Epigenetics and Obesity: A Relationship Waiting to Be Explained

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Introduction

Obesity can have multifactorial aetiologies. Although the primary factors are considered to be excess dietary intake when combined with limited physical activity \cite{1}, there are potentially numerous other contributors \cite{2} which may differ with development \cite{3}. Over the last decade, the worldwide increase in the incidence of obesity or ‘obesity epidemic’ has been accompanied by a parallel increase in academic research designed to both provide mechanistic explanations for excess fat deposition and identify potential new therapeutic targets. Whilst genetic research was expected to elucidate the pathways to excess fat deposition, the proportion of the variance in obesity explained by genetic variants remains low, despite extensive spending on genetic epidemiology studies by the National Institutes of Health (NIH). The emergence of the field of epigenetics now offers a new avenue with which to examine the causes of obesity. This article will argue that, despite the promise of epidemiological epigenetic studies, we need to understand function and practice caution in the interpretation of genome-wide association studies (GWAS) and related investigations.

Challenges for Genome-Wide Association Studies

Twin studies across a variety of ages and developed countries suggest that genetic variants contribute to between 40 and 85\% of the variation in body mass index.
(BMI) [4]. This provides the theoretical opportunity to use genetics to identify mechanisms contributing to BMI. As with many complex traits, initial candidate gene studies have not, to date, produced reliably validated associations with BMI [5]. The advent of GWAS was heralded as a breakthrough in overcoming this challenge. By combining the complete outline of common single-nucleotide polymorphisms (SNPs) and the correlational structure between them, GWAS enable approximately 80% of the genome to be screened using just 500,000–1,600,000 genotyped SNPs. Although numerous expensive GWAS have, to date, resulted in 32 validated loci associated with BMI [6], these genetic variants are only able to explain about 1–3% of the variance in BMI [7]. 1–3% is a non-trivial proportion of variance from a public health perspective and has the potential to identify intervention targets which can reduce BMI. Nonetheless, GWAS have not lived up to their initial promise, and we ask researchers to consider whether this approach will be more productive in finding the causes of obesity than mechanistic studies using, for example, in vitro cell types (discussed below).

The apparent failure of GWAS to explain the heritable variance in such complex traits as obesity has been termed the problem of the ‘missing heritability’ [8]. Whilst the reasons are unknown, plausible candidates include insufficient coverage of rare variants, the inability to account for environmental influences, poor statistical genetic methodology in some studies, and poor phenotype definition in traits with heterogenous and polygenic aetiologies, such as obesity.

**Epigenetics**

Epigenetics refers to the study of heritable and environmentally mediated changes to the genome that are mitotically stable and affect gene function but do not modify DNA sequence [9]. Although current epigenome-wide association studies (EWAS) do not use measured environmental factors as epigenetic changes such as methylation may reflect current diet, EWAS offer the potential of partially incorporating the role of the environment with genetics into a single paradigm. The main epigenetic marks are genomic DNA methylation, changes in chromatin organisation by histone modification, and noncoding RNAs or microRNAs [10]. Deviations from the base-pair consensus sequence result in altered gene expression, combining genetic and epigenetic information at loci could increase the amount ofheritable variance explained in obesity phenotypes [11]. For example, the obesity susceptibility locus fat mass and obesity-related gene (FTO) is involved in the demethylation of RNA [12]. Epigenetic studies have primarily emerged from the field of cancer [9], which might be considered as one extreme example of excess cell growth. It is therefore possible that the types of epigenetic adaptations found in cancer may represent one of a range of typical cellular responses to potentially life-threatening diseases.

Of note is that obesity, like cancer, is an example of excess and dysregulated cellular growth for which the risk of cancer is raised with obesity [13]. The extent to which comparable adaptations as in cancer are present in obesity represents a challenging question given its multi-system organ involvement and given that excess energy intake versus insufficient energy expenditure are considered to be the primary causes [14]. It is, however, worth noting that the process of expansion within adipose tissue with obesity represents a coordinated response amongst a range of cells that includes endothelial precursor and immune cells as well as preadipocytes that again share similarities with the growth of solid tumours [15].

