More than genes: the advanced fetal programming hypothesis

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\textbf{ABSTRACT}

Many lines of data, initial epidemiologic studies as well as subsequent extensive experimental studies, indicate that early-life events play a powerful role in influencing later susceptibility to certain chronic diseases. Such events might be over- or undernutrition, exposure to environmental toxins, but also changes in hormones, in particular stress hormones. Typically, those events are triggered by the environmental challenges of the mother. However, recent studies have shown that paternal environmental or nutritional factors affect the phenotype of the offspring as well. The maternal and paternal environmental factors act on the phenotype of the offspring via epigenetic modification of its genome. The advanced fetal programming hypothesis proposes an additional non-environmentally driven mechanism: maternal and also paternal genes may influence the maturating sperm, the oocyte, and later the embryo/fetus, leading to their epigenetic alteration. Thus, the observed phenotype of the offspring may be altered by maternal/paternal genes independent of the fetal genome. Meanwhile, several independent association studies in humans dealing with metabolic and neurological traits also suggest that maternal genes might affect the offspring phenotype independent of the transmission of that particular gene to the offspring. Considering the implications of this hypothesis, some conclusions drawn from transgenic or knockout animal models and based on the causality between a genetic alteration and a phenotype, need to be challenged. Possible implications for the development, diagnostic and therapy of human genetic diseases have to be investigated.

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The ‘fetal origin of disease’ hypothesis proposes that adulthood hypertension, insulin resistance, and dyslipidemia, leading to markedly increased rates of cardiovascular disease and non-insulin-dependent diabetes in adult life, originate through adaptation that the fetus undergoes when the environment (for example: nutrition) in early life is poor, caused by either maternal under-nutrition or placental insufficiency. These functional and structural changes of the newborn develop in likely different time windows, mainly during pregnancy, but also in very early childhood (Barker, 2004). It was proposed that an event occurring during a critical early period of life might permanently alter the organ structure and function in response to environmental factors. Such events may lead to cardiovascular/metabolic and renal diseases in later life.

Maternal under-nutrition or abnormal uteroplacental function reduces nutrient delivery to the fetus and may produce secondary adaptations in metabolism and gene expression that may be beneficial during intrauterine life, but that may contribute to disease risk in later life.

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A key factor in the pathogenesis of the metabolic syndrome in adult life and its consequences (cardiovascular diseases, hypertension and type 2 diabetes) is insulin resistance (Nolan et al., 2011). Thus, it was proposed that adverse events in early life – typically maternal undernutrition – might program insulin resistance, leading to cardiovascular diseases and diabetes in later life. This hypothesis was proven in humans in two independent populations (Li et al., 2011; Pfab et al., 2006) with different genetic backgrounds (Caucasians versus Asians) and different eating behavior (Asian food versus a Western diet). The study carried out in Southern China (Li et al., 2011), furthermore revealed for the first time that the low birth weight phenotype is not just low birth weight, but also disproportional intrauterine growth due to brain sparing. In line with our data is a recent study in an animal model of intrauterine growth retardation (IUGR) showing organ-specific effects on the expression of glucose transporters, facilitating better glucose transport to the growing brain, compared with other tissues. In other words, this animal model showed an organ-specific alteration of insulin resistance as a potential mechanism of brain sparing (Sadiq et al., 1999). Subsequent studies have demonstrated a strong correlation among fetal liver volume, gestational age, and fetal biometric parameters (abdominal circumference and others) (Dos Santos Rizzi et al., 2010; Vintzileos et al., 1985). Therefore, reduced abdominal circumference mainly reflects reduced liver size. An animal model of IUGR by uterine artery ligation was likewise characterized by low birth weight, lower liver weight, and lower liver glycogen storage (Bueno et al., 2010).

