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Genetic, nongenetic and epigenetic risk determinants in developmental programming of type 2 diabetes

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Abstract

Low birthweight (LBW) individuals and offspring of women with gestational diabetes mellitus (GDM) exhibit increased risk of developing type 2 diabetes (T2D) and associated cardiometabolic traits in adulthood, which for both groups may be mediated by adverse events and developmental changes in fetal life. T2D is a multifactorial disease occurring as a result of complicated interplay between genetic and both prenatal and postnatal nongenetic factors, and it remains unknown to what extent the increased risk of T2D associated with LBW or GDM in the mother may be due to, or confounded by, genetic factors. Indeed, it has been shown that genetic changes influencing risk of diabetes may also be associated with reduced fetal growth as a result of reduced insulin secretion and/or action. Similarly, increased risk of T2D among offspring could be explained by T2D susceptibility genes shared between the mother and her offspring. Epigenetic mechanisms may explain the link between factors operating in fetal life and later risk of developing T2D, but so far convincing evidence is lacking for epigenetic changes as a prime and direct cause of T2D. This review addresses recent literature on the early origins of adult disease hypothesis, with a special emphasis on the role of genetic compared with nongenetic and epigenetic risk determinants and disease mechanisms.

Abbreviations: GDM, gestational diabetes mellitus; LBW, low birthweight; MODY2, mature onset diabetes of the young; T2D, type 2 diabetes.

Introduction

In parallel with the global number of people with type 2 diabetes (T2D) approaching half a billion people (1), our understanding of its origins and fundamental underlying

Key Message

An adverse intrauterine environment – reflected by low birthweight and diabetes during pregnancy – is associated with an increased risk of developing type 2 diabetes mediated by genetic, nongenetic and epigenetic mechanisms.

disease mechanisms is becoming increasingly complex. From being considered a disease occurring among people with a genetic susceptibility when exposed to an affluent lifestyle, mediated primarily by inadequate insulin action (insulin resistance), we now know that T2D is a heterogeneous disease with a multifactorial etiology occurring as a result of organ dysfunctions in multiple tissues including the pancreas, liver, muscle, adipose tissue, gastrointestinal system and kidneys as well as the brain (2). The etiological causes range from genetics, with more than 50 known susceptibility genes, to a variety of nongenetic risk factors including age, obesity, physical inactivity and stress-induced metabolic perturbations including nonmetabolic diseases such as cardiac insufficiency and cancer, to adverse factors operating in fetal life.

The putative role of a fetal origin of T2D was introduced by Hales and Barker more than 20 years ago (3,4). Their hypothesis of early nutritional and other environmental factors adversely affecting growth and development of the fetus, and thereby permanently influencing risk of developing cardiometabolic disease later in life, has now become widely accepted and reproduced in several different populations (5–9). Several animal models have shown that global as well as protein undernutrition in pregnancy is associated with defects of glucose metabolism in the offspring resembling those seen in patients with T2D, including defective insulin secretion, insulin resistance and overt glucose intolerance (10–13), providing mechanistic proof-of-concept for the idea of developmental programming of T2D. However, epidemiological evidence for an association between low birthweight (LBW) and an increased risk for T2D and/or animal proof-of-concept studies, do not answer the crucial questions about the quantitative extent to which environmental factors, including nutrition in pregnancy, may contribute to the global propagation of T2D. Few studies have been able to directly link nutritional or other lifestyle factors in pregnancy to the risk of developing T2D in the offspring, and the epidemiological association between LBW and increased risk of T2D may be confounded by unknown genetic as well as nongenetic factors. As for potential confounding by genetic factors, it has been shown that genetic changes causing reduced insulin secretion, such as the mutation of the glucokinase gene causing mature onset diabetes of the young (MODY2), are also associated with reduced birthweight (2,14). Furthermore, potential nongenetic residual confounding of the epidemiological association between LBW and T2D cannot be excluded, and it needs to be emphasized that LBW is an unspecific marker of an adverse fetal environment that can be modified by a large variety of factors operating at different time points during, and even before or after, pregnancy, which may or may not directly influence the risk of T2D.

