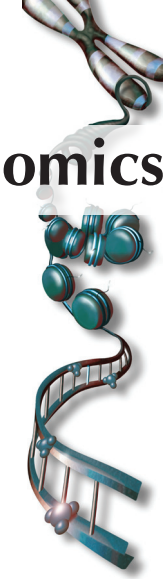
Foreword

Epigenomics



Effects of the *in utero* environment on the epigenome

"Overall, the manuscripts in this issue are aimed at informing both the novice and expert, reiterating the specific issues associated with epigenetic analyses and highlighting the need to carefully consider issues of study design and causality."

First draft submitted: 25 January 2017; Accepted for publication: 27 January 2017; Published online: 23 February 2017

Keywords: epigenetics • offspring • pregnancy • programming

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The role of epigenetics as a mediator of the early life 'programming' of health is becoming increasingly apparent. The proposed causal pathway begins with an exposure of varying dose or duration and requires sensitive tissue(s) and developmental window(s). The effect of the exposure is contingent on each of these factors but is also modulated by underlying genetic variation, which is also most likely in a tissue and time-specific manner.

This issue of Epigenomics provides valuable insights into aspects of epigenetic variation established *in utero*, with a focus on humans. We begin with the first definitive *in utero* exposure to reproducibly modify the developing human epigenome – namely maternal smoking in pregnancy. Rebecca Richmond and Bonnie Joubert, two leaders of the field in this regard, contrast the evidence for maternal smoking as an exposure with one-carbon donor (such as folate) status, essential for the production of methyl donors needed for a suite of epigenetic modifications. They highlight the importance of study design, particularly power to detect methylation signals, exposure assessment, controlling sources of variability, causal inference and the role of observed methylation changes in mediating downstream outcomes in the offspring [1]. There are clear differences in the reproducibility of findings across exposures, partly driven by study design and exposure measure heterogeneity, but also likely reflecting the differences in underlying sensitivity of genes and tissues to specific exposures in specific populations.

Part of the challenge in the field lies in untangling the various factors that together influence both early-life epigenetic profile and later phenotypic outcomes. Accurate exposure estimates are critical in this regard. A pioneer in the field, Stephanie London, and team highlight the value of considering exposure misclassification into epigenetic mediation modeling where possible. The problem of exposure classification in epigenetic analyses is exemplified by studies addressing the role of air pollution on infant epigenetic profile [2]. Burris and Baccarelli also highlight some of these issues, that include poorly defined measures of content and dosage, concluding that additional well-designed studies are required [3].

Kobor and co-workers summarize the evidence linking prenatal alcohol exposure to epigenetic variation in progeny. Importantly, they highlight the range of epigenetic processes potentially influenced by this important exposure, including DNA methylation, histone variants, post-translational modifications and ncRNAs [4]. Despite a large emerging of work, evidence to date remains somewhat fragmented and not as compelling

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as that associated with maternal smoking. The same can be said for the effects of maternal well-being on offspring epigenetic profile and later phenotype. Here, Ryan *et al*. summarize the evidence for a link, concluding that, although there is mounting evidence, further well-powered studies with well-defined exposure information are needed [5]. Shields takes this further, highlighting the importance of coordinated, strategic efforts across studies to empirically evaluate measures of key social and environmental exposures important for increased understanding of disease etiology. The focus on robust harmonized measures of exposures is clear, especially in epigenetic analyses, and the public health implications are elaborated [6].

Time is also an important consideration in epigenetic analyses, not only in terms of defining windows of sensitivity to exposures, but also in shaping the epigenome by a process of 'epigenetic drift.' The DNA methylation cock, first described by Steve Horvath, has been used to test for accelerated epigenetic ageing in a number of tissues and pathological conditions, primarily in adults. This is now being applied to early development. In this issue, Bianco-Miotto and co-workers apply this approach to the placenta and highlight evidence of 'accelerated' ageing in pre-eclampsia pregnancies [7]. This provocative finding extends the range of epigenetic 'curiosities' associated with the placenta and suggests that additional studies in related pathologies are warranted. However, as with all studies in this domain, there is an underlying assumption that cells of different types of the placenta 'age' at the same rate. For the placenta and other tissues, this needs to be determined on an empirical basis in order to rule out a change in cell composition over time as opposed to true intrinsic epigenetic ageing.

