Bugs in the program: can pregnancy drugs and smoking disturb molecular reprogramming of the fetal germline, increasing heritable risk for autism and neurodevelopmental disorders?

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Abstract

In a seeming paradox, the prevalence of autism spectrum disorder (ASD) has surged, while at the same time research has pointed to the strong heritability of this neurodevelopmental pathology. Here an autism research philanthropist suggests a biological phenomenon of exogenously induced ‘gamete disruption’ that could reconcile these seemingly contradictory observations. Mining information from her own family history and that of her fellow autism parents, while also engaging with the scientific community, she proposes that a subset of the autisms may be rooted in a variety of molecular glitches in parental gametes induced by certain acute exposures during the parents’ own fetal or neonatal development. These exposures include but are not limited to synthetic hormone drugs, tobacco, and general anesthesia. Consistent with this hypothesis, animal models have demonstrated adverse neurobehavioral outcomes in grandoffspring of gestating dams exposed to hormone-disrupting compounds, tobacco components, and general anesthesia. A recent epidemiological study showed a link between grandmaternal smoking and risk for ASD in grandoffspring through the maternal line. Given the urgency of the autism crisis, combined with the biological plausibility of this mostly unexplored paradigm, the writer contends that questions of nongenetic inheritance should be a priority in autism research.

Key words: autism spectrum disorder; epigenetic inheritance; germ cells; nongenetic inheritance; germline reprogramming; etiology of autism

Introduction

It has been said progress in science depends on asking the right questions. As a parent and research philanthropist, I am concerned that when it comes to causation of autism spectrum disorder (ASD) and related neurodevelopmental impairments, the questions we have been asking are incomplete. While the current paradigm tends to equate heritability to genetics [1] and limit environmental exposures of concern to the post-conception somatic [2], we have barely begun to probe the rather large territory where these realms intersect, that is where germline genes meet the environment. While perhaps lacking clear delineation as a scientific discipline, these phenomena sometimes are labeled nongenetic inheritance, epigenetic
inheritance, germ cell disruption, or germline exposures. And for the past several years I have been asking questions about them.

I did not become this inquiring mind by choice. I had been a lawyer and a real-estate investor when a remarkable series of events shifted my attention to the idea that certain toxicants could disrupt the molecular integrity of the vulnerable early germ cell, with implications for developmental integrity of offspring. What follows is an unexpected case study involving my own family, and many others, and how these stories have led me to fund and push for research into potential heritable etiologies of autism and abnormal neurodevelopment beyond the confines of conventional genetics and environmental epidemiology.

My husband and I have three children, born in 1997, 1999, and 2006. With each child there was no reason to worry. Our ancestries reflected nothing like autism or other serious mental or physical pathology, and my conceptions, pregnancies, and deliveries were low-risk and normal. My children were born robust and thriving, on their due dates, and without dysmorphology or birth defect. There is one caveat to that: at his 12-month check-up, my younger son was found to have an unusually large head circumference. His alarmed pediatrician ordered a CT scan, which came back normal.

Then, mysteriously, in their toddler years it became clear that the latter two of our children (pictured in Fig. 1) were affected by severe, nonverbal forms of autism. They are not just disabled, but extravagantly, catastrophically mentally disabled. They cannot read, write, or talk. They cannot say their own names. Though beautiful and possessed of striking athleticism, they cannot imitate even basic movements or gestures, beyond one or two signs from American Sign Language (ASL). They cannot dress themselves, engage in a simple conversation, or play with any toy. With few functional abilities, their lives are extremely limited and they will require 24-hour, 7-day-a-week care for the rest of their lives, imposing staggering costs not just on our family but on the society at large.

Clinicians and researchers could not provide an explanation for my children’s disabilities. Clearly their autism seemed to be genetic, given that I had not one but two children with such extreme impairments. But our family histories and genetic testing of the children, which included some exome sequencing and chromosomal microarrays, offered not a single clue.

A Mysterious Explosion of Autism

The calamitous pattern seen in my family has become increasingly common over the past three decades as autism rates have mysteriously soared. For example, California, where we live, 30 years ago counted roughly 3900 cases of autism deemed sufficiently severe to be eligible for state developmental disability services [3]. That number has soared beyond 100,000 today [4]. Based on a sampling from communities throughout the United States, approximately one in 68 children is now identified with ASD, according to estimates from Centers for Disease Control and Prevention (CDC)’s Autism and Developmental Disabilities Monitoring (ADDMD) Network [5]. The costs of autism are enormous and rising. A 2015 study from UC Davis estimated the US economic burden of autism to reach nearly $500 billion, potentially $1 trillion, by 2025 [6].