Twin studies represent a unique model to understand the role of the fetal environment in risk of developing T2D and associated dysmetabolic states of disease, but there is increasing concern that growth and development of twins in fetal life is different from that of singletons, and results from twin studies may therefore not be directly extrapolated to the general population (15).

Epigenetic mechanisms such as DNA methylation and histone modifications are considered to be important in phenotype transmission and the development of embryonic tissues. Therefore, epigenetic changes could represent a plausible mechanism by which gene functions can be permanently influenced by factors operating in fetal life. Nonetheless, we are only in the early stages of understanding how epigenetic mechanisms may influence the risk of common diseases in humans.

The aspiration of this narrative review is to provide an updated critical insight into recent developments in the field of developmental programming of T2D, the extent to which the hypothesis is challenged by genetic confounding, and to what degree epigenetic DNA methylation changes may still be considered a promising conceptual framework for explaining the link between adverse events operating in fetal life and subsequent increased risk of developing T2D. The prime focus will be on programming of T2D in humans through LBW and also to some degree by hyperglycemia in pregnancy.

Twin studies as a model for fetal programming

Some years after the proposal of the thrifty phenotype hypothesis by Hales et al. (3), we showed in a relatively small sample of genetically identical (monozygotic) twin pairs discordant for T2D, that twins with overt T2D displayed a small but significantly reduced birthweight compared with their genetically identical nondiabetic co-twins (16). The finding was important for the field of fetal programming, as it suggested that the association between LBW and increased risk of developing T2D may not solely be explained by one or more T2D susceptibility genes causing LBW as a result of impaired promotion of fetal growth by reduced insulin secretion, or action, in the fetus. Based on a similar theoretical argument of a genetically determined low-insulin promotion of fetal growth such as that launched in our twin study, Hattersley and Tooke a few years thereafter took an opposing viewpoint, launching the so-called "fetal insulin hypothesis" (17). To support the hypothesis that genetic impairment of insulin secretion may cause both LBW and increased risk of developing diabetes, Hattersley et al. used their discovery that carrying the glucokinase mutation causing MODY2 was associated with LBW (14). The

extent to which this finding may now be extrapolated to the much more common phenotype of T2D will be discussed later in this review. However, it needs to be stated here that the thrifty phenotype and the fetal insulin hypotheses are not mutually exclusive, and indeed both genetic and nongenetic factors causing LBW may explain or contribute to the well-established association between LBW and increased risk of developing T2D in humans.

By applying another twin approach we found a nongenetic association between LBW on the one hand and glucose intolerance and insulin resistance on the other in an unselected group of elderly twins with different glucose tolerance status (18). In another population of young and elderly nondiabetic twins, we applied reference standard measures for insulin sensitivity and once again confirmed the finding of a nongenetic association between LBW and insulin resistance independent of adult adiposity. Most interestingly, this association was only seen among elderly twins, suggesting an age-dependent effect of birthweight on insulin sensitivity (19). Frost et al. studied insulin secretion and insulin action using Homeostasis Model Assessments (HOMA) of fasting plasma insulin and glucose concentrations as well as oral glucose tolerance tests in a selected population of monozygotic twins with the most extreme intra-pair differences of birthweight (20). As expected, LBW was independent and significantly associated with insulin resistance and plasma glucose levels during oral glucose tolerance tests in this cohort. However, in the study there were no independent differences in insulin action or glucose tolerance status between the twins with the highest compared with lowest birthweight within the pairs (20). The discrepancies may have been a result of the association between birthweight and glucose tolerance status in different populations, which has consistently been shown to be U-shaped (21), meaning that selection of pairs with the largest birthweight discordance is likely to result in the recruitment of those twin pairs with a disproportionately increased T2D risk at both ends of the spectrum. Interestingly, the association between high birthweight and increased risk of T2D may occur mainly as a result of gestational diabetes mellitus (GDM) in the mother, as addressed below.

