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Transgenerational developmental programming

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BACKGROUND: The concept of developmental programming suggests that the early life environment influences offspring characteristics in later life, including the propensity to develop diseases such as the metabolic syndrome. There is now growing evidence that the effects of developmental programming may also manifest in further generations without further suboptimal exposure. This review considers the evidence, primarily from rodent models, for effects persisting to subsequent generations, and evaluates the mechanisms by which developmental programming may be transmitted to further generations. In particular, we focus on the potential role of the intrauterine environment in contributing to a developmentally programmed phenotype in subsequent generations.

METHODS: The literature was systematically searched at http://pubmed.org and http://scholar.google.com to identify published findings regarding transgenerational (F2 and beyond) developmental programming effects in human populations and animal models.

RESULTS: Transmission of programming effects is often viewed as a form of epigenetic inheritance, either via the maternal or paternal line. Evidence exists for both germline and somatic inheritance of epigenetic modifications which may be responsible for phenotypic changes in further generations. However, there is increasing evidence for the role of both extra-genomic components of the zygote and the interaction of the developing conceptus with the intrauterine environment in propagating programming effects.

CONCLUSIONS: The contribution of a suboptimal reproductive tract environment or maternal adaptations to pregnancy may be critical to inheritance of programming effects via the maternal line. As the effects of age exacerbate the programmed metabolic phenotype, advancing maternal age may increase the likelihood of developmental programming effects being transmitted to further generations. We suggest that developmental programming effects could be propagated through the maternal line *de novo* in generations beyond F2 as a consequence of development in a suboptimally developed intrauterine tract and not necessarily though directly transmitted epigenetic mechanisms.

Key words: development / fetal / programming / transgenerational / animal models

Introduction

It is now widely accepted that the early life environment can influence the long-term health of offspring in a variety of ways (Tarry-Adkins and Ozanne, 2011; Martin-Gronert and Ozanne, 2012a, b). The conditions for which a developmental basis is best described include insulin resistance (Martin-Gronert and Ozanne, 2012a, b), obesity (Cottrell and Ozanne, 2008) and hypertension (Chong and Yosypiv, 2012), but the list is rapidly expanding to include a variety of immunological (Bilbo and Schwarz, 2012), mental health (Raikkonen *et al.*, 2012) and reproductive (Sloboda *et al.*, 2011) problems. The concept of 'developmental programming' is supported by results from both animal models and human epidemiological studies. The timing of developmental programming interventions has been widely studied, leading to the proposal of critical windows during development, when programming takes place (Vickers, 2011).

A key element of developmental programming is the existence of 'transgenerational' effects, by which an early life exposure may affect the later-life health not only of the F1 generation, but also of future generations (F2 and beyond). The acceptance of transgenerational effects as an integral part of developmental programming is germane to a potential beneficial purpose of developmental effects in evolutionary terms. If the importance of transgenerational effects is accepted, then developmental programming could be described as a modifying, long-term influence promoting species survival according to the environment. The alternative view casts developmental programming as a pregnancy-by-pregnancy rapid adjustment of phenotype to the maternal surroundings, which may be revised on a generational basis without prejudicing the health of future offspring.

The definition of a transgenerational effect in the context of developmental programming models is not straightforward. When a programming intervention is applied to a mother (F0 generation) during pregnancy, it will directly influence the offspring developing *in utero* (F1 generation). However, the germ cells (future gametes) that will form the F2 generation develop during this pregnancy and hence will also be directly exposed to the suboptimal environment. Therefore, it can be argued that only effects on later generations (F3 and beyond) can truly be considered as transmitted across generations, not as a direct consequence of the original exposure (Jirtle and Skinner, 2007; Skinner, 2008). However, there are limited developmental programming studies that have followed a phenotype through to the F3 generation, and results show inconsistent phenotypic effects in the F3 generation (Drake *et al.*, 2005; Benyshek *et al.*, 2006, 2008; Harrison and Langley-Evans, 2009; Dunn and Bale, 2011).

Effect of maternal uterine environment

The mechanisms responsible for transgenerational effects in developmental programming are poorly understood. Several basic categories of mechanism exist: either transmission via alteration of the epigenome (either somatic or germline) (Ozanne and Constancia, 2007), transmission via components of the ooplasm (particularly mitochondria) (Knudsen and Green, 2004) or via development in a suboptimal uterine environment provided by the programmed F1 generation (Fig. 1). Development in a suboptimal uterine environment is known to produce an increased likelihood of disease later in life, for example, wild-type rat embryos transferred into a hyperglycaemic uterine environment become hyperglycaemic later in life (Gill-Randall *et al.*, 2004). However, it is not well established, however, whether developmental programming exposures in an F0 generation specifically affect the reproductive system or compromise maternal adaptations to pregnancy sufficiently in the F1 generation to establish *de novo* a developmental programming phenotype in the F2 and subsequent generations.

Maternal age is known to influence the uterine environment, and is a risk factor for adverse outcomes in pregnancy, including low birthweight and intrauterine growth restriction (Salem Yaniv *et al.*, 2011). Developmental programming effects may be magnified by the concurrent increase in co-morbidities in older mothers especially hypertension, obesity and diabetes (Gilbert *et al.*, 1999). It is possible that transmission of developmental programming effects to subsequent generations could be at least partially dependent on the extent of the maternal ageing at the time of pregnancy, and hence we have drawn attention to the age of the mother in transgenerational developmental programming experiments.

Here, we review the evidence that further generations are affected by developmental programming exposures, and assess the extent to which the uterine environment may have an impact on the transmitted phenotype.

Methods

PubMed searches were undertaken with keywords 'developmental programming', 'fetal programming' and 'transgenerational', 'trans-generational', 'intergenerational', 'generation' and 'F2 generation'. In all, 5565 papers were found using these search strategies. A preliminary title search identified 98 papers of relevance. These were then subjected to a more detailed abstract and language search. This identified 45 primary research papers using rodent models of developmental programming that bred and examined a phenotype in offspring at least as far as the F2 generation (Table I). Relevant studies dealing with transgenerational programming in humans and larger mammals were extracted from the same initial search. Toxicology studies carried out primarily to evaluate teratogenicity were classified as outside the remit of this review and thus excluded. However, where toxicology studies addressed transgenerational metabolic phenotypes, they were considered in the review.

