Developmental Programming and Transgenerational Transmission of Obesity

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Abstract

Obesity is an increasing global health concern that is commonly attributed to dietary and lifestyle changes, particularly intake of high calorie diets and reduced physical activity. Obesity and its related cardiometabolic disorders are also major issues in developing societies transitioning to Western-style diets and lifestyles. It is well established that alterations in the early life period and impacts upon sensitive periods of developmental plasticity can lead to a range of adverse phenotypic outcomes and predispose to later obesity. Human cohorts and experimental animal models have reported clear linkages between the early life environment, particularly the maternal nutritional milieu, and increased risk for offspring to develop offspring and related cardiometabolic disorders. Increasing evidence is also building around the paternal contribution to such programming

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effects, including paternal obesity. This process, via both the maternal and paternal lineage, is preferentially called "developmental programming" and sits under the Developmental Origins of Health and Disease (DOHaD) framework. Moreover, developmental programming is now regarded as a transgenerational phenomenon with a number of studies showing metabolic disorders passing to future generations, even in the absence of further environmental insults. Such disease transmission is commonly seen as a mode of "epigenetic inheritance" with both somatic and germline inheritance of altered epigenetic marks resulting in altered phenotypes that may persist across generations. In addition, there are data suggesting nongenomic components to transgenerational programming of obesity including direct effects on the developing fetus via a suboptimal intrauterine environment and maternal constraint. An increased understanding of how developmental programming effects are transmitted is essential to allow initiatives to be implemented to break the current cycle of obesity across generations.

Keywords

Developmental programming · Transgenerational · Epigenetic · Maternal diet · Paternal diet · Obesity · DNA methylation

List of Abbreviations

BPA	Bisphenol A
DOHaD	Developmental Origins of Health and Disease
GC	Glucocorticoid
GDM	Gestational diabetes mellitus
HFD	High-fat diet
LP	Low protein
MLP	Maternal low protein
NHP	Nonhuman primate

Background

Obesity is a significant public health concern commonly associated with dietary and lifestyle changes (increased intakes of calorie rich diets and sedentary behavior) and is a growing epidemic in developing societies transitioning to Western-style diets and lifestyles. Less attention, however, has been focused on the impact of the early life environment and the role of "developmental plasticity" on programming of later phenotype, including obesity, in adult life. This framework, preferentially termed the "developmental origins of health and disease" or "DOHaD" framework, has shown through extensive human and animal observations, that alterations in the early life environment can lead to an increased risk for developing cardiometabolic disorders in later life via the process of developmental programming (Gluckman et al. 2010). In this context, the DOHaD framework has recently been termed a new pathogenic paradigm to explain the current epidemiological transitions and worldwide increases in chronic diseases including obesity, diabetes, cancer, and cardiovascular disease

(Lopomo et al. 2016). In particular, both maternal under- and overnutrition have been shown to lead to an increased risk of obesity and related cardiometabolic disorders in offspring, thus perpetuating metabolic disorders across generations. As such, the process of developmental programming needs to be viewed as a transgenerational phenomenon with transmission of disease traits possible via both the maternal and paternal lineage (Aiken and Ozanne 2014; Vickers 2014).

Introduction

The original observations derived from epidemiological studies by Professor David Barker and others linked fetal growth restriction to later disease and thus implied that a nutritionally deprived fetal environment provided a strong stimulus for programming (Barker 2007a, b). These observations led to the development of a wide range of experimental models of intrauterine growth restriction which clearly showed a link between a poor maternal nutritional status and later obesity phenotype, effects which could, with varying degree, be transmitted across generations. However, more recently and given the rising obesity prevalence, a focus has turned toward models of parental obesity and the consequences of excess early life nutrition on the F1 generation and beyond (Hanson et al. 2016). Interestingly, both ends of the early life nutritional spectrum often manifest with similar programming effects in offspring with both maternal overnutrition and undernutrition giving rise to increased obesity in offspring. In part this can be attributed to "overnutrition" in fact being a form of malnutrition given the micronutrient deficiencies commonly associated with many obesogenic diets (Via 2012). However, whether the mechanisms underpinning the nutritional programming observed following either under- or overnutrition are similar remains poorly defined. It is also of note that with different exposure windows to altered nutrition a similar phenotype of obesity may manifest in offspring but they may originate via different mechanistic pathways (Howie et al. 2012, 2013; Thompson et al. 2007).

