Intergenerational influences on child development: an epigenetic perspective

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Abstract

The link between poor maternal nutrition and suboptimal outcomes in offspring is well established, but underlying mechanisms are not well understood. Modifications to the offspring epigenome are a plausible mechanism for the transmission of intergenerational signals that could extend to effects of paternal nutrition mediated by epigenetic modifications in sperm. The epigenome is extensively remodelled in the early embryo. Attention has therefore focused on the periconceptional period as a time when differences in parental nutrition might influence the establishment of epigenetic marks in offspring. So-called 'natural experiments' in The Gambia and elsewhere have highlighted loci that may be especially sensitive to periconceptional nutrition and some are associated with health-related outcomes in later life. There is speculation that some epigenetic signals could be transmitted across multiple generations, although this would require epigenetic marks to evade epigenetic reprogramming events at conception and in primordial germ cells, and evidence for this is lacking in humans. Effects on child development spanning one or more generations could impose an intergenerational 'brake' on a child's growth potential, limiting, for example the rate at which populations can escape from stunting.

Epidemiological evidence indicates that suboptimal maternal nutrition is associated with a wide range of adverse outcomes in offspring throughout the life course, but underlying mechanisms are poorly understood[1]. Evidence from animal models and some human data suggest that modifications to the developing offspring epigenome (Figure 1) offer a plausible mediating mechanism since i) they can be sensitive to the early environment in gestation; ii) they can influence gene expression and have been associated with numerous metabolic and other traits; and iii) there is the potential for intergenerational transmission of epigenetic signals[2]. However, the study of these effects in humans is challenging. Epigenetic marks are often tissue-specific and it may be difficult or impossible to access a disease-specific tissue of interest. Furthermore, associations may be confounded by the influence of genetic variation. Finally, causal pathways are difficult to elucidate in observational studies, and even randomised experimental designs are prone to confounding due to reverse causation effects[3].