Transgenerational effects in epidemiological studies

Transgenerational data relating to human populations is relatively sparse. The long generation times of humans, for prospective studies, and difficulty with the completeness of record keeping, for retrospective studies, make reliable data difficult to obtain. There are notable exceptions, for example, work from the isolated northern Swedish community of Overkalix (Bygren *et al.*, 2001; Kaati *et al.*, 2002) and from the Dutch famine cohort (Painter *et al.*, 2008) have both revealed composite measures of ill health in the second generation of offspring. The second generation of offspring from the Dutch famine cohort (babies whose maternal grandmothers were famine-exposed during pregnancy) were found to have an increased ponderal index compared with controls, giving evidence of a transgenerational effect via the maternal line (Painter *et al.*, 2008). In the Swedish Overkalix population, food availability to previous generations was inferred from contemporaneous records and was linked to the likelihood of developing type 2 diabetes and cardiovascular disease in subsequent generations. Interestingly, those whose grandfathers had a plentiful food supply in childhood had an increased likelihood of mortality related to type 2 diabetes as adults (Kaati *et al.*, 2002). Further evidence, from the same population, for the transmission of developmental programming via the paternal line linked grandparental food availability with the relative risk of mortality in grandchildren (Pembrey *et al.*, 2006). These effects were transmitted via the paternal line in a sex-specific manner, with the paternal grandfather's food supply linked to the relative mortality of male second-generation offspring, while the paternal grandmother's food intake was associated with the phenotype of the female second-generation offspring.

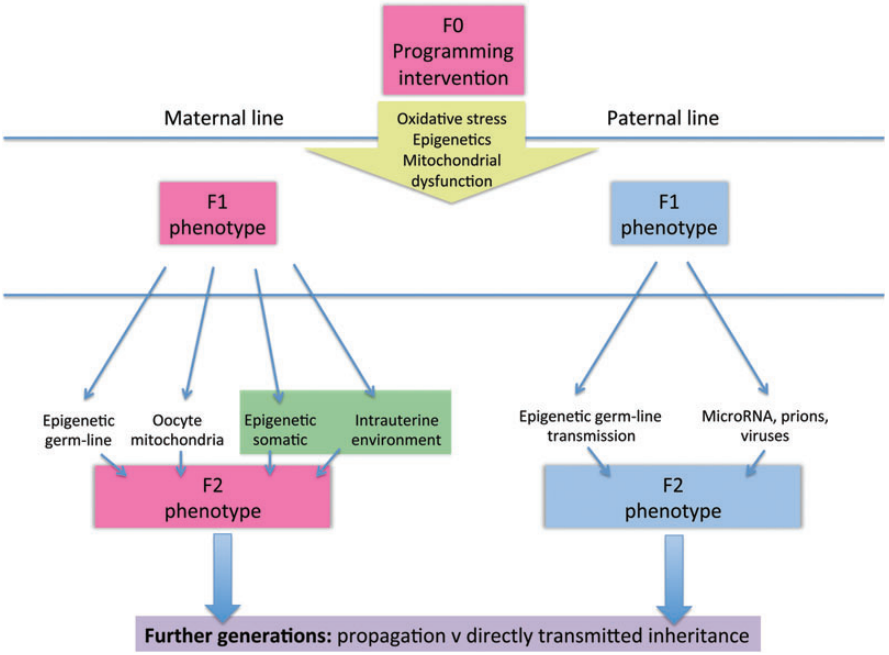

Figure 1 Potential mechanisms of transgenerational developmental programming via maternal (orange) and paternal (blue) lines. Mechanisms that involve phenotype propagation *de novo* in each subsequent generation are highlighted in green. Proposed mechanisms of developmental programming to F1 generations are shown in yellow.

Paradigms from toxicological studies

Aside from studies in which the primary aim was to determine developmental programming effects, useful observations regarding the mechanisms of transgenerational developmental programming may be derived from the field of toxicology research. In toxicology, the minimum required evidence to make breeding of further generations of rats unnecessary in terms of excluding an effect is reviewed in Beekhuijzen *et al.* (2009). The phenotypic effects seen in toxicology work are generally more substantial than those in developmental programming, particularly where the environmental stimulus provokes teratogenicity. The paradigmatic example of transgenerational developmental disruption occurs with the synthetic estrogen diethylstilbestrol (DES). DES is a potent endocrine disruptor, with profound effects on the development of estrogen-receptive tissues during *in utero* exposure. This leads to adverse F1 generation outcomes in human populations, including infertility (Palmer *et al.*, 2001) and tumour development (Herbst *et al.*, 1971). Effects are also thought to manifest in the F2 generation in humans (Titus-Ernstoff *et al.*, 2010), and have been demonstrated in mice (Newbold *et al.*, 2006). The mechanism of adverse effects caused by DES appears to be altered patterns of DNA methylation (McLachlan *et al.*, 2001). Further work with post-natal administration of soy isoflavones (which also mimic endogenous estrogens) in the mouse has shown similar F1 generation findings in terms of disrupting ovarian and uterine development, in particular, a higher incidence of uterine hyperplasia, in conjunction with increased bodyweight (Dinsdale *et al.*, 2011). Interestingly, although the effect of increasing bodyweight persisted to the F2 generation with soy isoflavone exposure, the reproductive effects were seen only in the F1 generation (Dinsdale *et al.*, 2011). Not all estrogen-like endocrine disruptors produce similar results; however, as the increasing dosage experiments with genistein and nonylphenol demonstrate (Ferguson *et al.*, 2009). Adult female rats showed only minimal dysregulation of sodium consumption despite exposure to dietary genistein and nonylphenol at relatively high doses (Ferguson *et al.*, 2009). The phenomenon of a sex-dependent response to an environmental stressor is also seen when the splenic immune response to early nonylphenol exposure is tested in rats, in terms of gender-specific changes in Natural Killer cell and splenic subpopulation cell numbers (Karrow *et al.*, 2004).

Further insight into the mechanisms underlying transgenerational effects has emerged from studies using the estrogen-like endocrine disruptor Bisphenol A (BPA; found commonly in plastic products). BPA exposure compromises male fertility and steroid receptor expression in the testes through to the F3 generation in the rat (Salian *et al.*, 2009). In mice, BPA exposure early in life has further been shown to lead to a range of epigenetic changes, such as DNA methylation patterns (Dolinoy *et al.*, 2007). Intriguingly, murine maternal behaviour may also be altered by BPA exposure (Palanza *et al.*, 2002), leading to potential propagation of some aspects of the phenotype through generations via behavioural mechanisms (Champagne *et al.*, 2006).

