

Issue 1 (Vol. 55) Latest Articles:

Multiomics-Identified Intervention to Restore Dysregulated Proteostasis

Biochemical, Cellular, and Proteomic Characterization of Hereditary Spherocytosis Among Tunislans

Scope & Aims

Cellular Physiology and Biochemistry is a multidisciplinary scientific forum dedicated to advancing the frontiers of basic cellular research. It addresses scientists from both the physiological and biochemical disciplines as well as related fields such as genetics, molecular biology, pathobiochemistry and cellular toxicology & pharmacology. Original papers and reviews on the mechanisms of intracellular transmission, cellular metabolism, cell growth, differentiation and death, ion channels and carriers, and the maintenance, regulation and disturbances of cell volume are presented. Appearing monthly under peer review, Cellular Physiology and Biochemistry takes an active role in the concerted international effort to unravel the mechanisms of cellular function.

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Original Paper

Analysis of Genomic DNA Methylation Levels in Human Placenta using Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry

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Key Words

Pregnancy • Placenta • Methylation • Global • LC-MS/MS • Fetal programming • Clinical

Abstract

Background: DNA-methylation is a common epigenetic tool which plays a crucial role in gene regulation and is essential for cell differentiation and embryonic development. The placenta is an important organ where gene activity can be regulated by epigenetic DNA modifications, including DNA methylation. This is of interest as, the placenta is the interface between the fetus and its environment, the mother. Exposure to environmental toxins and nutrition during pregnancy may alter DNA methylation of the placenta and subsequently placental function and as a result the phenotype of the offspring. The aim of this study was to develop a reliable method to quantify DNA methylation in large clinical studies. This will be a tool to analyze the degree of DNA methylation in the human placenta in relationship to clinical readouts. *Methods:* Liquid chromatography-electrospray ionization/multi-stage mass spectrometry (LC-ESI/MS/MS) technique was used for the guantification of the 5dmC/ dG ratio in placentas from 248 healthy pregnancies. We were able to demonstrate that this method is a reliable and stable way to determine global placental DNA methylation in large clinical trials. *Results/Conclusion:* The degree of placental DNA methylation seen in our pilot study varies substantially from 2% to 5%. The clinical implications of this variation need to be demonstrated in adequately powered large studies.

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Introduction

The 'fetal origin' hypothesis proposes that adulthood hypertension, insulin resistance, dyslipidaemia and non-insulin-dependent diabetes, which are connected to markedly increased rates of cardiovascular disease in adult life, originate through adaptation of the fetus to an early intrauterine environment. It has been suggested that not only maternal nutrition but also maternal exposure to toxins like alcohol, nicotine but also toxins in water and food in early life cause functional and structural changes of the newborn resulting in adulthood hypertension, insulin resistance and dyslipidaemia [1]. Epigenetics serves as an important mechanism capable of regulating gene transcription and linking events early in life to adult morbidity. It is comprised by heritable changes in chromatin that alter gene expression without altering the DNA sequence [2-4]. Throughout gestation, the placenta plays a crucial role in controlling growth and development of the fetus. The placenta acts as an interface between the growing child and its environment, the mother. Nutritions, toxins and any environmental challenge of the pregnant mother act on the newborn via the placenta. Moreover, the placenta also produces specific hormones affecting both the mother and the growing child [5]. The placenta itself develops within several weeks from pluripotent cells to a highly differentiated organ. Its epigenome is critical for normal placental function [6], and plays an important role in programming events occurring during early phases of pregnancy. After fertilisation, both paternal and maternal genomes undergo demethylation [7]. Establishment of correct epigenetic patterns in the trophoblast is crucial for the formation of the fetal side of the placenta and epigenetic factors play an important role in placental maturation and development [8]. It is known that placental function can be influenced by the environment throughout pregnancy, thereby impacting on the appropriate genetic programming needed to allow for proper fetal growth [4]. Exposure to environmental toxins and nutrition during pregnancy may alter DNA methylation of the placenta and subsequently placental function [9–11]. As a result this, can also trigger epigenetic changes in the offspring, ultimately altering its phenotype [12]. It is believed that these epigenetic alterations lead to a stable reset of key endocrine and structural properties of the offspring, thus potentially causing disease in later life [13].

DNA methylation, which is the best understood DNA epigenetic modification, may provide an attractive mechanism linking environmental cues to placental pathology, with consequences for fetal growth and adult life [4]. Methylation in vertebrate DNA is, in general, restricted to cytosine (C) nucleotides in the sequence Cytosin-Guanin (annotated CpG). The overall frequency of CpG dinucleotides in the vertebrate genome is low, but there are small stretches of DNA that are characterized by having CpG dinucleotides, extending for hundreds of bases, termed CpG islands [14-16]. The methylation status of cytosine residues, within CpG dinucleotides and in the context of CpG islands, provides an important mechanism for controlling gene expression activity [14, 16, 17]. Changes in the methylation status (hyper- or hypomethylation) have been associated with various health conditions including malignancies [18]. Besides affecting gene specific methylation patterns [11, 12], recent studies demonstrated that environmental cues impact on the global methylation status of the placenta [9–11, 19]. To shed more light on the consequences of this phenomenon, it is important to analyze the degree of placental DNA methylation in large clinical studies in relationship to clinical readouts. For doing so, the establishment of a suitable method to quantify placental DNA methylation is thus urgently needed. In the current study, we developed a reliable method to quantify DNA methylation in the human placenta based on previous work by Song and colleagues [20].

Materials and Methods

Clinical study

We used 248 placenta samples collected as part of the Berlin Birth Cohort study [21–23]. The study was approved by the local ethics committee. Biometric data of the newborn were measured during the routine

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postnatal examination. Gestational age at delivery was based on last menstrual period, anamnestically assessed during the first pregnancy examination. The following data of the newborn were added to the database: birth weight, birth length, head circumference, ponderal index, child sex, Apgar score 5 minutes postnatally, Apgar score 10 minutes postnatally and umbilical blood pH levels. Fetal blood was collected from the umbilical cord. Midwives collected maternal blood from a cubital vein in the delivery room or on the ward. Placenta was collected and frozen at -20°C immediately after the placenta was born.

Analysis of DNA Methylation

The deoxyribonucleosides 2'-deoxyguanosine (dG) monohydrate, 5-methyl-2'-deoxycytidine (5mdC) and 2'-deoxycytidine (dC) were purchased from ABCR (Karlsruhe, Germany). Hering sperm DNA was obtained from Sigma-Aldrich (Hamburg, Germany). Nuclease-free water for DNA extraction was purchased from Roth (Karlsruhe, Germany). LC-MS-grade water, methanol and formic acid were purchased from VWR international, Inc. (Dresden, Germany).

Preparation of stock solutions and calibration standards

Stock solutions of the standards were prepared by dissolving each analyte in LCMS-grade water at a concentration of 5 mM. All solutions were prepared freshly before analysis. The stock standard solutions were further diluted with water to yield the calibration standard concentrations of 0.05, 0.1, 0.5, 1, 2.5, 5 and 10% [5mdC]/[dG] with [dG] being 20 pmol. Finally formic, acid was analyzed after adding formic acid with 0.1% (v/v).