In a sample of 297 monozygotic and dizygotic elderly twins examined using oral glucose tolerance tests, we found a statistically significant increased prevalence of T2D compared with a control singleton sample recruited among healthy singleton spouses to the twins (22). However, spouses are not truly independent controls since a similar lifestyle is expected among the spouse and the twin. On the other hand, by including spouses, whom we expect to be genetically different but environmentally similar to the twins as a control group, the postnatal environmental "noise" in the comparison between twins and singletons was reduced.

Given the important role of age by itself in unmasking T2D in predisposed individuals, studies of T2D prevalence among elderly people are relevant. Another register-based Danish twin study, including both young and elderly twins, failed to reproduce our finding of an increased risk of T2D among monozygotic compared with dizygotic twins (23). However, in this study the diabetes diagnosis was based entirely on information from the Danish national registers, and the data were not supported by information on glucose tolerance status. Given the known selection bias towards more severe diabetes cases in registers, and as the sample of control cases may include a substantial proportion of people with undiagnosed T2D, it may not be surprising that this register-based study failed to reproduce our findings. Notably, our study showed that the increased prevalence of T2D among twins was carried predominantly by mild T2D cases (22).

Altogether, several lines of evidence support the risk of T2D among twins being influenced by the intrauterine environment in a complicated age- or time-dependent manner. Importantly, the influence of the intrauterine environment on risk of developing T2D in twins may differ both quantitatively as well as qualitatively from the influence of the intrauterine environment among singletons. Twin pregnancies may more often result in abortions compared with singleton pregnancies, and therefore it could be speculated that twins surviving a pregnancy may be genetically selected with respect to their capability to survive during metabolically challenging conditions. Twin studies should therefore be used with caution, and they may particularly not be used to assess the quantitative magnitude by which the intrauterine environment and LBW influence the risk of developing T2D in the general population.

T2D susceptibility genes

The availability and access to large-scale genome-wide screening platforms has substantially increased our knowledge of the role of genetics in T2D within the last decade. We now know of more than 50 independent genetic changes (single nucleotide polymorphisms), each of which is associated with a small but significantly increased risk of developing T2D in many different ethnic populations (24,25). Some of the major lessons learned are that the known T2D susceptibility genes are all extremely common in the general population, and furthermore they seem together to explain less than 20% of the total estimated heritability of T2D. The reason for this could of course be that there are many unknown T2D susceptibility genes still to be found, with a larger impact on the T2D risk in the general population. However, given the

extremely effective genetic screening platforms available, as well as the high prevalence of T2D in most societies, this does not appear to be the most plausible explanation. Other explanations for the unexplained heritability of T2D could be that heritability estimated from epidemiological studies of both twins and singletons has grossly overestimated the heritable component of the T2D disease due to factors such as more or less hidden common lifestyle factors and residual confounding by this in family studies. Importantly, these factors may include nongenetic exposures and lifestyle factors occurring at all stages in life. The limitations of heritability estimates from twin studies as a result of their special intrauterine environment have already been discussed, but also among singletons it is likely that a large proportion of the clustering of birthweights in families could result from shared nongenetic life conditions.

