



## Meeting report

### Executive Summary

To identify novel opportunities and mechanisms to accelerate research on environmental factors and autism, a diverse group of scientists came together on September 8, 2010 at the National Institute of Environmental Health Sciences (NIEHS) Keystone Campus in Durham, NC to share ideas and expertise. The meeting was co-sponsored by the NIEHS, a component of the National Institutes of Health, and Autism Speaks, a non-profit autism science and advocacy organization.

The participants held discussions in the following four broad areas:

- Lessons learned from other environmentally mediated disorders
- Novel tools and approaches in genomics and toxicology
- Cellular and molecular mechanisms
- Exposure science and epidemiology

For the purposes of the meeting and this report, autism and Autism Spectrum Disorder (ASD) are used interchangeably. The environment was defined broadly to include industrial and agricultural chemicals; endocrine active compounds; pharmaceuticals and medical exposures; and lifestyle, nutrition, and social environment.

The participants were charged with creating recommendations for (1) highest priority areas of research that address the contribution of environmental factors for risk and phenotypic expression of autism; (2) possible solutions for any barriers to progress identified in these areas; and (3) other resources needed for increasing the pace of this research.

Several overarching themes emerged during discussion. Understanding environmental influences to autism will require both discovery-based science as well as hypothesis-driven science in parallel. Collaborative approaches exploring gene-environment interplay should be encouraged. Strong interdisciplinary teams are needed to move findings back and forth from clinical and epidemiologic settings to mechanistic studies. Research needs and opportunities identified included expansion of epidemiology investigations to capitalize on existing resources and unique opportunities (e.g. special populations; studies with existing biorepositories and associated information), development of a range of model systems that can address the complexity of autism, exploration of bioinformatics and screening approaches to identify environmental chemicals of interest, increased emphasis on neuropathology, enhancement of capacity for measurement of environmental analytes, harmonization of exposure assessment instruments and mechanisms for expanding the workforce.

The conclusions reached by the meeting participants have been shared with the Interagency Autism Coordinating Committee (IACC).

## **Background**

In the last 20 years, autism or Autism Spectrum Disorder (ASD) has become an urgent public health crisis. From 1979-2009, its prevalence increased 600 percent. In 2010, more children will be diagnosed with ASD than AIDS, diabetes, and cancer combined. Put another way, 1 in 110 children suffer from ASD; it is four times more common in boys, who have ASD with the prevalence of 1 in 70. Autism costs the United States approximately \$35 billion per year, more than Type 1 diabetes, childhood leukemia, or cystic fibrosis.

Autism is a complex neurodevelopmental disorder with variability in symptom onset and presentation. The heterogeneity in autism phenotypes suggest that these syndromes are caused by multiple factors, much like what has been shown in cancer etiology. A complex interaction among environmental and genetic factors determines risk. Both the genetic and environmental contributions to autism risk are expected to vary among individuals. There is an urgent need to identify environmental risks for autism, as this information can be used to reduce harmful exposures and reduce the incidence of ASDs.

Recent discoveries using large, collaborative datasets and pooling efforts have identified several genes and gene loci that contribute to the risk of autism. While previous studies have focused on common variation (single nucleotide polymorphisms (SNPs)), more recent technological advances have identified an association of copy number variation (CNVs) with autism risk; many of genes implicated by CNV findings converge on pathways that control synaptic development, plasticity, neuron development and cell to cell signaling. It appears that some CNVs are rare variants that occur very infrequently or not at all in the general population. One line of reasoning suggests that each person with ASD may have a unique set of etiologically relevant CNVs. Some of these genetic variants are “highly penetrant” meaning that, if you carry that CNV, you very likely will develop ASD, whereas others raise the risk for ASD but need to combine with other genetic and/or environmental risk factors to cause ASD. Some of these CNVs are inherited, but many appear “de novo”, meaning that they exist in the affected child and not the parents.