The implication of DNA methylation in metabolic programming has coincided with the ready availability of genome-wide methylation arrays, which systematically screen the genome for methylation at locations where a cysteine and a guanine molecule are held by a phosphate bond (CpG islands). The addition of a methyl group at CpG islands, especially in the promoter regions, are likely to affect gene regulation through the reduced binding of transcription factors and repressors [16–18]. CpG methylation is also linked to histone acetylation through the methyl-CpG-binding protein 2 (MECP2) protein, which binds to methylated CpG sites and recruits histone deacetylases, causing regulatory consequences by influencing chromatin structure [16]. This technology has provided the potential to add to the search for causes of obesity by looking for epigenetic associations with BMI and gene function, from which we can infer the mechanistic insights needed to develop effective preventative interventions and treatments.

**Epigenetic Changes in utero and Later Obesity Risk**

Observations that prenatal exposure to maternal type 2 diabetes (T2D) in Native American Pima Indians predisposes offspring themselves to T2D and obesity led to the hypothesis that nutritional and other environmental exposures in utero can result in long-term changes which alter the function of our genes and give rise to a predispo-
position for diseases [19, 20]. Such ‘fetal origins’ hypotheses underpin current epigenetic research in this area, and subsequent animal and human studies have established that the molecular basis for such programming, centred around altered DNA methylation, could have functional consequences for gene expression [21, 22].

Although increased adiposity in pregnancy is associated with a greater risk for a range of pregnancy-related complications including increased birth weight in the offspring, as recently reviewed by Lawlor et al. [23], little high-quality human evidence exists to show such effects are mediated by epigenetic mechanisms. Some studies [24, 25] have shown an association between epigenetic variations and birth weight, but the extent to which this is causative and/or reflects changes in body composition is currently unknown. The main evidence supporting the role of epigenetic mechanisms in mediating the prenatal exposure to later health comes from the Dutch famine studies in which the significant prenatal nutritional stress was accompanied by substantial emotional stress [26]. These studies may have been confounded by events surrounding conception during this period of conflict, which may have resulted in increased stress and other adverse effects including the relatively late recognition of pregnancy [27]. The main outcome from these and related studies is therefore to emphasise the highly variable nature of methylation status across different genes [21, 28, 29].

It has also been proposed that nutritional manipulations prior to conception may have the greatest subsequent ‘epigenetic effect’ [21, 28]. However, supportive findings from more contemporary studies are currently confined to assisted reproduction [30] and large animal studies adopting embryo transfer [31]. It should be noted that the process of embryo transfer itself could be a major factor determining any long-term outcomes. For example, a combination of embryo transfer plus nutritional intervention can result in the opposite effect to the nutritional intervention alone, as summarised in table 1.

In developmental studies, the placenta is an obvious target for examining epigenetic responses with the number of methylated sites showing a modest increase as gestation progresses [32]. This is not unexpected given the pronounced changes in placental function that occur in order to meet the increase in the nutritional demands of the fetus as it grows [33]. The pathways affected also differ substantially with gestational age, thereby reflecting functional changes within the placental-fetal unit with development. Given the vital role of glucose in determining fetal growth and especially fat deposition, for which fetal supply is determined in part by placental glucose transporters (GLUTs), it is of interest to note that the methylation of each GLUT differs widely [34]. Methyla-

<table>
<thead>
<tr>
<th>Method of conception</th>
<th>natural mating [91]</th>
<th>embryo transfer with embryo taken from adult sheep [92]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, months</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Maternal body weight, kg</td>
<td>49</td>
<td>44</td>
</tr>
<tr>
<td>Nutritional intervention</td>
<td>2- to 3-fold increase in food intake</td>
<td>2-fold increase in food intake</td>
</tr>
<tr>
<td>Change in maternal body weight in pregnancy</td>
<td>approximately 20-kg increase</td>
<td>approximately 20-kg increase</td>
</tr>
<tr>
<td>Change in birth weight, %</td>
<td>approximately 10% increase</td>
<td>approximately 40% decrease</td>
</tr>
</tbody>
</table>

To date, two publications have attempted to relate DNA methylation in either cord blood or umbilical tissue to later fat mass in children of normal weight [37, 38]. The first study [37] showed that the degree of DNA methylation of the promoter region of 5 candidate genes was positively correlated with the fat mass in 78 children at 9 years of age, a finding that was only replicated for the RXRα in a separate cohort of 239 children at 6 years of age
when comparing 20% increments in total methylation. This type of reductionist approach may be too simplistic given the complex nature of infant growth which can be strongly influenced by many factors ranging from maternal diet and offspring gender [39, 40], to relative growth trajectory [41] that can all impact on body composition.

