

Endocrine Disruptors: Do Family Lines Carry an Epigenetic Record of Previous Generations' Exposures?

Endocrine disruptors are generally defined as chemicals in the environment that interfere with hormone signaling to such an extent that they cause adverse effects (*e.g.* Ref. 1). The possibility that the human population is incidentally exposed to environmental chemicals that interfere with hormone signaling during development is particularly concerning because of the importance of hormones in development and the potentially permanent affects for the individual. For example, fetal exposure to synthetic estrogens (*e.g.* diethylstilbestrol) can result in a higher incidence of reproductive tract cancers in adulthood (2). Recently, a new dimension to this issue has emerged—that the consequences of developmental exposure to an endocrine disruptor can be passed from one generation to the next with a high degree of fidelity (3). Two new papers in *Endocrinology* further develop this dimension and now show that the adverse affects of developmental exposure to a single endocrine disruptor extends to several organs and tissues, not just reproduction—all of which are transmitted through at least four generations (4)—and that this exposure is associated with altered methylation states of specific gene regulatory regions (5), providing a potential mechanism for the epigenetic transmission of the effects of endocrine disruptor exposure.

Michael Skinner and his research group at Washington State University reported earlier (2005) in *Science* (3) that the developmental exposure to the fungicide vinclozolin affects male rat fertility that is transmitted through four generations without further exposure to vinclozolin. Moreover, this report shows that DNA methylation patterns differ between the vinclozolin exposed and unexposed animals. Thus, the two new reports are a natural extension of this earlier work. Surprisingly, Anway and Skinner (4) now describe a number of disease states in the adult offspring of pregnant rats treated with vinclozolin on embryonic d 8–14. At 14 months of age, the vinclozolin-treated group exhibited a significant increase in abnormalities of the prostate, kidney, testis, and immune system, compared with the untreated control animals. These effects included various kinds of tumors (12–33%), prostatic lesions (45–55%), renal lesions (20–50%), and an increase in the incidence of infections (12–33%). These effects, at similar proportions, were observed in the F2-F4 generations as well. In addition, using selective breeding strategies, the authors show that disease prevalence is transmitted to the next generation through the male germ line. Thus, vinclozolin exposure to pregnant female rats can produce a wide range of effects on her offspring as well as that of four generations of her lineage.

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An important emerging literature in humans is based on the observation that birth weight is inversely associated with a cluster of metabolic disorders now identified as the metabolic syndrome (6). These disorders include obesity, hypertension, hyperlipidemia, and type 2 diabetes (7). Moreover, these maladies are transmitted transgenerationally (8). The mechanism(s) by which this syndrome is passed on to future generations is currently debated. The thrifty phenotype hypothesis proposed by Hales and Barker (7) essentially posits that poor fetal and infant nutrition initiates a cascade of events resulting in the metabolic syndrome in the adult offspring because the developmental responses to poor intrauterine nutrition alters the metabolic response to energy intake in the adult. This concept, in turn, requires that intergenerational transmission of the thrifty phenotype be mediated by epigenetic mechanisms. In contrast, the thrifty genotype hypothesis posits that the link between events resulting in lower birth weight and the increased risk of cardiovascular disease is primarily genetic (9, 10). This interpretation has led to the concept that genes linking poor maternal and neonatal nutrition to cardiovascular disease confer a selective advantage on the organism. Thus, the adaptive physiological responses of the fetus and neonate to malnutrition ensured survival and reproduction in an environment with low energy intake. However, in the presence of high energy intake, the metabolic syndrome is manifested.

Although these literatures do not directly overlap, it is important to recognize that there is empirical evidence of the intergenerational transmission of effects of the fetal environment in humans. Thus, the second contribution by Chang *et al.* (5) showing that vinclozolin exposure produces changes in the methylation pattern of 15 different genes (DNA regulatory regions) may provide important insight into the mechanism by which this intergenerational mechanism operates in both humans and in animals. The authors provide detailed evidence of specific patterns of methylation that are unique to vinclozolin-exposed lineages, and that some of the mRNAs regulated by these promoter/DNA sequences are expressed at significantly lower levels in the vinclozolin-exposed generations. Thus, vinclozolin exposure to the mother can affect the abundance of specific mRNAs in her offspring, and this abnormal expression pattern is transmitted through the next three generations.

It is currently unclear whether the effects of vinclozolin are mediated by effects on hormonal signaling during development. Vinclozolin is characterized as an antiandrogen (11), but vinclozolin and some of its metabolites also bind to the progestin, glucocorticoid, and mineralocorticoid receptors (12). Thus, vinclozolin may well interfere with endocrine signaling during development; if so, it will be important to identify the normal role of these hormonal signaling pathways on development, and whether these pathways might be

affected by other kinds of intrauterine environmental stressors. Moreover, it will be important to determine whether vinclozolin exposure in humans, either alone or in association with other compounds with similar modes of action, can produce effects on human health. Humans come into contact with vinclozolin because of its use as a fungicide on specific agricultural crops. Whereas the doses of vinclozolin used in the current work are not likely to be environmentally relevant, these mechanistic studies now published in *Endocrinology* will likely remain central to this important new dimension—that of the transgenerational effects of developmental exposure to endocrine disruptors. Moreover, considering the evidence that the effect of DES on reproductive cancers can be passed from one generation to the next (13), epidemiological studies should begin to incorporate this into their designs.

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