In addition to changes in the genome, autism has been linked to alterations in the epigenome, heritable changes in expression that are not attributable to DNA sequence. The best studied of these are DNA methylation and histone modification. Epigenetic changes in DNA transcription due to environmental influences have been well documented and markers for epigenetic alterations have been seen in both blood and postmortem brain samples in autism. These findings lead to the hypothesis that some instances of gene environment interplay in autism may arise through environmental exposures acting through epigenetic mechanisms.

While there have been major scientific advances in determining the genetic causes of autism, environmental risks and other non-genetic factors, and their interaction with genetic susceptibility, have been less well studied. Epidemiological studies conducted over the past decade have linked a number

of non-genetic factors to increased risk of autism. These can be categorized into three major areas: 1) demographic factors, 2) medical interventions and conditions and 3) xenobiotics. In addition, discoveries in immune system dysregulation and epigenomics have hastened discoveries on mechanisms by which environmental exposures may interact with genetic backgrounds. A few examples of findings which have advanced research in these areas are described below.

Some of the earliest reports of environmental influences in autism utilized cohort designs, especially those with rare exposures to study causal associations. Prenatal exposure to the teratogens thalidomide and valproic acid has been shown to increase risk for features of autism spectrum disorders. Epidemiologic studies in more recent years have shown more modest associations of autism risk with obstetric complications, gestational age and neonatal birth weight. Stimulated by the rising prevalence in autism in the past 20 years, attention has turned to industrial chemicals and other toxicants in the environment. Several studies have reported an increase in autism risk with rising parental age, and investigators have suggested that advanced parental age is a surrogate for the cumulative effects of environmental exposures. Many studies that have focused on postnatal ethylmercury exposure through thimerosal-preserved vaccines have failed to show an association with autism risk. A number of studies now underway are examining more broadly the possible linkage of autism risk with exposure to Hg and other heavy metals during critical periods of development.

One potential mechanism by which xenobiotic exposure may result in autism is perturbation of the immune system and consequent hyper- or hypoactivity of central and peripheral systems which control immune function. Several neuropathologic, epidemiologic, clinical and animal model studies have documented alterations in the immune system or markers of immune system challenge in individuals with autism. These findings have stimulated interest in compounds known to produce similar immunotoxicologic effects.

Against this background of newly emerging information about the function of genes associated with ASD, and rising interest in, and support for, a role of the environment, meeting participants were urged to consider the possibility of a targeted approach to identify exposures of interest by focusing on pathways of convergence or common biological mechanisms.

## **Lessons learned from other environmentally mediated disorders**

- **Benefits of looking both within and across disease boundaries.**

Autism spectrum disorders share with many other disorders a complex phenotype that presents challenges for studying environmental etiologies. One common approach has been to dissect the disease phenotype into homogeneous classes, with the assumption that environmental exposures may contribute differently to the defined subtypes. For example, genetic variation may be specific for particular subtypes of leukemia; this suggests that different forms of gene environment interplay may operate among subtypes. Looking more broadly at functional domains that do not adhere to strict disease boundaries may be beneficial as well. For example, recent genetic findings in autism indicate that specific genes or CNVs may contribute to risk not only for autism but also for related conditions such as schizophrenia. Likewise, exposure to pesticides has been associated with risk of Attention

Deficit Hyperactivity Disorder (ADHD) and there is suggestive evidence of linkage to autism/pervasive developmental disorder. These examples suggest that benefit could be gained from studying environmental and genetic risks in relation to phenotypes that cross disease boundaries.

- **Convergence of genetics and environment on common pathways.**

In many complex disorders, information about disease mechanisms has been critical for generating hypotheses about contributing environmental factors. For instance, in the area of Parkinson's Disease (PD), genetic findings point to disruption in protein trafficking. Environmental risks for PD interact with some of the same pathways and molecular targets that are altered in familial forms of the disease. The potential convergence of environmental and genetic risks underscores the value of research on genetics and neurobiology of autism for identifying potential pathways and targets for investigation in environmental risk factor research.

