Question 3: What Causes ASD, and Can Disabling Aspects of ASD Be Prevented or Preempted?

Aspirational Goal: Causes of ASD will be discovered that inform diagnosis, prognosis, and interventions and lead to prevention or preemption of the challenges and disabilities of ASD.

Since the last Strategic Plan update published in 2013, there have been substantial advances in the understanding of factors that contribute to a diagnosis of autism spectrum disorder. Few would dispute that the causes of ASD are many and include both genetic and environmental factors. There has been an increased appreciation in the last five years of the incredible complexity and interplay of these factors in the development of autism. Indeed, modifications in more than 100 genes are now known to increase the probability of an autism diagnosis [1,2] and very reasonable predictions are that 1,000 or more autism risk genes may ultimately be identified [1]. A plethora of potential environmental challenges have also been associated with autism, although studies in this area have not undergone the same exponential progress as genomics research. There has also been increasing emphasis on events during pregnancy, such as influenza infection, as potential causes of neurodevelopmental disorders. But, these studies have raised the interesting issue that environmental risks affect different people differently. This is the so-called "genes by environment" interaction. More and more modern medical problems are linked to the combination of a particular genome and a particular life history of environmental exposure (the exposome).

The title of this chapter, which has been modified from the 2013 Strategic Plan update, emphasizes the desire to understand the causes of the disabling aspects of autism spectrum disorder. These go beyond the core symptoms of deficits in social communication and the occurrence of restricted patterns of behavior or interests to include what are typically referred to as comorbid symptoms. In many cases, progress on the causes of these co-morbid symptoms is ahead of that for the core symptoms of autism. There is a growing appreciation that the causes of these medical problems urgently need to be addressed. They may be due to biological factors that are also causal of autism, manifestations of autistic behavioral problems such as poor diet that may lead to medical issues, or medical access issues which lead to poorer medical care. Regardless of the causes, this situation is beginning to receive much more research attention and efforts at resolution - though more effort in this area is needed.

The title to this chapter has changed (from "What caused this to happen and can it be prevented?") because the neurodiversity movement has had a great impact on the IACC and on the premises of the Strategic Plan enterprise. It is fully appreciated now that some features of autism should not necessarily be targets for prevention. As discussed above, it is the most disabling features of autism that are now the major targets of prevention or preemption. Discussions of the causes of ASD always ultimately touch on efforts at prevention. In the hypothetical situation that a known cause of autism is identified, the question arises whether the cause should be eliminated thus preventing some cases of autism. If the discussion were related to cancer, the answer would be clear. But, in autism it is not. There is clearly an increased sensitivity to any procedure or practice that would be directed at preventing the totality of autism, and this is reflected in the emphasis of this chapter.

Genetic Risk Factors

The application of genomic studies has significantly advanced the understanding of genetic risk factors for ASD. Dozens of new ASD susceptibility genes have been identified over the past 5 years and genome-

wide technologies have facilitated a molecular diagnosis in ~5-40% of ASD cases. This range in the rate of detection depends on the cohort examined and the technology used. Studies comparing individuals with ASD to typically developing controls have indicated that as much as 7-8% of individuals with ASD carry either a large pathogenic DNA deletion or duplication [5,6,7, 8]. Earlier results from DNA sequencing studies have further confirmed the role of gene-disruptive mutations to ASD [9,10,11,12], and more recent data also support and add to these findings [13,14,15]. The rates of *de novo* (spontaneous, non-inherited) likely gene-disruptive (LGD) and missense mutations (which result in amino acid changes) are significantly higher in individuals with ASD compared to their unaffected siblings (while the rate of synonymous mutations which do not alter amino acid sequence or biology does not differ) [14]. As much as 21% of autism diagnoses may be accounted for by *de novo* single-nucleotide variant (SNV) and insertion/deletion mutations [16]. Additionally, inherited LGD mutations have been found to show a preferential transmission from mothers to sons, indicating another potential risk factor contributing to an estimated 8% of autism diagnoses [17]. Recent whole genome sequencing studies of a large diverse ASD collection revealed a molecular basis in 11.2% of participants, including the finding that 7.2% carried copy number variations (CNVs; repeated sequences in the genome that vary between individuals) or chromosomal abnormalities [18], which further emphasize the need to use comprehensive genomic technologies [19]. Overall, a consistent observation emerging from genomic studies is the vast genetic heterogeneity involved in ASD [20, 21,22].