The fetal insulin hypothesis proposed that the association between LBW and risk of developing T2D could reflect that a gene was causing reduced insulin secretion and/or action, which could also explain the reduced growth of the fetus as a direct result of impaired insulin promotion of growth (17). Indeed, this was shown to be the explanation for the reduced birthweight in carriers of the MODY2/glucokinase mutation (14). We studied the extent to which any of the known common T2D susceptibility genes may affect birthweight in the Danish INTER99 cohort (26) and subsequently in a large consortium including 69 308 individuals of European descent from 43 studies (27). However, among the 47 known T2D susceptibility loci, only *ADCY5* and *CDKAL1* were associated with a minor reduction in birthweight. Hence, from these studies we can conclude that the consistent association between LBW and increased risk of T2D may at least not quantitatively be explained by the known T2D susceptibility genes. In a meta-analysis by Horikoshi et al., seven loci associated with birthweight as the primary phenotype were identified (27). These loci together accounted for a similar proportion of the birthweight variance as maternal smoking. However, besides *ADCY5* and *CDKAL1*, none of the identified birthweight loci were associated with a risk of developing T2D. The birthweight-lowering allele of *ADRB1* was associated with a reduced adult blood pressure. There is no way that this contributes to explaining the association between LBW and hypertension in the general population. The *HMGA2* and *LCORL* birthweight loci were associated with adult height, but not with any other cardiometabolic phenotypes. Interestingly, T2D susceptibility genes appear to influence the risk of diabetes in most populations by affecting distinct organ defects and in particular the pancreatic insulin secretion capability. This is in contrast to the effects of developmental programming influencing multiple organ functions relevant to the development of T2D (Figure 1).

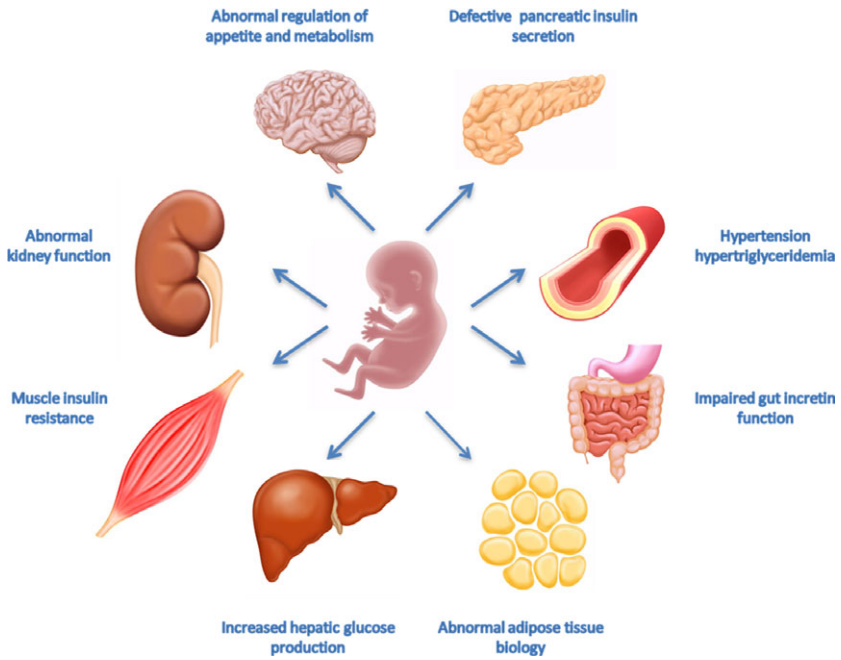

Figure 1. Type 2 diabetes is a multiple organ disease. The "thrifty phenotype hypothesis" represents a plausible and unifying explanation for the co-existence of all of the known metabolic organ dysfunctions in Type 2 diabetes. Reproduced with permission from (33) Vaag et al., 2012 ©Springer).

Lifestyle determinants in pregnancy and risk of T2D and associated dysmetabolic traits in offspring

The association between LBW and increased risk of developing T2D was originally thought to be mediated by undernutrition in pregnancy. This idea has been supported by studies of animals exposed to both global as well as protein undernutrition (13,28). In humans, the idea of undernutrition in pregnancy causing increased risk of T2D has to some extent been supported by results from the Dutch Famine Study (5,29), and more recently by follow-up studies of people surviving the extreme famine occurring in some parts of China between 1959 and 1961 (30). In contrast, the Leningrad Siege study only provided weak evidence of an association between intrauterine famine and low plasma insulin levels among adult offspring (31), and none of these epidemiological studies suggest that global intrauterine undernutrition may to any quantitatively important extent explain the global T2D epidemic we are now facing. Therefore, provided that developmental programming does represent an important player in T2D development, further knowledge of the exact exposure is needed. In that respect, there is an increasing awareness of the association between the fetal environment and risk of adult disease originating very early in pregnancy (32), and actually even at the time before or around conception (33), which could explain the limited impact of global undernutrition during the second and third trimesters of pregnancy on risk of developing T2D in the offspring. Interestingly, we recently found an association between a high glycemic index diet in pregnancy and adverse metabolic outcomes associated with increased risk of T2D among young adult offspring (34). The extent to which this may be mediated via high or low birthweight is currently unknown, but the Pedersen hypothesis of elevated maternal and subsequently fetal plasma glucose levels causing accelerated fetal growth could be part of the explanation for this association (35).

