPAG6	314	Sperm Associated Antigen 6	0.03	0.012	0.50	35.65
FAM156A	315	Family With Sequence Similarity 156 Member A	0.03	0.012	0.50	19.01
INTS4L1	316	Integrator Complex Subunit 4 Pseudogene 1	0.03	0.012	0.50	19.01
GMFB	317	Glia Maturation Factor Beta	0.03	0.012	0.50	19.20
TSPYL5	318	TSPY-Like 5	0.03	0.012	0.50	22.90
ZDHHC13	319	Zinc Finger DHHC-Type Containing 13	0.03	0.012	0.50	24.60
TAOK2	320	TAO Kinase 2	0.03	0.012	0.50	30.42
ZC3H13	321	Zinc Finger CCCH-Type Containing 13	0.03	0.012	0.50	28.67
PARD3	322	Par-3 Family Cell Polarity Regulator	0.03	0.012	0.50	24.05
CCNG1	323	Cyclin G1	0.03	0.012	0.50	35.58
EPHA7	324	EPH Receptor A7	0.03	0.012	0.50	9.72
SMS	325	Spermine Synthase	0.03	0.012	0.50	16.95
PTPRK	326	Protein Tyrosine Phosphatase, Receptor Type K	0.03	0.012	0.50	39.41
SYNE1	327	Spectrin Repeat Containing Nuclear Envelope Protein 1	0.03	0.012	0.50	39.24
NINL	328	Ninein Like	0.03	0.012	0.50	15.14
DERL2	329	Derlin 2	0.03	0.012	0.50	14.98
DYNC1I2	330	Dynein Cytoplasmic 1 Intermediate Chain 2	0.03	0.012	0.50	21.70
GRIK1	331	Glutamate Ionotropic Receptor Kainate Type Subunit 1	0.03	0.012	0.50	10.52
MEA1	332	Male-Enhanced Antigen 1	0.03	0.012	0.50	15.20
ECHDC2	333	Enoyl-CoA Hydratase Domain Containing 2	0.03	0.012	0.49	10.01
GNAS	334	GNAS Complex Locus	0.03	0.012	0.62	17.57
ZNF583	335	Zinc Finger Protein 583	0.03	0.012	0.61	31.60
ZNF343	336	Zinc Finger Protein 343	0.03	0.014	0.54	16.52
FOXB1	337	Forkhead Box B1	0.03	0.014	0.57	24.81
MBD3L1	338	Methyl-CpG Binding Domain Protein 3 Like 1	0.03	0.015	0.65	16.67
GTF2H2B	339	General Transcription Factor IIH Subunit 2B (Pseudogene)	0.03	0.012	0.52	62.46
ZNF234	340	Zinc Finger Protein 234	0.03	0.012	0.47	34.17
SLC13A3	341	Solute Carrier Family 13 Member 3	0.03	0.012	0.54	20.64
THAP1	342	THAP Domain Containing, Apoptosis Associated Protein 1	0.03	0.012	0.48	61.34
TFCP2	343	Transcription Factor CP2	0.03	0.012	0.47	23.19
HNRNPM	344	Heterogeneous Nuclear Ribonucleoprotein M	0.03	0.012	0.48	29.95
TMEM86B	345	Transmembrane Protein 86B	0.03	0.012	0.48	17.70
SH3BP1	346	SH3 Domain Binding Protein 1	0.04	0.012	0.49	33.77
FAF2	347	Fas Associated Factor Family Member 2	0.04	0.012	0.50	11.30
HSPA4	348	Heat Shock Protein Family A (Hsp70) Member 4	0.04	0.012	0.50	54.57
ACAD11	349	Acyl-CoA Dehydrogenase Family Member 11	0.04	0.012	0.50	44.23
RGS12	350	Regulator Of G-Protein Signaling 12	0.04	0.012	0.50	27.11
EIF4E	351	Eukaryotic Translation Initiation Factor 4E	0.04	0.012	0.50	16.72
GLRX2	352	Glutaredoxin 2	0.04	0.012	0.50	45.60
DAB2IP	353	DAB2 Interacting Protein	0.04	0.012	0.49	22.21

