Maternal hyperglycemia and foetal epigenetic adaptations

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Early Nutrition Project – The Power of Programming
Munich, Germany
March 13th, 2014

Note: for non-commercial purposes only
Outline

• Maternal hyperglycemia and risk of metabolic disorders in offspring
• What is epigenetics?
• Foetal epigenetic adaptations associated with maternal hyperglycemia
  – Candidate genes
  – Epigenome-wide approaches
• Limitations and future perspectives
Cumulative incidence of T2D in Pimas offspring according to maternal 2h-glucose at third trimester

Franks PW et al. Diabetes 2006
Gestational Diabetes Mellitus (GDM) association with childhood adiposity and blood pressure at 3 years old – Project Viva (Boston)

![Graph showing the association between GDM exposure and child outcomes including BMI z-score, sum of skinfolds, systolic blood pressure, and systolic blood pressure adjusted for skinfolds.](image)
Mechanisms involved in foetal metabolic programming

- Malleable – environmental exposure
- Durable – long lasting effect
Epigenetics:
Study of variations in gene expression caused by mechanisms other than the underlying DNA sequence
Epigenetics

- Most epigenetic phenomena are mitotically-stable and enduring, conveying long-term effects
  - Over decades in some humans studies
- Epigenetic phenomena can also be modulated by stochastic environmental stimuli
  - Modulated by environmental factors (pollutants, diet, smoking, etc.) during pre and post natal life
  - Particularly sensitive to in utero events
  - Tissue differentiation during foetal development
EPIGENETIC MECHANISMS
are affected by these factors and processes:
- Development (in utero, childhood)
- Environmental chemicals
- Drugs/Pharmaceuticals
- Aging
- Diet

DNA methylation
Methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes.

Histones are proteins around which DNA can wind for compaction and gene regulation.

HEALTH ENDPOINTS
- Cancer
- Autoimmune disease
- Mental disorders
- Diabetes

Histone modification
The binding of epigenetic factors to histone “tails” alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.
DNA methylation

• More likely at CpG site, enriched in promoters
  – Commonly in regions called CpG islands

• Highly methylated = low transcription
  – Most of the time, but not universal

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Adipokines: role in energy balance and glycemic regulation

- **LEP** encoding for leptin
  - ‘Adipostat’ role
  - lower levels = stimulate positive energy balance
  - Source: adipocytes, placenta during pregnancy

- **ADIPOQ** encoding for adiponectin
  - Potential role in insulin sensitivity
  - Higher levels associated with lower risk of T2D
Maternal hyperglycemia is associated with lower DNA methylation at *LEP* in foetal placental tissue.

\[ \rho = -0.44 \]
\[ P = 0.039 \]

N= 23 offspring of women with impaired glucose tolerance (IGT) during pregnancy.
Maternal glycemia is associated with DNA methylation at *ADIPOQ* in foetal placental tissue.


N= 98 offspring of women across the spectrum of glycemic regulation (NG–GDM)
Maternal glycemia and DNA methylation at other metabolism candidates genes

- **In lipid metabolism:**
  - **ABCA1**
    - Higher 2h-glucose = associated with lower DNA methylation in fetal circulating cells cord blood
  - **LPL**
    - Higher 2h-glucose = associated with lower DNA methylation in placenta on the foetal side

- **IGF pathways**
  - **IGF1R** and **IGFBP3** showed lower DNA methylation in foetal placenta associated with higher 2h-glucose

- **Energy regulation**
  - **PRDM16, BMP7** and **PGC1α** methylation levels in foetal placenta associated with fasting glucose and/or 2h-glucose

Houde AA et al. *Epigenetics* 2013
Houde AA et al. *Journal of DOHaD* 2014
Exposure to GDM and DNA methylation at candidates genes: imprinted loci and metabolic/inflammatory pathways

- Cord blood and placenta of offspring
  - GDM-diet (n= 88)
  - GDM-insulin (n=98)
  - Normoglycemia (n= 65)

- Tested list of candidate genes
  - 7 imprinted genes
    - Maternally: *LIT1, MEST, NESPAS, PEG3, and SNRPN*
    - Paternally: *H19 and MEG3*
  - Metabolic and inflammatory pathways
    - *NR3C1, PPARA, NDUFB6, IL-10, APC, LEP, and OCT4*
  - Global methylation markers
    - ALU and LINE1 repeats
Exposure to GDM and DNA methylation at candidates genes: imprinted loci and metabolic/inflammatory pathways

