**Catching diabetes**

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**Keywords:** Gestational diabetes mellitus; epigenetics; type 2 diabetes; transgenerational inheritance; metabolic syndrome; placenta; glucose tolerance

**Funding:** JDH is supported by the National Institute for Health Research through the NIHR Southampton Biomedical Research Centre

Epigenetics may be the mechanism by which gestational diabetes transmits risk for subsequent type 2 diabetes to the mother, child and future generations; can it also be utalised to design better interventions?

Gestational diabetes mellitus (GDM) is defined as new onset of glucose intolerance during pregnancy. About 1-15% of all pregnant women worldwide develop GDM and the prevalence is exponentially increasing as the risk factors of higher pre-conceptional body mass index (BMI) [1], hypertension [2] and gestational weight gain (GWG) [3] also increase [4, 5].

In the short term, women with GDM, especially at the higher range of glucose tolerance, are at increased risk for preeclampsia and other complications of labour. They are more likely to give birth to macrosomic or large for gestational age babies, which may in itself give rise to birth injuries for mother and baby [6, 7].

Although glucose tolerance tends to normalize after birth, approximately 50-% of women who had a gestationally diabetic pregnancies go on to develop type 2 diabetes (T2D) within the next ten years. This makes GDM the strongest risk factor for T2D [8]. This risk is proportional to preconceptional BMI and GWG [9]. It seems that GDM predisposes to T2D but it not clear if they two disorders share a common etiology or if one is causative of the other [10-13]. They share genetic risk variants [14].

Infants born to women with GDM are more likely to be over-weight in infancy and childhood [15] and have a higher risk for adult obesity and cardiovascular disease [16]. There is more controversial evidence linking GDM to offspring cognitive ability although exposure to maternal GDM has been shown to be an independent risk factor for long-term neuropsychiatric morbidity (such as autism spectrum disorder) [17]. GDM gives rise to a vicious cycle in which mothers with GDM have babies who are at greater risk of developing metabolic disease later in life, giving rise to a new generation of mothers with GDM and a higher risk for their offspring *ad infinitum* [18].

A leading hypothesis for how GDM confers a higher risk of metabolic disorder to the offspring is by persistent epigenetic change [19-23]. In mice, the male offspring of diabetic dams show differential methylation of H19 in their sperm, suggestive of transmission of the effects across 3 generations [24].

**DNA methylation correlates of GDM in birth tissues**

Research into postpartum epigenetic change as a consequence of GDM, has concentrated on the readily available samples from placental tissue and the umbilical cord blood. The placenta is the site of nutrient transfer between mother and child and hence an obvious site for mediation of the consequences of GDM for both mother and child. Its epigenome is unusual among human body tissues, being more malignant-like and having a preponderance of imprinted loci [25]. Interestingly, Houde *et al* (2013) showed concordant but opposite changes in DNA methylation relative to GDM status, at the *ABCA1* locus between the fetal and maternal sides of the placenta [26]. Petropoulos et al (2015) showed a substantial overlap between genes that were differentially methylated in the placenta and liver of rat offspring from GDM vs. normoglycemic control dams. They also showed some overlap of the differentially methylated rat genes with human orthologues differentially methylated in human placentas between offspring of GDM versus normoglycemic pregnancies [27]. This suggests that placental changes are relevant for later offspring physiology. However the overlaps were at the gene not the locus level and, as controls such as accounting for genotypic control of methylation were not implemented, we cannot be sure the overlaps are statistically significant. Similarly, Loke et al (2013) were able to show that maternal GDM was associated with low DNA methylation levels across the IGF2/H19 imprinted locus consistent in placental and non-placental offspring tissues and cells, such as buccal swabs, cord blood mononuclear cells, granulocytes and umbilical vein endothelial cells [28]. Their effect sizes were modest and again they did not account for possibly genotype driven changes. However genotype is probably less relevant for these known imprinted loci and the findings are in agreement with other studies showing aberrant methylation and expression *IGF2* and *H19* in umbilical cord bloods of offspring of GDM mothers compared to controls e.g. [29].

Other studies have found concordant changes in both placentas and umbilical cord blood, for instance decreased methylation of the maternally imprinted *MEST* gene, and the glucocorticoid receptor *NR3C1* gene in both cord blood and placenta in GDM offspring compared to controls [30]. Binder et al (2015) found differential methylation of the HLA-DOA locus, in maternal-side placentas from GDM pregnancies compared to pre-pregnancy BMI matched controls without pregnancy complication. They replicated the differential methylation of HLA-DOA relative to GDM, in placentas from an independent cohort and associated GDM and HLA-DOA methylation with placental RNA expression levels of major histocompatibility complex encoded transcripts [31].