To date, limited studies have explored the transgenerational inheritance of obesity risk in the setting of developmental programming, with most studies to date instead focusing on outcomes related to altered glucose tolerance, cardiovascular risk factors or epigenetic marks. Further, most studies only go on to characterize the F2 phenotype which still represents the initial insult and "memory" of the F0 generation environment.

What Defines "Transgenerational"?

There are an increasing number of studies examining transgenerational effects following an initial developmental programming stimulus. However, use of the term "transgenerational" is not straightforward – many studies report outcomes to the F2 generation only, which is not truly representational of transgenerational effects with outcomes in the F3 generation reflecting evidence for true disease trait transmission that are independent of the initial environmental insult (Aiken et al.

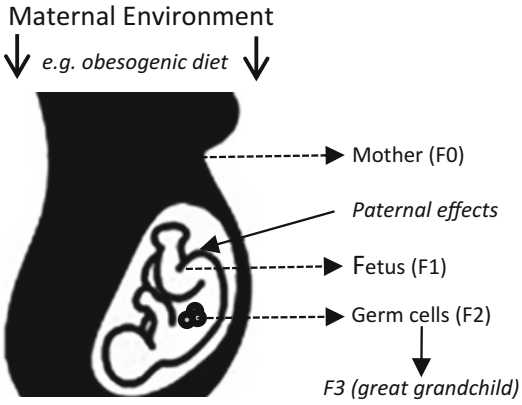

Fig. 1 Developmental programming is a complex transgenerational process – the initial maternal insult (F0) not only affects the fetus (F1) but also the germ cells that lead through to F2. F3 represents true transgenerational transmission as does not reflect the initial maternal influence

2016; Skinner 2008). When the mother is exposed to a poor environment (F0, altered maternal diet, for example), it directly effects fetal development in utero (F1) and also has effects on the germ cells that will ultimately form the F2 generation. Thus in the context of transgenerational effects only the F3 generation and beyond can truly be considered transgenerational as outcomes in F2 reflect the original maternal environment (Fig. 1). Thus, many studies use the terms "multigenerational" or "intergenerational." As an example, confirmation of a germline-based mechanism would require analysis of the F3 offspring to delineate any direct effects of developmental programming via the maternal nutritional environment and the transmission through the paternal line to avoid the potential confounds of maternal influences including changes in maternal behavior or an altered in utero environment (Vickers 2014). In this context, few studies to date have examined phenotypes through to F3 and those studies report varying, and in some cases conflicting, outcomes with some reporting a "wash-out" and resolution of the programmed phenotype by F3 (Aiken and Ozanne 2014, Benyshek et al. 2008) (see Table 1 for examples). For example, it has been observed that glucose homeostasis in F3 progeny of female rats undernourished in early development is also negatively impacted but less so than observed for F2 offspring. These data therefore suggest a trend toward a "resetting" or normalization in the F3 cohort when the nutrition of F2 mothers and nutrition of the F3 offspring postweaning was recuperated (Benyshek et al. 2006; Drake et al. 2005; Drake and Seckl 2011). These outcomes are then further confounded by the diversity in experimental approaches utilized from low protein (LP) exposure through to high energy diets and specific micronutrient deficiencies and sexually dimorphic responses to programming stimuli.

Evidence from Epidemiology and Human Cohorts

Various approaches utilizing family, twin, and adoption studies have led to the characterization of some genes causally linked to monogenic forms of obesity. However, the origins of the obesity pandemic cannot be considered as essentially

Table 1 Examples highlighting the variation in phenotypic outcomes in rodent models carried through to F3.LP low protein, GC glucocorticoid

Animal model	Primary outcome at F3	References
50% global undernutrition (Rat)	Hypertension and endothelial dysfunction persisted to F3 males	Ponzio et al. (2012)
Maternal LP and offspring on energy restricted diet (Rat)	Normalization of insulin sensitivity (male and female)	Benyshek et al. (2008)
Maternal GC exposure (Rat)	Low birth weight, changes in hepatic gene expression, and disturbed glucose homeostasis in F2 is resolved by F3 (males)	Drake et al. (2005)
Maternal LP (Rat)	Altered glucose tolerance – partial normalization in F3 generation when maternal diet of F2 dams and postweaning diet of F3 animals was recuperated (males and females)	Benyshek et al. (2006)
Maternal HFD (Mouse)	Paternally transmitted phenotype of increased body size to F3 offspring (females)	Dunn and Bale (2011)
Maternal LP (Rat)	Hypertension in F2 offspring resolved by F3 (males and females)	Harrison and Langley-Evans (2009)
Maternal exposure to endocrine disruptor	Transgenerational inheritance of differential DNA methylation in sperm of the F3 generation (males)	Skinner et al. (2015)
Maternal LP (Mouse)	Morphologic changes in pancreatic islets through to F3 (males)	Frantz et al. (2011)
Increased maternal dietary energy intake (25%)	Persistent changes in DNA methylation through to F3 (females)	Burdge et al. (2011)
Maternal obesity/diabetes (Mouse)	Attenuation of phenotype (defects in glucose and lipid metabolism) by F3 (males)	Cropley et al. (2016)
Maternal exposure to endocrine disruptor	Decrease in anxiety-like behavior in F# males, while females had an increase in anxiety-like behavior	Skinner et al. (2008)