While autism prevalence has markedly increased, in seemingly contradictory fashion, research has also shown autism to be highly heritable. Although no evidence suggests that autism is inherited ancestrally except in rare cases, the heritability among siblings is high. Studies have shown a sharply increased risk of autism if an older sibling has the condition [7]. Pooled data from collaborating sites in the Baby Siblings Research Consortium (BSRC) show that the familial recurrence of autism was 18.7% in a cohort of 664 high-risk siblings [8] and 19.5% in an expanded cohort of 1241 high-risk siblings [9].

As someone active in the autism community, I meet many parents like myself—with autism or related pathologies in two, or even three, of our children, but none of these conditions up our family trees. How could such a heritable condition increase so rapidly in prevalence? We are told that genes cannot evolve so quickly over such a short period of time.

Long ago I had given up on the idea that I would ever understand what led to my children’s disorders. But then between 2010 and 2013, almost out of the blue, I came into possession of three sets of documents that revealed a hidden history I had known nothing about.

A Surprising Discovery

In 2010 I obtained a few pages of my mother’s obstetric medical records from the time she was pregnant with me in Los Angeles.
in 1965. At age 45, it had never occurred to me that I had been prenatally exposed to anything in utero, as my mother did not smoke or drink or have any serious medical conditions. Indeed I remember in law school learning about the catastrophe of the synthetic estrogen ‘anti-miscarriage’ drug diethylstilbestrol (DES), and thinking, ‘Thank goodness I was never exposed to anything horrible like that’.

That fantasy ended when those old medical records revealed that my mother had been administered heavy and continuous doses of different synthetic steroid hormone drugs, including synthetic corticosteroids, progestins, and to a lesser degree, estrogens (not DES, however). More details were revealed in 2011 when I came across a 1977 study called ‘Prenatal Exposure to Synthetic Estrogens and Progestins: Effects on Human Development’, by June Reinisch, PhD. This landmark study described for the first time how fetal exposure to synthetic steroids can alter the personalities of the exposed children [10]. As I scanned the abstract on Google Scholar, my jaw dropped when I realized that, as fate would have it, I had been one of the 71 exposed study subjects. In a full-color flashback I finally realized why researchers had delivered all sorts of psychological tests to the 8-year-old me in my Beverly Hills childhood home.

In 2013, I received copies of papers generated by Dr Reinisch and her research team documenting in detail my prenatal drug exposures. These papers had been kept on file all these decades at the Kinsey Institute in Indiana, where Dr Reinisch had served as director. It felt like nothing short of a miracle. Almost no one of my era had any access to their own prenatal medical records, but now I had a goldmine, down to the last milligram of every drug my mother had been given. The papers indicated the drugs included Prednisolone from one month pre-conception through the first trimester, Deladroxate (a mix of the progestin dihydroxyprogesterone acetophenide combined with estradiol enanthate) from month 1 through 2, and Deluteval (a mix of the progestin 17α-hydroxyprogesterone caproate (17-GHPC) and estradiol valerate) from months 3 through 7.

Why had my mother been administered such an aggressive course of synthetic steroid treatment? Early fertility clinics and many obstetricians were motivated by the idea that a superabundance of novel, powerful sex steroids and corticosteroids could help prevent miscarriage in pregnancies considered to be at risk [11–13]. My mother had been considered at risk after experiencing two miscarriages, a history that now I had a goldmine, down to the last milligram of every drug my mother had been given. The papers indicated the drugs included Prednisolone from one month pre-conception through the first trimester, Deladroxate (a mix of the progestin dihydroxyprogesterone acetophenide combined with estradiol enanthate) from month 1 through 2, and Deluteval (a mix of the progestin 17α-hydroxyprogesterone caproate (17-GHPC) and estradiol valerate) from months 3 through 7.

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Other Family Stories

As I approached researchers for feedback about this idea, I also asked some autism friends if they happened to know of family exposures similar to mine. I quickly discovered that several friends with children with idiopathic autism or other neurodevelopmental disorders had also been subjected to ‘anti-miscarriage’ treatments when they were in utero. For example, I met a mother living near me in Northern California with three children with idiopathic autism, and who, like my husband and me, had no history of autism anywhere in her or her husband’s ancestry. It turned out that she too had been prenatally exposed to ‘anti-miscarriage’ treatment, and in fact had been born in the same Los Angeles hospital as me in 1965. Her brother was also prenatally exposed, and he, too, had a son with autism. Another local friend had a son with autism and severe mental illness, but no known risk factors for either condition. At my prompting, he asked his mother if she had undergone any anti-miscarriage treatments when he was in utero in 1969. Yes, she said, indeed she had. She had received weekly injections of hormones at a fertility clinic in New York City due to her having a D&C before that pregnancy. I heard similar exposure stories from others, including two mothers who had been exposed to anti-miscarriage treatments in utero and who each had three children on the autism spectrum. In almost all these cases, however, records were no longer available and exposure information was gleaned solely by hearsay.

