Fetal programming and epigenetics
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Abstract
Accumulating evidence suggests that the intrauterine environment can have an impact on long-term offspring health, so-called ‘fetal programming’. A number of environmental stressors have been studied in humans including maternal nutrition, smoking, substance misuse and mental illness. Although various biological mechanisms are likely to underpin fetal programming effects, there has been a particular focus on epigenetic modifications as potential mediators of observed associations between early environmental exposures and later health outcomes. In this review, we give an overview of evidence supporting a role for epigenetics in fetal programming, highlighting key human and animal studies. We also discuss challenges for research in this area, along with recommendations for future work, and potential therapeutic applications.

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Keywords
Fetal programming, Epigenetics, DNA methylation.

Introduction
Human and animal studies have demonstrated a link between in utero fetal stressors and altered risk of adult disease. Pioneering work in this field was conducted by David Barker who linked low birthweight to increased risk of metabolic disease and cardiovascular mortality in adulthood [1,2]. A diverse range of exposures in pregnancy have since been linked to life-long health outcomes in offspring including undernutrition and overnutrition, maternal mental illness and substance misuse [3–7].

Although the underlying mechanisms remain unclear, it has been hypothesised that certain intrauterine stressors may alter developmental trajectories in early life to ensure the highest chance of fitness and/or survival in later life as part of an adaptive response [8]. Barker postulated that poor nutrition in pregnancy caused physiological reprogramming resulting in an offspring well adapted to poor nutrition. Such an individual would then be at increased risk of metabolic disease in a nutritionally rich environment, the so-called ‘thrifty phenotype’ hypothesis [9].

Epigenetic modifications to the genome involve mitotically heritable changes that can influence gene expression without changing the underlying DNA sequence [10]. There is great interest in the potential for epigenetic modifications to mediate observed links between early environmental exposures and later health outcomes because they can be sensitive to the peri-conceptional and in utero environment and can persist throughout development [11]. In this short review we explore the role of epigenetic processes in mediating fetal programming using evidence from animal and human studies. We also highlight some of the challenges of research in this field and describe its potential use for identifying prognostic biomarkers and for designing therapeutic interventions, as well as suggesting avenues for future research.

Fetal programming and epigenetics
Epigenetic modifications include DNA methylation, histone modifications and RNA-based processes [10]. DNA methylation involves the addition of a methyl group to a cytosine base, most commonly where the cytosine is adjacent to a guanine, termed a ‘CpG’ site. CpG methylation at gene promoters typically leads to gene silencing although effects on transcription generally vary according to genomic context. Histone proteins act to compress DNA by coiling it up into units called ‘nucleosomes’. Histone modifications including methylation, acetylation and phosphorylation of histone ‘tails’ can influence gene expression by affecting the accessibility of the DNA and its packaging into areas of active and inactive transcription. There is increasing evidence that DNA methylation and histone
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DNA methylation plays a crucial role in embryonic and fetal development, most notably in establishing and maintaining cellular identity, in X-inactivation in women and in the regulation of genomic imprinting [12]. The first few days after conception constitute a particularly dynamic phase of epigenetic reprogramming, when epigenetic marks from parental gametes are largely erased to render cells into a ‘naive’ state, ready to acquire cell- and tissue-specific marks at gastrulation and beyond [13]. This cascade of early developmental processes which includes molecular mechanisms that maintain parent of origin-specific methylation at genomic imprints points to the periconceptional period as a ‘critical window’ for epigenetically mediated fetal programming effects. Indeed, many fetal programming studies focus on imprinted loci as potential hotspots of environmental influence in the human methylome [14].