arising due to genetic factors, because the human genome is unlikely to change over a relatively short time period as paralleled by the current epidemic (Lopomo et al. 2016). Clinical studies and epidemiological data has clearly shown that both environmental and genetic factors contribute to the increased propensity toward overweight/obesity and associated cardiometabolic disorders with epigenetic processes regulating the interplay between these factors (Elshhenawy and Simmons 2016). Reviews of the literature for both experimental animal models and human data have been undertaken by Aiken and Ozanne (2014), the influence of maternal prenatal nutrition on subsequent generations by Susser et al. (2012) and paternal transmission effects by Curley et al. (2011). Human data are understandably limited as a result of the long timeframe required to generate data over successive generations for prospective studies and quality issues around data derived from retrospective studies and, as per the caveat above, human studies to date have not yet extended to F3 (great grandchildren) as required to investigate true transgenerational transmission i.e. are "mutigenerational" in nature. Two of the most well-cited human cohort

studies are those of the Dutch Famine Cohort (Painter et al. 2008; Veenendaal et al. 2013; Lumey 1992) and the Overkalix and Avon Longitudinal Study of Parents and Children (ALSPAC) cohorts where both cohorts have reported transmission of poor health outcomes into the second generation (Pembrey et al. 2006).

In the ALSPAC/Overkalix cohorts over the period 1890–1920, transgenerational effects were reported via the male line. The paternal grandfather's nutritional intake was only linked to mortality risk in grandsons, while food supply to paternal grandmother's was only associated with mortality risk in granddaughters. These observations reflected adverse nutritional exposures during the periods of reduced growth (maternal and paternal grandparents) or fetal/neonatal life (grandmothers) but not during the pubertal period of either grandparent (Pembrey et al. 2006). Although these studies suggest environmental influences, current limited evidence does not offer direct support for epigenetic information being transferred via the gametes and it has been suggested that transgenerational effects of this nature could rather be elucidated via societal factors (Morgan and Whitelaw 2008).

Work on the Dutch Famine cohort did not indicate transgenerational effects of prenatal exposure to famine on birthweight nor on incidence of cardiometabolic disease. However, in utero famine exposure was associated with an increase in adiposity in F2 neonates and later adverse health outcomes in adulthood (Painter et al. 2008). Offspring of fathers undernourished prenatally, but not mothers, were heavier with increased adiposity compared to offspring born to parents who had not been exposed to famine in the prenatal period (Veenendaal et al. 2013). In a further study using this cohort, little evidence of transgenerational effects of famine exposure during pregnancy in the grandmother was found on health outcomes in the F2 offspring (Veenendaal et al. 2013). Reports on transgenerational programming effects arising from the Dutch Famine cohort have thus been variable. One study reported that F1 offspring exhibited lower birth weights and that these effects persisted to the F2 generation (Lumey 1992); however, a later study by the same group did not replicate some of the initial findings (Stein and Lumey 2000).

Multigenerational data have also been derived from a further two human cohorts; the Guatemalan cohort examining maternal nutritional supplements (Stein et al. 2003) and maternal famine exposure in China resultant from the Great Leap Forward Campaign in 1958 (Kim et al. 2016) which led to famine via disturbances in agricultural production. In the Guatemalan cohort, generations have been studied up to F2 with positive associations seen between improved maternal and F1 nutrition and improved F2 offspring developmental outcomes. However, mechanistic interpretation of these effects is limited due to lack of differentiation between prenatal (pregnancy) and postnatal (direct effects of breastfeeding and childhood supplement intake) factors (and interactions therein) on phenotype development. Re the China famine, almost all work to date has focused on adverse outcomes in the F1 generation. An exception was the report by Huang et al. which reported associations between famine exposure and F2 birth weights (Huang et al. 2010). Surprisingly, in rural areas, F2 offspring of famine exposed mothers had higher birth weights and lengths than did F2 offspring of unexposed mothers. This may be explained via higher mortality rates in rural areas contributing to survivor selection of healthier

mothers and offspring and thus survivor selection is an important consideration when interpreting outcomes derived from famine studies (Susser et al. 2012).

