Looking for Proof in the Wrong Generation?

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ABSTRACT

The article in this issue "Grandmaternal Perinatal Serum DDT in Relation to Granddaughter Early Menarche and Adult Obesity: Three Generations in the Child Health and Development Studies Cohort," by Cirillo and colleagues, is the first to report multigenerational health effects in granddaughters stemming from early life exposures to the pesticide o,p'-DDT in grandmothers. Health effects associated with F_0 environmental chemical exposures in multiple generations have been reported in rodent studies, but not in humans. The striking finding in this body of work by Cohn and her colleagues is that the granddaughters were never directly exposed to o,p'-DDT—only their grandmothers were, potentially when they were adolescents. The increased rise of obesity and early menarche due to o,p'-DDT exposures generations earlier may help explain why it has been so difficult to describe environmental contributors of disease. Have we been looking for exposures in the wrong generation?

See related article by Cirillo et al., p. 1480

It took one generation to discover the later life impacts of diethylstilbestrol (DES) on the developing fetus. DES, a synthetic estrogenic drug prescribed to pregnant women beginning in the late 1940s, was taken off the market in 1971 due to a strong association with clear-cell adenocarcinoma of the vagina and cervix in young adult daughters exposed in their mother's womb. Since then, numerous impacts of DES have been identified in both mouse models and epidemiologic studies of the children (1) and grandchildren, including menstrual aberrations (2).

If the initial DES finding had not been so strong and such a rare outcome in young women, would the intergenerational effect ever have been identified? The article in this issue "Grandmaternal Perinatal Serum DDT in Relation to Granddaughter Early Menarche and Adult Obesity: Three Generations in the Child Health and Development Studies Cohort" by Cirillo and colleagues leverages a unique resource to confirm for the first time in humans, the lasting impacts of the environmental chemical o,p'-DDT on obesity and pubertal milestones, health effects that are not so rare in young women today (3).

Over the last two decades there has been a steady and troubling rise in obesity, with 18.9% of 2- to 19-year-old U.S. children now considered obese (4). Some suggest that energy balance (sedentary lifestyle, video games, and poor diet) choices and basic genetics (obese parents have obese children) are to blame. While it is likely that energy balance choices are major factors, growing evidence in animal models report that environmental chemicals may contribute to elevated obesity incidence (5). Animal models have also shown multi-generational effects from a single chemical exposure, introducing the complex association between environmental exposures and additional factors that individuals cannot control (for example, DNA methylation status, histone modification, changes in expression of critical genes).

The first reports of a single chemical exposure leading to multigenerational health effects in rodent models was in 2005, when both the fungicide vinclozolin and the pesticide methoxychlor promoted undesirable male rat reproductive system outcomes over three generations (6). Since then, several other endocrine disruptors have been reported to adversely modify health endpoints in at least two generations (sometimes more), in addition to the exposed pregnant mothers. Bisphenol A, dibutyl- and diethylhexyl phthalates (7), methoxychlor (8), a hydrocarbon mixture (referred to as, jet fuel JP-8) (9), and DDT (10) have all been reported to cause intergenerational obesity in the rat model, in addition to some other health effects. More recently, tributyltin (TBT) was reported to induce obesity in F1-F3 offspring when pregnant mice were exposed to human-relevant (nmol/L) doses of TBT in drinking water (11, 12). These authors also exposed pregnant mice to the drug rosiglitazone, which activates PPARγ and causes lipid production in cultured fat cell systems and directly exposed animals, to demonstrate that typical adipogenic pathways were not the targets for multigenerational effects; rosiglitazone was without effect in multiple generations of offspring. These studies, together, highlighted potential mechanisms such as changes in chromatin organization, differentially methylated regions of DNA (sperm epimutation), overexpression of leptin and important metabolic genes in white adipose tissue, and that effects may be transmitted via the male or female germline. These mechanisms are difficult to delineate in humans.

Multigenerational epidemiology studies are valuable tools for discovery of intergenerational effects of chemical exposures – in the Child Health & Development Studies of o,p'-DDT, the outcomes were seen in granddaughters whose mother's eggs were exposed while they were in the grandmother's womb. Indirect exposures may also impact additional future generations through mutliple mechanisms, but those transgenerational impacts would need to be examined in the great grandchildren of women exposed during pregnancy (**Fig. 1**).