Tissue resistance to the effects of insulin is viewed as a fetal response by which in a situation of malnutrition, blood glucose concentrations are maintained, e.g., for the benefit of the brain, but at the expense of glucose transport into the muscles and insulin-mediated growth. This response may cause higher plasma glucose levels even as early as the end of pregnancy in children with low birth weight, and may thus explain the present findings (Li et al., 2011; Pfab et al., 2006).

The typical event causal for fetal programming is maternal undernutrition during pregnancy. This was first recognized in epidemiological studies and later confirmed in animal experiments (Barker et al., 1989; Ravelli et al., 1998; Gluckman et al., 2008; Vehaskari et al., 2000). Meanwhile, several other mechanisms caused by environmental conditions in early life leading to lifelong functional and structural alterations have been described, among them glucocorticoid exposure of the fetus due to 11 beta-hydroxysteroid dehydrogenase 2 deficiency of the placenta (Aufdenblatten et al., 2009; Seckl et al., 2000) or even a high protein diet during pregnancy (Thone-Reineke et al., 2006). Maternal cortisol secretion was shown to be inversely linked to fetal brain growth (Li et al., 2012). This study might therefore explain the finding that maternal stress during pregnancy, as analyzed by classical stress tests, is linked inversely to academic performance of the offspring (Li et al., 2012).

Moreover, recent studies indicate that the maternal immune environment is an etiological factor that can trigger fetal programming of (immune) diseases. An altered maternal immune adaptation to pregnancy can be related to placental insufficiently. Further, frequencies of regulatory T cells in the fetuses are synchronized with maternal frequencies. The maternal immune milieu is clearly involved in setting the fetal immune system in early life (for recent reviews of this interesting topic see: Martino et al., 2014; Hsu and Nanan, 2014).

The effects on the physiology described above are mainly facilitated via influences mediated via the mother. However, recent studies have shown that paternal environmental or nutritional factors affect the phenotype of the offspring as well. Paternal obesity in a rat high fat model (approximately 50–70% of the daily calories in the rat chow came from fat) permanently alters the insulin resistance of female offspring via the epigenetic alteration of beta cell function (Ng et al., 2010). There is already a human study showing that paternal programming exist as well (Chen et al., 2012). This study showed that paternal BMI affects growth of the male, but not the female offspring. Paternal BMI may thus be a risk factor for cardiovascular diseases of male offspring in later life. It remains to be demonstrated whether this is linked to paternal programming of cortisol secretion that is specific to the sex of the offspring. Interestingly, the effects of paternal under-nutrition on fetal programming of the offspring have not been analyzed so far.

Another mechanism responsible for programming events during intrauterine life may be related to maternal genes affecting the fetal phenotype independent of the fetal genome. Several independent association studies in humans suggest that certain maternal genes might affect the fetal phenotype independent of the transmission of that particular gene to the fetus (Hocher et al., 2000, 2001; Masuda et al., 2007; Miodovnik et al., 2012; Wang et al., 2002). The first study to show this, demonstrated an association between the maternal G protein β3 subunit 825 T allele and low birth weight of the offspring (Hocher et al., 2000, 2001). Subsequently, a Japanese study confirmed that the maternal G protein β3 subunit 825 T allele affects fetal growth independent of the transmission of that particular gene (Masuda et al., 2007). Another study demonstrated that the effect of maternal cigarette smoking on the birth weight of the offspring is modulated by a maternal metabolic gene polymorphism (Wang et al., 2002). A more recent study showed maladaptive behaviors in the male offspring of mothers who carried functional polymorphisms in the sex steroid pathway (Miodovnik et al., 2012). A fifth study demonstrated the same principle: the authors aimed to assess the role of the recently described type 2 diabetes gene TCF7L2 in birth weight. They genotyped the polymorphism rs7903146 in 15,709 individuals whose birth weight was available from six studies and in 8344 mothers from three studies. Each fetal copy of the predisposing allele was associated with an 18-g (95% confidence interval [CI] 7–29 g) increase in birth weight (p = 0.001) and each maternal copy with a 30-g (95% CI 15–45 g) increase in offspring birth weight (p = 2.8 × 10−5). Stratification by fetal genotype suggested that the association was driven by maternal genotype (31-g [95% CI
9–48 g] increase per allele; corrected \( p = 0.003 \) (Freathy et al., 2007).