MECOM	354	MDS1 And EVI1 Complex Locus	0.04	0.012	0.49	14.78
CIDEA	355	Cell Death-Inducing DFFA-Like Effector A	0.04	0.012	0.49	36.49
HSBP1	356	Heat Shock Factor Binding Protein 1	0.04	0.012	0.49	19.66
EXOC3L4	357	Exocyst Complex Component 3 Like 4	0.04	0.012	0.49	12.22
APH1A	358	Aph-1 Homolog A, Gamma Secretase Subunit	0.04	0.012	0.49	14.64
PLSCR2	359	Phospholipid Scramblase 2	0.04	0.012	0.49	52.12
ARMC1	360	Armadillo Repeat Containing 1	0.04	0.012	0.49	37.97
BRPF3	361	Bromodomain And PHD Finger Containing 3	0.04	0.012	0.49	27.67
ZNF385B	362	Zinc Finger Protein 385B	0.04	0.012	0.49	42.33
RASA1	363	RAS P21 Protein Activator 1	0.04	0.012	0.49	24.14
BEX5	364	Brain Expressed X-Linked 5	0.04	0.012	0.49	42.62
PPM1H	365	Protein Phosphatase, Mg2+/Mn2+ Dependent 1H	0.04	0.012	0.49	12.04
FUZ	366	Fuzzy Planar Cell Polarity Protein	0.04	0.012	0.49	25.97
MLPH	367	Melanophilin	0.04	0.012	0.49	29.85
NEUROG1	368	Neurogenin 1	0.04	0.012	0.49	35.72
RASGRF1	369	Ras Protein Specific Guanine Nucleotide Releasing Factor 1	0.04	0.012	0.48	29.72
KIF3A	370	Kinesin Family Member 3[a]	0.04	0.012	0.48	11.49
GUF1	371	GUF1 Homolog, GTPase	0.04	0.012	0.48	58.19
AQP11	372	Aquaporin 11	0.04	0.012	0.49	27.15
MSTO2P	373	Misato Family Member 2, Pseudogene	0.04	0.012	0.48	70.25
SCOC	374	Short Coiled-Coil Protein	0.04	0.012	0.48	54.22
WWC2	375	WW And C2 Domain Containing 2	0.04	0.012	0.48	34.28
PGAM1	376	Phosphoglycerate Mutase 1	0.04	0.012	0.48	46.40
SLC1A1	377	Solute Carrier Family 1 Member 1	0.04	0.012	0.48	53.79
RASA2	378	RAS P21 Protein Activator 2	0.04	0.012	0.48	53.83
PRR5-ARHGAP8	379	PRR5-ARHGAP8 Readthrough	0.04	0.012	0.48	21.17
ALDH3A2	380	Aldehyde Dehydrogenase 3 Family Member A2	0.04	0.012	0.48	10.56
RHOU	381	Ras Homolog Family Member U	0.04	0.012	0.48	23.16
SPIN3	382	Spindlin Family Member 3	0.04	0.012	0.47	42.43
EPG5	383	Ectopic P-Granules Autophagy Protein 5 Homolog (C. Elegans)	0.04	0.012	0.47	28.66
EHD3	384	EH Domain Containing 3	0.04	0.012	0.47	23.11
LYRM1	385	LYR Motif Containing 1	0.04	0.012	0.47	17.68
TMEM64	386	Transmembrane Protein 64	0.04	0.012	0.47	17.13
MRPS6	387	Mitochondrial Ribosomal Protein S6	0.04	0.012	0.47	30.31
AHCYL1	388	Adenosylhomocysteinase Like 1	0.04	0.012	0.47	63.38
CXCR4	389	C-X-C Motif Chemokine Receptor 4	0.04	0.012	0.47	35.54
GKAP1	390	G Kinase Anchoring Protein 1	0.04	0.012	0.47	51.55
FAM36A	391	COX20 Cytochrome C Oxidase Assembly Factor	0.04	0.012	0.47	47.96
HEATR2	392	HEAT Repeat-Containing Protein 2	0.04	0.012	0.47	19.60
CRELD2	393	Cysteine Rich With EGF Like Domains 2	0.04	0.012	0.47	17.94