- **Benefits from using a full range of model systems.**

Further investigation and development of suitable model systems are needed. In the area of autism, there has been some progress in developing rodent models, yet the full range of model systems has not been explored. Alternative models such as *Drosophila melanogaster* (fruit flies) and *Danio rerio* (zebra fish) have catalyzed discovery of important new information about numerous disorders, including neurodegenerative disorders such as Parkinson's and Alzheimer's disease, and sensitivity to substances of abuse such as ethanol and cocaine. The genetics of synapse formation and connectivity in these model systems is well established and easily manipulated, making them potentially useful for the study of autism-relevant cellular and molecular neurodevelopmental events, and their perturbation by environmental exposures. Model systems could be used to advantage in the study of both risk and protective factors for autism.

- **Advantages of using multiple epidemiologic approaches.**

Epidemiology has provided important clues for etiologic research in multiple complex disorders. While prospective longitudinal studies that measure environmental exposures before disease onset are ideal, many types of epidemiological study designs can be used to offset the weaknesses of any one approach. Combining data from multiple studies may be necessary to achieve populations of sufficient size. Results emerging from epidemiologic studies with different designs and populations can lend weight to etiologic hypotheses when they point to the same or similar risk factors and are consistent with findings from mechanistic laboratory-based studies. Expansion of existing large population-based studies to include diagnostic outcomes and exposures relevant to ASD provide opportunities for understanding environmental risk factors (e.g. National Children's Study). Studies of special populations with specific exposures (e.g., nutritional deficiencies, toxicants, and maternal infection, prematurity, use of assisted reproductive technology/in vitro fertilization) also offer the potential to better understand the contributions of specific environmental exposures to risk for ASD.

- **International opportunities**

The increasing development of international disease surveillance and research infrastructure provides opportunities to study autism among populations with unique exposures, nutritional profiles, and lifestyles. Breast cancer researchers, for example, were alerted to the importance of dietary factors by

studying such populations. Clues emerged from studying differences in breast cancer prevalence among populations in different countries. Of special note are studies that documented changes in disease risk observed when populations from non-Western countries with low breast cancer prevalence immigrated to Western countries associated with higher prevalence. As international surveillance infrastructure improves, it should be possible to create an autism “atlas” to examine differences in autism prevalence as a function of geography. Such analysis has proven useful in both cancer and asthma research. In addition to international opportunities, examples of populations within the US that may be useful for exploring autism risk or protective factors include Somali immigrants in Minnesota who have experienced a potential cluster of cases of autism, as well as the Amish community, which has been suggested to display a low prevalence of autism.

- **Understanding the role of comorbidities**

Autism appears to be associated with a greater frequency of several physiological conditions, including gastrointestinal dysfunction, sleep disturbance and early signs of fine motor and temperament difficulties. These associations are poorly studied, yet investigating them may reveal unexpected clues. For example, nonmotor features associated with Parkinson’s disease (e.g., olfactory deficits, autonomic abnormalities) often appear in the preclinical stage of disease and have yielded intriguing etiologic clues.

- **Creating and sustaining inter and transdisciplinary teams.**

Investigating relationships between genetic pathways, environmental risks, and clinical subtypes require sustained exchanges between basic and clinical scientists and epidemiologists. These crucial collaborations will require innovative funding mechanisms rather than traditional, single-investigator grants (i.e. R01s).

- **Applying tools and study designs from study of other environmental disorders.**

Adopting technologies, methods, and markers that have been developed and identified in the study of other diseases could prove fruitful for autism research. For instance, pesticide exposure and air pollution have been studied extensively in relation to other diseases and the knowledge gained could inform study design and analytic approaches in autism research.

## **Novel tools and approaches in genomics and toxicology**

- **Many genetics studies lack exposure information**

The genetic architecture of autism is complex. Some experts estimate that approximately ten percent of cases can be traced to single gene disorders or chromosomal rearrangements. Highly penetrant mutations in a number of synaptic genes have been associated with autism and rare copy number genic variants (CNVs) have been firmly linked to autism in several studies. Findings implicating common genetic variation (SNPs) in autism have been inconsistent and their contribution, relative to CNVs, to autism risk is currently unclear. The potential joint influence of environmental exposures with common genetic variation has been relatively unexplored. The lack of exposure information in many genetic studies may explain some of the variable findings, however, if gene environment interplay is operative. The impact of environmental exposures on CNV is another area that merits attention. Finally, potential differences in environmental risk for simplex vs. multiplex autism cases should be considered.