**Epidemiological Studies on Methylation and Obesity**

Two approaches have been used to study the associations between methylation and BMI: candidate gene studies and EWAS. Candidate gene approaches implicate a number of regions showing associations between altered methylation and BMI as a continuous trait or obesity as a categorical outcome [37, 38, 42–44]. However, as with initial candidate analyses, these studies have been blighted with a lack of replication [45] and methodological limitations such as sample sizes considered small for such genetic analyses. Although EWAS are emerging which implicate altered methylation in regions in the FTO gene [46] as well as the tripartite motif-containing 3 (TRIM3) and ubiquitin-associated and SH3 domain-containing A (UBASH3A) genes [47] with obesity, there still is a lack of independent replication. In addition to controversy over whether to look for rare or common variants in genetic research, there is currently no consensus as to which cell types to explore in EWAS; the so far published studies analyzed umbilical cord blood [42] and tissue [37], whole blood [42, 44, 46], saliva [48], and peripheral blood leukocytes [49] and lymphocytes [38, 50]. Even when standardizing across cell types, controlling for the effects of age on methylation remains a poorly understood statistical area. Monozygotic twin studies indicate that methylation changes with age, as do degenerative diseases such obesity [51, 52]. Finally, epigenetic studies face the same problems with statistical methodology and causal inference as genetic studies. Given these challenges, are there functional data to support a potential role for epigenetic, especially methylation, changes in the aetiology of obesity and enough empirical reasoning for justifying the time and expense of EWAS?

**Mechanistic Data on Epigenetics**

Animal studies offer the opportunity to examine the effects of epigenetic programming on obesity with a mechanistic approach. However, they are often confounded by suboptimal experimental design and interpretation [53]. In models of the development of obesity, many animal models use interventions which result in the rapid onset of obesity and are therefore of limited translational value [54] as this pattern of excess fat deposition in the months or weeks immediately before conception is not common at a population level.

Importantly, changes within adipose tissue itself underpin obesity. To date, the main processes considered to be strongly influenced by epigenetics relate to the actions of microRNAs [55] and long noncoding RNAs in the regulation of adipogenesis [56]. Fat depots have their own unique gene expression profiles [57] as well as developmental growth trajectories [58] which are not driven by changes in methylation [59]. This suggests that demonstrating a modest change in the methylation pattern of an individual gene is of limited value in our understanding of the process of obesity and its adverse long-term consequences. Indeed, our knowledge of adipose tissue biology is currently undergoing groundbreaking reassessment as it is now known that there are at least three different categories of adipocytes, i.e. brown, beige, and white adipocytes [60–62]. These may have very different lineages as, for example, brown fat appears to be closely related to muscle [55, 63] and holds a controversial role in the regulation of adult energy balance [64] with its potential to contribute to up to 20% of daily heat production when maximally stimulated [65].

**Adipose Tissue Growth and Its Response to Obesity**

Adipose tissue has a unique role as the primary energy-storing tissue of the body which enables virtually unlimited capacity to grow and expand [15]. Indeed, during early life, it can be the most rapidly growing organ within the body [66] and exhibits a number of adaptations that enable remodelling processes to occur [67]. These include a change in its inflammatory profile [68], which may facilitate its persistence under the hypoxic environmental conditions that occur during its proliferation and expansion [15].

Importantly, from a developmental perspective, the primary fat depot present in the fetus and newborn is brown adipose tissue, which is rapidly activated at birth following cold exposure to the extraterine environment [69]. As a result, the brown adipose tissue-specific uncoupling protein (UCP1) is present in maximal amounts and is maximally activated following intense endocrine stimulation such as that occurring at the time of birth [70].
The extent to which changes in brown fat function can contribute to changes in fat mass in later life also remains to be confirmed. It is of interest to note, however, that the onset of nonshivering thermogenesis in brown fat is compromised in those delivered by caesarean section [70] due to reduced neuroendocrine stimulation of UCP1 activation [71, 72]. This could explain, in part, the association between birth by caesarean section and overweight and obesity in later childhood and adulthood [73]. The activation of brown adipose tissue after birth is followed by its transformation to, and/or replacement with, white adipose tissue [66, 67], and this is likely to be accompanied by different methylation profiles and chromatin remodelling, as shown in vitro [74]. To date, however, epigenetic changes have not been shown to be major regulators of this process.