DNA extraction and hydrolysis

DNA was extracted using a QIAamp DNA Mini Kit from Qiagen (Hilden, Germany) together with an RNase A digestion according to the manufacturer's protocol. The content and purity of the collected RNA-free DNA was assessed spectrophotometrically at 230, 260 and 280 nm. Enzymatic DNA hydrolysis was performed using DNA Degradase Plus from Zymo Research (Freiburg, Germany). Briefly, 1 μ g of genomic DNA was mixed with 2.5 μ L 10X DNA Degradase Reaction Buffer, 1 μ L DNA Degradase Plus and filled up with water to a volume of 25 μ L. After 4 h at 37 °C the DNA digestion was stopped by adding 75 μ L of 0.1% formic acid. As a control 200 ng of the digested DNA was analyzed by agarose gel electrophoresis. 70 μ L DNA hydrolysis samples were further diluted with 280 μ L 0.1% formic acid to yield a final concentration of 2 ng digested DNA/ μ L.

LC-ESI-MS/MS Procedure

LC analysis was performed with an Agilent 1200 series HPLC system connected to an Agilent 6530 Accurate-Mass Q-TOF instrument with Jet Stream-Interface (Palo Alto, USA). For chromatographic separation a Waters (Milford, MA) X-BridgeTM C18 4.6 mm x 150 mm (3.5 µm particle size) protected by a Waters X-BridgeTM C18 4.6 mm x 20 mm guard column (5 µm particle size) was used. 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B) were chosen as mobile phases. The linear gradient elution was 4-20% of solvent B in 10 min at a constant flow rate of 0.5 mL/min. 50 µL diluted DNA hydrolysis samples were injected, typically containing 100 ng digested DNA. The optimized ESI-MS/ MS parameters in positive ion mode were as follows: gas temperature, 250 °C; drying gas flow, 8 L/min; nebulizer pressure, 60 psig; sheat gas temperature, 300 °C; capillary voltage 4000 V; collision energy 7 V for dC, 13 V for 5mdC and 10 V for dG. Quantification was accomplished in multiple reaction monitoring (MRM) mode by monitoring a transition pair of m/z 228.0979/112.0505 for dC, m/z 242.1135/126.0662 for 5mdC and m/z 268.1040/152.0780 for dG, which was used as an internal standard for the measurement. The scan time was 333 ms for each pair.

Percentage of methylation

The percentage of methylation was calculated as: Methylation % = [5mdC]/[dG] according to the calibration curve.

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Fig. 1. A: Full-scan spectrum (ESI-MS-spectrum) of a standard solution of 5mdC. Only one protonated [M+H]+ adduct of 5mdC is detectable at m/z 242.1135. B: A product ion spectrum of 5mdC (m/z 242.1135) can be found after the fragmentation, when the main [M+H]+ adduct of 5mdC appearsat m/z 126.0662.

Results and Discussion

Method development and validation Optimization of HPLC-ESI-MS/MS conditions

Figure 1A shows the full-scan spectrum (ESI-MS-spectrum) of a standard solution of 5mdC. With the optimized ESI-conditions only one protonated $[M+H]^+$ adduct of 5mdC is detectable at m/z 242.1135. No $[M+Na]^+$ but an $[2M+H]^+$ adduct occurred, having a sensitivity less than 3% of the main adduct. Figure 1B reports the product ion spectrum of 5mdC (m/z 242.1135). It can be found after the fragmentation, when the main $[M+H]^+$ adduct of 5mdC appears at m/z 126.0662. This ion originates due to the cleavage of the *N*-glycoside bond and transfer of a hydrogen atom from the sugar molecule [24]. Because these findings are comparable to the detection of dC and dG, the precursor/product ion pairs of m/z 228.0979/112.0505 for dC, m/z 242.1135/126.0662 for 5mdC and m/z 268.1040/152.0780 for dG were used as MRM transitions.

For high-sensitivity ESI-MS/MS measurements the ESI conditions were first optimized for the $[M+H]^+$ adduct of 5mdC, as shown in Figure 1A.

RNA contamination

Interference from RNA contamination poses a potential problem in the analysis of genomic DNA methylation [20]. In order to prevent those interferences, a sufficient DNA purification procedure with removal of RNA was chosen. In addition, an optimized gradient elution program with 0.1% formic acid in water and 0.1% formic acid in methanol was used to separate uracil and 5dmC, which typically elute at the same time. Figure 2 illustrates no interference of 5mdC with uracil.

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Fig. 2. A gradient elution program with 0.1% formic acid in water and 0.1% formic acid in methanol was used to separate uracil and 5dmC, which elute at the same time. There were no interference of 5mdC with uracil.



Fig. 3. Typical calibration curve of 5mdC. The limit of detection (LOD) of 5mdC (S/N = 3) was determined to be 10 fmol. The limit of quantification (LOQ) of 5mdC (S/N = 10) was found to be 50 fmol.



Calibration curves and sensitivity

Figure 3 shows a typical calibration curve of 5mdC. The limit of detection (LOD) of 5mdC (S/N = 3) was determined to be 10 fmol. The limit of quantification (LOQ) of 5mdC (S/N = 10) was found to be 50 fmol.

Calculation of genomic DNA methylation

Using an isotope labeled internal standard is one of the most accurate measurements of the methylation level in genomic DNA [25]. But it would get lost during sample preparation, where spin columns are used, which only adsorbs whole DNA molecules. Therefore usually, the global level of methylation is calculated as [5mdC]/([5mdC] + [dC]) using dC as internal standard. Since many modifications of dC are described, we decided to use the more stable dG instead, based on the assumption that in genomic DNA [dG] = [5mdC] + [dC] [20]. We calculated the grade of methylation in DNA as [5mdC]/[dG] and injected 100 ng of digested DNA, which conforms to ~ 20 pmol dG. For this reason calibration curves were prepared to imitate DNA hydrolysis products with 20 pmol of dG, dC and 0.5, 1, 2.5, 5 and 10% [5mdC]/[dG], as typically 2-6% of the mammalian DNA is methylated [26]. Figure 4 shows a LC-ESI-MS/MS chromatogram of a calibration mixture containing 20 pmol dG, dC and 2.5% [5mdC]/[dG].

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Fig. 4. LC-ESI-MS/MS chromatogram of a calibration mixture containing 20 pmol dG, dC and 2.5% [5mdC]/ [dG].



Fig. 5. A: Histogram showing the distribution of %mdC based on mdC/dG. B: Histogram showing the distribution of %mdC based on mdC/(mdC + dC).

Table 1. Intra-day precision of the LC-ES MS/MS method for the analysis of genom DNA methylation levels. a Relative standar deviation, b relative error

SI-		Human placenta DNA
ic	n	4
rd	Calculated mean [5mdC]/[dG] (%)	4.21
	RSD (%) ^a	0.06
	RE (%) b	0.03

Table 2. Robustness of the		Human placenta DNA	Hering sperm DNA	HeLa DNA
LC-ESI-MS/MS method for	n	6	3	3
the analysis of genomic DNA	Calculated mean			
mothylation lovals a Polativa	[5mdC]/[dG] (%)	4.27	2.63	7.10
ineuryiation levels. a Relative	RSD (%) a	0.16	0.23	0.26
standard deviation, b rela-	RE (%) b	0.06	0.13	0.15
tive error				

Accuracy, intra-day precision and robustness

Accuracy, intra-day precision and robustness of the LC-ESI-MS/MS method were assessed by measuring calibration curves and herring sperm DNA on five different days. Intra-day precision was determined by repeated analysis of standard curves at different

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times during the same day (n=2). Robustness was evaluated by repeated measurements of standard curves and hering sperm DNA on five different days. All in all, the method illustrates a very consistent and reliable method with overall low relative standard deviations (RSDs) and relative errors (REs) (Tables 1 and 2). The time interval between two different sample injections was 20 minutes. As the actual sample measurement is an automated process, this method is suitable for large sample sizes.