HACE1	394	HECT Domain And Ankyrin Repeat Containing E3 Ubiquitin Protein Ligase 1	0.04	0.012	0.47	38.01
SIRT1	395	Sirtuin 1	0.04	0.012	0.47	31.12
APEX2	396	Apurinic/Apyrimidinic Endodeoxyribonuclease 2	0.04	0.012	0.47	21.50
NUBP2	397	Nucleotide Binding Protein 2	0.04	0.012	0.47	39.48
PYDC1	398	Pyrin Domain Containing 1	0.04	0.012	0.47	18.93
RBBP7	399	Retinoblastoma Binding Protein 7	0.04	0.012	0.47	34.35
PKHD1L1	400	Polycystic Kidney And Hepatic Disease 1 (Autosomal Recessive)-Like 1	0.04	0.012	0.47	67.86
TNRC18	401	Trinucleotide Repeat Containing 18	0.04	0.012	0.47	34.26
ATRN	402	Attractin	0.04	0.012	0.47	38.27
RGS11	403	Regulator Of G-Protein Signaling 11	0.04	0.012	0.47	22.25
TNFRSF6B	404	Tumor Necrosis Factor Receptor Superfamily Member 6b	0.04	0.012	0.47	23.14
UBQLN4	405	Ubiquilin 4	0.04	0.012	0.48	29.60
C2ORF76	406	Chromosome 2 Open Reading Frame 76	0.04	0.012	0.49	10.08
TBCK	407	TBC1 Domain Containing Kinase	0.04	0.012	0.49	11.81
ZNF711	408	Zinc Finger Protein 711	0.04	0.012	0.49	22.41
FRG1	409	FSHD Region Gene 1	0.04	0.012	0.49	20.34

Supplemental Table 2. Gene Ontology (GO) analysis of differentially methylated genes (n=409). Significant enriched components are grouped according to three categories:
(a) Biological Processes; (b) Molecular function; and (c) Cellular components.

	Included Genes	*P*-value
(a) Biological Processes		
Intracellular signalling cascade	12	5.12E-05
Regulation of metabolic processes	121	6.1E-05
Cell development	7	7.35E-05
Cell Morphogenesis	32	3.7E-04
Embryonic Organ Development	15	4.9E-04
Macromolecule Biosynthesis	117	2.5E-04
(b) Molecular functions		
DNA Binding	266	1.1E-04
Transcription regulation	89	4.7E-04
(c) Cellular components		
Integral to membrane organization	7	4.5E-04
Intrinsic to plasma membrane	3	2.2E-04

Supplemental Table 3. Top-ranked (p<0.009) differentially hypermethylated genes included in the canonical pathways categorized by *P* value.

