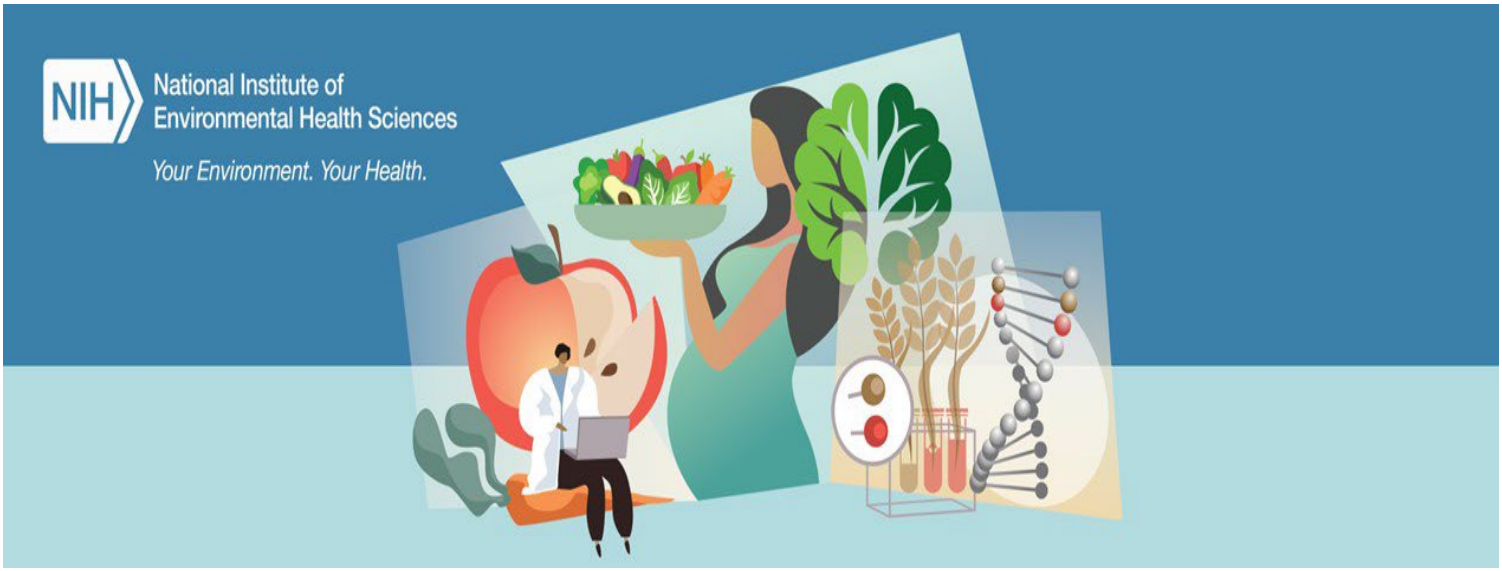


Can Nutrition Modify the Impact of Environmental Exposures on Autism Spectrum Disorder?

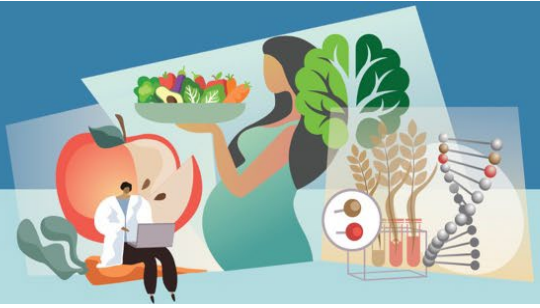
MEETING REPORT

June 21 – 22, 2022

NIEHS Division of Extramural Research Training Autism Program

National Institutes of Health • U.S Department of Health and Human Services

This report was developed by Avanti Corporation in collaboration with NIEHS meeting organizers.



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Introduction and Welcome

The National Institute of Environmental Health Sciences (NIEHS) hosted a virtual workshop titled "Can Nutrition Modify the Impact of Environmental Exposures on Autism Spectrum Disorder?" on June 21st - 22nd, 2022. The workshop was organized by the Division of Extramural Research and Training (DERT) Autism program. This workshop brought together environmental health scientists and nutrition researchers to cover a variety of topics, including mechanisms linking maternal nutrition and neurodevelopment, modifiers of environmental exposure, and methods for studying complex nutrient effects. Identifying mitigating influences of maternal diet and nutrition on the development of autism spectrum disorder (ASD) associated with other co-occurring environmental exposures could lead to important public health benefits. In cases where environmental exposures (e.g., air pollution) linked to ASD are difficult to reduce or prevent, targeted nutritional interventions may offer some protection against unavoidable chemical exposures.