Clinical data

The results of the analysis of 248 placenta samples from the BBC study cohort [21–23] are shown in Fgure 5A. The degree of DNA methylation ranges from 2% to 5%. This high variability of DNA methylation is remarkable given the fact that this tissue is just 9 months old. Epigenetic alterations are thought to be acquired upon environmental challenges. This may indicate that differences in environmental toxic and/or nutritional exposure of pregnant women translated into epigenetic modulation of the overall placenta DNA methylation degree. If DNA methylation is present in promoters of genes that control key placenta function, differences in DNA methylation should result in differences in the offspring's phenotype. This should be addressed in future larger studies being adequately powered for such questions. In any case, it is important to note that the degree of placental DNA methylation varies substancially although the age of the tissue is just 9 months. Two ways of calculating the methylation degree are shown in Figure 5A and 5B. We suggest calculating the degree of DNA methylation as dmC/dG, because many modifications of dC are described and dG is more stable. This is justified by the assumption that in genomic DNA [dG] = [5mdC] + [dC] [20].

Conclusions

In conclusion, High Performance Liquid Chromatography– Electrospray Tandem Mass Spectrometry (HPLC-ESI-MS/MS) based quantification of the dmC/dG ratio is a reliable, stable method to determine global DNA methylation in large clinical trials. The degree of DNA methylation varies substantially in the human placenta. The clinical implications need to be demonstrated in adequately powered studies. Environmental challenges in early life seem to affect multiple (hundreds) of genes. This explains why we could detect such differences in global DNA methylation in our cohort. It is unclear so far whether the pattern of global DNA methylation is different upon different environmental stimuli. We believe that this might be the case, since different environmental stimuli cause different phenotypes in the offspring (1-3). Studies in the future need to test this hypothesis. We suggest that the method described here is a suitable screening method to decide whether or not an environmental challenge in early life may cause fetal programming. If this is the case, not yet existing methods needs to be applied to describe the most likely very complex patterns of DNA methylation.

References

- 1 Barker DJP: The developmental origins of adult disease. J Am Coll Nutr 2004;23:588S–595S.
- 2 Nakao M: Epigenetics: interaction of DNA methylation and chromatin. Gene 2001;278:25–31.
- 3 Skinner MK: Role of epigenetics in developmental biology and transgenerational inheritance. Birth Defects Research Part C: Embryo Today: Reviews 2011;93:51–55.
- 4 Koukoura O, Sifakis S, Spandidos DA: DNA methylation in the human placenta and fetal growth (review). Mol Med Rep 2012;5:883–889.
- 5 Fowden AL, Forhead AJ: Endocrine interactions in the control of fetal growth. Nestle Nutr Inst Workshop Ser 2013;74:91–102.
- 6 Hemberger M: Genetic-epigenetic intersection in trophoblast differentiation: implications for extraembryonic tissue function. Epigenetics 2010;5:24–29.

Cellular Physiology	Cell Physiol Biochem 2014;33:945-952	
and Biochemistry	DOI: 10.1159/000358666 Published online: March 31, 2014	© 2014 S. Karger AG, Basel www.karger.com/cpb

- 7 Feng S, Jacobsen SE, Reik W: Epigenetic reprogramming in plant and animal development. Science 2010;330:622–627.
- 8 Gheorghe CP, Goyal R, Mittal A, Longo LD: Gene expression in the placenta: maternal stress and epigenetic responses. Int J Dev Biol 2010;54:507–523.
- 9 Nomura Y, Lambertini L, Rialdi A, Lee M, Mystal EY, Grabie M, Manaster I, Huynh N, Finik J, Davey M, Davey K, Ly J, Stone J, Loudon H, Eglinton G, Hurd Y, Newcorn JH, Chen J: Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preeclampsia, and obesity. Reprod Sci 2014;21:131–137.
- 10 Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyselaers W, Nawrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22.
- 11 Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais JP, Junien C: Sex- and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5. DOI: 10.1371/journal.pone.0014398
- 12 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572.
- 13 Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionarydevelopmental perspective. Int J Obes (Lond) 2008;32:S62–71.
- 14 Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168.
- 15 Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004.
- 16 Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69.
- 17 Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21.
- 18 Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245–254.
- 19 Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071
- 20 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504– 510.
- 21 Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta]3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242.
- 22 Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692.
- 23 Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324– 11329.
- 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769.
- 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531.
- 26 Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A Biol Sci Med Sci 2005;60:4–9.

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Introduction

The 'fetal origin' hypothesis proposes that adulthood hypertension, insulin resistance, dyslipidaemia and non-insulin-dependent diabetes, which are connected to markedly increased rates of cardiovascular disease in adult life, originate through adaptation of the fetus to an early intrauterine environment. It has been suggested that not only maternal nutrition but also maternal exposure to toxins like alcohol, nicotine but also toxins in water and food in early life cause functional and structural changes of the newborn resulting in adulthood hypertension, insulin resistance and dyslipidaemia [1]. Epigenetics serves as an important mechanism capable of regulatin 19 ene transcription and linking events early in life to adult morbidity. It is comprised by heritable changes in chromatin the 25 liter gene expression without altering the DNA sequence [2–4]. Throughout gestation, the placenta plays a crucial role in controlling growth and development of the fetus. The placenta acts as an interface between the growing child and its environment, the mother. Nutritions, toxins and any environmental challenge of the pregnant mother act on the newborn via the placenta. Moreover, the placenta also produces specific hormones affecting both the mother and the growing child [5]. The placenta itself develops within several weeks from pluripotent cells to a highly differentiated organ. Its epigenome is critical for normal placental function [6], and plays an important role in programming events occurring during early phases of pregnancy. After fertilisation, both paternal and maternal genomes undergo demethylation [7]. Es 40 lishment of correct epigenetic patterns in the trop 35 blast is crucial for the formation of the fetal side of the placenta and epigenetic factors play an important role in placental maturation and development [8]. It is known that placental function can be influenced by the environment throughout pregnancy, thereby impacting on the appropriate genetic program-9 ing needed to allow for proper fetal growth [4]. Exposure to environmental toxins and nutrition during pregnancy may alter DNA methylation of the placenta and subsequently placental function [9-11]. As a result this, can also trigger epigenetic changes in the offspring, ultimately altering its phenotype [12]. It is believed that these epigenetic alterations lead to a stable reset of key endocrine and structural properties of the offspring, thus potentially causing disease in later life [13].