Follow-up evaluation of individuals with ASD who have disruptive mutations to the same gene or genomic region, has further illuminated features and patterns of behaviors (sub-phenotypes) linked to these genetically defined subgroups. Clinical characterization of cohorts with disruptive gene mutations has revealed real, but subtle, phenotypic patterns tied to particular genes. Patterns of behavior linked to sub-phenotypes can prove helpful for establishing guidelines of care for clinicians. While major advances have been made through the application of genomic technologies, gaps exist in our understanding of the contribution of regulatory and other genomic regions to ASD risk. Whole genome sequencing will begin to illuminate the role of non-gene coding regions of the genome.

Heritability
ASD is highly familial, so that siblings of children with autism are 10-20 times more likely to receive an ASD diagnosis themselves than non-siblings [23, 24, 25]. Twin studies beginning in 1977 [26] have provided significant evidence that ASD is strongly associated with genetic factors. All 13 twin studies on autism to date have found genetic and environmental contributions to autism, although the proportions of the two factors and interpretations have varied substantially. One research team [27], for example, concluded that over 50% of the risk for autism in identical twins could be explained by shared environmental factors, whereas genetic heritability accounted for 37%. This somewhat surprising finding—that environmental factors contribute more substantially than genetics—has been challenged by a more recent, large-scale twin study [28], which found that the largest contribution to autism liability comes from additive genetic effects. A recent meta-analysis concludes that the causes of autism are due to strong genetic effects, and that shared environmental influences are seen only if the most severe forms of autism are included [29]. A recent survey of autism twin studies finds that concordance for monozygotic twins is roughly 45%, versus 16%for dizygotic twins [30]. Thus, twin and family data suggest that genetic variation between people accounts for a very substantial portion of the liability to ASDs at a population level. But, if autism had a completely genetic etiology, we would expect a much higher concordance rate in monozygotic twins; the lower rate may reflect, in part, that even monozygotic twins do not share an identical environment prenatally.

Genomic Architecture
The recent successes in ASD genetic studies have confirmed the importance of genetic risk factors. Similar to other common psychiatric disorders, the genomic architecture of ASD is complex, involving both common and rare forms of genetic variation [31], including common polygenic (multi-gene) variation, *de novo* variation, copy number variation, and inherited rare variation [32, 33, 13, 34, 14, 17, 21, 35]. Common polygenic variation may account for the greatest fraction of genetic influence, and approximately 20-50% of population liability. *De novo* variation accounts for less liability at a population level, but can have a very strong impact on the individuals who carry such variants [34, 14]. These data represent population risk; a crucial next step is integrating our understanding of rare variants of large effect with more common polygenic risk factors to more accurately predict ASD on an individual level.

Sex Differences
The overrepresentation of males among those diagnosed with ASD has been observed for decades [37, 38, 39]. Overall, the male to female (m:f) ratio is approximately 4:1, but that ratio varies substantially based on IQ and other features of ascertainment [37, 40, 41]. Specifically, in individuals with ASD and very low IQ, the male:female ratio is commonly estimated at 2:1 or 3:1; in individuals with ASD and high IQ, the m:f ratio become very large, often 7:1 or greater. This pattern has been consistently observed during the period of otherwise rapid changes in the epidemiology of ASD.

There are several theories as to why males and females might differ in their observed ASD liability [39]. Among biological theories, the extreme male brain hypothesis posits that the male brain is predisposed to many features that are on the ASD spectrum [42, 43]. The 'female protective effect' (FPE) hypothesis in ASD is amongst the most commonly investigated in recent genomics studies [44]. The FPE hypothesis suggests that females are 'protected' from ASD (for unspecified reasons) such that, on average, a greater aggregation of risk factors is necessary to produce a case. In the context of *de novo* and gene-disruptive inherited variation, that suggestion has been supported by the recent genetics literature [14, 45, 17, 8]. Deleterious CNVs are three times more likely to be identified in autistic females when compared to males [45], and loss-of-function mutations show a maternal transmission disequilibrium suggesting mothers are more likely to be carriers of such mutations than fathers [17]. Similarly, classes of *de novo* variation that are strongly associated with ASD risk are approximately twice as likely to be observed in female cases, as compared to male cases. Recent gene expression analysis demonstrates that autism risk genes, rather than being sexually dimorphic themselves, interact with pathways and cell types that themselves are sexually dimorphic [46].