In addition to compounds with estrogen-like activities, insight can be gained into the mechanisms of transgenerational reproductive dysfunction by studying the effects of anti-androgen endocrine disruptors, most notably vinclozolin (Anway *et al.*, 2006a, b). Modulation of the epigenome (as reflected by alterations in DNA methylation patterns in the testes) was observed in adult rat males of the F1 generation, and this was followed by DNA methylation disruption in the spermatozoa of the F2 and F3 generations (Anway *et al.*, 2006a, b). A pattern of down-regulation at key methylation sites in the testes was also observed through to the F3 generation (Anway *et al.*, 2008). The offspring phenotype in rat males following initial F0 vinclozolin exposure persisted to the F4 generation (Anway *et al.*, 2006a, b), and in females to the F3 generation (Nilsson *et al.*, 2008), although these findings have been disputed subsequently (Inawaka *et al.*, 2009). The phenotype included excess tumours and disrupted kidney development in both the male and female rat offspring (Anway *et al.*, 2008; Nilsson *et al.*, 2008). The vinclozolin paradigm also suggests a potential role of behavioural

Table I Primary research papers using rodent models of developmental programming where phenotype in offspring at least as far as the F2 generation was sought.

Author	Year	Organism	Programming intervention	Phenotype examined	Generation with phenotype
Anderson *et al.*	2009	Rat (SD)	F0 surgically induced placental insufficiency/F1 and F2 no intervention	Leptin, BP and reduced growth	F1 not F2
Anderson *et al.*	2006	Rat (SD)	F0 surgically induced placental insufficiency/F1 and F2 no intervention	Hypertension and arterial dysfunction	F2
Armitage *et al.*	2007	Rat (SD)	F0 high-fat diet/F1 and F2 no intervention	Aortic dysfunction, Na/K ATPase activity	Not F2
Beach *et al.*	1982	Mouse	F0 zinc deprivation/F1 and F2 no intervention	Depressed immune function	F2/F3
Benyshek *et al.*	2004	Rat (SD)	F0 low-protein or high-fat diet/F1 and F2 high-fat diet	Insulin resistance	Not F2
Benyshek *et al.*	2006	Rat (SD)	F0 low-protein diet/F1 and F2 energy restricted	Insulin/glucose metabolism	F2/F3
Benyshek *et al.*	2008	Rat (SD)	F0 low-protein diet/F1 and F2 energy restricted	Insulin/glucose metabolism	F2 not F3
Bertram *et al.*	2008	Guinea pig	F0 low-protein diet/F1 and F2 no intervention	HPA axis responses	F2
Blondeau *et al.*	2002	Rat (Wis)	F0 energy restriction/F1 and F2 no intervention	Beta cell mass	F2
Boloker *et al.*	2002	Rat (SD)	F0 surgical arterial ligation/F1 and F2 no intervention	Glucose tolerance, insulin resistance	F2
Brix *et al.*	2005	Mouse	F0 dietary milk exposure/F1 and F2 no intervention	Immune tolerance	Not F2/F3
Burdge *et al.*	2011	Rat (Wis)	F0 high-energy diet/F1 high-energy diet	Methylation status	F2/F3
Burdge *et al.*	2007	Rat (Wis)	F0 low-protein diet/F1 and F2 energy restricted	Methylation status	F2
Carone *et al.*	2010	Mouse	F0 low-protein diet/F1 and F2 no intervention	Hepatic cysteine metabolism	F2
Chernoff *et al.*	2009	Rat (SD)	F0 energy-restricted diet/no F1 and F2 intervention	Reproductive senescence	Not F2
Costantine *et al.*	2008	Mouse	F0 NOS3 knockout/F2 wild-type	Systolic blood pressure	F2
Crudo *et al.*	2012	Guinea pig	F0 glucocorticoid exposure/F1 and F2 no intervention	DNA methylation	F2
Csaba *et al.*	2005	Rat	F0 beta endorphin/F1 and F2 no intervention	Reduction in mast cell serotonin	F2 (>F1)
Ding *et al.*	2012	Mouse	F0 gestational diabetes/F1 and F2 no intervention	Decreased fasting insulin, decreased islet function	F2
Drake *et al.*	2011	Rat (Wis)	F0 dexamethasone administration/F1 and F2 no intervention	Fetal and placental weights	F2
Drake *et al.*	2005	Rat (Wis)	F0 dexamethasone administration/F1 and F2 no intervention	Birthweight, glucose tolerance and hepatic enzyme activity	F2 not F3
Dunn and Bale	2011	Mouse	F0 high fat/F1 and F2 no intervention	Bodyweight and glucose tolerance	F2/F3
Frantz *et al.*	2011	Mouse	F0 low-protein diet/F1 and F2 no intervention	Insulin secretion and pancreatic beta cell mass	F2/F3
Fullston *et al.*	2012	Mouse	F0 high-fat paternal diet/F1 and F2 no intervention	Impaired gamete development	F2
Garg *et al.*	2012	Rat (SD)	F0 no intervention/F1 caloric restriction/F2 embryo-transfer	Body weight, glucose tolerance	F2
Gauguier *et al.*	1990	Rat	F0 glucose infusion/F1 and F2 no intervention	Hyperglycaemia, hyperinsulinaemia, macrosomia	F2
Gniuli *et al.*	2008	Mouse	F0 and F1 high fat/F2 no intervention	Glucose tolerance, pancreatic beta cell dysfunction	F2
Harrison and Langley-Evans	2009	Rat (Wis)	F0 low-protein diet/F1 and 2 no intervention	Systolic blood pressure, nephron number, body composition	F2 not F3
Jimenez-Chillaron *et al.*	2009	Mouse	F0 50% caloric restriction/F1 and 2 no intervention	Birthweight, glucose tolerance and obesity	F2
Lam *et al.*	2000	Rat (Wis)	F0 alcohol consumption, F1 and F2 no intervention	Latency to right	F2
Mahsoudi *et al.*	2007	Mouse	F0 were derived from embryos cultured *in vitro*/F1 and F2 no intervention	Birthweight, litter size, reproductive performance	Not F2/F3

Continued

Table I *Continued*

Author	Year	Organism	Programming intervention	Phenotype examined	Generation with phenotype
Martin *et al.*	2000	Rat (SD)	F0 low-protein diet/F1 and 2 high fat or control diet	Plasma glucose and insulin	F2
Morgan and Bale	2011	Mouse	F0 prenatal stress/F1 and F2 no intervention	Methylation disruption	F2
Nascimento *et al.*	2012	Mouse	F0 vitamin D deficiency/F1 and F2 no intervention	Renal development	F2
Pallinger *et al.*	2006	Rat (Wis)	F0 beta endorphin/F1 and F2 no intervention	Increased histamine in peritoneal mast cells	F2
Peixoto-Silva *et al.*	2011	Mouse	F0 low-protein diet/F1 and F2 no intervention	Birthweight, glucose tolerance and adipocyte size	F2
Pentinat *et al.*	2011	Mouse	F0 early over-nutrition/F1 and F2 no intervention	Peripheral glucose tolerance	F2
Pinheiro *et al.*	2008	Rat (Wis)	F0 low-protein diet/F1 and F2 no intervention	Birthweight, glucose, leptin	F2
Pinto and Shetty	1995	Rat (Wis)	F0 exercise/F1 exercise or sedentary	Birthweight	F2
Radford *et al.*	2012	Mouse	F0 50% caloric restriction/F1 and F2 no intervention	DNA methylation	F2
Slamberova *et al.*	2005	Rat (SD)	F0 morphine exposure/F1 and F2 no intervention	Righting reflex	F2
Stone and Bales	2010	Prairie vole	F0 handling/F1 and F2 no intervention	Social behavioural indices	F2
Thamotharan *et al.*	2007	Rat (SD)	F0 50% caloric restriction/F1 and F2 no intervention	Glucose:insulin ratio, GLUT4	F2
Torrens *et al.*	2008	Rat (Wis)	F0 low-protein diet/F1 and F2 no intervention	Vascular endothelial changes	F2
Zambrano *et al.*	2005	Rat (Wis)	F0 low-protein diet/F1 and F2 no intervention	Glucose and insulin metabolism	F2