Gene Symbol	Gene Denomination	Chromosome Location & Number of CPG Islands	Function*	*P value*
NKX6-1[†‡]	NK6 homeobox 1	Chr 4, bp 85417332-85419573 (5 CpG)	β cell development and function	0.0002
CHRM2	Cholinergic receptor, muscarinic 2	Chr 7; bp 136553436-136557857 (3 CpG)	Cardiac function	0.0008
LMO2	LIM domain only 2 (rhombotin-like 1)	Chr 11; bp 33889459-33891595 (4 CpG)	Red blood cell development	0.001
ATG2B[†‡]	Autophagy related 2B	Chr 14, bp 96828029-96832550 (4 CpG)	Autophagy and energy homeostasis	0.002
MLLT3	Myeloid/lymphoid or mixed-lineage leukemia: translocated to, 3	Chr 9; bp 20616942-20625657 (4 CpG)	Transcription regulation	0.002
CLSTN2	Calsyntenin 2	Chr 3; bp 139652460-139654727 (4 CpG)	Neural function	0.002
KLF11[†]	Kruppel-like factor 11	Chr 2, bp 10180437-10189152 (4 CpG)	Apoptosis	0.003
SLC13A5[†‡]	Sodium-dependent citrate transporter, member 5	Chr 17, bp 6614780-6619231 (5 CpG)	Lipid metabolism	0.003
NRIP1[†]	Nuclear receptor interacting protein 1	Chr 21, bp 16434348-16438687 (6 CpG)	Transcription regulation, myogenesis	0.003
ZNF500	Zinc finger protein 500	Chr 16; bp4815731-4817858 (5 CpG)	Transcription regulation	0.003
GFOD1	Glucose-fructose oxidoreductase domain containing 1	Chr 6; bp 13486105-13488206 (4 CpG)	Nucleotide binding	0.004
LRP10	Low density lipoprotein receptor-related protein 10	Chr 4; bp 46938324-46940419 (4 CpG)	Internalization of lipophilic molecules	0.004
GTF2H2C	General transcription factor IIH, polypeptide 2C	Chr 5; bp 68855809-68856161 (4 CpG)	Transcription regulation	0.004
PYGO2	Pygopus family PHD finger 2	Chr1; bp154933928 – 154934735 (3 CpG)	Signal transduction	0.004
ZGPAT	Zinc finger, CCCH-type with G patch domain	Chr 20; bp 62339083-62341337 (4 CpG)	Transcription repressor	0.005
KCNK13	Potassium channel, subfamily K, member 13	Chr 14; bp90526475-90528701 (6 CpG)	Neural and heart metabolism	0.005
SOGA1	Suppressor of glucose, autophagy associated 1	Chr 20; bp 35489914-35492105 (5 CpG)	Autophagy	0.005
CALHM2	Calcium homeostasis modulator 2	Chr 10; bp 105195805-105204248 (5 CpG)	Calcium metabolism	0.006
SKAP2	Src kinase associated phosphoprotein 2	Chr 7; bp 26903474-26905663 (3 CpG)	Src signaling pathway	0.006
KPNA4	Karyopherin alpha 4 (importin alpha 3)	Chr 3; bp 160282616-160284700 (4 CpG)	Nuclear protein import	0.006
FMR1	Fragile X mental retardation 1	Chr X: bp146992741-146994927 (5 CpG)	Translation repressor	0.007
NKX2-5	NK2 Homeobox 5	Chr 5; bp 172662964-172665144 (4 CpG)	Heart development	0.008
ZNF182	Zinc finger protein 182	Chr 7; bp 148934520-14893861 (4 CpG)	Transcription regulation	0.008
ZNF436	Zinc finger protein 436	Chr 1; bp 23693745-23695831 (4 CpG)	Transcription regulation	0.008
ICOSLG	Inducible T-cell co-stimulator ligand	Chr 21; bp 45660794-45663032	Inflammation	0.008

*The most relevant functions of each gene are emphasized

[†]Analyzed by pyrosequencing

[‡] Validated by pyrosequencing

Supplemental Table 4. Top-ranked (p<0.009) differentially hypomethylated genes included in the canonical pathways categorized by *P* value.

Gene Symbol	Gene Denomination	Chromosome Location & Number of CPG Islands	Function*	*P value*
HIST1H2BI	Histone cluster 1, H2bi	Chr 6; bp 26272470-26272696 (1 CpG)	Transcription regulation, DNA repair and DNA replication	0.0002
UHRF2	Ubiquitin-like with PHD and ring finger domains 2, E3 ubiquitin protein ligase	Chr 9; bp 6411295-6413552 (4 CpG)	Cell cycle regulation	0.0003
MAPK8IP1†	Mitogen-activated protein kinase 8 interacting protein 1	Chr 11; bp 45906583-45908717 (3 CpG)	Pancreatic β-cell regulation	0.0004
CAMK4 †	Calcium/calmodulin-dependent protein kinase IV	Chr 5; bp 110558538-110560607 (4 CpG)	Regulation of glucose metabolim	0.0004
TOP3B	Topoisomerase (DNA) III beta	Chr 22; 22336948 – 22337333 (1 CpG)	DNA recombination and genome stability	0.001
STARD3NL	STARD3 N-terminal like	Chr 7; bp 38216648-38218788 (5 CpG)	Intracellular transport of cholesterol and sphingolipids	0.001
EIF2AK1	Eukaryotic translation initiation factor 2-alpha kinase 1	Chr 7; bp 6097361-6099530 (7 CpG)	Translational inhibitor	0.002
SFPQ	Splicing factor proline/glutamine-rich	Chr 1; bp 35657482-35659727 (8 CpG)	Post-transcriptional regulation	0.002
DOCK5	Dedicator of cytokinesis 5	Chr 8; bp 25041795-25042820 (1 CpG)	Guanine nucleotide exchange	0.003
GPR120 †‡	Free fatty acid receptor 4	Chr 10, bp 95325316-95327446 (4 CpG)	Lipid metabolism	0.003
PEX1	Peroxisomal biogenesis factor 1	Chr 7; bp 92157487-92158405 (1 CpG)	Peroxisomal transport	0.004
PRKG1	Protein kinase, cGMP-dependent, type I	Chr 6; bp 52750229-52752471 (6 CpG)	Cardiovascular and neuronal regulator	0.004
NKX3-2	NK3 homeobox 2	Chr 4; bp 13532661-13534792 (3 CpG)	Skeletal development	0.004
MAPK13	Mitogen-activated protein kinase 13	Chr 6; bp 36097402-36099496 (6 CpG)	Extracellular signaling	0.005

