Differential methylation of genes in individuals exposed to maternal diabetes in utero

Peng Chen, Paolo Piaggi, Michael Traurig, Clifton Bogardus, William C. Knowler, Leslie J. Baier, Robert L. Hanson

Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 1550 E. Indian School Rd, Phoenix, AZ 85014, USA.

Correspondence should be sent to: Robert L. Hanson, rhanson@phx.niddk.nih.gov

ESM Methods

Methylation measurement

DNA (1,000 ng) from peripheral blood leukocytes underwent sodium bisulfite treatment and recovery using the Zymo EZ DNA methylation Kit (Zymo Research, Irvine, CA). Converted DNA was analyzed for complete conversion and quantity by MethyLight [1]. Qualified DNA (15 µl) was analyzed using the Infinium HumanMethylation450K Beadchip technology (Illumina Inc., San Diego, CA, USA). The raw signal intensities from the Beadchip were extracted, corrected for background fluorescence and red-green dye bias, using the R (version 3.1.1, http://www.r-project.org/) package *methylumi* [2]. The beta value, which measures the extent of methylation at the CpG site covered by a probe, was calculated as $(m/(m+u))$, in which m and u refer to the mean methylated and unmethylated probe signal intensities respectively. Beta values for which the fluorescent intensity was not significantly above the background signal (detection *p* value >0.01) were considered missing. Probes whose sequence overlaps with a SNP or indel (minor allele frequency >0.5%), as determined by whole genome sequence data available on 272 Pima Indians, were excluded (N = 53,695 excluded). In addition, probes which directly target SNPs (N = 65), align to multiple genomic positions in Human genome build GRCh37.p13 (N = 3), map to the Y chromosome (N = 32) or provided a call rate <95% among all samples (N = 8,471) were also removed. The final analysis included 423,311 probes which mapped to an autosome or the X chromosome.

Mediation analysis

To assess the extent to which observed methylation differences may account for the increased diabetes risk in OMD, a formal mediation analysis was conducted [3]. This involved fitting the following regression models for methylation (M) and development of diabetes (D, by proportional hazards regression):

$$M = a * EXP + \Sigma$$

$$D = c * EXP + \Sigma$$

$$D = b * M + c' * EXP + \Sigma$$

where EXP represents intrauterine exposure (OMD = 1, OMND = 0) and Σ represents the effect of covariates. The significance of the mediation effect was assessed by comparing ab with its standard error ($= sqrt[a * SE_b^2 + b * SE_a^2 + SE_a^2 * SE_b^2]$) [3]. Percentage mediation, or the extent to which the excess risk in OMD is potentially explained by the methylation effect, was taken as $100[1 - c'/c]$ [4].

ESM Tables

ESM Table 1. The association of top CpGs with or without adjustment of pre-pregnancy maternal BMI.