Two sisters from Boston were the exception to that rule as they had succeeded in accessing some of their own prenatal exposure records from the 1960s. One of the sisters has a son and daughter with attention, conduct, processing, and learning disorders. Her daughter also had precocious puberty. She also had another daughter with Turner’s syndrome who died in utero. The other sister has a son on the autism spectrum who was also diagnosed with Attention Deficit Hyperactivity Disorder (ADHD), oppositional defiant disorder, and panic disorder. For a family with no history of any of these disorders, it was an avalanche of unprecedented mental disability.

The sisters explained that their mother has Type 1 diabetes and as a precautionary measure was heavily medicated during her pregnancies. In addition to insulin, records revealed that she had been given weekly injections of synthetic steroid hormones, and also, with respect to at least one of the sister’s gestations, sedatives, methamphetamine, cough syrup with codeine, tetracycline, diuretics, antihypertensives, anti-nausea drugs, thyroid hormone, an anticholinergic, aspirin, synthetic vitamin K, heartburn medications, vitamins, and more. This list might be shocking to the modern eye, but in that era, it was not unusual to so heavily medicate a pregnancy. As a small control group, the mother’s sister, who did not have diabetes, had unmedicated pregnancies, and her offspring and grand-offspring are all typically developing.

I also heard stories from other families invoking exposures that I had not previously considered. After I heard remarks like, ‘My mother smoked like a chimney when she was pregnant..."
with me’, over and over, I thought that perhaps heavy intrauterine exposure to tobacco smoke—documented to be mutagenic [15] and epimutagenic [16]—could possibly tamper with fetal gametes in some fashion. Just as with pregnancy drugs, toxicology had barely touched this question in spite of the high prevalence of pregnancy smoking during the post-war decades.

Other patterns also seemed to emerge. For instance, several autism parents spoke of their fetal or neonatal exposure to surgery or general anesthesia (GA). To share just one example, a mother of two boys with idiopathic autism shared that she had undergone two neonatal surgeries for the removal of a benign spinal tumor and a repair hernia, both presumably involving GA agents. Many parents with such GA exposures had more than one child with autism, suggesting to me that perhaps some GA agents could act as potent germline toxicants.

As I pondered this variety of family histories, it occurred to me that the concept of gamete disruption could possibly help explain, in part, a number of baffling patterns witnessed in autism research, including the following: the timing of the increase (observed to have begun with births in the 1980s [2]), one generation after sharply increased use of these drugs and the peak of maternal smoking; the regional, socioeconomic, and ethnic disparities (higher rates in higher socioeconomic status families [17, 18]); the puzzling 4:1 male: female sex ratio [19] (sex-specific intergenerational responses to exposures have been detected in human and animal studies [20-22]); the existence of the ‘broader autism phenotype’ among some parents of affected children [23] (prenatal exposure to synthetic sex steroids can cause shifts in personality and cognition [10, 24, 25]); and other phenomena that have eluded explanation, including the contrast between the strong heritability of autism and the surprisingly shallow findings from traditional genetics, often referred to as the ‘missing heritability’ in autism.

Hints in the Research

I have no pretense to being a scientist or performing research, and I am acutely aware that my collection of stories and sundry musings on nongenetic inheritance prove nothing. But in discussing my ‘gamete disruption’ ideas with researchers from fields as diverse as epigenetics, reproductive biology, mutagenesis, endocrine disruption, chromatin regulation, and germ cell biology, I was struck not so much by the paucity of possible pathways linking exposures, germ cell aberrations, and developmental pathologies, but by the long list of potential culprits. The possible molecular mechanisms suggested to me included impacts on DNA de-methylation, sex-specific DNA re-methylation and genomic imprinting, chromatin and transcription factor defects, mitochondrial effects, de novo mutagenesis, somatic mosaicism precipitated by impairments to the integrity of gamete DNA, and shifts in ncRNAs, among others.

While I remained agnostic about mechanisms, what became clear was a bottom line that gametes, particularly the early precursor primordial germ cells, are not at all like inert, imperturbable marbles of DNA, but more like dynamic, vulnerable mini organs that can be (and perhaps were even meant to be) responsive to environmental cues. The genetic determinant mindset that pervaded autism research did not account for either the historical biological context in which our germ cells developed or the complicated biological realities of molecular heritability. But I could hardly blame the autism research for side-stepping this field, as there is nothing easy about studying long-ago exposures or hunting for fleeting artifacts of a possible long-gone germ cell impact.