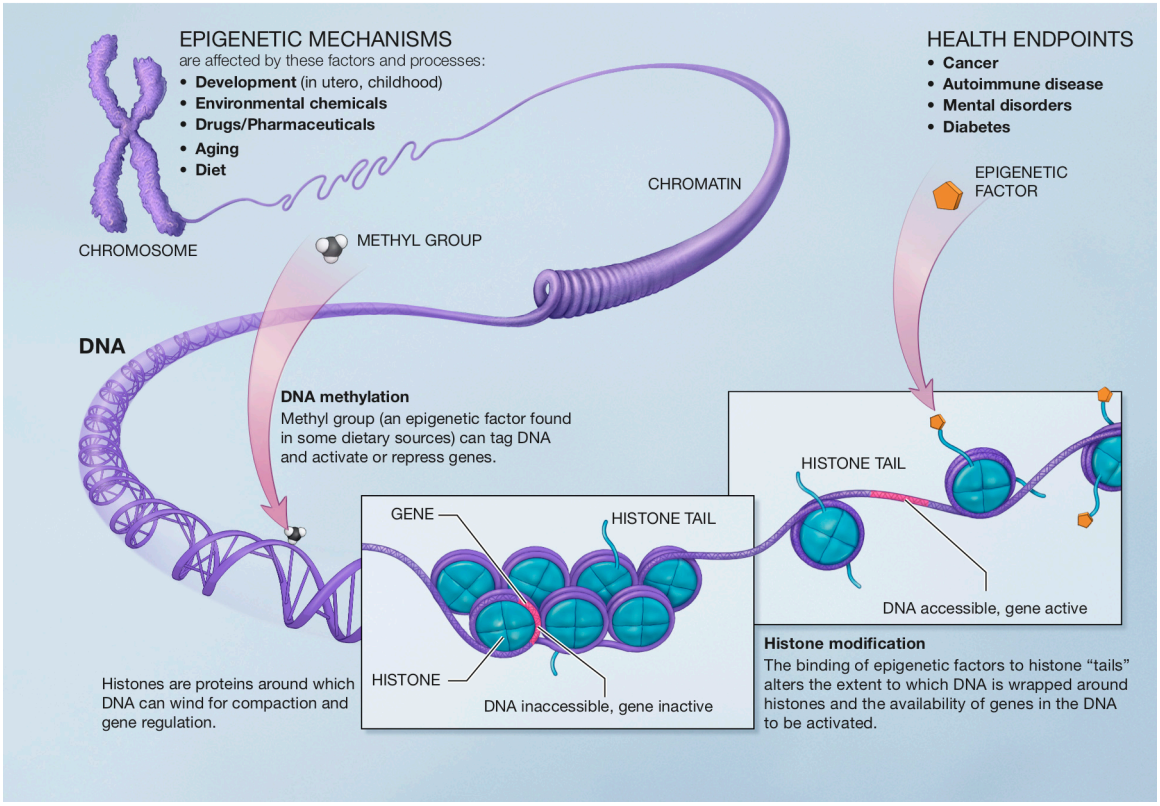

Figure 1. Epigenetic mechanisms may mediate links between early environmental exposures and health outcomes in later life (Image courtesy of US National Institutes of Health).

One widely-studied epigenetic process involves the addition of methyl groups to DNA. DNA methylation (DNAm) marks are faithfully copied across cell divisions in mitosis and can influence gene expression without altering the underlying DNA sequence. DNAm plays a key role in several cellular processes including the establishment and maintenance of cellular identity, X-chromosome inactivation and genomic imprinting. Importantly, DNAm marks are extensively remodelled in the very early embryo when maternal and paternal gametic methylation marks are erased in order to render embryonic cells into a totipotent state, ready to acquire tissue-specific marks at implantation, gastrulation and beyond. Attention has therefore focussed on the periconceptional period as a time when the establishment of epigenetic marks in offspring might be especially sensitive to differences in maternal (and potentially paternal) nutrition[4].

Nutrition plays a part in the establishment, maintenance and erasure of DNAm marks through the action of one-carbon and TET-mediated pathways that rely on so-called 'methyl donor' nutrients including folate and other B vitamins, choline, betaine and vitamin C that are derived from our diets[5]. Robust evidence linking early nutrition to health-related phenotypic effects mediated by DNAm changes comes from the Agouti mouse model, with methyl donor-supplemented dams producing an excess of obese, metabolically dysfunctional offspring, driven by DNAm changes in a cryptic Agouti gene promoter[6]. Evidence of *in utero* nutrient-offspring DNAm associations in humans is rapidly accumulating, with diverse although sometimes inconsistent effects reported from both observational and randomised study designs investigating a range of nutrient exposures throughout gestation[7].

Some major insights in the field of human epigenetic epidemiology have come from so-called 'natural experiments' that have shown intergenerational associations between parental or grandparental nutrition and offspring morbidity and mortality[8–11]. In these studies, individuals exposed to a range of early-life and peripubertal factors including famine, poor harvests and exposure to Ramadan are compared to controls, with the period in early gestation often highlighted as a window of heightened sensitivity. However, few of these studies, some of which rely on historical data stretching back to the 19th century, have been able to look at epigenetic effects directly, with the notable exception of investigations relating to *in utero* famine exposure in the 'Dutch Hunger Winter'[12].

A series of studies in The Gambia in sub-Saharan West Africa exploits another natural experiment whereby fluctuations in maternal energy balance and nutritional exposures show a distinct bimodal pattern corresponding to dry and rainy seasons. Season of birth has been linked to mortality in this population, with differential survival rates not manifesting until adolescence, suggesting the potential for an epigenetically-mediated effect of early-life adversity on later health[13]. Associations between *parental* season of birth and offspring anthropometry have also been observed[14]. Here, the discovery of an association with paternal season of birth is of particular interest since a patrilineal effect is most likely to be mediated through the father's sperm.