Interpreting DNA methylation association signals
Multiple factors complicate the interpretation of results from epigenome-wide association studies [15]. In a fetal programming context, this means that the identification of a statistically significant association between an early exposure and methylation at a particular locus is only a first step. Relevant factors include the extent (single CpG or regional, encompassing multiple CpG) and location (position with respect to regulatory features such as promoters or enhancers) of an association signal, both of which can inform its likely functional significance. The difficulty of assessing methylation changes in functionally relevant tissues is another consideration when attempting to assess functional relevance in human cohort studies. The observed effect size may also be important because, for example, large studies may be powered to detect small methylation differences that may be functionally unimportant. Ideally, exposure-related methylation changes should be linked to gene expression changes in functionally relevant tissues. Where these are unavailable, reference to external bioinformatics resources with expression and other epigenetic data can help to inform functional significance. Other considerations include the difficulty of establishing causal links between exposure, methylation and phenotype. This and the potential use of association signals as noncausal biomarkers of fetal programming effects are considered in a later section.

Animal studies
The ability to study mammalian development in relevant tissues and in tightly controlled environments means that animal studies have played an important part in establishing the role of epigenetically driven fetal programming in altering later disease risk. Studies in rodents have shown that maternal undernutrition and overnutrition, micronutrient status and stress can influence the physiology of the offspring, and that these changes are often accompanied by changes in the methylation of key metabolic or regulatory genes [16–20]. For example, feeding rats a protein-restricted diet during pregnancy induced hypomethylation of the glucocorticoid receptor and peroxisomal proliferator-activated receptor (Ppar)-α promoters in the livers of juvenile and adult offspring, which was associated with an increase in glucocorticoid receptor and Pparα mRNA expression [21,22], whereas Vucetic et al. [19] showed hypomethylation and increased expression of the μ-opioid receptor (Mor) and preproenkephalin (Penk) in the nucleus accumbens, prefrontal cortex and hypothalamus of mice from dams that consumed a high-fat diet during pregnancy.

Animal studies can also help identify details of the specific epigenetic mechanisms involved. Using a model of intrauterine ligation, which results in intrauterine growth restriction, Park et al. [23] found that the decrease in the expression of the pancreatic and duodenal homoeobox 1 (Pdx1) transcription factor was accompanied by changes in histone modifications around the pdx1 promoter which was subsequently followed by a change in DNA methylation, suggesting that DNA methylation may consolidate the change in expression.

Human studies
Early studies demonstrating a link between prenatal environment and altered epigenetic profiles in offspring were conducted in the offspring of mothers who were pregnant during the ‘Dutch Hunger Winter’ (DHW) [3,24]. This severe famine was caused by food shortages in Northwestern Holland during the Second World War. Initially, researchers noted that there was an increased risk of obesity in young men whose mothers were pregnant during the famine [3]. Further studies demonstrated altered DNA methylation up to six decades later, with the periconceptual and early pregnancy period identified as a critical period for famine exposure [24,25]. Methylation changes in blood were associated with genes related to growth and increased risk of metabolic disease in both male and female offspring [26].

Maternal BMI
A number of studies have demonstrated associations between maternal body mass index (BMI) and DNA
methylation in offspring. Evidence from candidate gene studies has identified links between maternal BMI and methylation in placenta, cord blood and child saliva at several genes with a known role in energy metabolism including PPAR and leptin (LEP) [27–29]. The advent of array-based platforms such as the Illumina 450 k and EPIC arrays has enabled the measurement of methylation genome-wide, although coverage with these arrays is still limited to 2–3% of CpGs in the genome. Analysis of 450 k methylation data from the UK Avon Longitudinal Study of Parents and Children (ALSPAC) cohort demonstrated an association between maternal BMI and altered offspring methylation at several loci in cord blood, and in peripheral blood at 6 and 15 years of age [30]. Stronger associations with maternal rather than paternal BMI supported an intra-uterine mechanism, and methylation changes were associated with differences in offspring adiposity. A meta-analysis of >10,000 mother–offspring pairs in the Pregnancy and Childhood Epigenetics (PACE) consortium found significant associations at large numbers of loci in newborn blood, although most were accounted for by changes in blood cell composition, with evidence for a causal uterine effect at 8 loci. There was some evidence for persistence of signals into adolescence [31].