- **Challenges for establishing and confirming genotype-phenotype relationships.**

Many challenges exist, including how to correlate mechanism at the gene expression level to genotype, and how to translate human phenotypes to cellular phenotypes. Scientists do not fully understand the connection between gene variation and clinical phenotype. For instance, variation in the SHANK3 gene may increase the risk of multiple disorders, with specificity likely to depend on other genes and on environmental exposures. Most genetic studies have been conducted using a stringent case definition that is not representative of the wide variation of clinical phenotypes that are found in the population with autism. The quality of genetic data is uneven. Standards must be set for genotyping, sequencing, and analysis. At a minimum, data should be made available with publication to enable independent analysis (e.g., using different criteria for defining CNVs). Ongoing studies (e.g., exome sequencing) and development of more rigorous and widespread quality control practices will generate more reliable clues. Genetic data of good quality will be essential for integration with exposure data and meaningful interpretation of joint effects.

- **Biologic pathways implicated by autism genetic findings**

Despite these uncertainties, when viewed in aggregate, some common functional themes have emerged from autism genetic studies, including disruption of synaptic homeostasis. Moreover, while there is still some debate, geneticists can agree on a limited number of gene loci that could be prioritized for functional studies. Development of targeted mice that express one or more of several disrupted genes relevant to autism could be useful. Such genes would include those implicated by CNVs within high-risk regions. Such models could take advantage of mild phenotypes to study additive (synergistic) influences of xenobiotics. Recent progress in developing a simple social test for use in mice (e.g., preference for being alone vs. in proximity to another mouse) will enhance the utility of transgenic mouse models. Differentiated cellular systems could be used to address synapse formation and connectivity. These approaches may better preserve the functional consequences arising from the complex genetics of autism risk and thus provide novel opportunities to study neurodevelopmental impairments relevant to ASD.

- **Consideration of maternal genes that affect xenobiotic metabolism or biologic response**

Maternal gene variation that contributes to the metabolism of, or biologic response to, xenobiotic exposure during pregnancy can impact autism risk through alteration of the fetal environment. This genetic interaction between the mother and fetus is not captured when the analysis is focused solely on the genotype of the affected child. Greater consideration of ‘teratogenic’ maternal alleles is needed to fully explore this form of genetic contribution to autism risk and its implications for xenobiotic exposure effects during gestation.

- **Bioinformatics/computational tools to explore relationships between toxicants, genes, proteins and diseases**

Bioinformatics approaches can be used to define potential relationships between genes and exposures and to identify environmental compounds for further evaluation. The National Toxicology Program (NTP) has recently explored the use of bioinformatics to identify genes/pathways associated with diseases/disorders of interest. Data arising from literature mining, functional genomics, toxicogenomics and genetics/genomics can be integrated to identify genes/pathways associated with disease. These

genes/pathways then become the basis for the development of chemical screening assays. Compounds that are hits in screening assays can be considered for more in-depth evaluation, with priority possibly given to compounds that have effects at very low concentrations, that hit multiple gene targets, or that are prevalent in the environment. A dedicated workshop on bioinformatics approaches is needed in the near future to further discuss the state of the science, whether it is mature enough for application in autism, and, if so, how to begin. Thalidomide and valproic acid, which have been associated with increased risk for autism, may be useful chemicals to consider in formulating initial strategies.

- **Environmental effects acting through epigenetics and epigenomics**

Advances in our understanding of epigenetics and epigenomics are highly relevant to understanding environmental contributors to autism, as epigenetic mechanisms act at the interface of genes and environment. Further development and application of sensitive assays to measure DNA methylations, histone modifications, higher order chromatin modifications and microRNAs are needed. Studies are needed to examine how exposures may act on germline and somatic cells via epigenetic mechanisms to alter gene expression in ways that contribute to the autism phenotype.