Evidence from Animal Studies

Given the relative paucity of data available from retrospective human studies, most insights into transgenerational programming of obesity have been derived from experimental animal models, particularly around alterations in maternal nutrition with data drawn from a range of small animal (rodent) models (including rat, mouse and guinea pig), as well as the sheep, pig, and nonhuman primate (NHP). These incorporate challenges including global nutritional restriction or overfeeding during preconception, gestation/lactation and early infancy, targeted micronutrient restriction, restriction of uterine blood flow, fetal exposure to high concentrations of glucocorticoids, and experimental models of gestational diabetes (GDM). Rodent models have been the primary model utilized to date due to the relatively short gestational length and timeframe required to generate offspring needed to generate cohorts representative of true transgenerational inheritance i.e. F3 and beyond. Although a number of studies have utilized small animal models to investigate at least through to the F2 generation, only a few have examined obesity as a primary endpoint with many focusing on markers related to insulin resistance/impaired glucose tolerance, cardiovascular dysfunction, or epigenetic changes (i.e., altered DNA methylation) (Aiken and Ozanne 2014).

It has been recently shown that standard control nutrition in utero in subsequent generations following high-fat diet (HFD) exposure in utero ameliorated, and standard nutrition in utero for three generations completely prevented, HFD effects on bodyweight and adipose tissue accrual and markers of metabolic dysfunction. These changes were associated with epigenetic changes in key adipocytokine genes (adiponectin and leptin) (Masuyama et al. 2015). Conversely, a study by Hardikar et al. showed that multigenerational LP exposure increased the risk of obesity and diabetes that could not be reversed with normalization of diet. This study showed that prolonged exposure to a LP/low energy diet "locked" the expression of a number of key regulatory genes that were resistant to reversal via exposure to a normal diet (Hardikar et al. 2015). Interestingly, the Hardikar study mirrors the human situation of those developing societies transitioning from relatively poor diets to high calorie diets and thus suggests there may be resilience to any corrective nutritional intervention. The discrepancies between such studies may lie in the background genetics and age of the animals used and the type and duration of nutrient exposures. An example of this was shown in work by Hoile et al. where fat/carbohydrate intake over multiple generations modified growth and cardiometabolic outcomes in female mice in an age-related manner (Hoile et al. 2015).

Massiera et al. reported that mouse dams fed a HFD gave rise to offspring who grew to have increased adiposity in the absence of further dietary manipulation (Massiera et al. 2010). Importantly, the effect of the maternal diet persisted and was

even amplified, up to (and beyond) the F4 generation. Thus, in the presence of genome stability and with a consistent environmental/dietary regimen over four successive generations, a Western-style HFD induced a gradual enhancement in fat depots weights, which experimentally mirrors the increasing prevalence of obesity observed in the current human setting.

In addition to studies in rodents, studies in larger model species also report transgenerational effects including those reported for the sheep, swine, and NHP (Braunschweig et al. 2012, Buchwald et al. 2012, Long et al. 2013). In an ovine model, maternal DEX administration to F0 mothers eliminated the neonatal leptin surge in female offspring. F2 DEX offspring displayed appetite dysregulation, an increased weight gain and fat mass during a feeding challenge paralleled by a decrease in insulin responsiveness following a glucose tolerance test (Long et al. 2013). In the pig, methylating micronutrient supplemented diets resulted in differences in gene expression, DNA methylation and carcass composition between F2 offspring of supplemented and nonsupplemented groups with the F2 control offspring trending toward an increase in fat mass as compared to F2 offspring of animals that were supplemented. These effects were shown to be transmitted down the male line (Braunschweig et al. 2012). Further work in the swine has also shown postnatal changes in adipose tissue mass and lipid profiles via transgenerational programming in pigs with obesity and hyperleptinemia (Gonzalez-Bulnes et al. 2014). In these piglets, pre- and postnatal development, adipose tissue mass and metabolic profiles were evaluated in the F2 generation, descendants of sows exposed to either over/undernutrition during gestation. The results suggested that these piglets exhibited early-onset increases in adiposity and dyslipidemia; features which are early symptoms characteristic of the metabolic syndrome, with hepatic tissue also showing early evidence of disease. These observations of early-life metabolic programming were more pronounced in males descended from overnourished grandmothers and during the transition from milk to solids. Less work has been undertaken in the NHP due to the long generation times required. It has been reported that utilizing a maternal DEX model in the NHP, higher cholesterol levels were observed in F2 and F3 offspring, with significantly more LDL cholesterol, although body weights were not affected (Buchwald et al. 2012).