Overlap with Other Disorders
Neuropsychiatric and developmental disorders share many genetic risk factors, and this varies depending on the specific disorders being compared [32, 49, 50]. ASD shares common risk genes with other neuropsychiatric disorders such as schizophrenia [PMID: 28540026] [PMID: 27145529], and autism is sometimes a feature of other neurodevelopmental syndromes such as Fragile X Syndrome, Rett Syndrome, Tuberous Sclerosis, and Phelan McDermid Syndrome [PMID: 9813777, 15070547, PMC2814423, 8132114]. The common, polygenic influences on ASD risk are similarly associated with multiple phenotypic outcomes (different combinations of risk genes can lead to different neuropsychiatric and developmental conditions). In recent studies, and in contrast to the *de novo* findings, common polygenic risk for ASD has been positively associated with general cognitive ability, logical memory, and verbal intelligence [51]. On average, ASD shares most risk with schizophrenia in the population, followed by bipolar disorder, but very little with substance abuse or major depression. It does not appear that this overlap involves the majority of common genetic risk for each disorder, and

the extent to which overlap occurs, and what biological factors it represents, remain under investigation.

Rare familial mutations that cause syndromic ASD and *de novo* (spontaneous) genetic influences on ASD risk are also strongly associated with intellectual disability, epilepsy, and global developmental delay [14, 52]; neurological comorbidities are identified in the majority of children with ASD [53, 54]. Some of the ASD-associated *de novo* events that result in protein truncation are more likely to be seen in cases of intellectual disability than in ASD itself. Similarly, several CNVs that increase risk for schizophrenia, also increase risk for ASD [55, 56, 57, 58, 59]. This is consistent with these mutations having major effects on brain development, which subsequently can manifest as different clinical outcomes. However, there are many intellectual disability genes that do not appear to increase risk for ASD [60], so understanding why some large-effect mutations that cause intellectual disability substantially increase risk for ASD, while others less so, remains an area of future investigation [61].

Genetic Testing and Communication of Risk
Genetic testing is recommended by the Accreditation Council for Graduate Medical Education for those at increased risk for ASD [62, 63]. This includes chromosomal microarray (CMA) followed by Fragile X testing and other specific tests depending on the phenotype. Several studies review the current recommendations for genetic testing in ASD [54]. In addition to its usage in research studies, whole exome sequencing (WES) has been shown to have a high yield in clinical populations with developmental disorders including ASD. Thus, we expect that WES will gradually supplant CMA [64, 65].

Given the incomplete penetrance of many large effect or familial genetic risk factors, care must be taken in pre-symptomatic or prospective risk counseling. Further understanding of the causal relationship between identified ASD risk genes and clinical outcomes is needed before guidelines for genetic counseling can be illuminated. Understanding parental concerns and attitudes when communicating complex genetic information that has an impact on family planning is also important [66, 67].

New Large-Scale Genetics Studies Launched in the Last 5 Years
Studies of the genetic architecture of ASD has resulted in the appreciation that much larger groups of subjects are needed to fully understand its complexity. In the last 5 years, several large-scale projects have been initiated. Recent large-scale efforts include MSSNG (funded by Autism Speaks), which seeks to sequence the genomes of 10,000 individuals affected by ASD, and the SPARK study (funded by the Simons Foundation), which seeks to sequence exomes of 50,000 families affected by autism. These studies are expected to not only identify additional autism risk genes but to also contribute to an understanding of the common variant patterns that enable expression of the mutations.

Policy Implications of Advances in Genomic Science
New technology and testing can also lead to increases in health care disparities; we must be vigilant to avoid this and support policies that enable access to all. Because of differences in population histories, understanding of genetic risk in one population may not be informative in others. This imparts an imperative to study diverse populations. Further, given the role of rare variants that will have very distinct frequencies in different populations, having information from diverse populations will be critical for the interpretation of genetic studies. When predictive testing is performed, care must be taken to ensure accurate prospective/predictive testing and that information about accurate probabilities of particular outcomes are communicated effectively and not mistakenly understood as absolutes. This requires genetic counselors or other professionals trained specifically in the communication of genetic risk to patients.

Environmental Risk Factors, Individually and in Combinations Over Time

The growing number of studies that are exploring environmental risk factors reflect an emerging consensus that non-genetic avenues of research are also likely to bear fruit. In this Strategic Plan, it is advantageous for the IACC to adopt a broad definition of studies on "environment" as encompassing research on all potentially non-heritable etiologic influences. This includes studies of exogenous exposures such as pesticides, endocrine disrupting and other industrial chemicals, pharmaceuticals, heavy metals, infectious agents, dietary factors, as well as other factors, such as parental age, maternal medical conditions, birth complications, and time between pregnancies. Some of these "environmental" factors might themselves be genetically influenced, while others might be mediating the effects of exogenous exposure.