- **Induced pluripotent stem cells.**

Induced pluripotent stem cell (iPSCs) lines or mesenchymal stem cell (mSC) lines from individual well-characterized patients with syndromic and idiopathic autism could also be used to advantage. Although it is not currently feasible to use these cell lines for large scale screening, they can serve as a tool to translate knowledge from clinical data to relevant cellular and molecular assays—translational toxicology. For example, cells obtained from individuals affected by autism may serve to identify critical cellular events, and their associated time periods, that are linked to specific disease manifestations. In turn, these cellular/molecular events can be evaluated for potential relevance to exposure susceptibility.

## Cellular and molecular mechanisms

- **Alterations in synaptic development and plasticity**

As noted in previous sections, the genetics findings point to the synapse as an important locus for understanding the underlying mechanisms of autism. Impairments in cell cell interactions, synaptic homeostasis, neuronal migration and excitatory and inhibitory neurotransmission are implicated. Development and application of cellular models that focus on these how environmental agents may alter the function of cellular adhesion molecules and related synaptic proteins are warranted.

- **Immune system alterations.**

Many of the recent large scale genetics studies have identified genes associated with cell signaling and neural development, while research involving epidemiological information and samples has identified many markers of immune system dysregulation in some individuals. Scientists have suggested that this may reflect a distinct phenotypic and etiologic subgroup of ASD, and that abnormalities of immune functioning are predisposing factors to adverse reactions to environmental exposures. The nature or mechanism of this interaction is not well understood, however.

Early studies of the immune response in autism primarily focused on the relative expression profiles of particular circulating cytokines, but it is unlikely that any individual cytokine (or group of cytokines)

directly influences postnatal neurodevelopment. Recent data assessing inflammatory events that occur in utero offer a promising new avenue to explore, though the success of this strategy will depend on the availability of robust animal model systems and informative clinical records.

The likely involvement of the immune system in autism etiology has important implications for the use of reductionist model systems (e.g., dissociated neurons). While much can—and has—been learned from simplified models, it is unlikely that they fully capture the complexity of the immune-CNS interaction that occurs in affected humans. Thus, in vitro models that better approximate this complexity (e.g., organotypic brain slice cultures) should be considered. Regardless of the specific techniques used, a tiered approach is desirable, with ongoing bidirectional exchange of information among molecular, cellular, organotypic and in vivo models.

- **Multiple mechanisms for environmental influences on autism.**

The search for a mechanistic understanding of environmental contributors to autism must include studies that address two kinds of questions. The first is what changes in gene and protein expression arise in autism and how does the environment contribute to those alterations? A related question is how do changes in gene and protein expression alter the response to toxicants? Both questions are germane to achieving an understanding of how environmental exposures combine with genetic variation to result in the autism phenotype.

- **Limitations of neuropathology findings**

Continued emphasis on studies that illuminate the basic neurobiology and neuropathology of autism will be important, as the results of these studies will inform decisions about which functional pathways to explore in toxicology studies. Unfortunately, the neuropathology of autism has been studied in relatively few cases, and the study has not been systematic. Resources are needed to augment availability of postmortem brain tissue for research. Enhancement of brain tissue repositories as well as a standard protocol for obtaining, storing and analyzing the brain tissue is crucially needed. NIH and Autism Speaks are now collaborating on an autism brain tissue repository, and further collaboration is welcomed. It was suggested that the collection of other types of tissues should be considered — extracranial tissue, brain stem, terminal ileum.

- **Considerations for mechanistic studies**

Based on the genetic pathways implicated in autism and the limited neuropathology data available, there is a need for mechanistic studies that focus on how dendritic and neurite growth, synapse formation and pruning are influenced by xenobiotics. There is a need to understand anatomical correlates of functional impairments such as network problems, not simply frank loss of cells. In testing compounds of interest with in vivo models, it will be important to use doses that produce body levels of the compound that are comparable to the levels in humans. Dosing is very different in animals versus humans, with much higher doses in animals needed to achieve levels comparable to those in humans. Model systems will also have to take into account differing sensitivities of organisms at various levels of development, for instance how the placental barrier affects exposure in utero. Studies that can address multiple exposures are also needed.