*The most relevant functions of each gene are emphasized

† Analyzed by pyrosequencing

‡ Validated by pyrosequencing

Materials

Subjects Inclusion and Exclusion criteria

Inclusion criteria were:

- Infants born from a singleton term pregnancy (37–42 wk) at Hospital Sant Joan de Déu, Barcelona; birth weight between 2.9 and 3.8 Kg for AGA infants (Z-scores between -1.1 and +1.1 for gestational age and sex), and between 1.9 and 2.6 Kg for SGA infants (Z-score below -2.0), as to obtain two distinct study subpopulations (*Ferrández-Longás A, Mayayo E, Labarta JI, Bagué L, Puga B, Rueda C, Ruiz-Echarri M, Labena C. Estudio longitudinal de crecimiento y desarrollo. Centro Andrea Prader. Zaragoza 1980-2002. In ERGON, ed. Patrones de crecimiento y desarrollo en España. Atlas de gráficas y tablas, pp 61-115. Madrid: 2004; Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. J Clin Endocrinol Metab 2007; 92:804-810)*.
- Placenta collected at delivery for research purposes; the inclusion rate was limited by logistic restraints (in particular for overnight collection).
- Written, informed consent in Spanish/Catalan language by at least one parent.

Exclusion criteria were:

- Maternal hypertension, pre-eclampsia, gestational diabetes [two or more altered values in the oral glucose tolerance test in the second trimester], alcohol abuse, smoking, or drug addiction.
- Birth weight below 1.9 Kg, since these infants are usually admitted to the Neonatal Unit.

- Complications at birth (need for resuscitation or parenteral nutrition), congenital malformations, or a syndromic or infectious etiology of low birth weight.

Cord blood and placenta collection

Venous cord blood was collected and processed before the separation of the placenta (*López-Bermejo A, Petry CJ, Diaz M, Sebastiani G, de Zegher F, Dunger DB, Ibáñez L. The association between the FTO gene and fat mass in humans develops within two weeks after birth. J Clin Endocrinol Metab 2008; 93:1501-1505*) and frozen at -80ºC until assay. After delivery, placentas were weighed and biopsies were then obtained from the maternal side, as described (*Díaz M, Bassols J, Lopez-Bermejo A, Gomez-Roig MD, de Zegher F, Ibáñez L: Placental expression of peroxisome proliferator-activated receptor-γ: relation to placental and fetal growth. J Clin Endocrinol Metab 2012; 97: E1468–1472*). We elected to assess the maternal side of the placenta because changes in epigenetic marks are expected to occur first in those cells rather that in placental fetal cells, in order to counteract a reduced nutrient supply. We followed a rigorous protocol aiming for sample uniformity in type and number of cells. Briefly, placentas were sectioned 4 cm around umbilical's cord insertion removing the amniotic and chorionic layers and obtaining fragments of about 1 cm^3 that were immediately rinsed three times in saline buffer to remove blood cells. To avoid degradation, biopsies were obtained within 5 minutes of delivery and were ultrafrozen in liquid nitrogen before storage at -80°C until DNA and RNA extraction.