Paternal Effects

There are many pathways by which the parent can influence offspring outcomes and recent work in the area of environmentally mediated epigenetic variation highlights the potential role of nongenomic mechanisms. In addition to DNA methylation changes observed as a result of mother-child interactions, there is growing interest in epigenetic mechanisms via which paternal influences can affect offspring outcomes. Research into paternal effects is now an expanding area of research via the recent inclusion of epigenetic approaches to the transmission of parental traits. This work has been reviewed in detail elsewhere by Curley et al. (2011).

Data from experimental animal models and (albeit limited) epidemiological evidence has suggested that paternal nutritional exposures in addition to paternal age and phenotypic variation can lead to variations in offspring phenotype and, in some studies, grand-offspring development. Masuyama et al. recently reported on transgenerational effects of paternal HFD-induced obesity in the preconception period in mice over successive generations on offspring outcomes, including bodyweight and adiposity, glucose homeostasis, lipid and adipokine profiles, hypertension and epigenetic changes. Consumption of a standard diet by male progeny during the generation following paternal HFD exposure ameliorated, whereas intake over two subsequent generations completely reversed the effect of paternal HFD intake on metabolic outcomes and epigenetic modifications in adipocytokine-related gene promoters. As the paternal HFD had a cumulative effect over two generations, this suggested that both maternal and paternal planes of nutrition may have effects on offspring health via epigenetic modifications of adipokine-related genes across multiple generations (Masuyama et al. 2016).

The work by Fullston et al. has also examined the impact of paternal obesity in programmed trait inheritance whereby paternal obesity resulted in metabolic disorders in two generations of mice despite incomplete transmission of the phenotype to F2 (Fullston et al. 2013). HFD-induced paternal obesity was observed to modulate miRNA content in sperm and germ methylation status. Work by Bale et al. has previously shown that a maternal HFD in mice leads to an increase in body mass concomitant with impaired insulin sensitivity that was shown to persist to the F2 generation via both parental lineages (Dunn and Bale 2009). However, as detailed above, as the primordial germ cells in the F1 generation may be directly affected by exposures during pregnancy, evaluation of the phenotype transmission to at least the F3 generation is essential to confirm that stable epigenetic programming has occurred. Examination of the F3 offspring in this model revealed that the outcomes were sex-specific with only females displaying an increase in body size at F3, and these traits were transmitted only via the paternal lineage. Paternal transmission of a phenotype through to F3 female offspring does support the potential for stable germline-based transgenerational inheritance, thus suggesting a role for gene imprinting in epigenetic developmental programming (Dunn and Bale 2011).

Potential Mechanisms

Human epidemiological studies of transgenerational inheritance are largely observational, and therefore may be considered of limited value in determining the mechanistic basis underlying these phenomena (Roseboom and Watson 2012) and animal studies have provided the most mechanistic evidence to date. There is now experimental evidence for a number of potential mechanistic pathways underpinning transgenerational programming or repropagation of developmental programming effects. Transgenerational effects can be transmitted through direct mechanisms via the maternal line including in utero constraint (Aiken et al. 2016) (Drake and

Seckl 2011; Danchin et al. 2011; Aiken and Ozanne 2014) as well as methylation of the gametes via the paternal and maternal lineage.

As obesity arises from both genetics and environmental influences, genome-wide association studies (GWAS) have revealed loci that can contribute to the obese phenotype (Chesi and Grant 2015). However, the total contribution to the risk of obesity from the loci identified to date appears relatively small. Clinical and epidemiological data clearly shows that the interactions between genetic and environmental factors contribute to an increased risk for obesity via epigenetic mechanisms (Elshhenawy and Simmons 2016; Bouret et al. 2015).

Environmentally mediated epigenetic transgenerational inheritance of obesity and related metabolic disorders involves a number of changes in phenotype, suggesting generalized alterations in genome activity with common epigenetic control regions coordinately regulating tissue-specific transcriptomes. During pregnancy and the early infant period, regulation of energy balance within a generation may be influenced by both the phenotype of the mother in the prior and the present pregnancy. These effects on phenotypic outcomes are associated with alterations in DNA methylation of specific genes that is consistent epigenetic marks being induced de novo across each subsequent generation (Burdge et al. 2011).

