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Novel insights, challenges and practical implications of DOHAD-omics research

Abstract

- Research investigating the developmental origins of health and disease (DOHAD) has never before had the technology to investigate physiology in such a data-rich capacity at such a micro-level.
- A symposium presented at the inaugural meeting of the DOHAD Society of Australia and New Zealand outlined the advantages and challenges of using "-omics" technologies in DOHAD research.
- DOHAD studies utilising "-omics" approaches to generate large, rich datasets were highlighted.
- Implications for policy and practise are discussed, and recommendations made to facilitate successful translation of results of future DOHAD-omics studies.

Studies exploring the developmental origins of health and disease (DOHAD) investigate how early life exposures increase susceptibility to later adverse health outcomes, both from a medical and public health perspective. This altered health risk appears to occur through reprogramming of physiological systems away from their normal developmental trajectories, highlighting the plasticity of organ systems in the perinatal periods (1). Recent research in this field has focused on the potential for these physiological changes to exert trans-generational effects, without the requirement for further exposures in subsequent generations (2). This appears to occur through genetic and environmental interactions, resulting in phenotypic changes that persist across generations.

The emergence of "-omics" biotechnologies has revolutionised physiological research in the DOHAD field. From the genome, to the epigenome, microbiome and metabolome – research investigating pathways leading to disease has never before had the technology to investigate physiology in such a high throughput, data-rich capacity. This commentary summarises this emerging research capability and its application in DOHAD studies to explain how environmental and social factors impact our physiology and become inherited, leaving a legacy of disease susceptibility for future generations. The current challenges of employing such technologies will be discussed, suggestions made to facilitate successful application of these platforms in future studies and to inform policy and practise.

The Epigenome

The epigenome refers to the changes made to the genome which result in altered transcriptional activity in the absence of DNA sequence alterations. This is a highly dynamic process, occurring in response to a number of external factors, with the changes stably maintained and enduring over multiple generations. Epigenetic mechanisms regulating gene expression include DNA methylation, histone modifications and the actions of small non-coding RNAs, each contributing to tissue specific gene expression and altered cellular phenotype. The introduction of high throughput sequencing and microarray techniques has facilitated the study of these epigenetic mechanisms in the DOHAD field.

The interaction between epigenetic inheritance and environmental exposures (for example diet, stress, drug exposures) has been recognised as an important determinant of phenotypic outcomes for their offspring (1). Exposures of the mother (F0 generation) can result in epigenetic modifications in both the developing fetus (F1) and the germline (F2) (3). Such transmission is not restricted to maternal exposures, with recent evidence that epigenetic modifications are also inheritable down the paternal line (4). Specifically, a murine model of paternal obesity has demonstrated altered methylation and microRNA profiles (4), highlighting the role of the father's contribution to inheritable disease susceptibility. While evidence supporting trans-generational epigenetic inheritance has primarily been derived from rodent studies, large multi-generational epidemiological studies support this transmission pattern. Data from the Överkalix Swedish tri-generation population study has demonstrated that the mortality risk ratio of grandchildren was associated with the food supply available to their same sex paternal grandparent (5). Whether an epigenetic mode of inheritance can contribute to such human outcomes is as yet unknown, but is expected given the strong parallels observed between the animal and human trans-generational studies. Further, changes in DNA methylation of genes well known to be involved in regulating fetal growth and development, such as the NR3C1 gene which encodes the glucocorticoid receptor, have already been demonstrated in humans following early life adverse exposures. For example, maternal depression in the third trimester of pregnancy is associated with increased methylation of the NR3C1 gene in cord

blood mononuclear cells, in conjunction with altered stress responses in the infants at three months of age (6). Increased methylation of this same gene is found in brain tissue of adolescents with a history of child abuse who later committed suicide (7), and in lymphocytes of 11-21 year olds following childhood maltreatment, and is associated with poor psychological health (8). Together, these studies provide evidence of the role of early epigenetic modifications in increasing vulnerability to poor long term health in humans.

The Microbiome

The human microbiome is the collection of micro-organisms that inhabit the human body, including commensal and symbiotic microbes. The study of the microbiome and its role in disease onset has been made possible by the introduction of large-scale sequencing techniques and gene expression arrays. These techniques have increased our ability to understand the contribution of the maternal microbiome to disease in subsequent generations. For example, altered bacterial colonisation of the alimentary tract of piglets following antibiotic and stress exposure in early life has been associated with immune development perturbations (9). This may have particular implications for preterm children, where both of these exposures are common in early life. Already, preliminary studies of the microbiome in preterm twins have demonstrated that an altered pattern of microbial gut colonisation precedes the development of necrotising enterocolitis (10). The potential for other common prenatal exposures to influence the maternal and neonatal microbiomes, including obesity (11) and smoking (12), as well as different modes of delivery (13), have also started to receive research attention in the DOHAD field. Further, the potential for an altered microbiome to contribute to epigenetic changes has been identified (reviewed in, 14).