Finer et al. (2015), examined genome-wide differential DNA methylation with respect to GDM on the the Illumina Infinium HumanMethylation 450K array (infinium450K) that measures DNA methylation at over 483,000 loci across the methylome [32]. They identified genome-wide significant differential methylation (FDR corrected p <0.05) with absolute methylation difference of more than 5% between groups, in 1373 loci from placenta n=25 vs. 18) and 1485 loci in cord blood (n=27 vs. 21) [33]. 378 differential loci were common across both tissues. Ruchat et al (2013) also examined placenta and cord blood from GDM pregnancies relative to controls on the infinium450K [34]. They did not find any genome-side significant loci but some of the differential loci they identified overlapped with those of Finer et al [35].

Studies of birth tissues in GDM are complicated by the likelihood of anatomical changes to the placenta and umbilical cord as a consequence of GDM or some of its co-morbidities such as inflammation. These are likely to be accompanied by cellular composition change, which will confound the DNA methylation signal, a problem encountered in many epigenetic studies [36]. Most of the studies do not take into account the fact that genotypic risk alleles may well differ between GDM and control groups and drive individual differences in methylation which are consistent across cell types. Also, criteria for diagnosis of GDM can be controversial and ethnic specific [37]. It may be more fruitful to look for DNA methylation changes proportional to glycemia scores across the normal and pathological ranges e.g. [38, 39]. Finally, suggestions of similar epigenetic profiles for GDM and pre-eclampsia are worrisome in that maybe the DNA methylation changes are related to inflammation and confounding causes of a suboptimal gestational environment rather than disease specific changes [40].

However, causality between maternal glycemia and infant DNA methylation status and phenotype was partially demonstrated by Allard et al (2015) [41]. In a dataset of 485 mother-infant pairs, they found that *LEP* DNA methylation levels in cord blood were associated both with cord blood leptin protein levels and maternal fasting glucose levels in the second trimester. Using a polygenic risk score for glycemia in the mothers as a genetic instrument they used mendelian randomization to show that the data were consistent with neonatal *LEP* methylation levels mediating the effects of maternal glucose tolerance on leptin protein levels. Maternal glycemia and cord blood *LEP* methylation was also associated with neonatal adiposity. Unfortunately, they were unable to identify a genetic instrument in the infant associated with *LEP* methylation levels and therefore compatible with the 2-step mendelian randomization protocol suggested by Relton et al [42]. Leptin levels and *LEP* DNA methylation have been implicated in programming for a wide range of metabolic disorders so although *LEP* methylation may well be causal for the increased leptin levels characteristic of neonates from GDM pregnancies, its biomarker signal is likely to be confounded by other metabolic complexities.

**Prognostic DNA methylation markers in the mother**

Michalczyk et al (2016) found differences in global histone H3 dimethylation levels in the white blood cells of women with and without GDM and differences again for those women with GDM who went on to develop T2D compared to those that didn’t, suggesting the heightened risk for T2D after GDM, could be transmitted epigenetically [43]. Wu et al (2016) [35] surveyed bloods from mothers prior to GDM development using the infinium450K. Methylation levels at particular CpGs were identified as significantly different between cases and controls prior to onset of disease and also differentially methylated in independent infinium450K experiments on placenta and cord blood after GDM pregnancy [33, 34].

**Utility of the epigenetic marks for prevention and treatment**

Identifying those methylation marks prognostic for GDM or implicated in transmitting diabetes across generations has great promise for assisting design of successful interventions. They can be used a “surrogate outcomes” assess the efficacy of an intervention rather than wait for the women to transition or not to T2D or for the offspring to grow up and develop T2D or not in middle age. Recent efforts to intervene in GDM via diet and exercise during pregnancy have met with some success in decreasing GDM pathology for the mother [44-48] and the neonate [49, 50]. However these rely on immediate phenotypes such as postpartum BMI, glucose tolerance or neonate birth weight. We look forward to new studies employing randomized controlled trails (RCTs) to determine if behavioural (e.g. the UPBEAT trial [45, 46]) or nutritional (e.g. the NIPPER trial <https://www.clinicaltrials.gov/ct2/show/NCT02509988>) intervention during pregnancy can prevent or reverse the epigenetic marks associated transmission of diabetes.

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