A number of experimental models have investigated alterations in methyl donor availability and effects on one-carbon metabolism as a mechanistic basis for transgenerational transmission of phenotypic traits. Supplementation of maternal intake with dietary methyl donors can generate a transgenerational phenotype with transmission characteristics that are sexually dimorphic (Dunn et al. 2011). In particular, it has been suggested that prenatal maternal nutritional exposures that affect the folate pathway may present a particularly useful domain to examine transgenerational phenotypes in human populations as we learn more about the interaction of folate with other micronutrients in the one-carbon pathway (Susser et al. 2012). Dysregulation of the folate pathway can impact on epigenetic stability and can have lasting transgenerational effects. Experiments utilizing embryo transfer techniques have revealed that methionine synthase reductase (MSR) deficiency in mice results in two differential phenotypes: adverse in utero effects on their wild-type daughters', resulting in growth disorders in grand-offspring, and observations of congenital defects that are independent of the maternal environment and can carry for up to five generations (Padmanabhan et al. 2013). The data on maternal folate status is less clear. There are data suggesting that supplementation of the maternal diet with folate can result in impaired insulin sensitivity and transgenerational transmission of respiratory disorders in offspring (Burdge and Lillycrop 2012, Hollingsworth et al. 2008).

It has been proposed that the epigenetic marks in the parent modify their "behavior" in a manner that can cause similar changes in epigenetic marks to be passed onto their offspring (e.g., alterations in DNA methylation in the hypothalamus); these behavioral changes recreating the epigenetic marks de novo with each subsequent generation (Danchin et al. 2011). An example of this relates to changes in maternal care (licking/grooming) where data suggest transmission of behavior during the postpartum period from the dams to their female offspring (Champagne

2008; Champagne and Meaney 2007). The mechanisms underpinning this transmission in the rat implicate differential methylation of hypothalamic estrogen receptors paralleled with estrogen-oxytocin interactions (Champagne 2008). Conversely however, a more recent report has also suggested that the obesity observed in offspring of obese mothers is independent of changes in maternal care although only effects in the first generation of offspring were examined (Connor et al. 2012).

In epigenetic inheritance via the germline, environmental influences during the sensitive period of developmental plasticity can lead to epigenetic changes within the F1 generation offspring's germline that is subsequently transmitted to F2 offspring and beyond (Danchin et al. 2011). In this setting, various environmental factors can promote the epigenetic inheritance of phenotypic variance (Skinner et al. 2013a). A well-cited example of this relates to environmental exposures to endocrine disruptors including that of bisphenol A (BPA). Male rats exposed to BPA in the perinatal period display an altered expression of testicular steroid receptor coregulators that can persist through to the F3 generation (Salian et al. 2009). Of interest, maternal behavioral changes may also manifest as a consequence of exposure to BPA (Palanza et al. 2002), thus potentially resulting in behavior-mediated effects in future generations (Champagne 2008). Ancestral exposure to insecticides including dichlorodiphenyltrichloroethane (DDT) has been reported in the rat to promote epigenetically mediated inheritance of obesity through to the F3 generation with over half of both males and females developing later obesity (Skinner et al. 2013b). The transgenerational transmission effects were via both the male and female germlines with the F3 generation displaying sperm epimutations and altered methylation profiles. Further, perinatal exposure to 4-Nonylphenol (4-NP) can result in obesity in both male and female F1 offspring. This phenotype is passed to the F2 via the maternal lineage and can be potentiated via a postweaning HFD (Zhang et al. 2014).

In addition to the role of epigenetic effects, the contribution of the uterine tract environment and maternal (mal)adaptations to pregnancy (e.g., maternal constraint) may be a key factor in programming via the maternal lineage. Poor nutrition in utero can cause DNA damage and lead to accelerated aging of the reproductive tract (Aiken et al. 2013). It has therefore been proposed that, in addition to direct epigenetic mechanisms, developmental programming could be mediated via the maternal line de novo in generations beyond F2 as a consequence of a suboptimal in utero environment (Aiken and Ozanne 2014). In addition, increasing maternal (and likely paternal) age may exacerbate programming effects being passed to future generations. Epidemiologic and experimental studies have now clearly shown that offspring of obese mothers are at elevated risk for obesity and cardiometabolic dysfunction in later adult life (Howie et al. 2009; Catalano 2003). In the current obesogenic environment, increasing rates of maternal overweight/obesity translates to birth of offspring that are themselves predisposed toward obesity in their reproductive years thus perpetuating the cycle of obesity. Work in mouse models with a genetic predisposition toward obesity show that the effects of maternal obesity can compound with each successive generation and lead to a shift in distribution of the population toward increased body weight and adiposity.