The chief of the Genes, Environment and Health Branch in DERT, Cindy Lawler, Ph.D., delivered the opening remarks and welcomed all participants. She described the workshop agenda before elaborating on the goals and vision for the workshop. She stated the following reasons for organizing this workshop:

- **Complexity of exposure effects - genetic, lifestyle, and social context matter:** Environmental factors such as chemical exposures do not occur in isolation, but always in the context of genetic susceptibility, stage of development, other chemical exposures, psychosocial, and lifestyle factors. Therefore, to understand how environmental influences affect health, researchers need to study how all of these factors act together.
- **Intriguing, preliminary research on maternal nutrition and chemical exposures in autism:** There are some preliminary, very intriguing findings that the contribution of environmental exposures such as pesticides and air pollution on the development of autism can be affected by maternal diet and nutrition. One example is folate intake during the period of conception. Currently, these findings are restricted to a few studies.
- **Opportunities to incorporate current concepts and approaches from nutrition fields:** Meeting organizers wanted this workshop to be a means for a broader discussion about current concepts and nutrition and their application to other health conditions, with the goal of helping to infuse those current emerging approaches and concepts into the autism research supported by NIEHS.
- **Limited understanding of underlying biology of protection:** The primary autism findings in influence of maternal diet come from observational epidemiology studies and with the caveats about making causal connections. However, researchers are learning more about the underlying biology of autism. This presents an opportunity to look at that biology for clues about how, where, and when these environmental and nutritional factors may be converging.
- **Strong potential for translation through protective exposures:** Meeting organizers also had an aspirational goal of thinking about how these connections among maternal diet and nutrition, environmental exposures, and their role in altered neurodevelopment can be translated to public health.

Keynote Sessions

Daniel Geschwind, Ph.D., delivered the keynote lecture on the first day. He provided an overview of genetics and systems biology of ASD. He described the complexity of genetic architecture of ASD as it involves many genes in many combinations. Genetic heterogeneity makes it difficult to identify causal genetic factors. Using network analysis, his lab identified a convergent pattern of pathology in post-mortem brain from subjects with ASD. This included down regulation of genes related to synaptic vesicle and signaling function; some GABAergic markers; splicing regulation and up-regulation of microglial and astrocyte genes. Their research also indicated that the most sensitive period for developing ASD is during the mid-fetal period. His lab developed in vitro tools for modelling the effect of genetic and environmental factors to better understand disease mechanisms.

A question-and-answer session that followed the keynote presentation provided workshop participants an opportunity to ask questions. In response to a question about studying gene – environment (GxE) interactions, the speaker noted that GxE was a tough way to look at ASD and expressed concern about most GxE study conclusions from psychiatric genetics due to small sample sizes. As an example, he explained there were drawbacks in his own research using samples from Danish biobanks due to limited disparities in environmental exposures in Denmark. When asked about the difference in prevalence of ASD between males and females, the speaker stated that their research concluded that autism risk genes were not sex differentially expressed. However, it was likely that genetic risk was interacting with differential vulnerability, which is due to the underlying sex differential neurobiology of how the brain works and may have something to do with glial neuronal interactions. He also informed participants that this was an important question and that multiple research groups were working on identifying a possible mechanism.

Joshua Roffman, M.D., M.M.Sc., delivered the keynote lecture on the second day of the workshop. He discussed the neuroprotective effects of prenatal folic acid across cells, and neural circuits, and how this research may be translated into public health interventions. His lab used brain organoid models to demonstrate that pre-treatment with folic acid, in combination with maternal immune activation, affects gene expression in certain developing neurons. They also used brain imaging data from adolescents to show increased cortical thickness and reduction in emerging psychiatric disease after increased periconceptional folic acid consumption. However, translating these findings into public health interventions has been challenging. Their research identified a gap in knowledge and implementation in both patients and health care providers. He further discussed barriers to research translation in susceptible populations.

During the question-and-answer session that followed the keynote presentation, the speaker was questioned about an observed increase in ASD incidence despite the roll-out of folic acid fortification policy in both the U.S. and Nicaragua. He stated that researchers do not understand why rates of ASD were increasing, but it was possible that the rates would have been worse in the absence of folic acid fortification. He also noted that it was difficult to compare data across different countries due to variance in diet and public health policies. When asked about the best ways to inform women from high-risk populations due to socio-economic background, the speaker described a knowledge gap and an

implementation gap. He listed several practical issues that may prevent women from taking folic acid supplements during pregnancy. He also called attention to the legacy of mistrust among certain populations towards biomedical researchers. Additionally, he reminded participants of some system level changes that may increase intake of folic acid among women of childbearing age.