- **Sex ratio in autism: is this a clue?**

The much higher prevalence of ASD among males suggests the need for further study of functional pathways that differ among genders as well as toxicants that have greater effects on males. For example, estrogen is important in the development of males, and many endocrine-active compounds are estrogen mimics. In addition, the glucocorticoid pathway is involved in both stress responses and immune responses, and the immune response, especially autoimmunity, shows major gender differences. Prevalent exposures such as bisphenol A (BPA) and polybrominated diphenyl ethers (PBDEs) merit special attention because of their known effects on hormonal systems.

- **Microbiome**

Some studies have identified larger populations of detrimental intestinal bacteria among children with autism, and to a lesser degree, their family members. More comprehensive studies of the microbiome in autism are needed. Such studies are important in light of not only the frequency of gastrointestinal disorders in people with autism, but also the gastrointestinal system's large role in the body's immune function.

- **Mitochondrial function**

An additional topic requiring further attention is the potential role of mitochondrial dysfunction in autism. Some data suggest that a significant portion of children with autism have mitochondrial impairments. The relationship of these impairments to the etiology of autism and/or to environmental stressors is unknown.

- **Strategies for selection of chemicals to study**

The selection and prioritization of chemicals to study in model systems is a complex issue and may benefit from unbiased bioinformatics approaches discussed in the previous section. While it may be useful to considering prioritizing studies of toxicants for which exposure has increased over the past 20 years, coinciding with the increase in autism prevalence, the likely involvement of epigenetic mechanisms in autism means that the relevant exposures could have happened much earlier (i.e., to grandparents rather than parents).

## **Exposure science and epidemiology**

- **Capitalizing on existing studies**

A range of epidemiologic study designs is needed. In addition to developing new studies that focus on autism, investigators should consider opportunities to conduct retrospective analyses for autism outcomes on populations enrolled in previous studies of other diseases, or to capture data from existing cohorts for whom follow-up is ongoing and for which exposure data is already collected or could be added. Examples of such cohorts include the Nurses' Health Study and occupational cohort studies such as textile workers in China. Ancillary studies conducted under the auspices of the National Children's Study will provide unique opportunities as well. For example, questions related to immune challenges could be addressed by examining effects on offspring when women are infected with influenza during pregnancy. The role of vaccines as immune challenges, their effects on the maternal immune system and risk for autism in offspring merits additional study as well. Another useful comparison may be

children who attend daycare vs. those who do not, as there may be differences in the rate of infection associated with time spent in the daycare setting.

Leveraging existing studies designed for other purposes to ask questions about autism presents two major challenges. These are the differing standards for defining autism and the likely small proportion of subjects with autism in such studies. Harmonization of phenotyping and exposure assessment approaches could be useful for combining data from different studies of small sample size. Currently, diagnosing autism is labor intensive and requires significant expertise. Development of screening questionnaires that could make diagnosis quicker and less costly is needed, especially to capitalize on ongoing studies as well as international opportunities. The NIH as well as Autism Speaks currently fund development of such questionnaires.

Capitalizing on existing studies should be viewed as one of many approaches applied to autism. Use of existing studies would be low cost and may yield surprisingly robust findings. For instance, many currently accepted associations with Parkinson’s disease were found by examining cohorts that were originated to study other diseases, such as cancer. As long as the exposures are well-characterized and the disease outcomes well defined, then this approach could lead to valuable information about possible associations that could be validated in other, more focused cohorts.

- **Body burden and source of exposure**

Monitoring of the external environment (ambient air, for example), as well as the internal environment (blood and tissue levels of particular chemicals) will be equally important to a full understanding of how environmental exposures contribute to disease. Finding high levels of a chemical in an individual’s blood during etiologically relevant time periods does not reveal the source of exposure. Such data will be particularly important for informing prevention strategies.