Little is known around transgenerational effects on the gut microbiome and obesity risk. It has been reported that supplementation to mice of beneficial microbes is sufficient to reduce the transgenerational risk for obesity regardless of past dietary history (Poutahidis et al. 2015). Western populations exhibit a reduced diversity in the gut microbiome compared to that of populations living more traditional lifestyles. This raises the question of which environmental factors have led to such a change in microbiota profiles arising due to modernization and an obesogenic environment (Sonnenburg et al. 2016). Using a humanized mouse model, Sonnenburg et al. modeled the impact of changes in dietary fiber on the gut microbiome. Intake of a diet low in fiber manifests as a progressive reduction in microbial diversity over each successive generation. This decay in microbial diversity across generations may partially explain the observed decline in microbiome diversity that has accompanied modernization (Attar 2016).

Summary

Experimental evidence now strongly supports the concept of developmental programming as representing a transgenerational phenomenon (Fig. 2). Experimental models have shown that maternal diet prior to and during pregnancy/lactation and/or preconception paternal nutrition can influence the health of grandchildren and later generations. In humans, however, there is not yet sufficient evidence to draw conclusions but the data derived from animal models highlights the need to address

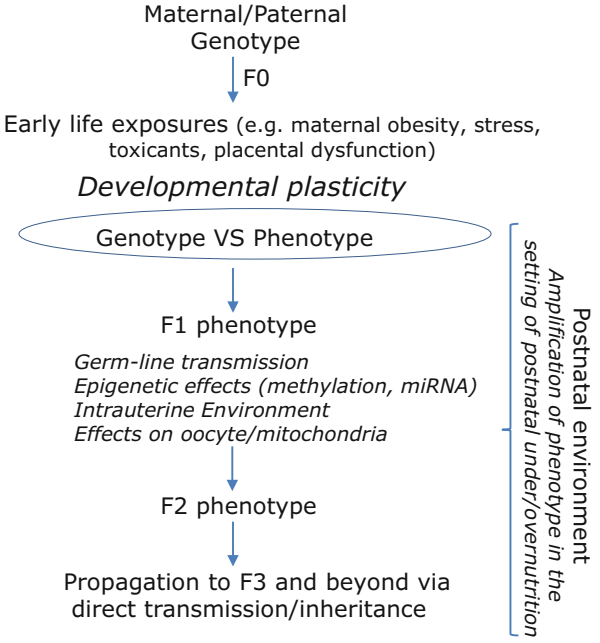

Fig. 2 Potential mechanisms by which an initial maternal insult can lead to transmission of disease traits across future generations

this question in prospective studies in human populations (Susser et al. 2012). In humans and other animals, genetic selection probably does not completely explain transgenerational effects from mother through to F3. As a general rule, far more than four generations will be required for genetic selection to change DNA sequence enough to produce detectable phenotypic effects (Susser et al. 2012).

Maternal and/or paternal exposures to adverse environments including obesogenic diets or starvation result in a diverse range of phenotypes that can have different transmission characteristics as they carry through subsequent generations. There have been reasons proposed for why such mechanisms have evolved to facilitate transgenerational transmission of maternal phenotypes (Susser et al. 2012). One is the capacity for phenotypic plasticity that is adaptive in times of nutritional variability, for example, famine. Another is the need for a wide range of stochastic variation during early development to allow for survival under changeable conditions with potential implications for phenotypes of subsequent generations. From an evolutionary standpoint, programming and epigenetic events must be adaptive. However, when do they become maladaptive? (Reynolds and Vonnahme 2016). Epigenetic transmission of traits allows for the next generation(s) to be "maximally competitive" in the environment they anticipate derived from early life cues (Dunn and Bale 2011). Under such assumptions, adaptive gene programs that are acquired by the parents during their life course can persist into subsequent generations, thus enabling existence in a potentially suboptimal environment. However, evidence to date suggests that early environmental exposures, including suboptimal early life nutrition, can result in maladaptive changes that can be subsequently passed onto offspring. This concept is known as "mismatch" whereby when the expected versus actual nutritional environments differ, adaptations made in utero can result in a thrifty offspring phenotype that is maladaptive for a postnatal obesogenic environment (Godfrey et al. 2007). These epigenetic traits may result in manifestation of a phenotype over several generations at a population level – such transmission may explain the rapid onset of overweight/obesity phenotypes currently observed in human populations (Dunn and Bale 2011), particularly in those societies undergoing nutritional transitions. Current studies also suggest a potential for paternal epigenetic germline inheritance. However, it is necessary to consider the interactions between maternal (e.g., in utero constraint) and paternal influences (McPherson et al. 2015) when examining epigenetic variation within the germline as a mediator of these effects (Curley et al. 2011).