aspects when considering transgenerational propagation of environmentally induced effects. Rat males exposed to prenatal vinclozolin and their descendants showed increased anxiety traits and decreased reproductive behaviours, which may be important when considering how effects are disseminated through lineages (Crews *et al.*, 2007).

Other endocrine disruptors, including polychlorinated bisphenyls also show profound transgenerational effects in female reproductive tract development through to the F2 generation. Polychlorinated bisphenyl exposure in the female rat results in particularly suppressed surges of luteinizing hormone prior to ovulation, and reduced uterine and ovarian weights in F1 and F2 offspring. It is unclear whether the changes in reproductive physiology themselves may be responsible for subsequent generation effects or whether these are a result of epigenetic modification (Steinberg *et al.*, 2008). A dose-dependent toxicity effect of caffeine-exposure *in utero* is also seen in the F2 generation, although the mechanism of the transgenerational effect is unclear (Pollard and Claassens, 1992). Transgenerational programming via the paternal line in mice is demonstrated in toxicological programming, with the endocrine toxicant benzo(a)pyrene (BaP) producing adverse effects on spermatogenesis through to the F2 generation following an initial paternal F0 exposure (Mohamed el *et al.*, 2010). Interestingly, an F3 effect was specially sought but not detected in this study, demonstrating that the transgenerational paternal effect was limited in this case to the F1 and F2 generations (Mohamed el *et al.*, 2010).

Animal models of transgenerational programming

The overwhelming majority of developmental programming studies that span more than two generations have been performed in mice and rats, the earliest of which were conducted over 30 years ago (Zamenhof *et al.*, 1971; Stewart *et al.*, 1975; Beach *et al.*, 1982). Recently, however, more studies have described transgenerational developmental programming effects in large animals. In particular, striking data have become available on paternal-lineage transgenerational programming in the pig (Braunschweig *et al.*, 2012). There is also evidence of a significant adverse effect on lipid metabolism persisting to an F2 generation following prenatal dexamethasone administration in the marmoset (Buchwald *et al.*, 2012). There have also been data from rodents other than mice and rats; several studies have shown second-generation effects in the guinea-pig, including prenatal dexamethasone administration inducing methylation changes persisting into a second generation (Crudo *et al.*, 2012) and F0 protein restriction impairing the hypothalamic–pituitary–adrenal (HPA) axis in the F2 offspring (Bertram *et al.*, 2008). A second-generation effect of food restriction leading to delayed neurodevelopment was also reported in hamsters (Liang *et al.*, 2007).

Within rat and mouse models, there is a great range of developmental programming interventions that have been studied in terms of second-generation effects. These include exercise (Pinto and Shetty, 1995), surgical models (Boloker *et al.*, 2002; Anderson *et al.*, 2006, 2009), alcohol and opiate exposure (Lam *et al.*, 2000; Slamberova *et al.*, 2005) and embryo culture *in vitro* (Mahsoudi *et al.*, 2007). The majority of studies, however, focus on maternal dietary interventions, primarily calorie restriction (Blondeau *et al.*, 2002; Thamotharan *et al.*, 2007; Chernoff *et al.*, 2009; Jimenez-Chillaron *et al.*, 2009; Radford *et al.*, 2012), high-fat (Armitage *et al.*, 2007; Gniuli *et al.*, 2008; Dunn and Bale, 2011; Fullston *et al.*, 2012) and low-protein (Torrens *et al.*, 2003; Benyshek *et al.*, 2006; Burdge *et al.*, 2007; Pinheiro *et al.*, 2008; Harrison and Langley-Evans, 2009; Carone *et al.*, 2010; Frantz *et al.*, 2011; Peixoto-Silva *et al.*, 2011) diets. Micronutrient deficiencies have also been reported to produce second-generation phenotypes,

including maternal zinc (Beach *et al*., 1982) and vitamin D (Nascimento *et al*., 2012) deficiencies. The range of outcomes reported in the second generation is also wide, with many studies measuring multiple parameters of growth and glucose/insulin metabolism (Pinto and Shetty, 1995; Martin *et al*., 2000; Boloker *et al*., 2002; Drake *et al*., 2005; Thamotharan *et al*., 2007; Benyshek *et al*., 2008; Pinheiro *et al*., 2008; Pentinat *et al*., 2010; Peixoto-Silva *et al*., 2011; Dunn and Bale, 2011; Ding *et al*., 2012; Garg *et al*., 2012). Other studies report effects on blood pressure and vascular dysfunction (Anderson *et al*., 2006; Armitage *et al*., 2007; Costantine *et al*., 2008; Torrens *et al*., 2008; Harrison and Langley-Evans, 2009). Apart from components of the metabolic syndrome, the studies reviewed here include those with outcomes relating to immune function (Beach *et al*., 1982; Brix *et al*., 2005; Csaba *et al*., 2005; Pallinger *et al*., 2006), reproduction (Chernoff *et al*., 2009; Fullston *et al*., 2012) and neurological aspects of development (Lam *et al*., 2000; Slamberova *et al*., 2005; Stone and Bales, 2010). Despite careful analyses of these parameters in different studies, there remains significant variation in the phenotypic characteristics transmitted to the F2 generation; these may be diet or timing dependent, or may be influenced by the rodent (rat v mouse) or strain used. Examples include the phenotypic variation amongst surgical arterial ligation models, with effects on leptin and bodyweight present only in the F1 generation (Anderson *et al*., 2009), but effects on arterial dysfunction and glucose tolerance persisting into the F2 generation (Boloker *et al*., 2002; Anderson *et al*., 2006). Within maternal low-protein diet rat models (one of the most common model types studied), the phenotype has variously been reported to extend into the F3 generation (Benyshek *et al*., 2006), or to stop at the F2 generation (Benyshek *et al*., 2008; Harrison and Langley-Evans, 2009), depending on the precise details of the dietary model and the outcomes studied. In terms of embryo-transfer experiments, there was no effect on birthweight persisting into an F2 generation when a mouse model was used (Mahsoudi *et al*., 2007), but increased body weight in the F2 generation was reported in a rat model (Garg *et al*., 2012).