Children born after maternal bariatric surgery have improved cardiovascular risk profiles compared with their unexposed siblings, and a number of studies have investigated potential epigenetic mediators. A sibling study explored the impact of maternal bariatric surgery on blood methylation levels of genes associated with cardiometabolic pathways in offspring born before and after maternal bariatric surgery and identified multiple differentially methylated loci [32]. A similar study found that maternal bariatric surgery is associated with methylation differences at genes involved in glucose and leptin signalling [33].

### Maternal diabetes

DNA methylation changes in offspring have also been associated with maternal diabetes with differential methylation at some loci linked to metabolic changes in offspring. One study in 485 mother–child dyads found evidence that changes in cord blood methylation at the leptin gene mediated the relationship between maternal hyperglycaemia and neonatal leptin levels [34]. A study of 388 Pima Indian children found significant associations between exposure to intrauterine maternal type two diabetes mellitus and DNA methylation in blood at multiple genes, some of which were linked to impaired insulin secretion, weight gain and increased risk of type 2 diabetes [35]. An analysis of offspring from the US Exploring Perinatal Outcomes among Children (EPOCH) cohort that were exposed to gestational diabetes mellitus also found alterations in blood methylation at a number of genes, one of which was associated with multiple adiposity-related outcomes [36]. A recent Danish study with more than 1200 cases and controls also found associations between gestational diabetes mellitus and blood methylation changes in children, although most were confounded by maternal BMI, and only one CpG was validated in a replication cohort [37]. Results from array-based studies assessing genome-wide methylation are generally inconsistent, perhaps reflecting differences in inclusion criteria, time of methylation measurement and adjustment covariates used (e.g. maternal BMI).

### Maternal nutrition

Nutrition can influence DNA methylation through the action of one-carbon pathway and associated metabolites including folate, riboflavin, choline and betaine [14]. Multiple studies, including follow-ups to randomised controlled trials with maternal folate interventions, have looked at the effect of folate in pregnancy on offspring DNA methylation [38–41]. These have found many positive associations, but with no consistent pattern of associated genes or effect direction emerging, although this may be due to variation in windows of exposure and differences in times of measurement in postnatal tissues. Other evidence comes from so-called ‘natural experiments’ such as the DHW studies. From a fetal programming perspective, a notable DHW study of 885 subjects found evidence that a number of methylation loci measured in whole blood mediated a relationship between exposure to maternal famine in utero and adult BMI and serum triglycerides, including at genes related to glycolysis and adipogenesis, with methylation-associated gene expression changes in an external data set [42]. A series of studies in The Gambia in sub-Saharan West Africa exploited another natural experiment, whereby a population largely dependent on subsistence farming experiences annual patterns of rainy and dry seasons, with corresponding significant seasonal differences in one-carbon nutrients measured in maternal plasma. These studies have found DNA methylation changes associated with season of conception and certain one-carbon nutrients at human metastable epialleles — loci with independent evidence of establishment in the early embryo [43,44]. Notably, methylation at one region within the POMC gene with evidence of nutrient-sensitive establishment in the early embryo and altered methylation in the arcuate nucleus of the hypothalamus has been associated with obesity in German children and adults [45,46].

### Maternal smoking

Multiple studies have identified consistent changes at specific methylation loci measured in offspring blood that are associated with maternal smoking during
pregnancy, making this one of the most robust epigenetic biomarkers of an early environmental exposure. Large studies reveal that smoking-associated changes are present at birth in cord blood and appear to persist at least into adolescence [47,48]. More recently, a series of studies have used causal analysis techniques such as Mendelian randomisation to explore links to health outcomes and related traits in offspring. A meta-analysis of five prospective birth cohorts with participant ages ranging from 16 to 48 years identified several loci measured in blood that were associated with maternal pregnancy in smoking, with evidence of a causal link to increased risk of inflammatory bowel disease and psychiatric morbidity in offspring [49]. Another study linked smoking-related loci in blood identified methyl-ation loci in blood linked to prenatal smoking that were associated with several cardiovascular risk factors [51].