Prevention or Amelioration of Disabling Aspects of ASD
Research on environmental contributors to ASD should routinely collect and make use of data on specific ASD symptoms, the levels of symptoms and impairment, as well as co-occurring conditions. As linkages between exposures and specific impairing aspects of ASD are revealed, public health strategies can be tailored to prevent or mitigate these features by reducing harmful exposures and/or increasing factors that confer protection or resilience. Additionally, improved understanding of what role environmental factors play in ASD severity (including risk for co-occurring conditions) might eventually inform strategies for identifying children in need of specific types of early intervention services.

Susceptible Periods During Development
The concept of windows of susceptibility, a central principle in environmental health sciences, is relevant to studies of environmental risks in ASD. Many lines of evidence point to prenatal origins of ASD [68, 69, 70, 71, 72, 48]. In addition, very large epidemiological studies link maternal bacterial or viral infection during specific times of pregnancy to increased risk for ASD in the offspring [73].

The periods of prenatal development that are most relevant to environmental risks for ASD are incompletely understood, however, and may be dosage- and/or exposure-dependent [74]. When considered together, many existing ASD studies suggest that preconception and early gestation are vulnerable periods for environmental exposures. This has been supported by previous reports linking autism symptoms to maternal ingestion of drugs such as thalidomide (25, 26) and valproic acid (26, 27). Other factors associated with ASD risk include preterm birth, (35–38) advanced maternal and paternal age at conception (39–45) and short inter-pregnancy interval (46, 47). In addition, the preconception/periconception period may be critical for the observed association of decreased ASD risk with maternal folate intake [75, 76].

A few of the studies on air pollution exposure suggest an enhanced risk in the later part of pregnancy [77, 78]. Evidence from the broader neurotoxicology literature [82], also indicates that exposures in the late prenatal and early postnatal periods can exert significant effects on a wide range of brain and behavior phenotypes during the first years of life. All of these time windows cover critical stages of rapid brain development and are also characterized by immaturity of both the immune system and metabolic detoxification mechanisms. These features combine to offer vulnerability and provide biological plausibility for environmental impact on ASD risk extending from preconception into the early postnatal period. Additional attention to the timing of exposures relative to the cascade of events that unfold during brain development is needed to identify and understand the molecular basis of exposure-

associated ASD risk. With this in mind, study designs and biomarkers of exposure should be chosen to capture prenatal and early life exposures.

Studies in Large and Diverse Populations
While the number of ASD epidemiology studies and the resulting data are growing through efforts such as the Early Autism Risk Longitudinal Investigation (EARLI) and Markers of Autism Risk in Babies-Learning Early Signs (MARBLES), most potential environmental risk factors have not been investigated sufficiently to draw firm conclusions [83]. The limitations inherent to observational studies mean that multiple studies in different populations and settings, with high quality measures of exposure and adequate controls, are needed to reconcile disparate findings and establish robust linkages of an environmental exposure to ASD risk. The likelihood that many different factors, each with modest effect, will contribute to ASD means that large sample sizes may be needed to detect associations with exposure, especially for those exposures with low prevalence.

Under-represented minority communities and low income communities often face disproportionate exposure to harmful environmental chemicals [84, 85, 86]; additional attention is needed to ensure that these populations are represented in ASD research and that disparities in environmental risk factor exposure are addressed. Inclusion of these vulnerable population subgroups in ASD studies may, in some regions, be particularly challenging when studies recruit from young children with a past ASD diagnosis. Data from the Autism and Developmental Disabilities Monitoring (ADDM) Network indicate that non-Hispanic black children (NHB) are significantly less likely than non-Hispanic white (NHW) children to receive an early comprehensive developmental evaluation [87], and Hispanic children are less likely to receive an ASD identification by age 8 in comparison to NHW and NHB children. These findings underscore the need to carefully consider case ascertainment strategies that do not rely solely on previous ASD diagnosis in designing studies of ASD risk factors.