Maternal versus paternal line transmission of programming effects

Many rodent models, in common with human studies (Bygren *et al*., 2001; Pembrey *et al*., 2006; Veena *et al*., 2007; Painter *et al*., 2008), have investigated whether an F2 generation effect can be produced via the maternal line, paternal line or both. The 'parent of origin' of further generation programming effects is an important question, particularly given the existence of sex differences in the F1 generation phenotype in many developmental programming models (Aiken and Ozanne, 2012).

Paternal line transmission. Experimental designs testing the transmission of developmental programming effects through the paternal line are less common than those investigating maternal transmission (Carone *et al*., 2010; Pentinat *et al*., 2010; Dunn and Bale, 2011; Morgan and Bale, 2011; Fullston *et al*., 2012). Impaired glucose tolerance in the F1 and F2 generations has been demonstrated in response to paternal over-nutrition in the F0 generation (Pentinat *et al*., 2010). Impaired glucose tolerance with effects paternally transmitted to the F3 generation was seen in the offspring of F1 males who had been exposed to a maternal high-fat diet in the F0 generation (Dunn and Bale, 2011). Paternal F0 exposure to a high-fat diet also altered the epigenome of sperm in subsequent generations (Fullston *et al*., 2012). Exposure of F0 generation males to a low-protein diet with transmission via the paternal line has also generated F2 effects, manifest as changes in DNA methylation at various loci in liver cells, including loci potentially involved in regulation of lipid metabolism (Carone *et al*., 2010). There is little published evidence regarding caloric under-nutrition or non-dietary models transmitting metabolic phenotypes via the paternal line, although an effect of reduction in miRNA expression in the F2 perinatal brain in response to F0 generation paternal stress exposure has been shown (Morgan and Bale, 2011).

Maternal line transmission. A greater number of studies have demonstrated transgenerational programming effects transmitted via the maternal line (Pinto and Shetty, 1995; Lam *et al*., 2000; Pinheiro *et al*., 2008; Frantz *et al*., 2011; Martin *et al*., 2011; Peixoto-Silva *et al*., 2011; Nascimento *et al*., 2012). These include such diverse exposures as exercise-induced stress (Pinto and Shetty, 1995), zinc deprivation (Beach *et al*., 1982) and maternal alcohol consumption (Lam *et al*., 2000). A maternal high-fat diet is reported to result in F2 offspring phenotypes, including glucose intolerance and pancreatic dysfunction (Gniuli *et al*., 2008). However, the F2 generation does not appear to show adverse effects of a maternal high-fat diet in terms of the cardiovascular risk factors observed in the F1 generation (Armitage *et al*., 2007). A number of offspring outcomes have been shown in response to F0 dietary protein restriction in the female, including effects on glucose/insulin metabolism (Zambrano *et al*., 2005; Benyshek *et al*., 2008; Pinheiro *et al*., 2008; Peixoto-Silva *et al*., 2011), adiposity (Peixoto-Silva *et al*., 2011), DNA methylation in the liver (Burdge *et al*., 2007), pancreatic islet mass (Frantz *et al*., 2011), nephron number (Harrison and Langley-Evans, 2009) and cardiovascular effects (Torrens *et al*., 2008; Harrison and Langley-Evans, 2009). However, global maternal under-nutrition gives less consistent results. Maternal under-nutrition was found to decrease beta cell mass in the F1 generation with effects on beta cell development also apparent in the F2 generation (Blondeau *et al*., 2002), and to impair glucose/insulin metabolism (Thamotharan *et al*., 2007). However, maternal under-nutrition was reported to have no discernible effect transmitted to the F2 generation in terms of reproductive system function (Chernoff *et al*., 2009).

Transmission via either parental line. Other studies report the ability to induce an F2 phenotype through both the maternal and paternal lines (Drake *et al*., 2005, 2011; Jimenez-Chillaron *et al*., 2009; Ding *et al*., 2012; Radford *et al*., 2012). In an F0 caloric-restricted mouse model, breeding of the F2 generation from either an exposed F1 mother or father gave rise to impaired glucose tolerance in the F2 generation (Jimenez-Chillaron *et al*., 2009). However, birthweight of the F2 offspring was only low when the exposure was via the paternal line, and later obesity of the F2 offspring was an effect transmitted only via the maternal line (Jimenez-Chillaron *et al*., 2009).

Notably, a mouse model of gestational diabetes demonstrated transmission of the same developmental programming effect via a potentially different mechanism depending on whether the intervention was transmitted via the maternal or paternal line (Ding *et al*., 2012). The F2 generation displayed impaired glucose tolerance via either the F0-exposed mother or father, but the effect was markedly increased when the father was the exposed parent (Ding *et al*., 2012). Similarly, in a rat model of F0 prenatal dexamethasone exposure, the F2 generation showed decreased fetal and placental weight at E20 where the exposed F1 parent was the father, but increased fetal and placental weight where the mother was the exposed parent. This effect was associated with differing patterns of placental gene methylation. Ultimately, however, birthweight was reduced in both the maternally and paternally exposed groups (Drake *et al*., 2011).

F3 and subsequent generations in rodent models

Few rodent developmental programming studies have examined a phenotype in the F3 generation (Table II). Those that have carried out this analysis have found varying and often conflicting results. Overall analysis of these data is complicated by the range of interventions and models used, as well as difficulty in interpreting studies in which further interventions have been applied to F1 and subsequent generations.

A phenotype of increased insulin:glucose ratio was detected in the F2 offspring of rats subjected to protein restriction during pregnancy, which persisted to the unexposed F3 generation (Benyshek *et al*., 2006). Notably, however, little effect was observed in the F1 generation, whose fasting insulin:glucose ratio was decreased relative to controls. This result implies an

Table II Primary research papers using rodent models of developmental programming where phenotype in offspring at least as far as the F3 generation was sought.