Messages from Sessions

The workshop was divided into three different sessions distributed over two days. Each session included five presentations. The final session of the workshop was followed by a moderated panel discussion. This report is broken down by workshop sessions and provides a brief overview of workshop presentations and the panel discussion.

Scientific Session One: Mechanisms Linking Maternal Diet and Neurodevelopment

The session opened with a talk by Kristen Lyall, Sc.D., who presented an overview of existing scientific evidence linking maternal dietary factors to neurodevelopmental outcomes and identified areas of research specific to autism spectrum disorder that have remained less studied. She presented findings on prenatal fish intake, dietary polyunsaturated fatty acids, and key nutrients including vitamin D and folic acid associated with autism-related traits. She identified the need for more research on the comprehensive effects of combinations of nutrients or foods. Lyall emphasized that because multiple biological pathways link nutrients to neurodevelopment, there is an opportunity to look at combined effects, both on the genetic landscape and with other environmental exposures.

Kelly Ferguson, Ph.D., M.P.H., gave the second talk of the session, which looked at maternal oxidative stress during pregnancy and the potential to mitigate harmful outcomes through dietary factors. This talk focused on 8-isoprostaglandin-F2alpha as a biomarker for oxidative stress. Ferguson reported on the association between 8-iso and adverse pregnancy outcomes, including preeclampsia and preterm birth, which have been strongly linked to neurodevelopmental outcomes including ASD. She also presented evidence from The Infant Development and the Environment Study (TIDES), which showed that omega-3 fatty acid supplementation can reduce oxidative stress. She discussed the potential for phthalate exposure to counteract the benefits of dietary exposure. She also identified several complications in this line of research including confounding variables of sociodemographic factors, dose dependency, and the use of biomarkers versus questionnaires. When asked about the different pathways of oxidative stress, she noted that the primary mechanisms of 8-iso production is through free radicals reacting with ionic acid in cell membranes. There is also debate over whether a minor amount could be created through upregulation of COX-2 pathways.

The third talk of the session, presented by Elinor Sullivan, Ph.D., was focused on the impact of maternal nutrition and metabolic state on the in-utero environment and how these prenatal factors influence child development and outcomes of psychiatric disorders. She presented results from a non-human primate study showing that offspring from mothers fed a western-style diet were more likely to develop inflammation, anxiety, aggression, impaired social behavior, and obesity. This study generated much conversation on the impact of prenatal factors on child and mother. There was some indication of maternal behavioral differences across diets, but not at later time points. Preliminary data have indicated associations between the microbiome and offspring behavioral outcomes relating to gut

serotonin and tryptophan metabolism. Sullivan's team also conducted a human study that showed how increased maternal BMI predicted increased offspring negative valence behavior and identified omega-3 fatty acid as a protective factor. Sullivan also provided new evidence for the association of obesity-induced maternal inflammation with altered serotonin-kynurenine pathways in pregnancy, which may be a mechanistic link between maternal obesity and adverse child neurobehavioral developmental outcomes.

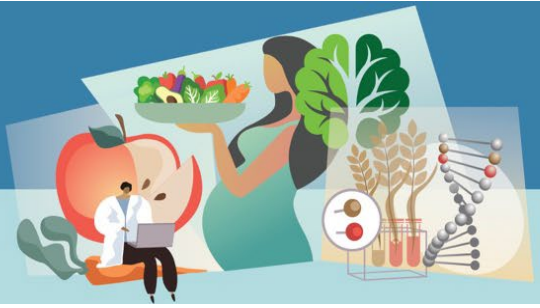
Hehuang "David" Xie, Ph.D., gave the fourth talk of the session, which explored the potential harm of high prenatal folate intake and its association with ASD. He summarized the combined findings from two studies that sought to identify the epigenetic and epitranscriptomic mechanisms that link maternal intake of folic acid and ASD. He also identified folate deficiency as a risk factor for Fragile X Syndrome. Xie highlighted key findings from these studies, which included the potential for optimized maternal folate levels to improve child neuro-developmental outcomes. When asked about folic acid exposure in cell culture experiments, he noted that future work will include an assay for blood and brain tissue in the mouse model.

Paul Curtin, Ph.D., closed the session with a discussion of three new conceptual frameworks for characterizing the role of metal dysregulation in ASD and the role of timing and context of exposures. Using the tooth biomatrix as a model, he highlighted the significance of retrospective biomarkers as a non-invasive means to assess early life exposures. He provided evidence to suggest that the dysregulation relevant to a disease may not be in the exposure level of an element, but in how that element is regulated over time. He stressed that while homeostatic dynamics were tightly regulated across studied groups, these processes were highly disrupted in ASD. A challenge in combining different cohorts for this analysis is the potential for marked differences in exposure levels (e.g., severe deficiencies versus marginal deficiencies), but Curtin noted that in measuring rhythmicity and focusing on cycling in homeostatic dynamics, tightly regulated biological systems were roughly consistent across populations.