Although a large number of studies across a range of models have now shown transmission of phenotypic traits through to F2, transmission to F3 or beyond is less defined with some studies reporting a resolution or amelioration of the phenotype by F3. In an analysis of those studies that did carry through to F3, over half failed to show any effect (Aiken and Ozanne 2014) (examples of these are shown in Table 1). Defining the mechanistic pathways underpinning transgenerational programming is an area of urgent research and of particular importance to those populations undergoing nutritional transitions and uptake of Western diets and lifestyles. Some traits resolve over generations whereas others persist. This indicates that divergent mechanisms of transmission may be involved and that those traits that do persist can be

transmitted via the male lineage (Dunn and Bale 2011). Human data remains limited with the strongest evidence for transgenerational inheritance is data that has been derived from the rodent models (Morgan and Whitelaw 2008). Research targeted toward breaking the transgenerational cycle of developmental programming and disease risk is essential in reducing the rates of obesity and related cardiometabolic disorders.

Mini-dictionary of Terms

- Developmental programming: a stimulus or insult operating at a critical period of development that results in a long-standing or lifelong effect on the offspring.
- DNA methylation: is the process by which methyl groups are added to the DNA molecule and can change the activity of a DNA segment without changing the sequence.
- Genotype: relates to the set of genes in our DNA which is responsible for a particular trait.
- Methyl donor: a methyl donor is any substance that can transfer a methyl group to another substance. These include folate, methionine, and choline.
- miRNA: microRNA; small noncoding RNA molecule (containing about 22 nucleotides) that functions in RNA silencing and posttranscriptional regulation of gene expression.
- Phenotype: characteristics of an individual resulting from the interaction of its genotype with the environment.
- Transgenerational: acting across multiple generations.
- Transgenerational epigenetic inheritance: transmission of information from one generation to the next (e.g., mother–child) that affects offspring traits without alteration of the primary structure of DNA.

Key Facts

- An altered early life environment (including poor nutrition) can lead to an increased risk of later obesity in later life through the process of developmental programming.
- Human and experimental evidence has shown that developmental programming needs to be viewed as a transgenerational phenomenon with both maternal and paternal effects on transmission of disease traits reported.
- A number of mechanisms may underlie the potentiation of obesity risk across generations including epigenetic effects or altered maternal adaptations to pregnancy including a poor reproductive tract environment.
- Most evidence to date has been derived from experimental animal models with only limited human evidence where effects have been shown through to the F2 generation.

- Many studies report effects up to the F2 generation whereas true transgenerational effects are in the F3 generation and beyond as do not reflect the initial environmental insult – F3 data are limited and conflicting with some showing a resolution of the programmed phenotype over time.

Summary Points

- The process of developmental programming can lead to an increased risk of overweight/obesity being not only transmitted from parent to offspring but to future generations.
- Data from limited human studies and extensive experimental models indicate that obesity and related cardiometabolic disorders and adverse environmental influences may be perpetuated to the F2 and F3 generations via both maternal and paternal backgrounds.
- The mechanisms are still poorly defined but it is well known that environmental factors can modulate genome activity through several processes. Of these, the most widely studied is around epigenetics and the influence of the maternal phenotype and intrauterine environment on regulation of fetal energy metabolism via alterations in DNA methylation of specific genes including adipocytokines.
- In addition to epigenetic effects, the transmission of disease traits across generations can result from direct maternal influences including maladaptation to pregnancy, maternal constraint, and a suboptimal reproductive tract.
- Increasing evidence points toward paternal effects on propagation of disease traits across generations, including the effect of paternal obesity on offspring obesity risk.
- Exposures to environmental stressors, including poor early life nutrition, can manifest as maladaptive epigenetic traits passed onto offspring – over several generations these have the potential to lead to a population-level phenotype.
- There is an urgent need to define the mechanisms that underpin the transgenerational effects observed in the developmental programming framework in order to modulate or "reprogram" the phenotype of subsequent generations.

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