Commentary

MOLECULAR METABOLISM

Anti-hypertensive treatment in pregnancy impacts offspring growth and metabolism: Q&A*

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The *DOHaD* (Developmental Origin of Health and Disease) and Transgenerational Epigenetic Inheritance (TEI) concepts describe how the environment, including parental and early-life exposures, induces changes during early development that influence adult health and disease risk. Some of these developmental alterations result from epigenetic changes in the control of gene activities. Once identified, these epigenetic mechanisms will help to explain the rise in incidence and burden of chronic diseases.

In this issue of *Molecular Metabolism*, Oelkrug et al. [1] describe that anti-hypertensive treatment in pregnancy generates long-lasting metabolic phenotypes in offspring. This work belongs to a bigger group of studies aiming to highlight acquired and heritable phenotypes, and to uncover molecular mechanisms for DOHaD and TEI in mammals. The work is an important contribution to the field, as these phenomena are still not completely understood. Notably, the work also presents several important clinical implications, as it questions the safety of anti-hypertensive drugs during pregnancy and suggests that studies addressing pregnancy safety should be extended to monitor intergenerational health.

Using the study of Oelkrug et al. as a starting point, this commentary will discuss three general aspects to consider when developmental programming and/or epigenetic inheritance studies are performed: (1) The Experimental Unit; (2) The choice of proper controls; and (3) The approach to phenotypic and mechanistic workup.

The experimental and statistical unit (EU) is defined as the smallest division of the experimental material such that any two experimental units can receive different treatments [2]. In other words, the single or group of animals assigned as treated or control is to be considered the EU. Therefore, in the case of parental inheritance including *in utero* effects, such as in Oelkrug et al. [1], the parents are the EU. That said, a proper study design is critical to determine the size of the experimental groups needed to identify epigenetic phenotypes, which, in many cases, are subtle or partially penetrant [3]. Oelkrug et al. used 3–4 animals per group (mothers – n = 3–4) and were able to identify multiple significant phenotypes in male offspring (including glucose intolerance and dwarfism). These findings suggest that a general mechanism *in utero* has been driving the observed developmental programming. Interesting – though not surprising – female offspring showed resistance to the reprogramming.

Another critical point in designing and performing developmental programming and/or epigenetic inheritance studies is the choice of proper controls. Especially in the case of maternal inheritance, many confounding variables have to be considered. Mothers can influence offspring health through germline epimodifications [4], *in utero* effects [5], and lactation and maternal care [5]. Many of these can be controlled by *in vitro fertilization* (IVF) (which excludes non-germline dependent effects) and cross-fostering (which excludes post-delivery effects). Given that Oelkrung et al. did not focus on germline mediated inheritance, there was no need to conduct IVF. However, the absence of cross-fostering in the study design does not exclude that lactation and maternal care contribute to the observed phenotypes. Likely excluding lactation effects is the observation that only a very small amount of the active molecules appear in breast milk [6], thus making anti-hypertensive treatment during lactation safe for children. What remains to be controlled is the effect of maternal care. The authors show that treated mothers have increased body temperature towards the end of pregnancy and, as a consequence, increased food intake. Both parameters affect offspring metabolism [7], thus potentially confounding the observed phenotypes. Also, in Oelkrug et al., paternal effects are not completely ruled out, though the exposure time of the fathers is likely too short to have a substantial impact.

The third and final point is more philosophical and deals with the need of changing our mindset when approaching phenotypic and molecular analyses, especially those coming from studies of acquired inheritance. The mechanistic knowledge we have on acquired inheritance does not allow us to predict offspring phenotypes from parental exposures. Also, one epimodification can give rise to several disparate offspring phenotypes [8], and some extreme environmental exposures (e.g., overt obesity or severe growth retardation) induce similar offspring phenotypes ("U-shape responses"). That said, the ideal would be to approach offspring phenotyping by letting animals go through a comprehensive phenotyping pipeline and be assayed for developmental and adult traits. The same applies to mechanistic

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dissections. Focused phenotypic analysis may highlight only a subset of the existing phenotypes (and maybe not the strongest). Restricting the mechanistic workup, with established and accessible technologies, may shed light on one single aspect and miss or misinterpret the overall picture.

The study from Oelkrug et al. provides an example of comprehensive phenotyping. Prazosin treatment has not been reported to worsen metabolic homeostasis in rodents [9], yet Oelkrug et al. found that offspring of prazosin treated mothers are dwarfs, glucose intolerant, and insulin resistant. This could not have been possible without a comprehensive metabolic phenotyping, which — though hypothesis driven — included tests for circadian behavior, glucose homeostasis and thermoregulation.

The same is not true for the mechanistic workup the authors have presented to explain the observed phenotypes. Stemming from the dwarfism (not the strongest observed phenotype), the authors analyzed molecular contributors to body size and found that the GH/IGF1 axis was altered at the level of the *Ghr*, the expression of which was significantly reduced upon maternal prazosin treatment. As a molecular explanation to this, Oelkrug et al. analyzed DNA methylation levels of selected CpG sites within regulatory elements of the IGF1 and Ghr genes, and claimed that a mild difference (maybe 5% — not really appreciable from the figure) in the DNA methylation level of a single CpG site (constitutively hypermethylated — >80% basal methylation level) explains the difference in gene expression and the downstream phenotypes. Despite the fact that it remains questionable whether differential methylation of a single CpG site can influence transcription [10], these data are purely correlative and need further investigation. *How many more avenues might have been opened by an unbiased approach?*

To conclude, Oelkrug et al. [1] present a new model of acquired inheritance and highlight that toxicology has to keep an open eye on drug effects within and across generations. Though presenting interesting and far reaching findings, some aspects of the study need further investigation.

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