DNA extraction and modification

DNA was extracted from placenta and cord blood samples by the phenol-chloroform method (Promega, Madison, Wisconsin, USA), following the

manufacturer's protocols. DNA quality and concentration were assessed using a UV-VIS spectrophotometer (Nanodrop 1000, Agilent Technologies, Wilmington, DE, USA); 300 ng of placental and cord blood DNA were bisulfite-modified using the Methylcode Bisulfite Conversion Kit (Invitrogen, Carlsbad, CA, USA) following manufacturer's guidelines.

Microarray data pre-processing

Probe intensities were extracted from scan images as raw signals with no background subtraction, using Agilent's Feature Extraction Software (v.10.7). The raw data were imported into *Agilent Genomic Workbench v6.5* BATMAN algorithm (Bayesian Tool for Methylation Analysis), and normalized to control probes present on the array. Outlier features on the arrays were flagged by the same software package. Values were $\log_2$-transformed and logged data were used for principal component analysis (PCA) (data not shown), and for statistical analysis. The Welch T-test was used for identification of differentially methylated genes. An individual probe was considered differentially methylated if its *p*-value was < 0.05 (not corrected for multiple testing), and if the associated difference of means between the AGA and SGA subgroups was at least 0.25. The resulting *P* values for each gene were then corrected for multiple testing by calculating their false discovery rate (FDR). A gene promoter was considered differentially methylated if its FDR was < 0.1. To investigate the biological relevance of these genes, we performed a Gene Ontology (GO) analysis (http://www.geneontology.org). Significantly enriched ($p<0.01$) GO terms in SGA *versus* AGA newborns were obtained and subdivided into three categories "Biological Processes", "Molecular functions", and "Cellular components". Gene networks and canonical pathways representing key genes were identified using the KEGG (Kyoto

Encyclopedia of Genes and Genomes) software (*Altman T, Travers M, Kothari A, Caspi R, Karp PD. A systematic comparison of the MetaCyc and KEGG pathway databases. BMC Bioinformatics 2013;14:112*).

Validation of DNA methylation patterns by pyrosequencing (see below) was limited to n=8 genes: *GPR120* (G-protein coupled receptor 120), *ATG2B* (autophagy-related 2B), *NKX6.1* (NKX6 homeodomain 1), *SLC13A5* (Mammalian Indy homologue), *KLF11* (Kruppel-like factor 11), *NRIP1* (Nuclear receptor interacting protein 1); *MAPK8IP1* (mitogen-activated protein kinase 8 interacting protein 1) and *CAMK4* (Calcium/Calmodulin-Dependent Protein Kinase IV). The rationale as well as the subsequent steps undertaken to come to this selection are detailed in the Results section.

Validation of DNA-methylation patterns by bisulfite pyrosequencing

Specific pyrosequencing primers and pre-designed PCR primers for quantification of CpG methylation (Qiagen, Germantown, USA) were used for validation in the 51 placenta-cord blood pairs (30 AGA; 21 SGA), selecting primers flanking the same CpG islands assessed in the array from the promotor regions of the following loci: *MAPK8IP1* (PM00152586), chr 11, bp 45906583-45908717 (3 CpG sites); *CAMK4* (PM00117530), chr 5, bp 110558538-110560607 (4 CpG sites); *GPR120* (PM00044758), chromosome 10, bp 95325316-95327446 (4 CpG sites); *ATG2B* (PM00164570), chr 14, bp 96828029-96832550 (4 CpG sites); *KLF11* (PM00011942), chr 2, bp 10180437-10189152 (4 CpG sites); *NKX6.1* (PM00019299), chr 4, bp 85417332-85419573 (5 CpG sites); *NRIP1* (PM00203259), chr 21, bp 16434348-16438687 (6 CpG sites); *SLC13A5* (PM00067039), chr 17, bp 6614780-6619231 (5 CpG sites). We used 1 µl of bisulfite- converted DNA as template for each

PCR in a final volume of 25 µl. Hot-start PCR was performed using the PyroMark PCR kit (Qiagen, Germantown, USA) and the correct size of the PCR product was verified by gel electrophoresis. Pyrosequencing was performed using the PyroMark Q96 ID system and evaluated using the PyroMark software 2.5 (Qiagen, Germantown, USA). The target CpG sites were evaluated by converting the resulting pyrograms to numerical values for peak heights. The methylation percentage of each locus was calculated as the mean of all CpG analyzed.