- Imprinted gene *MEST* was hypomethylated in GDM exposed (diet or insulin) compared to control group in both placenta and cord blood cells
  - Lower methylation at *MEST* in circulating blood cells was associated with adult obesity in independent cohort of case-control (age-sex matched)

- *MEST* potential role – animal studies
  - Potential role in fetal and placental growth; adult behavior, particularly in maternal care
  - *MEST* expression is up-regulated by early post-natal overnutrition
  - *MEST* overexpression = enlargement of adipocytes and fat expansion
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Suggestive differential methylation in cord blood cells

El Hajj N et al. Diabetes 2013
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1% lower in both tissues
3% lower in fetal placenta
3% higher in cord blood cells

El Hajj N et al. Diabetes 2013
Epigenome-wide association study (EWAS) in GDM-exposed case-control study

Newborns
N= 30 GDM-exposed
N= 14 controls

Illumina
450k
Beadchip

Type 1 diabetes

Diabetes mellitus

N=51
ABCC8, APOM, B2M, BACH2, BRD2, C6orf10, CCDC101, CDK4, C1ITA, COL11A2, CPT1A, CUX2, DDX39B, DPCR1, EHMT2, GABBR1, GAD2, GPSM3, HCG4, HIST1H4A, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQB1, HLA-DQB2, HLA-DRA, HLA-L, HSPA1L, ICA1, ITPR1, LRP1B, LY6G5C, MICA, NOTCH4, PPT2, PSMB8, PSMB9, PSORS1C1, PTPN11, TAP1, TCF19, TLR5, TNF, TNFRSF1B, TNXB, TRIM26, TRIM31, UBAH3A, ZNRD1.

N=42
ATF6, ATP10A, CA5A, CACNA1C, CACNA1D, CAMTA1, CHI3L1, CHRM2, CNR1, CNTNAP2, CPLX2, CYP2E1, DAB1, DIP2C, DLGAP2, DRD4, FGFR1, FOXC1, FRMD4B, GABRA1, GABRB3, GABRD, IGF1R, IGFBP2, KCNQ1, KLF11, LGALS3, mir-125, PDE3A, PDE4D, PHLP1, PTGIR, RBFOX1, RBMS1, RNF220, SLC6A3, SORCS2, SPATA5, STK32C, TCF7L2, VWA3B, ZBTB16.

N=13
CACNA1E, CCK, CDKN1A, DLK1, FGFR2, HFE, HTR1A, NMU, NR1H4, OBP2A, PASK, PBX1, PPP1R3C

Genes classified in metabolic diseases pathways

Glucose metabolism disorder
Limitations of current studies and future challenges

• Often based on candidate genes
• Small sample size
• Access to tissues
  – Cord blood, placenta
• Assessment at birth only, need for longitudinal studies
• Integration of genetics, epigenetics, transcriptomics, metabolomics
Epigenetics: potential mechanism of foetal metabolic programming

ENVIRONMENTAL TRIGGERS
- Exercise
- Smoking
- Toxins
- Diet
- Stress
- Cold
- Heat
- Drugs
- Infection

GENETIC MACHINERY
- Binding of methyl-CpG binding proteins
- Recruitment of HDACs & co-repressors

DNA variation
- Histone modification
- mRNA / miRNA
- Protein

PHENOTYPE
- (e.g. adiposity, glucose intolerance, insulin resistance, diabetes)

CELL

Altered cellular environment

Franks P.W. & Ling C., BMC Med. 2010
Acknowledgements

- Research participants
- Personnel of the Blood Sampling in Pregnancy Clinic (CRCEL)
- Research team and students: M Doyon, MC Battista, J Moreau, M Gerard, J Menard, M Lacroix, C Allard, G Lacerte, L Guillemette, AA Houde, V Desgagne, S Cote, SM Ruchat
- Collaborators at U. Sherbrooke: L Bouchard, P Perron, AC Carpentier, JC Pasquier, JL Ardidouze, MF Langlois, JP Baillargeon
- Colleagues and collaborators HPHCI: M Gillman, E Oken, A Baccarelli
Questions
Genes classified in immunological pathways