Scientific Session Two: Dietary Factors as Modifiers of Environmental Exposures

Rebecca Schmidt, Ph.D., opened the session by providing an overview of current research on the associations between prenatal nutrition and ASD. She described results from the Childhood Autism Risk from Genes and Environment (CHARGE) study, which provided early research on associations between ASD risk and folic acid supplements, pesticide exposure, and PFAS exposure. She identified several mechanisms that exposures share and emphasized the need for additional studies on these mechanisms. While there is evidence to support optimizing prenatal nutrients to attenuate the risk of ASD, Schmidt noted that findings are complicated by factors such as reporting bias and confounding. Schmidt emphasized the importance of education and medical oversight when consuming prenatal vitamins, as some studies have shown a potential U-shaped association between autism and folic acid.

In the second talk of the session, Amanda MacFarlane, Ph.D., presented her findings on folate mediated one-carbon metabolism as a mechanism for the association between maternal nutrition and ASD. She identified several explanations for conflicting results in studies of dietary exposures and ASD, including



the method used to assess exposure. She discussed the differences between dietary intake assessments, supplement use, and biomarkers of intake and status and what those differences mean for data interpretation.

In the third session talk, Edwin van Wijngaarden, Ph.D., presented results from the Seychelles Child Development Study to examine the influence of background levels of methylmercury (MgHg) exposure from fish consumption on neurodevelopment. He detailed the three cohorts, assessed as part of this study, and results showing a range of beneficial, adverse, and null associations. There was no overall association between prenatal MeHg exposure and neurodevelopmental outcomes. There were child development benefits associated with fish consumption, including the potential for the nutrients in fish to mask MeHg toxicity. Van Wijngaarden also presented the results of genetic studies that have been conducted on these cohorts to explain the mechanisms behind these results. When asked about the potential for microplastics to change the relative benefits versus risks of fish consumption, van Wijngaarden noted that research collaborators have found minimal contamination of ocean fish with microplastic thus far.

Audrey Gaskins, Sc.D., gave the fourth talk of the session and provided an overview of the current research examining the potential for periconceptional nutrition to attenuate the reproductive toxicity of air-pollutant exposure. She discussed the potential for folate, and other methyl donors, to offset this toxicity. Documented associations include nitrite exposure and pregnancy loss and congenital heart defects, as well as folic acid supplementation and several reproductive health outcomes. While folate is unlikely to be selectively protective for conditions such as autism, it may be more generally protective by influencing a range of reproductive endpoints. Gaskins identified inflammation, oxidation stress, and epigenetics as potential mechanisms of toxicity.

The final talk of the session was given by Rachel Thornton, M.D., Ph.D., who provided an overview of health equity and health disparity research. She presented historical data and analysis on persistent health disparities and the pervasive nature of such disparities across a person's lifetime. Thornton highlighted the importance of socioeconomic status and the myriad of related exposure factors on developmental outcomes for children. She also identified policy interventions that can promote or undermine health. A key takeaway from her presentation was the idea that racism acts as a separate, structural force, which drives marginalization and health disparities even after controlling for socioeconomic factors.

Scientific Session Three: Methods for Addressing the Role of Dietary Factors, Interactions with Environmental Chemicals, and Tools to Expand the Research

Anna Maria Siega-Riz, Ph.D., began the session by presenting an overview of epidemiological approaches for examining the relationship between periconceptual diet and disease. She highlighted the role of dietary assessments in epidemiological studies and the pregnancy specific factors that should be considered. She also provided resources with guidelines for conducting dietary assessments. There are several different types of dietary assessments, and the appropriate tool is determined by the specific window of interest and the research question.

The second talk of the session was given by Wei Perng, Ph.D. Perng shared her research using metabolomics to explore and characterize the relationship between diet and health outcomes. She presented several types of dietary biomarkers and the key considerations for their use in assessing exposure. She also detailed a study on the association of sugar-sweetened beverage intake with cardiometabolic health in adolescents. Perng emphasized the need for continued advancements in traditional nutritional epidemiology, particularly in the context of biomarker research.

The third talk of the session was given by Onyebuchi Arah, M.D., Ph.D., who provided an overview of modern methods for effect modification, interaction, and mediation analysis for the study of dietary factors associated with ASD. Arah presented the four types of effect modifications and emphasized the role of causal assumptions in linking environmental exposures to ASD and methods to evaluate those assumptions.