An affluent and unhealthy Western lifestyle is commonly associated with subclinical inflammation (36), which could represent another adverse exposure in pregnancy affecting risk of developing T2D in the offspring. However, measurements of four key markers of subclinical inflammation in pregnant women were not associated with adverse metabolic health outcomes among young adult offspring (37). Increased physical activity may improve metabolic health and reduce risk of developing GDM in pregnant women. However, in contrast to our a priori hypothesis of a more healthy metabolic profile in young adult offspring of pregnant women with a high physical activity level, we actually found some indications for the opposite scenario (38). In summary, we are only at the beginning of understanding to what extent distinct diet and lifestyle conditions in pregnancy affect the risk of developing T2D among offspring. There is an urgent need to understand the importance of timing of the adverse exposures, and furthermore there is a need to understand to what extent alternative adverse exposures during pregnancy such as infections, anemia and endocrine-disrupting chemicals may influence the risk of developing T2D in the offspring.

Developmental programming by GDM in the mother

One adverse exposure influencing the T2D risk in the offspring is maternal GDM. Several studies have shown that women with previous GDM have an increased risk of T2D in the years following pregnancy (39,40), and an estimated 30–70% risk of developing diabetes within 15 years (40–42). Most importantly, data strongly indicate that the intrauterine hyperglycemic and hypermetabolic environment in mothers with GDM may place the offspring at a substantially increased long-term risk for T2D at a magnitude exceeding that known for intrauterine undernutrition and LBW. Studies in selected groups of GDM offspring have shown an increased risk of overall and central obesity (43,44), and data from a Danish GDM offspring cohort have shown that young GDM offspring exhibit up to an eight-fold elevated risk of T2D, a two-fold higher risk of overweight and a four-fold higher risk of metabolic syndrome (45,46). Experimental animal studies have supported the notion that elevated plasma glucose levels in pregnancy may directly influence organ development and subsequently the risk of T2D development in the offspring (47). This is important given the theoretical possibility that genes shared between the mother and her offspring may contribute to the association between GDM and risk of T2D development among offspring in humans. Women born with an LBW exhibit increased risk of developing GDM (48), and it has been suggested that transmission of increased risk of T2D over several generations may be initiated by fetal undernutrition and LBW in the first generation, subsequently maintaining an increased risk of T2D in the next generations due to GDM in women affected by either LBW or GDM in their own mothers (49). The extent to which LBW and GDM may influence the risk of developing T2D in different developing or developed societies, and/or if the role of LBW versus GDM in causing T2D may change over time within societies, is currently unknown. For instance, it may be speculated whether the T2D epidemics occurring today in developing countries could reflect fetal undernutrition in the present population, whereas the

T2D epidemics in developed societies could to a greater extent be a result of GDM or obesity.

Developmental programming and epigenetics

The increasing awareness of a prime role of epigenetics, including DNA methylation and histone modification, as well as small noncoding regulatory microRNAs, has provided ideas and novel tools to look for mechanisms underlying developmental programming of organ defects relevant to insulin resistance and T2D. An important example of epigenetic discoveries from animal studies is the documentation of transcriptional regulation by promoter DNA methylation and histone modifications of the key pancreatic proliferation and transcription factor *PDX1* by fetal undernutrition (50).