Nonetheless, far from the walls of autism research, work in the field of germ cell mutagenesis, chromatin biology, epigenetics, and hormone disruption suggested that environmental agents can produce a variety of molecular alterations to germ cell precursors, leading to a form of nongenetic heritability that can ultimately affect germline integrity and the neurobiology and behavior of the offspring generation. For example, certain hormone-disrupting and toxic chemicals can cause abnormal behaviors and changes in brain gene expression in later generations in animal models. Gestational exposure to the fungicide vinclozolin altered the physiology, behavior, metabolic activity, and transcriptome in discrete brain nuclei in descendant males, causing them to respond differently to chronic restraint stress [26]. Gestational exposure to bisphenol-A (BPA) imposed generational effects on mRNA in the brain and on social behaviors [27]. Intrauterine exposure to BPA was also found to have transgenerational effects on imprinted genes in brain [28]. Even where hormone-disrupting chemicals were not observed to exert transgenerational effects (those appearing in generations following the direct germ cell exposure), direct germline and imprinted gene impacts were detected [29].

With respect to tobacco, the smoke component benzo[a]pyrene increases the mutation burden in the sperm of fetal mice [30], and fetal nicotine exposure promotes hyperactivity in next-generation mice [31]. In another study, the only one I could find that examined generational impacts of GA, gestating mice exposed to GA bore grandpups that suffered learning retardation, suggesting to the researchers the existence of an exogenously imposed germline genetic effect [32]. In human epidemiological studies, DES is seen to increase the risks for adverse effects in male and female grandoffspring of the mothers given the synthetic hormone drug [33, 34]. A recent epidemiological study partly underwritten by my research fund to probe for potential association between grandmaternal pregnancy smoking and autism in grandoffspring found a link between that exposure and risk of autism and also of autism-related social communication and repetitive behavior traits in grandchildren via the exposed maternal line [35]. Given the high rates of post-war pregnancy smoking in western countries and the known genotoxicity of tobacco smoke, it was surprising that this study was the first to examine the potentially important connection between that pervasive germline exposure and neurodevelopmental pathology in offspring today.

My efforts at private grantmaking can only touch a toe on this sprawling unexplored continent of questions relating to whether exogenously induced molecular germ cell perturbation raises risk for neurodevelopmental dysregulation in offspring. The list of intensive exposures, molecular mechanisms, and phenotypic outcomes worth considering is long, but many research angles are available. Human cohorts could be explored in attempts to trace possible connections between acute parental prenatal exposures and impairments in their offspring. Animal models can demonstrate next-generation effects of pregnancy drugs, smoking, and other exposures of concern, such as radiation. The models could interrogate germline mutagenic and epimutagenic signatures, gene expression patterns, characteristics of brain development, and behaviors of the ensuing offspring. In vitro studies can test how induced primordial germ cell-like cells respond to common pregnancy toxicants. I hope that in the coming years research priorities will expand beyond pure genetics and somatic exposures to encompass this area where germline genes meet the environment.
Perspective and Conclusions

Fifty years ago, leading scientists raised alarm bells about a possible ‘genetic emergency’ caused by the post-war influx of synthetic chemicals, concerned about subtle impairments in human germ cells that could affect the developmental integrity of future generations [36]. Yet today, those alarms barely echo. The FDA, for example, altogether ignores the existence of the fetal germ line in its pharmaceutical risk assessment protocols. The idea of exogenously informed germine disruption is almost completely off the radar of autism and developmental disorders research.

I shared my story and hypothesis in the hope that researchers and funders might take a cue from the reproductive biologists and epigeneticists who understand germ cells not merely as an enclosure for a protein-coding template, but as complicated, highly dynamic biological entities that contain many layers of heritable information that can reshape gene expression and ultimately, brain development and behaviors. As I have learned, generational research presents no shortage of challenges, but a responsible and biologically informed approach to public health often depends on asking difficult questions and then answering them.

Jill Escher is an autism research philanthropist, a real-estate investor who provides low-income housing for adults with developmental disabilities, president of Autism Society San Francisco Bay Area, a former lawyer, and the mother of two children with nonverbal autism. Learn more about the work of the Escher Fund for Autism at GermlineExposures.org. This commentary was adapted from a talk delivered at the Transgenerational Epigenetic Inheritance: Impact for Biology and Society conference in Zurich on 28 August 2017.

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