DNA methylation, which is the best understood DNA epigenetic modification, may provide an attractive mechanism linking environmental cues to placental pathology, with nsequences for fetal growth and adult life [4]. Methylation in vertebrate DNA is, in general, restricted to cytosine (C) nucleotides in the sequence Cytosin-Guanin (annotated CpG). The overall frequency of (30) dinucleotides in the vertebrate genome is low, but there are small stretches of DNA that are characterized 8 having CpG dinucleotides, extending for hundreds of bases, termed CpG islands [14–16]. The methylation status of cytosine residues, within CpG dinucleotides and in the context of CpG islands, 39 wides an important mechanism for controlling gene expression activity [14, 16, 17]. Changes in the methylation status (hyper- or hypomethylation) have been associated with various health conditions including malignancies [18]. Besides affecting gene specze: methylation patterns [11, 12], recent studies demonstrated that environmental cues impact on the global methylation status 6 the placenta [9-11, 19]. To shed more light on the consequences of this phenomenon, it is important to analyze the degree of placental DNA methylation in large clinical studies in relationship to clinical readouts. For doing so, the establishment of a suitable method to quantify placental DNA methylation is thus urgently needed. In the current study, we developed a reliable method to quantify DNA methylation in the human placenta based on previous work by Song and colleagues [20].

Materials and Methods

Clinical study 21 We used 248 placenta samples collected as part of the Berlin Birth Cohort study [21–23]. The study was approved by the local ethics committee. Biometric data of the newborn were measured during the routine

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Putra et al.: DNA Methylation Levels in Human Placenta

postnatal examination. Gestational age at delivery was based on last menstrual period, anamnestically assessed **34** ng the first pregnancy examination. The following data of the newborn were added to the database: birth weight, birth length, head circumference, ponderal index, child s**13** Apgar score 5 minutes postnatally, Apgar score **13** minutes postnatally and umbilical blood pH levels. Fetal blood was collected from the umbilical cord. Midwives collected maternal blood from a cubital vein in the delivery room or on the ward. Placenta was collected and frozen at -20°C immediately after the placenta was born.

Analysis of DNA Methylation

The deoxyribonucleosides 2'-deoxyguanosine (dG) monohydrate, 5-methyl-2'-deoxycytidine (5mdC) and 2'-deoxycytidine (dC) were purchased from ABCR (Karlsruhe, Germany). Hering sperm DNA was obtained from Sigma-Aldrich (Hamburg, Germany). Nuclease-free water for DNA extraction was purchased from Roth (Karlsruhe, Germany). LC-MS-grade water, methanol and formic acid were purchased from VWR international, Inc. (Dresden, Germany).

Preparation of stock solutions and calibration standards

Stock solutions of the standards were prepared by dissolving each analyte in LCMS-grade water at a concentration of 5 mM. All solutions were are further diluted with water to yield the calibration standard concentrations of 0.05, 0.1, 0.5, 1, 2.5, 5 and 10% [5mdC]/[dG] with [dG] being 20 pmol. Finally formic, acid was analyzed after adding formic acid with 0.1% (v/v).

20 A extraction and hydrolysis

DNA was ext 27 ed using a QlAamp DNA Mini Kit from Qiagen (Hilden, Germany) together with an RNase A digestion according to the manufacturer's protocol. The content and purity of the collected RNAfree DNA was assessed spectrophotometrically at 230, 260 and 280 nm. Enzymatic DNA hydrolysis was rformed using DNA Degradase Plus from Zymo Research (Freiburg, Germany). Briefly, 1 µg of genomic DNA was mixed with 2.5 µL 10X DNA Degradase Reaction Buffer, 1 µL DNA Degradase Plus and filled up with water to a volume of 25 µL. After 4 h at 37 °C the DNA digestion was stopped by adding 75 µL of 0.1% formic acid. As a control 200 ng of the digested DNA was analyzed by agarose gel electrophoresis. 70 µL DNA hydrolysis samples were further diluted with 280 µL 0.1% formic acid to yield a final concentration of 2 ng digested DNA/µL.

LC-ESI-MS/MS Procedure

LC analysis was performed with an Agilent 1200 series HPLC system connected to an Agilent 6530 Accurate-Mass Q-TOF instrument with Jet S 23 m-Interface (Palo Alto, USA). For chromatographic separation a Waters (M33 rd, MA) X-BridgeTM C18 4.6 mm x 150 mm (3.5 µm pa 3 cle size) protected by a Waters X-BridgeTM C18 4.6 mm x 20 mm guard column (5 µm particle size) was used. 0.1% formic acid in water (solvent A) and 0.1% for 13 acid in methanol (solvent B) were chosen as mobile phases. The linear gradient elution was 4-20% of solvent B in 10 min at a constant flow rate of 0.5 mL/min. 50 µL diluted DNA hydrolysis samples were injected, typically containing 100 ng digested DN 7 The optimized ESI-MS/ MS parameters in positive ion mode were as follows: gas temperature, 250 °C; drying gas flow, 8 L/min; nebulizer pressure, 60 psig sheat 12 temperature, 300 °C; capillary voltage 4000 V; collision energy 7 V for dC, 13 V for 5mdC and 10 V for dG. Quantification was accomplished in multiple reaction monitoring (MRM) mode by monitoring a transition pair of m/z 228.0979/112.0505 for dC, m/z 242.1135/126.0662 for 5mdC and m/z 268.1040/152.0780 for dG, which was used as an internal standard for the measurement. The scan time was 333 ms for each pair.

Percentage of methylation

The percentage of methylation was calculated as: Methylation % = [5 mdC]/[dG] according to the calibration curve.



Fig. 1. A: Full-scan spectrum (ESI-MS-spectrum) of a standard solution of 5mdC. Only one protonated [M+H]+ adduct of 5mdC is detectable at m/z 242.1135. B: A product ion spectrum of 5mdC (m/z 242.1135) can be found after the fragmentation, when the main [M+H]+ adduct of 5mdC appearsat m/z 126.0662.

Results and Discussion

Method development and validation

Optimization of HPLC-ESI-MS/MS conditions

Figure 1A shows the full-scan spectrum (ESI-MS-spectrum) of a standard solution of 5mdC. With the optimized ESI-conditions only one protonated $[M+H]^*$ adduct of 5mdC is detectable at m/z 242.1135. No $[M+Na]^*$ but an $[2M+H]^*$ adduct occurred, having a sensitivity less than 3% of the main adduct. Figure 1B reports the product ion spectrum of 5mdC (m/z 242.1135). It can be found after the fragmentation, w15 the main $[M+H]^*$ adduct of 5mdC appears at m/z 126.0662. This ion originates due to the cleavage of the *N*-glycoside bond and transfer of a hydrogen atom from the sugar molecule [24]. Because these findings are comparable to the detection of dC and dG, the precursor/product ion pairs of m/z 228.0979/112.0505 for dC, m/z 242.1135/126.0662 for 5mdC and m/z 268.1040/152.0780 for dG were used as MRM transitions.

For high-sensitivity ESI-MS/MS measurements the ESI conditions were first optimized for the $[M+H]^*$ adduct of 5mdC, as shown in Figure 1A.

RNA contamination

Interference from RNA contamination poses a potential problem in the analysis of genomic DNA methylation [20]. In order to prevent those interferences, a sufficient DNA purification procedure with removal of RNA was chosen. In addition, an optimized gradient elution program with 0.1% formic acid in water and 0.1% formic acid in methanol was used to separate uracil and 5dmC, which typically elute at the same time. Figure 2 illustrates no interference of 5mdC with uracil.

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Fig. 2. A gradient elution program with 0.1% formic acid in water and 0.1% formic acid in methanol was used to separate uracil and 5dmC, which elute at the same time. There were no interference of 5mdC with uracil.



Fig. 3. Typical calibration curve of 5mdC. The limit of detection (LOD) of 5mdC (S/N = 3) was determined to be 10 fmol. The limit of quantification (LOQ) of 5mdC (S/N = 10) was found to be 50 fmol.