Progress has also been made in identifying the effects of Gambian seasonality on DNAm. We have shown for example that season of conception and levels of certain nutritional biomarkers in maternal blood plasma at conception predict DNAm in infants at a number of putative metastable epialleles - genomic regions where methylation is established in the early embryo[15–19]. Several of these loci have been associated with molecular traits and health outcomes including obesity, immune function and cancer[17,18,20]. The link to obesity involves a variably methylated region of the proopiomelanocortin (*POMC*) gene that is involved in the regulation of appetite. Evidence that *POMC* methylation is established in early life, is associated with periconceptional environment, is stable thereafter, and is associated with *POMC* expression, positions this locus as a strong candidate for mediating links between early-life nutrition and the regulation of bodyweight in later life in humans[18,21].

This and related work in human nutritional epigenetics focusses on the potential for DNAm changes to mediate 'intergenerational' signals, that is methylation changes that arise as a result of direct exposure of the embryo or fetus to maternal factors. Intergenerational epigenetic effects additionally encompass paternal and grandmaternal exposures, since germ cells that give rise to the current generation could have been exposed *in utero* (Figure 2, grey shaded areas).

There is currently a great deal of interest in the possibility that epigenetic signals might also be transmitted across multiple generations in so-called 'transgenerational epigenetic inheritance', where subsequent generations have not been directly exposed (Figure 2, green shaded areas). Epidemiological evidence for putative transgenerational effects comes, for example, from the Swedish Överkalix and Uppsala studies that show an association between grand paternal exposure to poor nutrition and male grandchild all-cause mortality[8,9]. However, there is scepticism that such observations could be mediated by epigenetic modifications since this would require epigenetic states to escape two waves of epigenetic

reprogramming, the first at conception, and the second during the development of primordial germ cells in gestation. Evidence for this is currently lacking in humans.