CpG	Gene/CpG island	Effect	P	Effect_mBMI	P_mBMI
cg21192468	*LHX3*	4.8	1.7×10^{-08}	4.5	1.3×10^{-07}
cg14381623	*LHX3*	3.2	6.9×10^{-07}	2.9	5.0×10^{-06}
cg15796459	*SHROOM2*	-1.6	4.8×10^{-06}	-1.4	2.2×10^{-05}
cg20749955	*PPP1R3B*	-3.6	1.3×10^{-05}	-2.9	3.9×10^{-04}
cg12140144	*PRDM16*	1.0	1.1×10^{-04}	1.0	1.6×10^{-04}
cg06717221	*ATP8B3*	-2.7	1.1×10^{-04}	-2.7	1.3×10^{-04}
cg15833797	*LHX3*	2.5	6.7×10^{-06}	2.6	1.9×10^{-06}
cg14605520	*LHX3*	2.8	7.6×10^{-05}	3.0	1.5×10^{-05}
cg10772621	*chr19:54411376-54411968*	-1.6	2.0×10^{-04}	-1.6	2.0×10^{-04}
cg25952247	*LHX3*	3.3	3.0×10^{-06}	3.1	1.3×10^{-05}
cg20345234	*chr10:65800729-65801528*	-2.3	6.6×10^{-05}	-2.1	2.3×10^{-04}
cg00762450	*ANKRD20A4*	1.4	4.1×10^{-05}	1.5	1.7×10^{-05}
cg13700073	*FSCN2*	-1.7	3.0×10^{-05}	-1.6	7.1×10^{-05}
cg20769177	*WNT9B*	2.4	9.5×10^{-05}	2.1	6.8×10^{-04}
cg08292290	*GLRX5*	-0.6	2.4×10^{-05}	-0.5	2.4×10^{-04}
cg06268875	*PIEZO2*	5.1	1.0×10^{-05}	4.9	1.9×10^{-05}
cg04350311	*ELFN2*	1.5	3.2×10^{-04}	1.4	4.4×10^{-04}
cg15183961	*ANKRD20A2*	1.8	1.7×10^{-05}	1.7	4.3×10^{-05}
cg26671988	*chr5:102090439-102091241*	2.1	8.7×10^{-05}	2.0	2.1×10^{-04}
cg17186803	*SCN4B*	1.2	4.1×10^{-04}	1.2	4.8×10^{-04}
cg24049468	*AK3*	2.3	2.8×10^{-04}	2.4	2.2×10^{-04}
cg27222147	*CACNA1C*	-0.7	8.7×10^{-04}	-0.6	3.5×10^{-03}
cg08370430	*chr17:12927455-12928747*	1.5	1.5×10^{-05}	1.5	1.8×10^{-05}
cg15618978	*TRIM59*	1.5	3.3×10^{-05}	1.4	1.5×10^{-04}
cg07464358	-	0.9	3.8×10^{-05}	0.9	3.3×10^{-05}
cg24996440	*chr2:3583550-3584833*	-2.1	4.1×10^{-05}	-1.9	1.5×10^{-04}
cg03862414	*PLEKHH3*	2.4	2.2×10^{-05}	2.2	7.7×10^{-05}
cg05772155	*chr10:65800729-65801528*	-3.2	6.4×10^{-04}	-2.9	1.1×10^{-03}
cg21172615	*LHX3*	2.6	1.4×10^{-04}	2.4	4.2×10^{-04}
cg20941258	*TDGF1*	2.9	7.2×10^{-06}	2.7	2.5×10^{-05}
cg08414676	*SORD*	-2.5	9.8×10^{-05}	-2.3	3.6×10^{-04}
cg00509616	*GCOM1*	0.9	1.9×10^{-03}	0.9	4.1×10^{-03}
cg09674170	*CLDN9*	3.3	1.1×10^{-05}	3.0	1.1×10^{-04}
cg07993743	*WNT9B*	2.1	6.0×10^{-05}	2.0	7.3×10^{-05}
cg04645534	*STC1*	-2.6	4.6×10^{-04}	-2.4	1.3×10^{-03}
cg04413090	*SBK1*	1.2	6.8×10^{-06}	0.9	3.2×10^{-04}
cg13427473	-	-0.7	1.6×10^{-04}	-0.7	1.6×10^{-04}

cg16426215	*chr16:56709677-56709953*	-1.2	2.0×10^{-04}	-1.0	2.6×10^{-03}
cg24503407	*PM20D1*	-4.3	1.3×10^{-05}	-4.0	3.1×10^{-05}
cg12875241	*GPR143*	4.0	2.8×10^{-04}	3.3	2.9×10^{-03}
cg14732789	*chr20:29534910-29535208*	-1.4	3.6×10^{-04}	-1.4	3.8×10^{-04}
cg14105781	*TBL1X*	1.4	5.0×10^{-04}	1.1	2.2×10^{-03}
cg08911291	*chr9:44118137-44120175*	2.6	8.9×10^{-05}	2.8	3.5×10^{-05}
cg05806645	*PPP1R3B*	-2.3	1.8×10^{-04}	-1.8	2.8×10^{-03}
cg25629768	*LMNB2*	1.5	1.0×10^{-04}	1.3	4.8×10^{-04}
cg16482344	*LINC00839*	-4.0	7.5×10^{-05}	-4.0	8.6×10^{-05}
cg25949304	*PCDHGA4*	-2.7	6.5×10^{-04}	-2.6	1.1×10^{-03}
cg27073142	*SORD*	-4.3	2.0×10^{-04}	-3.7	1.0×10^{-03}

All results are shown for the 296 individuals who had data on pre-pregnancy maternal BMI. Effect represents the difference in percentage of DNA methylation in OMD compared with OMND. Effect_mBMI represents the difference after adjusting for maternal pre-pregnancy BMI (P_mbmi is the corresponding p value).

ESM Table 2. Differentially methylated pathways and genes.