We have demonstrated that the fetal environment affects epigenetic prints in human tissues, including skeletal muscle and subcutaneous fat, thereby reinforcing fetal life as a critical time for establishment and maintenance of epigenetic marks. For example, we found that young LBW men exhibit increased methylation in the promoter region of the transcription factor *PPARGC1A* in both skeletal muscle and subcutaneous fat compared with normal birthweight controls (51,52). Furthermore, we recently investigated global DNA methylation in both muscle and fat tissue obtained from normal birthweight and LBW individuals following 5 days of overfeeding. We found that in skeletal muscle, overfeeding induced widespread DNA methylation changes in normal birthweight controls affecting genes involved in inflammation, metabolism and cancer (53). However, similar changes in DNA methylation were not seen in LBW individuals, suggesting inflexibility of short-term changes of DNA methylation in response to overfeeding (54) (Figure 2).

Furthermore, an immature fat stem cell phenotype seems to accompany LBW. We found increased DNA methylation in the leptin promoter region in differentiating pre-adipocytes from LBW individuals compared with matched controls (55). This finding was supported by a markedly reduced gene expression and secretion of leptin in adipocyte stem cells from LBW individuals (Figure 3). In addition, we found decreased gene expression of the adipocyte differentiation markers *FABP4/aP2*, *PPARγ2* and *GLUT4* gene expression in LBW pre-adipocytes, all together indicative of an immature stem cell phenotype (55).

Regarding the potential role of small noncoding RNAs, we have found increased expression of microRNA (miR)-483-3p in subcutaneous adipose tissue from LBW humans and in rats that were protein undernourished in utero (56). Subsequent analyses showed that miR-483-3p

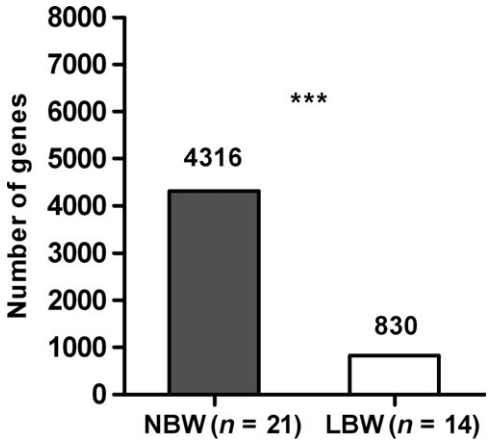

Figure 2. DNA methylation response to 5 days of high-fat overfeeding in skeletal muscle from normal birthweight (NBW, grey bars) and low birthweight (LBW, white bars) men. Number of genes with at least one CpG site that shows a DNA methylation difference with $p < 0.05$ after the high-fat overfeeding diet compared with the control diet are shown on the *y*-axis for all subjects examined. Total number of genes on the array: 14 475. ***$p < 0.001$ (χ^2). (Reproduced with permission from (53) Jacobsen et al., 2014 ©Springer).

influences the protein expression level of the GDF3 protein, which in turn is involved in the development and maturation of adipose tissue. We showed that the GDF3 protein was downregulated in adipose tissue samples from both rats that were undernourished in fetal life as well as in young LBW men (56). This may lead to impaired development of the subcutaneous adipose tissue and may reduce the capacity to store fat in the adipose tissue leading to fat deposition in other tissues including muscle, liver and the pancreatic beta cells. This in turn may impair the metabolic function of these organs by an effect known as lipotoxicity, thereby adversely influencing the risk of developing T2D.