Calibration curves and sensitivity

Figure 3 shows a typical calibration curve of 5mdC. The limit of detection (LOD) of 5mdC (S/N = 3) was determined to be 10 fmol. The limit of quantification (LOQ) of 5mdC (S/N = 10) was found to be 50 fmol.

Calculation of genomic DNA methylation

Using an isotope labeled internal standard is one of the most accurate measurements of the methylation level in genomic DNA [25]. But it would get lost during sample preparation, where spin columns are used, which only adsorbs whole DNA molecules. Therefore usually, the global level of methylation is calculated as [5mdC]/([5mdC] + [dC]) using dC as internal standard. Since many modifications of dC are described, we decided to use the more stable dG instead, based on the assumption that in genomic DNA [dG] = [5mdC] + [dC] [20]. We calculated the grade of methylation in DNA as [5mdC]/[dG] and injected 100 ng of digested DNA, which conforms to ~ 20 pmol dG. For this reason calibration curves were prepared to imitate DNA hydrolysis products with 20 pmol of dG, dC and 0.5, 1, 2.5, 5 and 10% [5mdC]/[dG], as typically 2-6% of the mammalian DNA is methylated [26]. Figure 4 shows a LC-ESI-MS/MS chromatogram of a calibration mixture containing 20 pmol dG, dC and 2.5% [5mdC]/[dG].

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Fig. 4. LC-ESI-MS/MS chromatogram of a calibration mixture containing 20 pmol dG, dC and 2.5% [5mdC]/ [dG].



Fig. 5. A: Histogram showing the distribution of %mdC based on mdC/dG. B: Histogram showing the distribution of %mdC based on mdC/(mdC + dC).

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Table 1. Intra-day precision of the LC-ES MS/MS method for the analysis of genom DNA methylation levels. a Relative standar deviation, b relative error

	Human placenta DNA
n	4
Calculated mean [5mdC]/[dG] (%)	4.21
RSD (%) a	0.06
RE (%) b	0.03

Table 2. Robustness of the		Human placenta DNA	Hering sperm DNA	HeLa DNA
LC-ESI-MS/MS method for	n	6	3	3
the analysis of genomic DNA	Calculated mean	5.00 m	and the set	11.000-214
methylation levels a Balativa	[5mdC]/[dG] (%)	4.27	2.63	7.10
methylation levels, a Relative	RSD (%) a	0.16	0.23	0.26
standard deviation, b rela-	RE (%) b	0.06	0.13	0.15
tive error				

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Accuracy, intra-day precision and robustness

Accuracy, intra-day precision and robustness of the LC-ESI-MS/MS method were assessed by measuring calibration curves and herring sperm DNA on five different days. Intra-day precision was determined by repeated analysis of standard curves at different

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times during the same day (n=2). Robustness was evaluated by repeated measurements of standard curves and hering sperm DNA on five different days. All in all, the method illustrates a very consistent and reliable method with overall low relative standard deviations (RSDs) and relative errors (REs) (Tables 1 and 2). The time interval between two different sample injections was 20 minutes. As the actual sample measurement is an automated process, this method is suitable for large sample sizes.

Clinical data

The results of the analysis of 248 placenta samples from the BBC study cohort [21–23] are shown in Fgure 5A. The degree of DNA methylation ranges from 2% to 5%. This high variability of DNA methylation is remarkable given the fact that this tissue is just 9 months old. Epigenetic alterations are thought to be acquired upon environmental challenges. This may indicate that differences in environmental toxic and/or nutritional exposure of pregnant women translated into epigenetic modulation of the overall placenta DNA methylation degree. If DNA methylation is present in promoters of genes that control key placenta function, differences in DNA methylation should result in differences in the offspring's phenotype. This should be actor reserves in future larger studies being adequately powered for such questions. In any case, it is important to note that the degree of placental DNA methylation degree are shown in Figure 5A and 5B. We suggest calculating the degree of DNA methylation as dmC/dG, because many modifications of dC are described and dG is more stable. This is justified by the assumption that in genomic DNA [dG] = [5mdC] + [dC] [20].

Conclusions

In conclusion, High Performance Liquid Chromatography– Electrospray Tandem Mass Spectrometry (HPLC-ESI-M 33 MS) based quantification of the dmC/dG ratio is a reliable, stable method to determine global DNA methylation in large clinical trials. The degree of DNA methylation varies substantially in the human placenta. The clinical implications need to be demonstrated in adequately powered studies. Environmental challenges in early life seem to affect multiple (hundreds) of genes. This explains why we could detect such differences in global DNA methylation in our cohort. It is unclear so far whether the pattern of global DNA methylation is different upon different environmental stimuli. We believe that this might be the case, since different environmental stimuli cause different phenotypes in the offspring (1-3). Studies in the future need to test this hypothesis. We suggest that the method described here is a suitable screening method to decide whether or not an environmental challenge in early life may cause fetal programming. If this is the case, not yet existing methods needs to be applied to describe the most likely very complex patterns of DNA methylation.

References

- Barker DJP: The developmental origins of adult disease. J Am Coll Nutr 2004;23:588S-595S.
- Nakao M: Epigenetics: interaction of DNA methylation and chromatin. Gene 2001;278:25–31.
- Skinner MK: Role of epigenetics in developmental biology and transgenerational inheritance. Birth Defects Research Part C: Embryo Today: Reviews 2011;93:51–55.
- 4 Koukoura O, Sifakis S, Spandidos DA: DNA methylation in the human placenta and fetal growth (review). Mol Med Rep 2012;5:883–889.
- 5 Fowden AL, Forhead AJ: Endocrine interactions in the control of fetal growth. Nestle Nutr Inst Workshop Ser 2013;74:91–102.
- 6 Hemberger M: Genetic-epigenetic intersection in trophoblast differentiation: implications for extraembryonic tissue function. Epigenetics 2010;5:24–29.