RNA isolation and retro-transcription

Between 100 and 125 mg of frozen placenta was homogenized using a Polytron benchtop homogenizer (Kinematica Ag, Littau-Luzern, Switzerland). Total RNA was isolated from placental homogenates and from 1 mL cord blood samples using Tripure reagent (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's protocol. The quantity of isolated RNA was determined with a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). In addition, 1 µg of total RNA was run on a 1.0% agarose gel using SYBR safe staining to test the integrity of the RNA. Samples with partially degraded RNA (n=8) were rejected. 2 µg of total RNA was reverse-transcribed to cDNA using the high capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA), and subsequently diluted with nuclease-free water (Sigma, St. Louis, MO, USA) to a concentration of 20 ng/µl cDNA. Retrotranscription products were stored at -20ºC until use.

Assays & Neonatal Body Composition

Circulating glucose was measured by glucose oxidase method. Insulin and IGF-I were assessed by immuno-chemiluminescence (DPC IMMULITE 2500, Siemens,

Germany), the respective detection limits being 0.4 μU/mL and 25 ng/mL; intra- and inter-assay coefficients of variation (CV's) were <10%. Circulating HMW adiponectin was measured with an ELISA kit (R&D Systems, Minneapolis, MN, USA); intra- and inter-assay CVs were <9%. Samples were stored at -20ºC until assay.

Body composition was assessed at the neonatal age of approximately 2 weeks (range, 11-17 days), by dual X-ray absorptiometry with a Lunar Prodigy, coupled to Lunar software (Lunar Corp, Madison, WI), adapted for assessment of infants; body fat and lean mass were assessed during natural sleep; coefficients of variation (CVs) were <3% for lean and fat mass (Ref. 4 of the manuscript).

Data

Correlation analyses

Cord blood glucose showed a positive correlation with insulin levels (r=0.35; p=0.01). No correlations were found between maternal BMI, cord blood insulin, and body composition in infants.

In placenta, hypermethylation in *ATG2B, NKX6.1* and *SLC13A5,* and hypomethylation in *GPR120* associated with lower birth weight, birth length, lean mass and circulating IGF-I (P<0.04 to P<0.0001), and to less total and abdominal fat at 2 weeks (P<0.02 to P<0.0001). These associations were essentially the same in cord blood (all P<0.01 to P<0.0001) (Supplemental Table 5).

In placenta, lower expression of *ATG2B, NKX6.1* and *SLC13A5,* and higher expression of *GPR120* associated with lower birth weight, birth length, total and abdominal fat (P<0.03 to P<0.0001). In cord blood, expression levels of *GPR120, NKX6.1* and *SLC13A5* were positively associated with birth weight and serum IGF-I

(P<0.01 to P<0.0001) while those of *GRP120* and *SLC13A5* directly correlated with total and abdominal fat (P<0.003 to P<0.0001) (Supplemental Table 5).

All correlations were maintained after adjusting for sex, gestational age, type of delivery and pre-gestational BMI.

Multiple regression analyses

Multivariate analysis adjusting for sex and gestational age showed that birth weight was independently explained by *GPR120* methylation (β=0.558, P<0.001) and both *NKX6.1* methylation and expression in placenta (β=-0.294, P=0.02; β=0.484, P<0.001, respectively) (Supplemental Table 6a). In cord blood, *GPR120* methylation explained 49% of birth weight variability (β=-0.699, P<0.001) (Supplemental Table 6b).

In addition, total and abdominal fat at age 2 weeks were independently explained by placental *GPR120* and *SLC13A5* methylation (β =0.771, P<0.001; β =0.400, P=0.03 for total fat; β= 0.748, P<0.001; β=0.511, P=0.008 for abdominal fat, respectively) and by placental *NKX6.1* expression (β=0.442, P=0.006 for total fat; β=0.509, P=0.002 for abdominal fat) (Supplemental Table 7a). In cord blood, *GPR120* expression was the only predictor of total and abdominal fat (β=0.413, P=0.01, and β=0.330, P=0.04, respectively) (Supplemental Table 7b).

Supplemental Table 5. Bivariate correlations between placental and cord blood gene methylation and expression and selected parameters in all subjects.