Authors	Year	Organism	Programming intervention	Phenotype examined	Generation with phenotype
Beach *et al.*	1982	Mouse	F0 zinc deprivation/F1 and F2 no intervention	Depressed immune function	F2/F3
Brix *et al.*	2005	Mouse	F0 dietary milk/F1 and F2 no intervention	Immune tolerance	Not F2/F3
Burdge *et al.*	2011	Rat (Wis)	F0 high-energy diet/F1 high-energy diet	Methylation status	F2/F3
Drake *et al.*	2005	Rat (Wis)	F0 dexamethasone/F1 and F2 no intervention	Birthweight, glucose tolerance and hepatic enzyme activity	F2 not F3
Benyshek *et al.*	2006	Rat (SD)	F0 low-protein diet/F1 and F2 energy restricted	Insulin/glucose metabolism	F2/F3
Mahsoudi *et al.*	2007	Mouse	F0 embryos cultured *in vitro*/F1 and F2 no intervention	Birthweight, litter size, reproductive performance	Not F2/F3
Benyshek *et al.*	2008	Rat (SD)	F0 Low protein/F1 and F2 energy restricted	Insulin/glucose metabolism	F2 not F3
Harrison and Langley-Evans	2009	Rat (Wis)	F0 protein restriction/F1 and F2 no intervention	Systolic blood pressure, nephron number, body composition	F2 not F3
Dunn and Bale	2011	Mouse	F0 high fat/F1 and F2 no intervention	Bodyweight and glucose tolerance	F2/F3
Frantz *et al.*	2011	Mouse	F0 low protein/F1 and F2 no intervention	Insulin secretion and pancreatic beta cell mass	F2/F3

Each of these papers is included in the 45 papers in Table I.

alteration of the phenotype between generations, which seems incompatible with a germline epigenetic modification. A further study by the same group failed to detect an F3 phenotype at all when F1 and F2 generations were maintained on energy-restricted diets, including during pregnancy and lactation (Benyshek *et al.*, 2008). A phenotype in the females of the F3 generation was detected using a maternal high-fat diet, with exposure only in the F0 generation (Dunn and Bale, 2011). The female F3 offspring in this study showed no difference in insulin sensitivity compared with controls, but there was a phenotype of increased body size transmitted only via the paternal line. No phenotypic effects were seen in the male F3 offspring, or in either sex transmitted via the maternal line (Dunn and Bale, 2011). In a severe (5%) F0 protein restriction mouse model, with generations F1–F3 maintained on normal diet, an F3 phenotype of reduced basal insulin levels and decreased pancreatic beta cell mass was detected (Frantz *et al.*, 2011).

Important insight into the mechanisms of transgenerational developmental programming effects is also provided by studies where an effect on F2 or later generations has been explored but not detected (Drake *et al.*, 2005; Mahsoudi *et al.*, 2007; Benyshek *et al.*, 2008; Harrison and Langley-Evans, 2009; Burdge *et al.*, 2011). Notably, Drake *et al.* sought a phenotype in an F3 generation of rats following F0 exposure to exogenous glucocorticoid administration. Although an offspring effect was detectable in both F1 and F2 generations, this did not persist to F3, importantly the first generation not to be exposed to the intervention *in utero* (Drake *et al.*, 2005). A similar result was obtained from a rat model where protein restriction during pregnancy and lactation was applied to the F0 generation, and caloric restriction in adult life, extending through pregnancy and lactation was applied to subsequent (F1, F2 and F3) generations. This produced insulin resistance in the F1 and F2 generations, but not in the F3 generation. The F3 generation had normal glucose tolerance regardless of their adult diet (Benyshek *et al.*, 2008). The effect of an F0 protein restriction in the rat was also tested in a separate study without any intervention applied to subsequent generations (Harrison and Langley-Evans, 2009). This study also failed to find a phenotype in the F3 generation, having looked for evidence of elevated systolic blood pressure, nephrogenesis and body composition effects (Harrison and Langley-Evans, 2009). These results add weight to the possibility that many reported transgenerational effects are results of the original exposure, rather than true epigenetic transmission of developmental programming (Skinner, 2008). The lack of phenotype in the F3 generation where only an F0 insult is applied contrasts with the results from a model where the developmental programming intervention was sustained between generations. Rats with sustained exposure to a high-fat diet over three generations had epigenetic changes in all exposed generations; however, the authors concluded that the changes they detected were programmed *de novo* in each generation. There was no evidence for a substantial direct contribution of F0 effects to the F3 generation epigenetic changes (Burdge *et al.*, 2011).

In the related field of toxicology research, it is more common to continue studies where teratogenic effects are found to include second, third and fourth generations. Particularly of relevance to developmental programming research is evidence of multi-generational reproductive dysfunction following exposure to potent endocrine disruptors (vinclozolin and methoxychlor, a fungicide and pesticide, respectively) (Anway *et al.*, 2008). Transmission of these effects via the paternal line has been demonstrated to be associated with alteration of DNA methylation patterns in the male gametes, persisting into an F3 generation (Chang *et al.*, 2006).

Potential mechanisms of transmission of intergenerational effects

Many authors have argued in favour of an epigenetic basis for transgenerational programming (Thamotharan *et al.*, 2007). Epigenetic mechanisms include alterations in DNA methylation, histone modification or small RNA molecules. Attention has focused largely on changes in DNA methylation patterns invoked by nutritional or other environmental stimuli (Reik *et al.*, 2001), and such changes are sought in numerous transgenerational developmental programming models (Burdge *et al.*, 2007, 2011; Crudo *et al.*, 2012). DNA methylation occurs primarily via the binding of methyl groups to cytosine residues within CpG islands (Yang *et al.*, 2004). Methylation patterns are thought to be reset twice during normal early

mammalian development, initially being removed during preimplantation development asynchronously from the paternal and then maternal alleles (Rivera and Ross, 2013). New methylation patterns subsequently emerge during early post-implantation development. DNA methylation patterns are also replaced in the primordial germ cells during embryonic development (Lees-Murdock and Walsh, 2008). It has been hypothesized that dietary availability of methyl donors can alter early methylation patterns, and transmissible epigenetic changes in an F1 generation in response to dietary manipulation have been demonstrated in experiments with agouti mice (Morgan *et al*., 1999; Waterland and Jirtle, 2003). Strong evidence for the role of epigenetics rather than the intrauterine environment comes from embryo-transfer experiments. Thamotharan *et al*. (2007) generated an F2 phenotype of glucose:insulin ratio changes and GLUT4 alterations following an F0 dietary intervention, despite transferring the F2 blastocysts to a control uterus for development. This finding, however, is not consistent across studies (Gill-Randall *et al*., 2004; Martin *et al*., 2011; Garg *et al*., 2012).

Programming via histone modification is less well described. A number of possible modifications to histones are possible, including methylation, phosphorylation and acetylation, all of which can change the interaction between histones and DNA to alter gene expression (Zentner and Henikoff, 2013). Alterations in histone modification have been demonstrated as a result of exposure to heavy metals [arsenic (Zhou *et al*., 2008) and nickel (Broday *et al*., 2000)] *in vitro*, although not in an *in vivo* developmental programming model. It has further been postulated that the effects of steroid co-receptor expression dysregulation caused by early life exposure to BPA, may be due at least in part to the actions of steroid co-receptors as histone modifiers (Walker and Gore, 2011).