 Part at J. DAM Methylation Letters Part at J. DAM Methylation Letters Peng S, Jacobsen SE, Reik W: Epigenetic reprogramming in plant and animal development. Science 2010;330:622–627. Gheorghe CP, Goyal R, Mittal A, Longo LD: Gene expression in the placenta: maternal stress and epigenetic responses. Int J Dev Biol 2010;55:657–523. Nomura Y, Lambertini L, Riddi A, Lee M, Mystal EY, Grabie M, Manaster J, Huynh N, Finik J, Davey M, Davey K, Ly J, Stone L, Loudon H, Eglinton G, Hurd Y, Nevcore JH, Chen E (Jobah methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preeclampsia, and obesity. Reprod Sci 2014;21:131–137. Jamsen BG, Godderis L, Pieters N, Poels K, Kid Ski M, Cuypers A. Fierens E, Penders J, Plusquin M, Gyselaers W, Navrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:202. Gallou-Kabani C, Gabory A, Tost J, Karrini M, Nayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Bernde C, Vieau D, Ekstrom TJ, Jiais JP, Junien C. See-and Dies S, Specif Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5::D101: 101377 Journalpone. 0014398. Filiberto AC, Maccani MA, Koestler D, Wihlem-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marst C: Brithweight is associated with DNA promoter methylation of the glucocorticol receptor in human placenta. Epigenetics 2011:66:65–72. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary: developmental partspreve. Int J Obes Chong 2003;25:27-11. Nafer TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BIOG 2006;15:15:156-16. Trasler JM: Gamete imprinting setting epigenetic pathways to baseity: an evolutionary: developmental and epigenetic methylaton of the cyten	3 3 7.0	and Biochemistry	DOI: 10.1159/000358666	© 2014 S. Karger AG, Basel	Q
 Feng S. Jacobsen SE, Reik W: Epigenetic reprogramming in plant and animal development. Science 2010;3:30:622–627. Gheorghe CF, Goyal R, Mittal A, Longo LD: Gene expression in the placenta: maternal stress and epigenetic responses. Int J Dev Biol 2010;5:4:507–523. Nomura Y, Lambertini L, Ralali A, Lee M, Mystal EY, Grabie M, Manaster J, Huynh N, Finik J, Davey M, Davey K, Ly J, Stone J, Loudon H, Eglinton G, Hurd Y, Newcorn JH, Chen J: Global methylation in the placenta and umblical cord blood from pregnancies with maternal gestational diabetes. preeclampsia, and obesity. Reprod Sci 2014;21:131–137. Joansen BG, Godderis L, Pieters N, Poels K, Kird Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyzelaers W, Navrot TS: Placental DM hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22. Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Rousens B, Remacle C, Vieau D, Elström TJ, Jais JP, Junien C. Sze- and Diet-Specific Changes of Imprinted Gene Expression and DMA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: DOI: 10.1371/journal.pone.0014398 Filterb AG, Maccard MA, Kosselt CP, Wilhelm-Benartzi C, Avissar-Wihting M, Banister CE, Gagne LA, Marsit CJ: Birthweight & associated with DNA promoter methylation of the glucocorticol receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmenta. Being episters 3: 3rd ed New York, London, Garland Science, 2004. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Finsler JM: Gametei imprinting: setting epigenetic pathways to obesity entropic hervite developmenta. Jul Science 19: 39: 303–324–34. Wenhoranta H, Li S, Sala		and blochernistry	Putra et al.: DNA Methylation Levels in	Human Placenta	5.
 Feng S, Jacobsen SE, Reik W: Epigenetic reprogramming in plant and animal development. Science 2010;330:622-627. Gheorghe CJ, Goyal K, Mittal A, Longo LD: Gene expression in the placenta: maternal stress and epigenetic responses. Int J Dev Biol 2010;54:507-523. Nomura Y, Lambertin I, Raidi A, Lee M, Mystal EY, Grabie M, Manaster J, Huynh N, Finik J, Davey M, Davey K, Ly J, Stone J, Loudon H, Eglinton G, Hurd Y, Newcorn JH. Chen J: Global methylation in the placenta and umbilical corb blood from pregnancics with maternal gestational diabetes, preeclampsia, and obesity. Reprod Sci 2014;21:131-137. Janssen BG, Godderis L, Pitzerts N, Poels K, Kici Ski M, Cuypers A, Fierens F. Penders J, Plusquin M, Gyselaers W, Navrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fiber Toxicol 2013;10:22. Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacke C, Vieau D, Ekström TJ, Jais P, Junien C. See: and Dite-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet PLoS One 2010;5. DOI: 10.1371/journal.pone.0014398 Filberto AC, Maccani MA, Koessler D, Wilhelm-Benarti C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit G, Birthweight is associated with DNA promoter methylation of the gluccoerticoid receptor in human placenta. Epigenetics 2011;6:566–572. Gulcuman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmenta. Epigenetic sol 10:000:2003;2:S62–71. Nariee TM, Farrell WL, Garrell UN, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJ0G 2008;115:158–168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Finsler M: Camere li					
 2010;330:622-627. 87. Gheorghe CF, Goyal R, Mittal A, Longo LD: Gene expression in the placenta: maternal stress and epigenetic responses. Int J Dev Biol 2010;54:507-523. 99. Nonura Y. Lambertini L, Rialdi A, Lee M, Mystal EY, Grabie M, Manaster J, Huynh N, Finik J, Davey M, Davey K, Ly J, Stone J, Loudon H, Eglinton G, Hurd Y, Newcorn JH, Chen J: Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabets, preeclampsia, and obesity. Reprod 62: (2014;21:131-137. 91. Janssen BG, Godderis L, Pieters N, Pools K, Kit Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyselaers W, Nawro TS: Placental DM Nypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22. 91. Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Brreton C, Reussen B, Remacle C, Vieuau D, Ekström TJ, Jais JP, Junien C: Sex- and Diet Specific Changes of Imprinted Gene Expression and DNA Methylaton in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5:: D01: D13771/journal.ponc.0014398 91. Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsti C; Burthweight is associated with DNA promoter methylation of the glucocorticol receptor in human placents. Epigenetics 2011;6:566-572. 91. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental persetty. Int JOB 66:65-727. 91. Rafer JM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJ0G 2008;115:158-168. 91. Strachah T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. 91. Trasler JM: Ganneti pathetras and epigenetic memory. Genes Dev 2002;166-21. 91. Bird A: DNM ant Epigenetic regulation of gene expression: how the genomic integrates intrinsic and environmental signals. Nat Genet 2003;3	▶7	Feng S, Jacobsen SE, Reik W: Ep	igenetic reprogramming in plant a	nd animal development. Science	
 Onto apple CP (2007) IC mitro Longe JDS concerned expression in the placetime intervent in stress into placetice responses. Int J Dev Biol 2010;54:507–523. Nomura Y, Lambertini L, Rialdi A, Lee M, Mystal EY, Grabie M, Manaster J, Haynh N, Finik J, Davey M, Davey K, Ly J, Stone L, Loudon H, Eglinton G, Hurd Y, Nevcorn JH, Chen J: Global methylation in the placetta and umbilical cord blood from prognancies with maternal gestational diabetes, preeclampsia, and obesity. Reprod Sci 2014;21:131–137. Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyselaers W, Nawrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fiber Toxicol 2013;10:22. Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Visau D, Ekström TJ, Jia JP, Jhuten C: Soc: and Diet/Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placents under a High-Fat Diet PLoS One 2010;5. DOI: 10.1371/journal.pone.0014398 Filibreto AC, Maccani MA, Koester D, Wildhen-Benarzti C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticol receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:562–71. Nafer TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJ0G 2008;115:159–168. Strachan T: Human molecular genetics 3.3rd ed New York, London, Garland Science, 2004. Trasler JM, Gameet Bundowska T, Andresson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-QG Hypermethylation patterns and epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: D	8	2010;330:622-627. Cheorghe CP Goval R. Mittal A	Longo ID: Gene expression in the	alacenta-maternal stress and enigenetic	
 Nomura Y, Lambertini L, Rialdi A, Lee M, Mystal EY, Grabie M, Manaster L, Huynh N, Finik J, Davey K, Ly J, Stone J, Loudon H, Eglinton G, Hurd Y, Newcorn JH, Chen J: Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preclamspia, and obesity. Reprod Sci 2014;21:131–137. Jonssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyselaers W, Nawrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22. Galou-Kabani C, Gabory A, Tos J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais JP, Junien C: Sex- and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fa Diet PLoS One 2010;5: DOI: 10.1371/journal.pone.0014398 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsti CJ: Birthweight is associated with DNA promoter methylation of the gluccocritoid receptor in human placenta. Epigenetics 2011;6:566–572. Gluckama PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:S62–71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KM: Epigenetic control of fetal gene expression. BJOG 2006;115:158–168. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Tracher JM: Manethylation patterns and epigenetic memory. Genes Dev 2002;16:6-21. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21. Jense KR, Katzani K, Kari AK Specific methols of the next generation. Reprod Fertil Dev 2006;18:63-69. Torchar L, Sziamio K, Spittskowska T: Andresson M. Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG	- 0	responses. Int J Dev Biol 2010;5	54:507–523.	Sacenta. Inaternal suless and epigenetic	