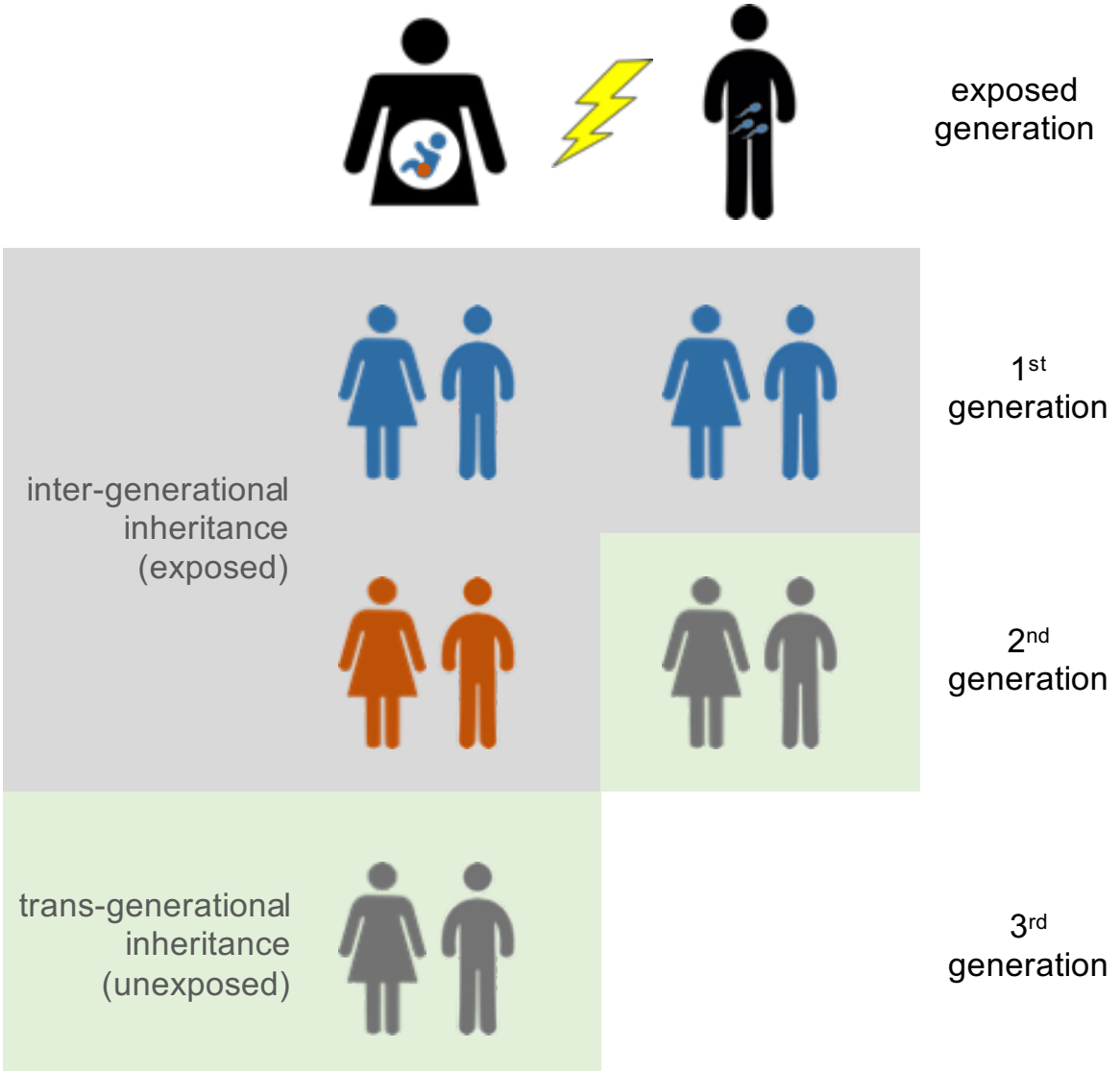

Figure 2. Inter- and trans-generational epigenetic inheritance through the maternal (left) and paternal (right) lines.

Despite this, efforts are ongoing to identify examples of transgenerational epigenetic inheritance in mammals, motivated in part by robust examples of this phenomenon in plants and lower animals. For example, transgenerational epigenetic mechanisms have been shown to mediate the inheritance of corn kernel colours in maize plants through the action of small interfering RNAs[22], and transgenerational epigenetic inheritance has also been observed to mediate the multi-generation effect of heat exposure in nematode worms, in this case through the action of histone modifications[23]. Work in mammals is focussed on the mouse. Examples typically involve severe exposures such as postnatal trauma and exposure to endocrine disruptors, leading to widespread metabolic or developmental pathologies, with regions of DNAm that escape reprogramming and sperm RNAs emerging as leading candidate epigenetic mechanisms[24–26].

Depending on context, DNAm and other epigenetic changes that are driven by environmental exposures in early life or in preceding generations could be classified as epigenetic errors or as adaptations. The latter case is often referred to as *epigenetic programming* or as an example of a *predictive-adaptive-response* (P-A-R)[27]. In the context of early maternal nutrition, we might speculate that DNAm or other epigenetic marks that are responsive to the periconceptional nutrition milieu could have evolved to sense the environment, record the information and adapt the organism to its anticipated postnatal environment. Where there is a mismatch between pre and postnatal environments this would lead to maladaptation and potentially to disease. In this way regions of epigenetic variability at a population level could provide a substrate for adaptation to rapidly changing environments in a manner not amenable to adaption through Darwinian genetic evolution which typically operates over much slower time scales[28]. Importantly, the evolution of such a mechanism would require that epigenetically variability at environmentally-responsive loci is under genetic control. In support of this, evidence is emerging that increased variability at certain DNAm loci may be driven by genotype[20,29,30], suggesting that some intergenerational DNAm signals might fall within the P-A-R paradigm.

In conclusion, a diverse range of epigenetic mechanisms might influence the growth and development of children. Nutritional and other environmental insults affecting parents could plausibly affect their children, potentially providing an 'intergenerational brake' that would limit the rate at which individuals (and hence populations) can escape from the effects of an adverse family history. The possibility of longer-range effects spanning several generations cannot be ruled out, but currently lacks convincing evidence in humans. Either scenario would encourage a counsel of patience when judging the success of interventions attempting to enhance the growth of children through either pre- or post-natal dietary supplementation, since a proportional gain over several generations might be the best that can be expected. Furthermore, to the extent that epigenetic mechanisms may underly predictive adaptive responses, where there is a mismatch between a child's early- and later-life nutritional experiences, a gradualist approach might offer the optimal trajectory for moving populations from nutritional poverty.

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