Pathway	Enrichment	Gene symbol	Gene full name
Metabolic pathways	O=37;adjP=0.0002		
		UGP2	UDP-glucose pyrophosphorylase 2
		SORD	sorbitol dehydrogenase
		XYLT1	xylosyltransferase I
		PIGH	phosphatidylinositol glycan anchor biosynthesis, class H
		NOS1	nitric oxide synthase 1 (neuronal)
		COX4I1	cytochrome c oxidase subunit IV isoform 1
		COX10	COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast)
		PIP5K1C	phosphatidylinositol-4-phosphate 5-kinase, type I, gamma
		FLAD1	FAD1 flavin adenine dinucleotide synthetase homolog (S. cerevisiae)
		DBH	dopamine beta-hydroxylase (dopamine beta-monooxygenase)
		ADI1	acireductone dioxygenase 1
		GALNT9	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 9 (GalNAc-T9)
		INPP5A	inositol polyphosphate-5-phosphatase, 40kDa
		ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase
		PAPSS1	3'-phosphoadenosine 5'-phosphosulfate synthase 1
		BDH1	3-hydroxybutyrate dehydrogenase, type 1
		FUT1	fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase, H blood group)
		SUCLG2	succinate-CoA ligase, GDP-forming, beta subunit
		PTDSS2	phosphatidylserine synthase 2
		DEGS2	delta(4)-desaturase, sphingolipid 2

		NDUFA10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 10, 42kDa
		HSD17B12	hydroxysteroid (17-beta) dehydrogenase 12
		SEPHS1	selenophosphate synthetase 1
		SUCLA2	succinate-CoA ligase, ADP-forming, beta subunit
		AK5	adenylate kinase 5
		PSAT1	phosphoserine aminotransferase 1
		GUSB	glucuronidase, beta
		BCKDHB	branched chain keto acid dehydrogenase E1, beta polypeptide
		FUT4	fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)
		CYP2E1	cytochrome P450, family 2, subfamily E, polypeptide 1
		GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)
		AKR1D1	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)
		CSGALNACT1	chondroitin sulfate N-acetylgalactosaminyltransferase 1
		MDH1	malate dehydrogenase 1, NAD (soluble)
		DHCR7	7-dehydrocholesterol reductase
		B4GALT7	xylosylprotein beta 1,4-galactosyltransferase, polypeptide 7
		PSPH	phosphoserine phosphatase
Wnt signaling pathway	O=11;adjP=0.0004		
		SMAD3	SMAD family member 3
		PRKCG	protein kinase C, gamma
		WIF1	WNT inhibitory factor 1
		SFRP2	secreted frizzled-related protein 2
		WNT10A	wingless-type MMTV integration site family, member 10A
		FZD9	frizzled family receptor 9
		WNT7B	wingless-type MMTV integration site family, member 7B
		TBL1X	transducin (beta)-like 1X-linked
		NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
		CHP2	calcineurin-like EF hand protein 2
		WNT9B	wingless-type MMTV integration site family, member 9B
Protein digestion and absorption	O=8;adjP=0.0005		
		ATP1A1	ATPase, Na+/K+ transporting, alpha 1 polypeptide
		KCNK5	potassium channel, subfamily K, member 5
		SLC7A9	solute carrier family 7 (glycoprotein-associated amino acid transporter light chain, bo,+ system), member 9
		SLC9A3	solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3
		COL18A1	collagen, type XVIII, alpha 1
		KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1
		SLC6A19	solute carrier family 6 (neutral amino acid transporter), member 19

COL6A3	collagen, type VI, alpha 3

O, the number of differentially methylated genes in the pathway; adjP, the false discovery rate.

ESM Table 3. Developmental role of the 11 genes among the 39 genes with genome-wide significance.

Gene	Developmental Role	Literature
LHX3	Motor neuron and interneuron specification Pituitary development Spinal cord development	[5] [6, 7] [8]
PRDM16	Brown adipocyte tissue mess	[9]
WNT9B	Kidney tubule development Upper jaw and lip development	[10, 11] [12]
AK3	Cardiac differentiation	[13]
CACNA1C	Timothy syndrome skeletal muscle development	[14] [15]
TDGF1	Cardiomyogenesis	[16, 17]
STC1	Growth plate chondrogenesis Osteoblast development and bone formation Bone and muscle development	[18] [19] [20]
PM20D1	Birth weight	[21]
SBK1	Brain development	[22]
TBL1X	Fetal brain development	[23]
LMNB2	Nervous system development	[24]

ESM References

[1] Campan M, Weisenberger DJ, Trinh B, Laird PW (2009) MethyLight. Methods in molecular biology 507: 325-337

[2] Triche TJ, Jr., Weisenberger DJ, Van Den Berg D, Laird PW, Siegmund KD (2013) Low-level processing of Illumina Infinium DNA Methylation BeadArrays. Nucleic acids research 41: e90

[3] Baron RM, Kenny DA (1986) The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. Journal of personality and social psychology 51: 1173-1182

[4] Preacher KJ, Kelley K (2011) Effect size measures for mediation models: quantitative strategies for communicating indirect effects. Psychological methods 16: 93-115