 K, Ly J, Stone J, Loudon H, Eglinton G, Hurd Y, Newcorn JH, Chen J: Global methylation in the placenta and umbilical cord blood from pregnancies with maternal gestational diabetes, preeclampsia, and obesity. Reprod Sci 2014;21:131–137. Janssen BG, Godderis L, Pieters N, Poels K, Kid Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyselaers W, Navro TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22. Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais JP, Junien C: Sex- and Die-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: D010: 10:1371/journalpone.0014398 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit C, Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;5:66–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond 2008;2:562–71. Mafee TM, Farrell WE, Carroll WD, Fryer AA, Ismali KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird & Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Gene 2003;33:2542–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K. Non -Q6 hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Com03:33:2542–254. Venhoranta H, Li S, Salamon S	▶9	Nomura Y, Lambertini L, Rialdi	A, Lee M, Mystal EY, Grabie M, Man	aster I, Huynh N, Finik J, Davey M, Davey	
 umbuicht cord blood Program Degrancies with maternal gestational diadeets, Preclampsa, and obesty. Reprod Sci 2014;211-137. Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A, Fierens F, Fenders J, Plusquin M, Gyselaers W, Navro TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22. Gallou-Kabani C, Gabory A, Tos J, Karimi M, Mayeur S, Lesage J, Boudal E, Gross MS, Taurelle J, Vigé A, Brreton C, Reusens B, Remacle C, Vieua D, Ekström TJ, Jais PJ, Junien C: Sex and Die-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: DOI: 10.1371/journal.pone.0014398 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whitting M, Banister CE, Gagne LA, Marsit C, Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:62–71. Nafeer TM, Farrell WE, Carroll UD, Fryer AA, Ismali KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete Imprinting setting epigenetic memory. Genes Dev 2002;16:6–21. Jaentsch R, Bird & Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245–254. Venhorantat H, Li S, Salamon S, Filskowska T, Andersson M, Switonski M, Kind A, Schnieke A, Filskowski K: Non-QG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foretuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.birce.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation b		K, Ly J, Stone J, Loudon H, Eglint	ton G, Hurd Y, Newcorn JH, Chen J:	Global methylation in the placenta and	
 Janssen BG, Godderis L, Pieters N, Poels K, Kici Ski M, Cuypers A, Fierens F, Penders J, Plusquin M, Gyselaers W, Nawrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fiber Toxicol 2013;10:22. Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais JP, Junien C: Sex- and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placentu under a High-Fat Diet. PLoS One 2010;5: D01: 10.1371/journal.pone.0014398 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avisar-Whiting M, Banister CE, Gagne LA, Marrit CJ: Birthweight Is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572. Guchema PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:S62–71. Nafeer TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2006;18:63–68. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33::2245–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson H, Switonski M, Kind A, Schnieke A, Flisikowski K: No-CyG hypermethylation in Jaecenta of mutation-induced intrautering growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination		Reprod Sci 2014:21:131–137.	nancies with maternal gestational	diabetes, preeclampsia, and obesity.	
 Gyselaers W, Nawrot TS: Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol 2013;10:22. III Gallou-Kahani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais JP, Junien C: See: and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: DOI: 10.1371/journal.pone.0014398 Filberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obsc (Lond) 2008;32::S62–71. Mafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMI: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Stracham T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33::245–254. Wenhoranta H, Li, S. Salamon S, Filiskowska T. Andresson M. Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Sowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] Subwinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] Subwinski T, Sto	▶10	Janssen BG, Godderis L, Pieters	N, Poels K, Kici Ski M, Cuypers A, I	Fierens F, Penders J, Plusquin M,	
 early life. Part Fibre Toxicol 2013;10:22. P11 Gallou-Kabani C, Gabory A, Tost J, Karimi M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Vigé A, Breton C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais JP, Junien C: Sex- and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: DOI: 10.1371/journal.pone.0014398 P12 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in huma placenta: Epigenetics 2011;6:566-572. P13 Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:562-71. P14 Nafee TM, Farrell WC, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158-168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. P15 Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2020;16:6-21. P16 Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:245-254. P 4 Nahoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced Intrautering growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DDI: 10.1016/j.bbrc.2014.01.071 P20 Song L, James SK, Kazim L, Kazyf AK: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504-510. P14 Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241-1242. P16 Th Slowinski T,		Gyselaers W, Nawrot TS: Placen	tal DNA hypomethylation in associ	iation with particulate air pollution in	
 Gallou-Kabani L, Gabory A, Tost J, Kamm M, Mayeur S, Lesage J, Boudadi E, Gross MS, Taurelle J, Yige A, Bretor C, Reusens B, Remacle C, Vieau D, Ekström TJ, Jais PJ, Junien C. Sex- and Diet-Specific Changes of Imprinted Gene Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: D01: 10.1371/journal.pone.0014398 Filberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary- developmental perspective. Int J Obes (Lond) 2008;32:562–71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2000;115:158–168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental ajgnals. Nat Genet 2003;33:2545–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; Jobi: 10.1016;Jobrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504– 510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] S subun		early life. Part Fibre Toxicol 201	3;10:22.		
 breson Expression and DNA Methylation in Mouse Placenta under a High-Fat Diet. PLoS One 2010;5: D01: 10.1371/journal.pone.0014396 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6::566-572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond J 2008;32::562-71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115::158-168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic memory. Genes Dev 2002;16:6-21. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245-254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Kon-CpG hypermethylation in placenta of mutation-induced intrautering growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504-510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] subunit 8257 allele with low birthweight. The Lancet 2000;352:1241-1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fe	▶11	Gallou-Kabani C, Gabory A, Tost Breton C, Beusens B, Bemacle C	: J, Karimi M, Mayeur S, Lesage J, Bo Vieau D, Ekström TL Jais IP, Junio	oudadi E, Gross MS, Taurelle J, Vigé A,	
 2010;5. DOI: 10.1371/journal.pone.0014398 Fillberto AC, Maccani MA, Koestler D, Wihlelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:562–71. Mafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jennorantental signals. Nat Genet 2003;33:245–254. Venhorantat H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] submit 8257 aldee with low birthweight. The Lancet 2000;351:241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Girculation 2006;114:1687–1692. Natr AV, Hocher B, Verkaart S, van Zeelan		Imprinted Gene Expression and	DNA Methylation in Mouse Placer	ita under a High-Fat Diet. PLoS One	
 Filiberto AC, Maccani MA, Koestler D, Wilhelm-Benartzi C, Avissar-Whiting M, Banister CE, Gagne LA, Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566–572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:562–71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Strachan T: Human molecular genetics 3: 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic memory. Genes Dev 2002;16:6–21. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;32:542–524. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SK, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T. Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Piba T, Slowinski T, Stolze T, Pleschka A, Neumayer HB, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Piba T, Slowinski T, Golze S, Pieschka A, Neumayer HB, Halle H: Association of Tradioxascular diseases in later life, is al		2010;5. DOI: 10.1371/journal.p	one.0014398	0	