The Metabolome

The metabolome is the complete set of metabolites, or low molecular mass compounds, found in biological samples that regulate cell and tissue growth, development, survival, maintenance and responses to the environment. As the downstream product of gene expression and transcriptional activity, the potential for metabolomic profiling to provide a phenotypic signature of pathophysiology has been recognised (15). Methods used to assess the metabolome rely on high resolution analytics including mass spectrometry (coupled with compound separation processes), nuclear magnetic resonance spectrometry and Fourier transform infrared spectroscopy. Unlike both the epigenome and the microbiome, the metabolome can be highly dynamic, able to change in short time frames, ranging from seconds to minutes. The choice of sampling material is therefore an important consideration (e.g. blood samples reflect highly dynamic responses while hair samples reflect prolonged exposure and are therefore a more stable phenotype (16)), and will be specific to the research question and critical to the interpretation of results. The large volume of data generated by such techniques can provide insight into interactions between metabolites, genes, transcripts and proteins (17). As such, these data can be highly informative of mechanisms leading to disease and understanding the impact of environmental exposures on system physiology. The potential for metabolomics platforms to be used to identify biomarkers predicting pregnancy outcome is already becoming apparent. These include observations of differences in the neonatal blood metabolome across gestational age which are dependent on postnatal age at sampling (18), specific pathology and illness severity (19), studies linking the maternal hair metabolome with fetal growth restriction (16) and the ongoing prospective study for the early prediction of pre-eclampsia (20, Trial Number NCT01891240).

Challenges to "–omics" approaches in DOHAD research

Utilisation of these emerging platforms in DOHAD research shows clear promise in expanding our current knowledge of the mechanisms driving the intergenerational transmission of disease and heightening disease susceptibility in individuals following specific exposures in early development. While the provision of such large volumes of biological data using these "–omics" approaches provides enormous opportunity, a number of challenges remain in their application and interpretation. The first clear challenge relates to identifying the appropriate time for tissue sampling, given the current limited use of these approaches in this field. Furthermore, "healthy" ranges are yet to be established. This limitation exists with any advance in technology and will be overcome through public sharing of "-omics" data. In order to establish these ranges, sampling from multiple time points and multiple tissues will be necessary. This information will benefit the design of future studies where sampling can then occur at single time point during tissue-specific sensitive periods to yield the most meaningful (reliable, valid and interpretable) data. The development of "normative" ranges will also help address many of the other current unknowns in this area. These include understanding what sample size is needed to identify meaningful effects; understanding and predicting the stability of an "–omics" profile; identifying how a "second-hit" or multiple exposures impact; understanding whether the duration or timing of each exposure important in determining outcome; and understanding whether a genetic susceptibility is needed for the intergenerational transmission of poor outcomes or whether this is a highly conserved process. These questions are of fundamental importance in the design of DOHAD studies, to ensure they are conducted in a cost- and time-effective manner.

Once we have identified biomarkers or signatures predictive of poor maternal, fetal or neonatal outcomes, the next critical step is to use this information to identify how to normalise these effects. This will necessitate an understanding of how postnatal factors influence (normalise or exacerbate) the "–omics" profile induced by the early life environment. Longitudinal studies of twins have provided some preliminary evidence of environmental influences, exploring the stability of the epigenome across the first 18 months of life and the degree of epigenetic discordance between siblings with a shared genetic and environmental background (21). Continued longitudinal assessments of these children will increase our understanding of the role of the environment on the epigenome across the life course. Further, the impact of additional exposures in pregnancies of these subsequent generations has yet to be identified. As few studies have assessed the potential for "–omics" profiles to be modified beyond the F2 generation (2), there is a clear lack of data informing this latter challenge.

Recommendations for future studies

The emerging evidence shows clear promise in expanding our understanding of the developmental origins of health and disease. Collaborative studies are highly recommended, integrating data derived from multiple "–omics" platforms, collected from samples across early development and linked to clinical health outcomes. Analysis of samples derived from current and planned randomised controlled trials will allow the impact of standard care and interventions to be assessed concurrently. Together these studies will facilitate our understanding of disease susceptibility, onset and progression to a degree that has not previously been possible. This is an exciting time for biological research in the DOHAD field – offering the potential to understand complex biological interactions between systems that contribute to health outcomes across generations.

Implications for Policy and Practice

The basic tenet of DOHAD research is that perinatal health behaviours of both the mother and father, as well as those of the child in early life, can significantly impact on the future health of the child and that of subsequent generations. This implies that effective interventions applied at critical periods of development can substantially reduce future disease burden. The potential for this research to be translated into tangible health benefits for child health and future generations is therefore enormous, aligning perfectly with the growing demands of national health regulatory bodies to focus efforts on preventative health care. The integration of –omics technologies into DOHAD research will increase our understanding of biological mechanisms underlying the transgenerational transmission of disease, and how interventions normalise these effects. The outcomes of this research can then be used by health advocates to inform policy and practice, by clinicians and health workers to promote and support healthy perinatal behaviours, and be communicated to the wider community to optimise future child health.

While information on DOHaD and early life healthy behaviours is becoming more readily available, it is unclear whether this is being effectively communicated to the health care providers that need it most, i.e. those in direct contact with women who are pregnant or planning a pregnancy. For example, surveys of general practitioners reveal a limited knowledge of nutritional requirements for pregnancy, with many feeling uncomfortable providing this information due to lack of confidence (22). Knowledge gaps such as this must be urgently addressed to optimise the health of future populations. Similarly, while the internet is teeming with websites offering advice for pregnant and breastfeeding women, these often contain inaccurate or misleading advice and conflicting information. Evidenced-based, on-line resources to which women can be directed for accurate health information are needed. Overcoming these, along with other challenges in translating the concepts of DOHaD effectively into policy and practice, forms the core agenda of the newly formed Australia and New Zealand DOHaD Society.

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