The issue of tissue specificity of DNA methylation profile in birth samples is highlighted by De Carli *et al*. who apply various modelling approaches to try and develop a predictor of placental DNA methylation from cord blood methylation datasets [8]. Generally speaking, there is very little correlated methylation, highlighting the importance of choosing a target tissue relevant to exposure/outcome of interest, and also the need for cautious interpretation of findings. Although compelling, it is important that these issues be directly tested across a variety of additional tissues.

Perhaps not surprisingly, sex-specific epigenetic variation established *in utero* is emerging as a potential contributor to the well-established sexual dimorphism observed in response to specific environmental exposures. In this issue, Fry and co-workers confirm the widespread epigenetic differences between placental methylomes of male and female pregnancies. As anticipated, the majority of differences are attributable to the X chromosome, but interestingly several of these regions are not subject to X-chromosome inactivation and, in fact, show higher methylation in males. Furthermore, a small number of these differentially methylated regions are autosomal loci. The associated genes are plausibly linked to placental function and may lead a sex-dependent environment for the developing fetus, with potential longer term implications for health and disease [9].

The ultimate goal of research in early-life epigenetics in the context of DOHaD is to build evidence for each step of the proposed causal pathway, including associating epigenetic variation to postnatal phenotypes. Here, Regine Steergers-Theunissen and team present the first EWAS data related to perimembranous ventricular septal defects in children. They identify differential methylation at specific loci in blood and discuss some of the limitations of their findings according to the criteria laid out by Michels *et al*. [10]. This includes the relevance of the assayed tissue to the specific pathology under investigation, cell heterogeneity, small effect size and lack of functional data [10]. Inherent in any study such as this are uncertainties related to the role of identified methylation variation in the pathogenic process. Circumstantial evidence for a link is often presented, but only additional replication, longitudinal and functional studies in relevant tissue(s), and complementary analyses in animal models can provide confidence for a role in disease. Longitudinal studies commencing very early in life are particularly important in identifying the presumed early-life exposures that trigger the pathway to disease. In this sense, it is important that negative findings from well-designed studies also find a home in the literature to avoid publication bias.

Overall, the manuscripts in this issue are aimed at informing both the novice and expert, reiterating the specific features of epigenetic analyses and highlighting the need to carefully consider issues of study design and causality. Although there are inherent limitations in what can be done in humans, the careful and considered work of experts, such as these, will undoubtedly continue to build the evidence base needed to gain a more complete picture of epigenetics as a mediator of *in utero* exposure effects on offspring health outcomes.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Richmond RC, Joubert BR. Contrasting the effects of intra-uterine smoking and one-carbon micronutrient exposures on offspring DNA methylation. *Epigenomics* 9(3), 351–367 (2017).

2 Valeri L, Reese S, Zhao S *et al.* Misclassified exposure in epigenetic mediation analyses. Does DNA methylation mediate effects of smoking on birthweight? *Epigenomics* 9(3), 253–265 (2017).

3 Burris HH, Baccarelli AA. Air pollution and *in-utero* programming of poor fetal growth. *Epigenomics* 9(3), 217–221 (2017).

4 Lussier AA, Weinberg J, Kobor MS. Epigenetics studies of fetal alcohol spectrum disorder: where are we now? *Epigenomics* 9(3), 291–311 (2017).

5 Ryan J, Mansell T, Fransquet P, Saffery R. Does maternal mental well-being in pregnancy impact the early human epigenome? *Epigenomics* 9(3), 313–332 (2017).

6 Shields AE. Epigenetic signals of how social disadvantage 'gets under the skin': a challenge to the public health community. *Epigenomics* 9(3), 223–229 (2017).

7 Mayne BT, Leemaqz S, Smith A, Breen J, Roberts CT, Bianco-Miotto T. Accelerated placental aging in early onset preeclampsia pregnancies identified by DNA methylation. *Epigenomics* 9(3), 279–289 (2017).

8 De Carli MM, Baccarelli AA, Trevisi L. Epigenome-wide cross-tissue predictive modeling and comparison of cord blood and placental methylation in a birth cohort. *Epigenomics* 9(3), 231–240 (2017).

9 Bommarito PA, Martin E, Fry RC. Effects of prenatal exposure to endocrine disruptors and toxic metals on the fetal epigenome. *Epigenomics* 9(3), 333–350 (2017).

10 Michels KB, Binder AM, Dedeurwaerder S *et al.* Recommendations for the design and analysis of epigenome-wide association studies. *Nat. Methods* 10(10), 949–955 (2013).