A further epigenetic mechanism by which transgenerational epigenetic effects may be mediated is via alteration of microRNA (miRNA) expression. miRNAs are small, non-coding sequences than can influence translation through binding sequence specifically to the 3′ untranslated region of mRNA transcripts (Hou *et al*., 2011). miRNA expression is known to be modulated in response to environmental factors, including cigarette smoke (De Flora *et al*., 2012) and dietary factors (Parasramka *et al*., 2012). There is little evidence yet, that such changes can be propagated through generations.

Transgenerational programming via the paternal line

Evidence from both human and rodent studies suggests that transgenerational developmental programming effects can be transmitted down the paternal line (Drake *et al*., 2005, 2011; Chang *et al*., 2006; Ding *et al*., 2012; Fullston *et al*., 2012). Germline epigenetic modification is the most often cited explanation for this effect. Direct transmission of epigenetic methylation patterns via the germline is postulated despite evidence of extensive demethylation during both germ cell formation and zygotic development (Goldberg *et al*., 2007; Jirtle and Skinner, 2007; Sasaki and Matsui, 2008). Transgenerational inheritance of DNA methylation patterns has been demonstrated via both the maternal and paternal lines at the A^{vy} and $Axin^{Fu}$ alleles in the mouse (Morgan *et al*., 1999; Rakyan *et al*., 2003), loci that can be affected in both F1 and F2 generations by maternal diet (Cropley *et al*., 2006). It may be the case that other endogenous loci are similarly responsive to dietary intervention and behave in the same way as the A^{vy} and $Axin^{Fu}$ alleles, however, this evidence of a potential mechanism does not demonstrate that epigenetic transmission of effects via the germline can actually occur. An elegant array study of DNA methylation patterns demonstrated perturbed imprinted gene expression in F1 and F2 generations of prenatal undernourishment in the mouse (Radford *et al*., 2012). The findings of this study suggest that fetal alteration in imprinted gene expression is 're-programmed' on a generation-by-generation basis rather than representing epigenetic germline modification (Radford *et al*., 2012).

Study of the sperm of the F1 generation is of obvious interest where the F2 exposure is via the paternal line. In a mouse model with impaired fasting insulin in the F2 generation, both Igf2 and H19 expression were found to be down-regulated in the F1 sperm (Ding *et al*., 2012), giving a potentially important insight into the mechanism of transmission. F1 sperm gene expression changes were also reported in a low-protein mouse model, leading to paternal line transmission of alterations in hepatic cysteine metabolism (Carone *et al*., 2010). However, other models where a paternal line transmission of developmental programming effects is postulated failed to find any epigenetic alterations to the F1 sperm (Drake *et al*., 2011; Radford *et al*., 2012). This implies that mechanisms other than direct germline transmission must be sought, even though the paternal line.

The difficulties with germline epigenetic modification have led to examination of mechanisms other than via the alleles contributed by the father. Indirect modification of the conceptus or conceptual environment by other paternal influences, for example, via transmission of prions (Shorter and Lindquist, 2005), viruses or miRNA (Ostermeier *et al*., 2004) has been postulated, but these remain poorly understood. The increasing understanding of the role of microRNAs in paternal gametes may lead to the proposal of an alternative mechanism (Gluckman *et al*., 2007).

Transgenerational programming via the maternal line

Disentangling the relative contribution of the different mechanisms to transgenerational effects via the maternal line is more complex. Four methods have been identified by which the mother may influence the phenotype of a subsequent generation (Lie, 2007). The first method, as discussed for the paternal lineage, is by providing half the fetal genes and associated epigenetic markings (Drake *et al*., 2005; Jimenez-Chillaron *et al*., 2009). The same reservations regarding intergenerational epigenetic reprogramming will apply for programming via the maternal lineage. The other methods by which a mother may influence an offspring phenotype are via somatic epigenetic reprogramming, via the ooplasmic contribution to the fetus and via the provision of the intrauterine environment.

Somatic epigenetics. Rodent studies provide evidence that somatic epigenetic modification may also be transmitted between mother and offspring via maternal behavioural differences during the post-natal period. The work of Weaver *et al*. (2004) demonstrated via cross-fostering studies that high levels of nursing and grooming behaviour by the rat mother promotes epigenetic alterations (both DNA methylation and histone modification) in the pups, leading to a less acute glucocorticoid and behavioural response to stress in adult life. Such somatic epigenetic modifications can be self-perpetuating between generations by behavioural mechanisms (Champagne *et al*., 2006). Thus, pups of mothers that exhibit high levels of nursing and grooming behaviours will themselves exhibit these behaviours with their subsequent litters (Francis *et al*., 1999). A similar behaviourally mediated transgenerational effect has been observed in prairie voles, but with the interesting difference that the behaviour leading to developmental programming (alloparental behaviour) could be 'set' in the F0 generation by experimental intervention (Stone and Bales, 2010).

Ooplasmic programming. Despite the obviously important contribution to the mechanism of transgenerational programming effects made by epigenetic modifications, consideration must also be given to the role of non-genomic aspects of embryonic and fetal physiology and attention has focused particularly on the contribution of the mitochondria. The mitochondria and mitochondrial DNA (mtDNA) inherited by the developing conceptus are derived from the oocyte cytoplasm in humans and rodents, and therefore inherited via the maternal line (Cummins, 2002). The mitochondrial response to energy flux within tissues is being increasingly understood (Crescenzo *et al*., 2006). mtDNA copy number changes dramatically within individual tissues in response to oxidative stress during early gestation, and

could therefore provide a mechanism for developmental programming by maternal dietary intervention (Aiken *et al*., 2008). It has previously been demonstrated that rats fed a high-fat diet exhibit altered oocyte mitochondrial biogenesis and redox status (Igosheva *et al*., 2010), providing a potential mechanism for transmission of developmental programming effects via the cytoplasm rather than DNA of the developing conceptus. Early reprogramming of mitochondrial function has been suggested elsewhere as an important mediator of developmental programming effects (Theys *et al*., 2009).

The role of the intrauterine environment. The role of the intrauterine environment in setting the phenotype of future generations is demonstrated by studies of embryo-transfer of control embryos into abnormal uterine environments. Transfer of control embryos into hyperglycaemic uteri gives a phenotype of diabetes in the adult offspring (Gill-Randall *et al*., 2004). Similarly, the transfer of wild-type embryos into the uteri of mice subjected to maternal ghrelin deficiency caused their implantation to be impaired, a result likely due to defective endometrial proliferation in the F1 uterus (Martin *et al*., 2011). Conversely, the phenotype in a second generation of caloric-restricted rat offspring can be 'rescued' by blastocyst transfer to a control uterus (Garg *et al*., 2012). These results imply that the uterus is not a passive partner in establishing the phenotype of the F2 generation, and that a germline modification is not required to transmit a programming phenotype in the presence of a modified reproductive tract.