- **Systemic autoimmune syndrome**
  - N=13
  - AIRE, CD22, CD44, EGR3, ETS1, HIST1H4A, IL23A, IRF5, ITGAM, LPP, MMP8, NMNAT2, XKR6

- **Type 1 diabetes**
  - N=19
  - ABCC8, BACH2, CCDC101, CDK4, CPT1A, CUX2, GABBR1, GAD2, HCG4, HLA-A, HLA-B, HLA-L, ICA1, PTPN11, TAP1, TCF19, TLR5, UBASH3A, ZNRD1

- **Rheumatoid arthritis**
  - N=29
  - ALPL, APLP2, ARG1, ATF6B, ATXN1, BLNK, BRD2, CCHCR1, CD247, CELF2, CELF4, CHI3L1, CNR1, COL4A2, CYP2B6, DDX39B, DIP2C, DOK6, F10, FMN2, FOXO1, FTH1, G0S2, GABRA1, GABRB3, GABRD, GRB10, HIST1H2AC, HLA-DMB, HLA-DRB1, HLA-G, HLA-J, HOXC4, JAM3, KIAA1908, KIR3DL2, LCP1, LTF, MLLT6, MLN, MMEL1, MPO, NELL1, NTRK2, PBX2, PIK3CG, PRDM1, PRTN3, PTPRC, PTPRN2, RIMS1, SND1, SYNE1, TAGAP, TCF7L2, TRIM15, VIM, WDR46

- **Rheumatoid arthritis**
  - N=3
  - BAT1, BRD2, HIST4H4

- **Type 1 diabetes**
  - N=3
  - DBI, DDX39B, DIP2C, DOK6, F10, FMN2, FOXO1, FTH1, G0S2, GABRA1, GABRB3, GABRD, GRB10, HIST1H2AC, HLA-DMB, HLA-DRB1, HLA-G, HLA-J, HOXC4, JAM3, KIAA1908, KIR3DL2, LCP1, LTF, MLLT6, MLN, MMEL1, MPO, NELL1, NTRK2, PBX2, PIK3CG, PRDM1, PRTN3, PTPRC, PTPRN2, RIMS1, SND1, SYNE1, TAGAP, TCF7L2, TRIM15, VIM, WDR46
LEP promoter DNA methylation and mRNA expression of leptin on fetal side of the placenta

Bouchard L et al. Diabetes Care 2010
LEP encoding leptin

Energy balance regulation
Adipokines
hormones/cytokines produced by the adipose tissue potentially implicated in obesity and diabetes
Candidates genes

• Other genes investigated by Bouchard lab
  – ABCA1 cord blood DNA methylation levels are negatively correlated with maternal glucose 2 h post-OGTT (r = -0.26; P = 0.02)
    • Ref: Houde AA Epigenetics 2013
  – LPL-CpG1 and CpG3 were also negatively correlated with maternal glucose (2-h post OGTT; r = –0.22; P = 0.02) and HDL-cholesterol levels (third trimester of pregnancy; r = –0.20; p = 0.03), respectively
    • Ref: Houde AA et al, Journal of DOHaD 2014
  – Both *IGF1R* and *IGFBP3* were hypomethylated in placentas exposed to IGT compared to NGT (12.9% vs. 17.2%; p=0.02 and 10.1% vs. 12.6%; p=0.01 respectively) and negatively correlated with 2h-glucose in the overall population (r=-0.23; p=0.01 and r=-0.20; p=0.03 respectively)
    • Veronique Desgagné
  – *PRDM16* and *PGC1α* DNA methylation was also correlated with the fasting glycemia at the end of the 2nd TofP (r=-0.19, p=0.048 and r=0.32, p=0.001; respectively) whereas DNA methylation levels at *BMP7* and *PGC1α* genes were correlated with the 2h post-OGTT glucose concentration (r=-0.20, p=0.029 and r=0.26, p=0.006; respectively)
    • Sandra Côté
Genome-wide approach

• Using 27k illumina platform
• Circulating blood cells – children 8-12yo from the EPOCH study (white, Kaiser)
  – N = 11 offspring exposed to GDM
  – N= 10 normoglycemic mothers (controls)
• No findings reaching FDR p-value

West NA et al. *Immunometabolism* 2013