It is generally believed that epigenetic changes are tissue-specific, meaning that these changes in tissues of primary relevance to the development of T2D including muscle, fat, liver and the pancreatic beta cells, may not be mirrored by similar epigenetic changes in blood cells. In that respect, the recent report of DNA methylation alterations of the *HIF* gene, as seen both in blood cells as well as in adipose tissue biopsies from obese individuals, may be of great importance (57). Indeed, this supports the rationale for ongoing studies of epigenetic changes in blood cells, including cord blood samples, in people exposed to an adverse fetal environment. Studies from the Dutch Famine Study reported a decreased DNA methylation of the *IGF2* gene in blood from offspring of severely undernourished mothers compared with their unexposed, same-sex siblings (58). This association was

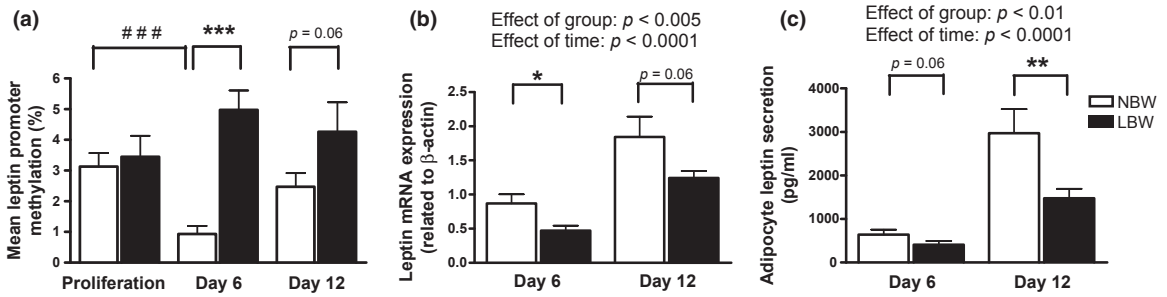

Figure 3. Stem cells isolated from subcutaneous adipose tissue biopsies of young men with a low birthweight exhibit reduced gene expression (b) and release (c) of the leptin hormone when cultutred in vitro compared with stem cells (pre-adipocytes) from normal birthweight subjects. Importantly, the reduced leptin gene expression level in cells from low birthweight subjects is likely to be caused by increased leptin promotor DNA methylation (a). (Reproduced with permission from (55) Schultz et al., 2014 ©American Diabetes Association.)

specific for periconceptional exposure, reinforcing that early human development is a crucial period for establishing and maintaining lifelong epigenetic marks.

In addition to studies of undernutrition in pregnancy, studies on overnutrition exemplified by GDM, have also supported epigenetics as a link between fetal programming and later risk of developing T2D. Recently published data from genome-wide methylation analysis on blood samples from 21 children whose mothers had GDM, showed that increased methylation of the *PYGO1* and *CLN8* genes was associated with increased vascular cell adhesion molecule 1 levels (involved in atherosclerosis) in the offspring of GDM mothers (59). Another recent small-scale study reported that global methylation levels were reduced in placentas from GDM women, whereas no major differences in methylation levels were detected in cord blood samples (60). Furthermore, the maternally imprinted *MEST* gene, the nonimprinted glucocorticoid receptor *NR3C1* gene, and interspersed *ALU* repeats, showed significantly decreased methylation levels in both placental tissue and cord blood from GDM mothers (61). Taken together, recent studies have provided increased evidence for the finding that epigenetic changes can be detected in primary tissue samples as well as in blood samples from people affected by an adverse intrauterine environment such as is seen in connection with LBW or in the offspring of GDM women. Although studies of immature cell types from these individuals in particular appear promising, we are only at the very beginning of understanding the role of epigenetics in developmental programming of T2D.

Conclusion

In conclusion, T2D is a multifactorial disease affecting multiple organ functions involved in insulin and glucose homeostasis. The known T2D susceptibility genes may only explain a small proportion of the background risk of developing the disease, and there is increased evidence that developmental programming by nongenetic mechanisms is a significant player in the origin of T2D. People born with LBW and offspring of pregnancies affected by GDM are the main groups at increased risk of T2D, as a result of an adverse intrauterine environment. Studies have emerged to support that epigenetic mechanisms including DNA methylation, as well as small noncoding microRNAs, are involved in the development of T2D, and in particular recent studies of pre-adipocyte stem cells have suggested that general immature cell differentiation influenced by epigenetic changes may contribute to the development of T2D in individuals born with LBW.

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