In the final talk of the session, Jorge Chavarro, M.D., Sc.D., presented work analyzing the interaction between health effects and diet as the route of environmental exposure. There was a focus on pesticide exposure through residues in foods, particularly foods that are generally considered to be part of a healthy diet. The talk highlighted the adverse health outcomes associated with pesticide exposure and the methods used to identify these associations. Methods included using the pesticide residue burden score, a metric calculated by the USDA Pesticide Data Program to score overall contamination for any fruits or vegetables consumed in a month.

Session three closed with a question-and-answer discussion. Siega-Riz was asked to share specific features that research apps could incorporate from consumer apps. She mentioned user-friendliness, ease of data entry and retrieval, and immediate feedback for users. She was also asked to comment on the unique confounding challenges associated with biomarker-based data compared to other dietary assessments. She emphasized the importance of data triangulation and noted that no one biomarker could be used to assess all exposures of interest. In response to a question related to biological versus socioeconomic confounding, Chavarro argued that our assumptions about nutrition and the environment may be flawed. Arah also commented on the auto-physiological considerations associated with biomarker data. He stated that continuing down the causal chain may mean encountering intermediates with their own confounding variables. When asked about group-based approaches other than LASSO, Perng noted that she has used elastic net and unsupervised approaches like PCA in the past.

Challenges and Opportunities

Scientific session three was followed by a panel discussion. The discussion was moderated by NIEHS Kimberly Gray, Ph.D., from NIEHS, and panelists included Youssef Oulhote, Ph.D., José Cordero, Ph.D., Julie Daniels, Ph.D., Rosalind Wright, Ph.D., and Andres Cardenas, Ph.D. All panelists shared their insights about the different scientific sessions and addressed current needs and possible opportunities in various areas of research related to autism and gene-environment and gene-nutrient interactions.

The following are some of the research needs identified by panelists:

- **Knowledge Gaps:**

  - Researchers need to embrace complexity. Current research is still in exploratory territory with very few studies; studying this in a holistic way will require researchers to embrace complexity.
  - Researchers need to understand interindividual variability and its source. This may help with precision prevention approaches of getting to subgroups, if not individuals.
  - There is a need to identify trans-diagnostic pathways, features that manifest early but are related to many serious mental health or behavioral issues down the line.
  - There is a need to identify early-life biomarkers that highlight epigenetic programming during sensitive periods of development.
- **Research needs:**
  - Panelists identified a need for larger sample sizes and identified collaboration between different consortia as an opportunity to attain them. Larger sample sizes will enable large-scale 'omics studies and help clarify gene-environment and gene-nutrient interactions. It is difficult to determine an appropriate sample size as it depends on the technology being used.
  - Most of the research is from studies in the U.S., Europe etc. Researchers need to leverage studies in low- and middle-income countries that may have higher environmental exposures but also a much wider range of nutrient intakes.
- **Research to action needs:**
  - Identifying steps to improve autism outcomes will need a very strong trans-disciplinary group that covers basic science understanding of mechanism, translation of research into action, and incorporating social determinants of health into research and translation.
  - There is a need to consider different ways of community engagement based on different populations and social determinants of health. Looking at access to care and early diagnosis is important to autistic individuals and families.

The following are opportunities identified by panelists:

- **Research opportunities:**
  - Exciting new tools such as metabolomics, epigenomics, and exposomics are being developed to understand the complexity of environmental mixtures. These may enable researchers to test especially complex hypotheses.
  - There are opportunities in studying placental biology that are coming from other areas of research.
  - By having more inclusive samples in consortiums like Environmental Influences on Child Health Outcomes and using current technology and data science metrics, researchers might be able to understand heterogeneity in results across different studies.
  - It is important to understand timing and dose of exposures and nutrients. This may open multiple opportunities to intervene and prevent adverse outcomes.
  - Bringing exposure mixture models together, both on the environmental side and nutritional side, presents a great opportunity to move these fields forward.

- There is a lot of overlap and blurred lines among different neurodevelopmental outcomes and focusing only on particular endpoints or outcomes may be limiting. Researchers may need to consider outcome compositions that are more on a continuum. They could focus on subtle specificities that might go across different outcomes and may be relevant to certain mechanisms.

- **Implementation opportunities:**
  - The medical field needs to collect nutrition data systematically. Current real time assessment tools may be useful for this.
  - Reporting results back to cohorts builds community trust and encourages participation in research. Feedback from research participants is helpful in developing new questions.
  - It is important to consider social determinants of health during research translation. The care-giving environment and the social environment are two important levers because cultural background has significant implications for human health outcomes.