Translational research between animal model toxicology and observational human epidemiology studies must be leveraged to identify more subtle environmental risks sooner to protect future generations. Examples of epidemiology cohorts that could be considered for future research on third-generation effects of environmental exposures are listed in **Table 1** – some of which are planning now for those future phases. For example, the current phase of ELEMENT, the E3Gen, was launched in February 2019 to capture the third generation of participants (13).

Ideally human cohorts will need to be large as each generation becomes harder to contact and re-enroll. Persistent or chronic

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Table 1. Examples of human cohorts that could be leveraged to look for multigenerational effects of chemical exposures.

Study name	Type	Exposure years	Size	Country	Key publication
US National Cancer Institute (NCI) DES Combined Cohort Follow-up Study	Children of women prescribed DES during pregnancy	1940–1971	5,707	US	15
Child Health and Development Studies	Birth cohort	1959–1967	15,000	US	16
Michigan PBB Registry	Population exposed to polybrominated biphenyl	1973	6,000	US	17
Seveso Women's Health Study	Population exposed to dioxin	1976	981	Italy	18
Avon Longitudinal Study of Parents and Children (ALSPAC)	Birth cohort	1991–1992	14,000	England	19
Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT)	Birth cohort	1994–2005	850	Mexico	13
Danish National Birth Cohort	Birth cohort	1996–2002	100,000	Denmark	20
Norwegian Mother, Father and Child Cohort Study (MoBa)	Birth cohort	1998–2008	90,000	Norway	21
Generation R	Birth cohort	2002–2006	9,778	Netherlands	22

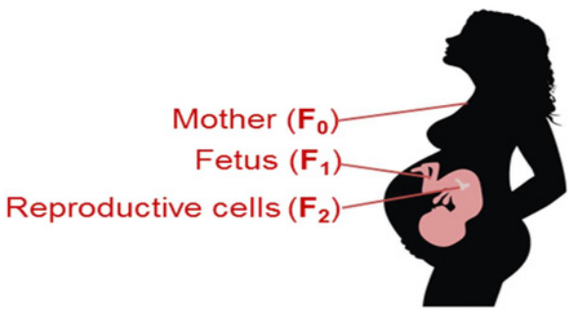

Figure 1.
Multigenerational inheritance of health effects following a gestational exposure. This differs from transgenerational exposure in that one more generation would be needed for evaluation; that generation would have had no exposure. Modified with permission from Walker and colleagues (23).

environmental exposures, such as DDT, brominated flame retardants, legacy perfluorinated chemicals, and dioxins/PCBs will be easier to evaluate than transient exposures or chemicals with short half-lives, such as many chemicals in personal care products. Many of the current birth cohorts were not explicitly designed to evaluate environmental exposures, so prospective collection of biological samples or questionnaires may not be ideal. In the absence of biological samples, some exposures may be able to be reconstructed (e.g., air pollution, water biomonitoring). Better tracking of participants through electronic medical records, particularly in countries with large integrated health care systems, and *a priori* designs to detect multigenerational health effects (collecting appropriate samples and data over time) may facilitate research on future generations.

In the work by Cirillo and colleagues, there are important complexities evident that might only have been discovered in human studies – the risk of obesity in granddaughters was dependent on the obesity status (body mass index, BMI) of the grandmother, the adult BMI of the F1 mother, being African-American, and the o,p'-DDT level measured in the grandmother during her pregnancy. Studies in rodents are rarely conducted in obese vs. normal weight groups. This report also contributes important data to help develop ideas on what may be contributing to the "early puberty" epidemic that is happening globally (14). Is it possible that the increased environmental burden that our grandparents experienced, has affected not only their own health, but also that of generations to come? Importantly, this three generation study had retained enough participants over its many years of existence to evaluate effects by race – African-American granddaughters were more affected by their grandmothers' o,p'-DDT exposures than other races. This is yet another aspect of environmental exposures that is rarely evaluated in toxicology studies.

This work by Cirillo and colleagues should make all of us pause to ask if exposures to persistent chemicals such as o,p'-DDT generations earlier may help explain why it has been so difficult to describe environmental contributors of disease such as the increased global rise in obesity prevalence and reports of early menarche – have we been looking for exposures in the wrong generation? In considering existing studies that may be able to add a third generation, "perfect" cannot be the enemy of good. It is likely that no study will be perfect – some may have exposure assessments that are not to modern standards or sample sizes that may be regarded as small – but if the study findings are consistent with controlled studies in animal models, they may provide sufficient translational evidence to draw conclusions on public health risk.

Authors' Disclosures

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