 Marsit CJ: Birthweight is associated with DNA promoter methylation of the glucocorticoid receptor in human placenta. Epigenetics 2011;6:566-572. Gluckman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:562-71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158-168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63-69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21. Jaenisch R, Bird A: Epigeneti regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245-254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karryf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504-510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241-1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1667-1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schillmgnam KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced	▶12	Filiberto AC, Maccani MA, Koest	tler D, Wilhelm-Benartzi C, Avissar	-Whiting M, Banister CE, Gagne LA,	
 Iniman placenta. Epigenetics 2011;6:566-572. Iakexman PD, Hanson MA: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. Int J Obes (Lond) 2008;32:562-71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158-168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63-69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:5245-254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504-510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241-1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Girculation 2006;114:1687-1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schilingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc		Marsit CJ: Birthweight is associa	ated with DNA promoter methylati	ion of the glucocorticoid receptor in	
 developmental perspective. Int J Obes (Lond) 2008;32:562–71. Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Bienisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:5245–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schilingmann KP, Schaller A, Gallati S, Bindels BI, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu J,	▶13	Gluckman PD Hanson MA: Dev	111;6:566-572. elonmental and enigenetic nathwa	vs to obesity: an evolutionary-	
 Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KMK: Epigenetic control of fetal gene expression. BJOG 2008;115:158–168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:5245–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu H, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance	15	developmental perspective. Int	J Obes (Lond) 2008;32:S62–71.	ys to obesity, an evolutionary	
 2008;115:158–168. Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245–254. Yenhoranta H, Li S, Salamon S, Flisk kowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Te	▶14	Nafee TM, Farrell WE, Carroll W	/D, Fryer AA, Ismail KMK: Epigenet	tic control of fetal gene expression. BJOG	
 Strachan T: Human molecular genetics 3. 3rd ed New York, London, Garland Science, 2004. Trasler JM: Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006;18:63–69. Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;9		2008;115:158-168.			
 Piol Trainer JM. Gameer implificing secting epigenetic patterns for the hext generation. Reprod Feb theor 2006;18:63–69. Pird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:5245–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504– 510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324– 11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess geno	15	Strachan T: Human molecular g	enetics 3. 3rd ed New York, Londo setting enigenetic patterns for the	n, Garland Science, 2004.	
 Bird A: DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6–21. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:5245–254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;87:2765–2769. Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–45	10	2006:18:63-69.	setting epigenetic patterns for the	next generation. Replot Per til Dev	
 Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33:S245-254. Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commu 2014; DOI: 10.1016/j.bbrc.2014.01.071 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504-510. Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] subunit 825T allele with low birthweight. The Lancet 2000;355:1241-1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687-1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324-11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;87:8765-2769. Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526-4531. Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-R	▶17	Bird A: DNA methylation patter	ns and epigenetic memory. Genes	Dev 2002;16:6–21.	
 environmental signals. Nat Genet 2003;33:S245–254. 19 Venhoranta H, Li S, Salamon S, Flisikowska T, Andersson M, Switonski M, Kind A, Schnieke A, Flisikowski K: Non-CpG hypermethylation in placenta of mutation-induced intrauterine growth restricted bovine foetuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 20 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. 21 Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta]3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. 22 Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. 23 Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. 26 Kyoung Kim H, Kyong Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular S	▶18	Jaenisch R, Bird A: Epigenetic re	egulation of gene expression: how	the genome integrates intrinsic and	
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 fortuses. Biochem Biophys Res Commun 2014; DOI: 10.1016/j.bbrc.2014.01.071 20 Song L, James SR, Kazim L, Karpf AR: Specific method for the determination of genomic DNA methylation by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. 21 Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. 22 Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. 23 Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. 26 Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 	1 9	K: Non-CnG hypermethylation i	n placenta of mutation-induced in	15KI M, KIND A, SCHNEKE A, FIISIKOWSKI trauterine growth restricted hovine	
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 by liquid chromatography-electrospray ionization tandem mass spectrometry. Anal Chem 2005;77:504–510. 21 Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. 22 Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. 23 Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. 26 Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 	▶20	Song L, James SR, Kazim L, Karp	of AR: Specific method for the deter	rmination of genomic DNA methylation	
 510. 21 Hocher B, Slowinski T, Stolze T, Pleschka A, Neumayer HH, Halle H: Association of maternal G protein [beta] 3 subunit 825T allele with low birthweight. The Lancet 2000;355:1241–1242. 22 Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. 23 Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. 26 Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 		by liquid chromatography-elect	rospray ionization tandem mass sp	pectrometry. Anal Chem 2005;77:504-	
 Petal 3 suburits 25 Tallele with low birthweight. The Lancet 2000;355:1241–1242. Pfab T, Slowinski T, Godes M, Halle H, Priem F, Hocher B: Low birth weight, a risk factor for cardiovascular diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687–1692. Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 	▶21	510. Hocher B. Slowinski T. Stolze T.	Pleschka A. Neumaver HH. Halle H	· Association of maternal Corrotain	
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 diseases in later life, is already associated with elevated fetal glycosylated hemoglobin at birth. Circulation 2006;114:1687-1692. 23 Nair AV, Hocher B, Verkaart S, van Zeeland F, Pfab T, Slowinski T, Chen YP, Schlingmann KP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324-11329. 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765-2769. 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526-4531. 26 Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 	▶22	Pfab T, Slowinski T, Godes M, Ha	alle H, Priem F, Hocher B: Low birth	n weight, a risk factor for cardiovascular	
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 Nair AV, Hocher B, Verkaart S, Van Zeeland F, Prab J, Stowinski J, Chen FP, Schlingmann RP, Schaller A, Gallati S, Bindels RJ, Konrad M, Hoenderop JG: Loss of insulin-induced activation of TRPM6 magnesium channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 	D 22	2006;114:1687-1692.	an Zaaland F. Dfah T. Claudaaki T. C	kan VD Caklin awa an VD Cakallan A	
 channels results in impaired glucose tolerance during pregnancy. Proc Natl Acad Sci USA 2012;109:11324–11329. 24 Hu J, Zhang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. 25 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. 26 Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 	- 25	Gallati S. Bindels RI. Konrad M.	an Zeeland F, Prab I, Slowinski I, C Hoenderop IG: Loss of insulin-indu	iced activation of TRPM6 magnesium	
 11329. Lugardian Marken Ma		channels results in impaired glu	cose tolerance during pregnancy.	Proc Natl Acad Sci USA 2012;109:11324–	
 Lang W, Ma H, Cai Y, Sheng G, Fu J: Simultaneous determination of 8-hydroxy-2'-deoxyguanosine and 5-methyl-2'-deoxycytidine in DNA sample by high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2765–2769. Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 		11329.			
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 Friso S, Choi S-W, Dolnikowski GG, Selhub J: A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. Anal Chem 2002;74:4526–4531. Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 		2010;878:2765-2769.	mass spectrometry. J em omatogr	D Analyt reentor bioned bie ser	
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 2002;74:4526–4531. Kyoung Kim H, Kyoung Kim Y, Song IH, Baek SH, Lee SR, Hye Kim J, Kim JR: Down-Regulation of a Forkhead Transcription Factor, FOXO3a, Accelerates Cellular Senescence in Human Dermal Fibroblasts. J Gerontol A 		high-performance liquid chrom	atography/electrospray ionization	n mass spectrometry. Anal Chem	
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