[5] Seo SY, Lee B, Lee S (2015) Critical Roles of the LIM Domains of Lhx3 in Recruiting Coactivators to the Motor Neuron-Specifying Isl1-Lhx3 Complex. Molecular and cellular biology 35: 3579-3589

[6] Kelberman D, Dattani MT (2009) Role of transcription factors in midline central nervous system and pituitary defects. Endocrine development 14: 67-82

[7] Colvin SC, Mullen RD, Pfaeffle RW, Rhodes SJ (2009) LHX3 and LHX4 transcription factors in pituitary development and disease. Pediatric endocrinology reviews : PER 6 Suppl 2: 283-290

[8] Park S, Mullen RD, Rhodes SJ (2013) Cell-specific actions of a human LHX3 gene enhancer during pituitary and spinal cord development. Molecular endocrinology 27: 2013-2027
[9] Dempersmier J, Sambeat A, Gulyaeva O, et al. (2015) Cold-inducible Zfp516 activates UCP1 transcription to promote browning of white fat and development of brown fat. Molecular cell 57: 235-246
[10] Karner CM, Chirumamilla R, Aoki S, Igarashi P, Wallingford JB, Carroll TJ (2009) Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. Nature genetics 41: 793-799
[11] Karner CM, Das A, Ma Z, et al. (2011) Canonical Wnt9b signaling balances progenitor cell expansion and differentiation during kidney development. Development 138: 1247-1257
[12] Jin YR, Han XH, Taketo MM, Yoon JK (2012) Wnt9b-dependent FGF signaling is crucial for outgrowth of the nasal and maxillary processes during upper jaw and lip development. Development 139: 1821-1830
[13] Dzeja PP, Chung S, Faustino RS, Behfar A, Terzic A (2011) Developmental enhancement of adenylate kinase-AMPK metabolic signaling axis supports stem cell cardiac differentiation. PloS one 6: e19300
[14] Papineau SD, Wilson S (2014) Dentition abnormalities in a Timothy syndrome patient with a novel genetic mutation: a case report. Pediatric dentistry 36: 245-249
[15] Tanaka H, Furuya T, Kameda N, Kobayashi T, Mizusawa H (2000) Triad proteins and intracellular Ca2+ transients during development of human skeletal muscle cells in aneural and innervated cultures. Journal of muscle research and cell motility 21: 507-526
[16] D'Aniello C, Lonardo E, Iaconis S, et al. (2009) G protein-coupled receptor APJ and its ligand apelin act downstream of Cripto to specify embryonic stem cells toward the cardiac lineage through extracellular signal-regulated kinase/p70S6 kinase signaling pathway. Circulation research 105: 231-238
[17] Parisi S, D'Andrea D, Lago CT, Adamson ED, Persico MG, Minchiotti G (2003) Nodal-dependent Cripto signaling promotes cardiomyogenesis and redirects the neural fate of embryonic stem cells. The Journal of cell biology 163: 303-314
[18] Wu S, Yoshiko Y, De Luca F (2006) Stanniocalcin 1 acts as a paracrine regulator of growth plate chondrogenesis. The Journal of biological chemistry 281: 5120-5127
[19] Yoshiko Y, Aubin JE, Maeda N (2002) Stanniocalcin 1 (STC1) protein and mRNA are developmentally regulated during embryonic mouse osteogenesis: the potential of stc1 as an autocrine/paracrine factor for osteoblast development and bone formation. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society 50: 483-492
[20] Jiang WQ, Chang AC, Satoh M, Furuichi Y, Tam PP, Reddel RR (2000) The distribution of stanniocalcin 1 protein in fetal mouse tissues suggests a role in bone and muscle development. The Journal of endocrinology 165: 457-466
[21] Haworth KE, Farrell WE, Emes RD, et al. (2014) Methylation of the FGFR2 gene is associated with high birth weight centile in humans. Epigenomics 6: 477-491
[22] Nara K, Akasako Y, Matsuda Y, et al. (2001) Cloning and characterization of a novel serine/threonine protein kinase gene expressed predominantly in developing brain. European journal of biochemistry / FEBS 268: 2642-2651
[23] Han K, Gennarino VA, Lee Y, et al. (2013) Human-specific regulation of MeCP2 levels in fetal brains by microRNA miR-483-5p. Genes & development 27: 485-490
[24] Coffinier C, Chang SY, Nobumori C, et al. (2010) Abnormal development of the cerebral cortex and cerebellum in the setting of lamin B2 deficiency. Proceedings of the National Academy of Sciences of the United States of America 107: 5076-5081