- **NIH Genes Environment and Health Initiative**

Efforts such as the NIH Genes Environment and Health Initiative are aimed at improvements in methods for characterizing personal exposures—diet, medications or recreational drugs, air pollutants, and the spatial and temporal nature of those exposures. The initiative is developing wearable sensors to capture such personal exposures as physical activity and diet, as well as particulate matter and other combustion byproducts. Prototypes for these sensors are now available and undergoing field testing and validation. Although the measurement of multiple exposures in real time will provide important information not otherwise available, cost considerations will likely limit their use to a subset of study participants. Additional work is needed to make these sensors useful for young children and infants. Another component of the Genes, Environment and Health Initiative focuses not on environmental exposures *per se*, but on markers of biologic response to exposure; successful products developed from these efforts could be applied to studies of autism risk.

- **Measurement of environmental exposures**

At present, access to and capacity for environmental analysis in biospecimens and other relevant media (e.g., dust) are limited and need significant expansion. Also needed is standardization and sharing of exposure instruments and protocols. A tiered approach could be used, with a brief exposure

questionnaire for use in studies where the focus is not on environment and an expanded form for environmental epidemiology studies. The National Academy of Sciences exposure assessment group is developing a list of recommendations that can guide addition of environmental exposure assessment to existing studies.

- **Issues to consider in biospecimen collection, storage and analysis.**

Additional attention to strategies for the collection, storage and analysis of biospecimens is needed. One approach is to measure every exposure possible and collect and store every biospecimen possible. The rationale for this approach is that sufficient evidence to identify compounds of interest may emerge long after samples have been obtained. Studies could benefit from expanding the types of biospecimens collected to include diapers, cord blood and newborn blood spots. Issues to be addressed in biospecimen collection and storage include developing standard protocols, determining how long samples should be stored to remain viable, and limits on the number and amount of samples that can be obtained from infants and young children. A related question concerns when to use biospecimens, which are considered precious and rare resources. What threshold marks sufficient evidence to warrant depleting them to test for the presence of environmental compounds of interest? The answer may be when suspicion emerges from several different avenues—evidence in animal models as well as in cellular models, and prevalence of the exposure in the “real world.” Development of technology and methods to reduce the amount of sample used in analytic assays will be helpful and should be encouraged.

- **Harnessing expertise of practicing clinicians**

To find clues to environmental contributors as well as possible treatments for further study, investigators should consider careful biological and behavioral characterization of children with autism during regressive periods, as well as children who show symptom improvement, including remission or recovery. Efforts to more fully characterize regression and improvement and the implications for treatment will require more substantial efforts to engage clinicians. Clinicians know patients well and can make very interesting observations, but there is not a formal mechanism for funneling those observations into hypotheses for rigorous scientific study. Autism Speaks operates an Autism Treatment Network that includes a database of detailed characterization from clinicians of more than 3,000 children with ASD. This kind of approach lends itself also to the study of cases with comorbidities such as gastrointestinal dysfunction and natural experiments that are already ongoing, such as children who are treated with gluten-free diets and other nutritional interventions. Studies that examine which phenotypes benefit from such diets and other interventions may be useful. Involving environmental health scientists in that network may be fruitful.

- **Involvement of biostatisticians**

Large-scale studies of the role of gene by environment interactions in disease will pose analytical challenges, so it will be important to collaborate with biostatisticians from the outset. These challenges include the uncertainty in ascertainment of cases, errors in exposure assessment, and integrating different data sets (e.g., genetics, epigenetics, metabolomics). The use of case-only designs (when needed assumptions are met) to detect the presence of a gene by environment interaction may provide

efficiency. In making all these analytical decisions, it will be important to collaborate with the biostatisticians in study design stage, rather than as an afterthought.

## **Other Ways to Accelerate Progress**

Suggested mechanisms to attract the best and brightest scientist to autism research include funding for predoctoral and postdoctoral fellowships, as well as programs to inspire undergraduates. Sharing postdoctoral fellows among disciplines may be a unique avenue for promoting the multidisciplinary work needed to discover solutions to autism. Development of research capacity in countries outside the United States (US) should include efforts to support training of international scientists and collaborations between US and international scientists.