**Rodent Models of Epigenetic Programming**

One early-life programming model which has been interpreted as showing that the maternal diet had an impact on the incidence of obesity in the offspring is that of the agouti mouse, in which changes in coat colour can be linked to differences in body weight and, by inference, fat mass [75]. The finding that gross changes in the micro-nutrient content of the diet (i.e. approx. 9-fold increase in choline and folic acid, approx. 3 times more methionine, and approx. 60 times more vitamin B12 compared to control diets [76, 77]) altered the relative distribution of coat colour in the offspring has been widely cited as indicative of an epigenetic cause of obesity. However, the actual proportion of offspring which were of the lean phenotype was barely altered by the dietary intervention [78]. Further publications using this model have also suggested a generational effect [79], but this outcome may have been confounded by a mismatch in the distribution of sex between groups [78]. This is clearly important as across a majority of species, females tend to possess more fat than males and, in rodents, this difference is exemplified by different sex-specific growth trajectories which culminate in females attaining their mature body weight much earlier than males [53].

A number of other nutritional models using rodents have implicated epigenetic adaptations, although to date these have primarily focused on nutrient imbalances which are accompanied by some degree of fetal growth retardation [33]. The main driver of the adverse phenotype of excess weight gain in such models is accelerated postnatal growth. This is particularly important in the rodent as it occurs over the periods in which maturation of the hypothalamic-pituitary-adrenal axis, thermoregulatory control, and activation of nonshivering thermogenesis with brown adipose tissue occur [33]. The results from rodent models are also difficult to interpret as they are frequently presented as a percentage change in methylation above baseline. As noted earlier, since it is the actual methylation state primarily across the promoter region of the gene of interest that will determine gene function [80], such results may have little, if any, functional relevance.

The most comprehensive studies in epigenetic programming have been conducted on the pancreas showing progressive epigenetic silencing of Pdx1 [81]. These studies have utilised uterine artery ligation as an experimental intervention, resulting in a significant reduction in fetal blood supply but not always in intrauterine growth retardation [82]. In addition, although approximately 50% of the offspring show postnatal accelerated growth, whether this predicts later adverse outcomes has not been reported. Recently, investigators utilising this model have employed a bioinformatics approach and have demonstrated that, rather than being adaptations within single genes, the overall pattern of DNA methylation is reset [83]. Consequently, the majority of responses were found within gene-encoding regions and promoters as well as noncoding DNA regions, suggesting structural changes within the chromatin [84]. These adaptations were seen as early as 7 weeks of age, prior to the onset of obesity, highlighting the critical role that disturbed insulin action has in metabolic programming [83].

Complementary studies on the pancreas using a low-protein/high-carbohydrate diet have further shown an abnormal epigenetic regulation of a promoter-enhancer interaction at the gene encoding HNF-4α [85]. This is a key transcription factor required for pancreatic β-cell differentiation and glucose homeostasis and leads to transcriptional downregulation and silencing of the Hnf4α locus. Interestingly, the effect appears to not be disrupted by ageing [86]. Whilst disturbances in both insulin secretion and action underpin many of the adverse outcomes following fetal programming [87], whether epigenetic adaptations are their cause or consequence remains to be established.

The complexity of adipose tissue development is becoming more apparent with at least 50 factors in rodents now being shown to affect brown and/or beige adipocyte development and function, often in a depot-specific manner [62]. Moreover, the extent to which each depot can be quantified and manipulated will have important conse-
quences for its place as a potential therapeutic target [88]. To unravel the relationship between early development, epigenetics, and risk of later obesity, the routine use of techniques for functional assessment, such as those recently demonstrated for brown adipose tissue [89, 90], will be required in relevant cohorts of subjects.

Towards the Future of Epigenetic Research in Obesity

Obesity has a multifactorial aetiology with the potential for psychological, behavioural, sociological, and other contributors. At its core, however, obesity is a disorder of excess fat deposition. Many of the poor health outcomes associated with obesity are driven by the metabolic results of excess fat accumulation. Great strides have been made in understanding adipose tissue biology, and investigators should not lose sight of this vital work when looking for contributors to obesity. EWAS, to be useful, are likely to have to be conducted on the same scale as GWAS, which, to date, have made valuable but small contributions in explaining the individual variation in BMI. Now we are beginning to understand why their utility has thus far been limited: a lack of statistical refinement and a lack of functional phenotypes, to name but two candidates. Given the similarity of the first wave of EWAS results to the first wave of GWAS results, we would suggest there is much to be learned from the history of obesity research as we move forward with future studies.

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Epigenetics and Obesity Research Considerations

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