	Methylation				Expression			
	Placenta		Cord Blood		Placenta		Cord Blood	
ATG2B	**β**	***P***	**β**	***P***	**β**	***P***	**β**	***P***
Birth weight	-0.316	0.03	-0.441	0.002	0.522	<0.0001	-	-
Birth length	-0.358	0.013	-0.435	0.002	0.495	<0.0001	-	-
IGF-I	-0.293	0.045	-	-	-	-	0.338	0.023
Fat Mass	-	-	-	-	0.442	0.001	-	-
Abdominal fat	-	-	-	-	0.416	0.003	-	-
Lean Mass	-0.334	0.022	-0.509	<0.0001	0.428	0.002	-	-
NKX6								
Birth weight	-0.358	0.025	-0.593	<0.0001	0.553	<0.0001	0.399	0.007
Birth length	-0.395	0.001	-0.581	<0.0001	0.473	<0.0001	0.329	0.027
Insulin	-	-	-0.310	0.046	-	-	-	-
IGF-I	-0.328	0.002	-0.420	0.003	0.448	0.001	0.327	0.028
Fat Mass	-0.492	0.001	-	-	0.324	0.02	-	-
Abdominal fat	-0.417	0.008	-0.313	0.031	0.325	0.02	-	-
Lean Mass	-0.324	0.044	-0.560	<0.0001	0.476	<0.0001	-	-
SLC13A5								
Birth weight	-0.560	<0.0001	-0.505	<0.0001	0.415	0.002	0.661	<0.0001
Birth length	-0.463	0.001	-0.477	0.001	0.498	<0.0001	0.597	<0.0001
Insulin	-	-	-0.330	0.031	-	-	0.346	0.027
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
Lean Mass	-0.557	<0.0001	-0.512	<0.0001	0.430	0.002	0.647	<0.0001
GPR120								
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	-	-
Insulin	0.349	0.019	-0.325	0.036	-	-	-	-
IGF-I	0.475	<0.0001	-0.414	0.004	-	-	0.445	0.004
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
Lean Mass	0.617	<0.0001	-0.615	<0.0001	-0.309	0.029	0.320	0.034

IGF-I: insulin-like growth factor I

Supplemental Table 6. Multivariate linear models of birth weight in placenta (a) and cord blood (b).

(a)			
Placenta	**Birth weight**		
	Beta	Sig.	R^2
GPR120 methylation	0.558	<0.001	30%
NKX6.1 methylation	-0.294	0.02	62%
NKX6.1 expression	0.484	<0.001	54%
(b)			
Cord Blood	**Birth weight**		
	Beta	Sig.	R^2
GPR120 methylation	-0.699	<0.001	49%

(a) Non-predictive variables: sex, maternal body mass index (BMI), *ATG2B* methylation, *ATG2B* expression, *SLC13A5* methylation, *SLC13A5* expression and *GPR120* expression.

(b) Non predictive variables: sex, maternal body mass index (BMI), *GPR120* expression, *ATG2B* methylation, *ATG2B* expression, *SLC13A5* methylation and *SLC13A5* expression.

Supplemental Table 7. Multivariate linear models of fat and abdominal fat mass at age 2 weeks in placenta (a) and cord blood (b).

(a)						
Placenta	**Total Fat (g)**			**Abdominal Fat**		
	Beta	Sig.	R^2	Beta	Sig.	R^2
GPR120 methylation	0.771	<0.001	30%	0.748	<0.001	22%
SLC13A5 methylation	0.400	0.03	51%	0.511	0.008	51%
NKX6.1 expression	0.442	0.006	41%	0.509	0.002	35%
(b)						
Cord Blood	**Total Fat (g)**			**Abdominal Fat**		
	Beta	Sig.	R^2	Beta	Sig.	R^2
GPR120 expression	0.413	0.01	17%	0.330	0.04	11%

(a) Non-predictive variables: sex, maternal body mass index (BMI), *GPR120* expression, *SLC13A5* expression, *NKX6.1* methylation, *ATG2B* methylation or expression.

(b) Non-predictive variables: sex, maternal body mass index (BMI), *GPR120* methylation, *SLC13A5* methylation or expression, *NKX6.1* methylation or expression, *ATG2B* methylation or expression.