Funding mechanisms are needed that encourage collaborations among clinicians and basic scientists or among exposure scientists and people who develop unique animal models (zebrafish). Such mechanisms may attract scientists who are new to the field of autism.

An internet-based clearinghouse of multidisciplinary research findings and tools would serve as a place to “connect the dots.” For instance, perhaps PhenX (a project funded by the National Human Genome Research Institute (NHGRI) to help integrate genetics and epidemiology data) can be informed from an ASD perspective. The Autism Genetics Resource Exchange (AGRE) has an environmental questionnaire and this could be shared and used to enrich data collection in clinical studies.

Efforts to increase analytical capacity and core facilities are needed. For example, adding an environmental, immune or animal models core to an already existing multidisciplinary team that studies autism would be beneficial. Access to these core facilities and services could encourage individual scientists to expand the scope of their studies to address environmental hypotheses.

A multidisciplinary advisory group focused on the environment could help to evaluate progress and shape future efforts (e.g., initiatives). Such a group could provide constant feedback, which may be more effective than one-time meetings.

## **Summary of research and resource recommendations**

- **Epidemiology**
  - Expand epidemiology studies to include international or other special populations – varying in exposure or prevalence
  - Capitalize on existing epidemiology studies that could be mined or expanded to identify autism cases and make use of exposure information and biosamples that have been collected and stored (e.g., placenta, diapers, blood spots, cord blood)
  - Standardize and disseminate environmental exposure data collection instruments and analysis protocols to enable pooling data and/or valid comparisons among studies
  - Engage biostatisticians to develop and apply efficient and sensitive methods to detect joint effects of genes and environment

- Assemble large data sets allowing mapping of detailed genetic, detailed environment and detailed phenotypic information—also should include medical comorbidities, inflammatory markers etc.
- **Novel approaches and technologies**
  - Epigenomics—this should be given high priority, as environmental influences may operate through epigenetic mechanisms that alter chromatin structure and ultimately, gene expression; consider both germline and somatic effects
  - Explore bioinformatics approaches to identify environmental risks—convene a one day workshop to determine whether the science is ready and how to begin.
  - Explore use of induced pluripotent stem cells as a translational tool
  - Explore how to reach out to clinicians who treat individuals with autism to mine their observations—this is occurring in the Autism Treatment Network, but could be expanded to include more systematic data collection on environment
  - Support incorporation of personal exposure sensors and biomarkers of response to exposure as they become available
  - Explore potential differences in microbiome of individuals with autism vs. comparison groups
- **Topics of special interest**
  - Regression--aggressively study regression as it occurs to identify potential environmental triggers
  - Mitochondrial impairment—explore role of mitochondrial impairment in autism, including developing less invasive ways to measure
  - Sex differences--support studies that are guided by sex differences in autism risk to identify environmental contributors
- **Model systems**
  - Support development and refinement of animal models and model systems—these should be conducted in parallel with human genetic and epidemiology studies so that rapidly emerging knowledge of autism biology and pathways can be pursued in models
    - Models enabling study of how environment influences synaptic proteins implicated by genetic findings
    - Models incorporating neuro-immune interactions are needed
    - Develop full range of models, including use of alternative model organisms
- **Resources**
  - Need to expand national resources for measurement of environmental compounds—current resources are operating at capacity
  - Develop relevant facility/service Cores to support investigators who could pursue environmental hypotheses
    - immune profiling
    - animal models
    - epigenomics

- More neuropathology—tissue can be examined for pathology and for presence of toxicants. Requires greater awareness, education, development of protocols for preparation and storage; consider collecting extracranial tissues
- **Workforce**
  - Consider initiatives that encourage collaboration across disciplines/consortium approach
  - Develop easy to use web-based clearinghouse of information about existing data sets, laboratory assays and other resources to orient individuals new to the field
  - Build international capacity—initiatives that encourage collaboration with international investigators or training targeted at international scientists
  - Funding for shared postdocs to build long term collaborations between PIs
  - Teaching/outreach beginning early (e.g., guest lectures in schools)

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