These studies support the advanced fetal programming hypothesis (Fig. 1), proposing that a gene of the mother – and most likely also of the father – in a human may influence the physiology of the offspring without being present in this particular individual. The general concept that the genes of one organism might influence the physiology of another organism came originally from bacterial and viral infections. It was shown that bacteria/viruses may alter their host’s metabolism, e.g., to stimulate nutrient supply to the parasite (Clive et al., 2007). Interaction of one organism with the metabolism of another organism of the same species is seen in mammals mainly during pregnancy, where the placenta serves as an interphase between the two individuals (Clive et al., 2007). This new concept that maternal genes might influence offspring physiology during later life without being present in the offspring is currently based on clinical association studies only (see above). Causal animal experiments addressing this concept and aiming to explore the underlying molecular pathways are lacking so far. To test this hypothesis, we bred female heterozygous eNOS knockout mice with male wild-type mice and compared the physiology of the resulting wild-type offspring with the physiology of mice derived from the breeding of female and male wild-type mice. We have chosen eNOS (endothelial nitric oxide synthase) knockout mice to test this hypothesis, as it plays a pivotal role in placental function (Kakui et al., 2003; Skarzinski et al., 2009). This enzyme synthesizes nitric oxide from L-arginine. Nitric oxide reduces vascular resistance in the placenta, thus improving placental blood flow.

Maternal eNOS may thus influence this interphase between fetus and mother, leading to cardiovascular and/or metabolic effects in later life (Hocher et al., unpublished data). Also, paternal genes may act in a similar way. They may affect the maturation of the sperm without being present in the genome of the offspring. This ‘advanced fetal programming hypothesis’ is illustrated in Fig. 1. This hypothesis has several implications. It breaks with the classical laws of inheritance according to Mendel. A parental gene affects the phenotype in the offspring without being present in the offspring and possibly also in the next (F2) generation. The implications for our understanding of human diseases are potentially huge, but clinical studies are needed to fully understand them.

In modern biomedical science we often use transgenic animal models or rodent models where a certain gene is deleted (knockout mice, for example). We are using this approach because we believe in the causality between a genetic alteration and a phenotype. However, according to the advanced fetal programming hypothesis, the observed phenotype is altered by maternal/paternal genes with them not necessarily also being present in the affected organism. Therefore, many conclusions based on either transgenic animal models or knockout models need to be challenged.

The assumption that these types of experiments prove the causal relationship between a certain gene and the resulting phenotype is likely to be at least partially misleading. The maternal and paternal factors (such as; genes, macro- and micronutrition, exposure to toxins, and stress hormones) act on the phenotype of the next generation, either directly via altering the early life hormonal environment (Miodovnik et al., 2012) or the toxin environment.
(Wang et al., 2002), or via epigenetic modification of the offspring DNA.

The implications of this hypothesis might as well play a role for disease manifestation in human genetic diseases. The phenotype, prognosis, and treatment of human genetic diseases may be affected by parental genes as well – not just by the genes of the index patient. This will make giving patients a genetic diagnosis and proper advice more complex.

So far, three classical epigenetic mechanisms have been described: DNA methylation, histone acetylation, and expression of micro-RNA (Gluckman et al., 2008). These mechanisms affect the transcription rate of certain genes involved in the pathogenesis of cardiovascular diseases. Examples of epigenetically regulated genes involved in the pathogenesis of cardiovascular and metabolic diseases are the genes of the renin-angiotensin system (RAS), the peroxisome proliferator-activated receptors (PPAR) system, or the glucocorticoid receptor (Bogdarna et al., 2007; Jiang et al., 2012).

References