Direct modulation of the reproductive tract of the F1 generation has been demonstrated in several rodent studies. Normal reproductive tract function encompasses multiple anatomical and physiological elements, many of which can be disrupted by developmental programming interventions [reviewed in (Sloboda *et al*., 2011)]. An ovarian phenotype in the F1 generation has been demonstrated in a number of studies, in terms of both follicular number (Bernal *et al*., 2010) and estrous cycling (Chernoff *et al*., 2009; Sloboda *et al*., 2009). It has been suggested, by Leese *et al*., that the oviductal and uterine fluid composition can be affected by developmental programming exposures. The oviductal fluid may be crucial in defining the delicate relationship between the preimplantation conceptus and the reproductive tract environment (Leese *et al*., 2008), influencing further embryonic development of the F2 generation.

There is also evidence of a developmental programming effect on the uterus. Uterine vascular adaptations to normal pregnancy are disrupted by maternal diet in both maternal dietary restriction (Hemmings *et al*., 2005) and maternal low-protein (Torrens *et al*., 2003) rat models. Defective endometrial proliferation and uterine gene expression has been demonstrated in the wild-type F1 offspring of ghrelin-deficient mice (Martin *et al*., 2011). Any of these disruptions to the F1 reproductive function could potentially generate a suboptimal environment sufficient to perpetuate a programming phenotype to an F2 generation.

The contribution of the pregnancy itself to exacerbating an F1 phenotype is also worthy of consideration. The physiological demands of a pregnancy have been viewed as a 'stress test', unmasking sub-clinical tendencies such as type 2 diabetes and vascular dysfunction in the mother (Verier-Mine, 2010; Bilhartz *et al*., 2011). 'Programmed' animals may fail to respond to the challenges of pregnancy due to decreased physiological reserve, even if the reproductive tract itself is not affected by the developmental intervention. F1 animals with a relatively normal phenotype outside pregnancy may nonetheless adapt poorly to gravidity, and subsequently transmit adverse effects to an F2 generation. This effect is best described in gestational diabetes (Aerts and Van Assche, 2006), where the effects of maternal hyperglycaemia on the offspring developing *in utero* are well understood. Transmission of gestational diabetes via the maternal line in the rat to an F2 generation was one of the earliest-described transgenerational developmental programming phenotypes reported (Gauguier *et al*., 1990). Other systemic aspects of pregnancy physiology, including cardiac output distribution (Ahokas *et al*., 1984) and vascular adaptations (Brawley *et al*., 2004), are also disrupted by maternal dietary interventions in the rat. Preventing the usual maternal adaptations to pregnancy could be a crucial element in determining the early life environment to which subsequent generations are subjected.

Influence of age at breeding in the F1 generation

The debate regarding the relative contribution of the ageing gametes and ageing intrauterine environment is yet to be resolved (Baird *et al*., 2005; Balasch and Gratacos, 2012). The age of the F1 mother at the time of breeding the F2 generation is a plausible confounding factor in interpreting the results of transgenerational breeding studies, yet it is usually neglected in the interpretations and age is often not reported. Of the 42 transgenerational rat and mouse studies included in this review, 30% did not state the age at which the F1 generation was mated. Three of the studies gave a maternal weight range for breeding (230–300 g) (Drake *et al*., 2005; Anderson *et al*., 2006, 2009), but as the developmental programming phenotype often includes accelerated weight gain in the F1 generation, this strategy may not produce age-matched control and experimental groups. The majority of studies that did state the breeding window for the F1 generation within the methods section bred between 2 and 3 months of age (25/40 studies; 16 at 2 months, 9 at 3 months). Only one study reported a breeding age ~3 months, maintaining the F1 generation to 8 months (Blondeau *et al*., 2002). The mean age of breeding from all studies where the information was available was 77 days and 80 days for mouse and rat, respectively. The rationale for breeding younger animals is clear, particularly within the practical confines of long and expensive transgenerational animal studies. Sexual maturity in the female mouse is usually reached by 8 weeks of age (Laboratory, 1976) and in the rat by 8–10 weeks (Gill-Randall *et al*., 2004), and from an economic perspective to maximize breeding capacity, it is clear that most studies will start their breeding programme soon thereafter.

However, if the non-genomic component of the conceptus (mitochondria and other cytoplasmic components) or the maternal reproductive tract environment is a significant contributor to developmental programming phenotypes, then the age of the F1 mother is potentially an important factor. Developmental programming phenotypes may be more pronounced in second-generation (F2) offspring when the additive effects of the F0 intervention and F1 maternal ageing converge.

Conclusions

Even accounting for a possible negative publication bias (only 4 out of 48 reviewed papers failed to find an F2 phenotype), the weight of evidence suggests that developmental programming should be regarded as a transgenerational phenomenon. The evidence for transmission of a phenotype to an F3 or subsequent generation is less secure (nine studies were identified and five failed to show an F3 effect), but has nonetheless been demonstrated and is also identified in toxicology studies. Furthermore, there is no universal agreement on the mechanism by which developmental programming effects can be passed between generations.

The debate as to whether an adverse F1 uterine environment, generated by an F0 developmental programming stimulus, is sufficient to programme an F2 or subsequent phenotype remains undetermined. However, the idea that that the conceptus itself might not be the vehicle of programming represents a distinct shift in principle from the direct transmission of a programming intervention through germline modification. The *de novo* regeneration of the programming phenotype via the maternal reproductive tract with each generation has been referred to elsewhere as a 'vicious cycle' of developmental programming (Gabory *et al*., 2009). However, this description fails to take into account the potential benefits of developmental programming, situations

where future generations adapt better to their environments. We suggest instead the term 'propagational programming' to distinguish those models in which the phenotype is not directly transmitted, but instead 're-programmed' in subsequent generations. This class of model includes not only those where the uterine environment is thought to be the agent of propagation, but also somatic epigenetic re-programming by behavioural (Francis *et al*., 1999; Weaver *et al*., 2004) or other mechanisms. Several authors also present evidence that even where a germline epigenetic modification is found, that this is propagated rather than transmitted directly to subsequent generations (Burdge *et al*., 2011; Radford *et al*., 2012). Defining the relative contribution of propagational mechanisms of developmental programming to subsequent generations is an area that urgently requires further research, with implications for the ideas of developmental programming as a field.

Authors' roles

The review was designed by S.E.O. and C.E.A.; C.E.A. performed the comprehensive literature search. The manuscript was drafted and edited by both S.E.O. and C.E.A.

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