Challenges, opportunities and avenues for future research
Establishing an epigenetically mediated causal pathway from exposure through to disease in human cohorts is challenging. Analyses are prone to confounding, and in contrast to genetic associations studies, epigenetic changes may be driven by reverse causation effects, where a disease or trait causes changes in the epigenome. A deeper understanding of links between early-life exposures and health outcomes therefore requires careful appraisal of existing evidence. Diverse study designs including the use of longitudinal cohorts, negative controls and in the case of analysis of modifiable exposures, randomised controlled trials are warranted, along with other techniques for strengthening causal inference such as Mendelian randomisation [52].

Furthermore, epigenetic assays have their own biases and limitations. For example, genome-wide DNA methylation arrays used in most human cohort studies cover a small fraction of the human methylome. They are often designed to focus on specific functional elements such as gene promoters and enhancers, so that potentially important signals, for example, in intergenic regions, will be missed. Unbiased approaches such as whole-genome bisulfite sequencing are currently prohibitively expensive for population studies. Methylation array-based assays are also prone to systematic errors arising from their design. These must be accounted for during statistical analysis, along with potential confounding due to tissue- and cell-specific methylation patterns in heterogenous samples [15].

Genetic variation also has a strong influence on the epigenome so that an understanding of the interplay between genotype, epigenotype and environment in genetically heterogenous human populations is required to fully elucidate drivers of fetal programming effects, alongside functional work in animal and cell models that now forms an important component of work in molecular epidemiology.

It is also important to note that the majority of human methylation studies are conducted in European and North American populations, limiting power to detect ethnic differences in fetal programming effects, and few studies consider sex interactions, despite evidence that sexual dimorphism may in some cases be epigenetically regulated [53].

Epigenetic association studies are increasingly benefitting from techniques such as ATAC-seq and ChIP-seq that can link observed methylation changes to functional outcomes at the molecular level, and from access to public bioinformatic resources mapping tissue-specific chromatin accessibility and gene expression patterns [54,55].

Despite the many challenges, robust exposure-methylation associations have potentially been used as biomarkers of exposure in research studies and clinical medicine. Epigenetic signatures could be used in epidemiological studies as a quantitative biomarker of exposure, improving our understanding of the role of gene—environment interactions in disease risk [56]. Epigenetic signatures could also be used to guide personalised preventative medicine strategies in individuals and whole populations [57,58].

In addition, some groups are investigating the potential to mitigate or prevent adverse epigenetic changes during pregnancy through nutritional supplements or parenting and bonding support interventions [59,60]. This concept is most advanced in the context of cancer therapies, where agents such as inhibitors of DNA methyltransferase and histone deacetylase are being used to slow the progression of tumours and aberrant haematopoiesis [61]. It is possible that some of these techniques could one day be applied to mitigate or reverse adverse effects of fetal programming, although correction of suboptimal developmental trajectories that are already set in early life may not be amenable to postnatal therapies.

Conclusion
Epidemiological evidence strongly supports the notion that adverse prenatal exposures can have negative conse-quences for life-long health through fetal programming. There is mounting evidence that some of these effects are mediated by environmentally driven changes to the epigenome, with early gestation emerging as a particular window of sensitivity.
Conflict of interest statement
Nothing declared.

Acknowledgements
KS is supported by a National Institute of Health Research (NIHR) Aca-
demic Clinical Fellowship at the London School of Hygiene and Tropical
Medicine. KAL is supported by Diabetes UK (16/0005544) and the NIHR South-
hampton Biomedical Research Centre. MJ is funded by the UK Medical
Research Council (MRC) under the MRC/DfID Concordat agreement (MRC Pro-
gram MC-A760-5QX00).

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