Given the clear differential in ASD risk in males and females, studies which examine risk and protective factors within sex-specific subgroups are especially important; however, given the lower ASD prevalence in females, nearly all studies to date have not had a sufficient sample of females to conduct such analyses. Thus, additional efforts are needed to increase representation of females in ASD studies to enable meaningful analyses of sex-specific differences and the role of both genetic and environmental factors in affecting those differences. The Environmental Influences on Children's Health Outcomes (ECHO) initiative of the NIH is combining data from more than 78 cohorts comprising approximately 50,000 children and 40,000 women. Although the extent of ASD-related measures that are, or will be, included in ECHO has not yet been established, this initiative represents an exceptional opportunity to study ASD-related traits in large and diverse populations.

Exposure Science
One of the most significant obstacles facing epidemiologic studies of environmental risks for ASD is exposure assessment. In many studies, exposure measures are not readily available for very early developmental periods and rely on indirect methods (e.g., participant recall of prior exposures), or utilize one or two biologic measurements of compounds with very short half-lives. Direct exposure assessment, such as through personal monitoring or use of an adequate time-course of exposure biomarkers, is expensive and burdensome for participants. Consequently, deep characterization of exposure during etiologically relevant time periods is typically limited to studies with small numbers of participants, yielding low power.

The recent development and application of the concept of the "exposome" [88, 89] represents a key advance that could accelerate progress in identifying environmental risks for ASD. The concept of the exposome calls attention to the totality of exposures across an individual's lifespan [90, 91]. In addition to the universe of external environmental factors, the exposome concept can be extended to include endogenous biomarkers of exposure response – internal exposures that originate from metabolism and other cellular processes – as well as more general external factors that constitute social determinants of health. Measuring the exposome comes with challenges in capturing and integrating many individual measures over time. Recognizing that no single approach or tool likely will suffice, the field is embracing a multi-faceted strategy, using multiple tools to help characterize the exposome. For example, use of personal sensors and mobile devices can be harnessed to capture many aspects of the exposome in real time. Refinement of more targeted, conventional exposure assessment tools also has a place in characterizing the exposome.

General "omics" approaches such as transcriptomics, proteomics, metabolomics, and epigenomics show promise in identifying molecular response profiles that can be linked to exposures [92, 93, 94], and in some cases, these profiles persist over time. These downstream biomarkers may suggest groupings of exposures that operate by similar pathways. Linking direct measures of either individual or classes of exposures or the broad exposome with early "omic" markers of biological response in both targeted and non-targeted data analyses can provide complementary information of potential etiologic relevance.

Because exposomic approaches have the potential to generate high-dimensional exposure data, discovery-based analytic methods analogous to those being used in genomics can potentially be applied to uncover novel environmental risk factors – ones that would be missed by approaches that focus on a small number of established or suspected neurotoxicants. An exposomic approach also is well-suited to the simultaneous consideration of multiple exposures and risk factors; although, as the genomics field has learned, very large samples are needed to achieve significance in these kinds of analyses.

Linkages between Genes and Environment

Gene and Environmental Studies

Despite general agreement that both environment and genetics contribute to ASD risk, only modest progress has been made in identifying gene-environment interactions. A few epidemiology studies, such as the Childhood Autism Risk from Genes and Environment (CHARGE) study, have reported an interaction of exposures with common or rare structural genetic variation [4, 76, 95, 96, 97]. However, each study focused on different combinations of exposures and genes, they lack independent replication, and two of these studies addressed severity of symptoms rather than impacts on incidence [4, 97]. Many large ASD genetic collections have been assembled but most include minimal or no exposure information. On the other hand, studies focused on environmental risks often feature deep exposure assessment and have incorporated some genetic information, but smaller sample sizes constrain the power of gene-environment interaction analyses.

A concerted effort is needed to enrich existing ASD studies by adding genetic data collection to environmental studies and exposure measures to genetic studies. Identifying and accounting for genetically driven effects on exposure levels is critical for interpretation of ASD-exposure associations. In the autism literature, it is notable that the genes implicated in the folic acid association with decreased ASD risk have not emerged in any of the genetic studies using genome-wide screening; only in mothers who had low folic acid intake do those one-carbon metabolizing genes appear to play an

etiologic role [95]. At this stage, it appears that the presence of a 'main effect' (an association not dependent on another factor) is not always detectable and that pursuit of interactions should not require main effects of a specific environmental factor or gene/SNP, a priori.

Availability of low-burden exposure measures that can be incorporated in large-scale genetic studies, perhaps leveraging innovations in exposomics or epigenomics, is a high priority. Once these data exist in concert in large sample sets, new statistical and analytic approaches for gene-environment discovery in human population research can be applied [100]. Polygenic risk scores have seen increasing use in complex disease studies and can yield improved efficiency for detecting interaction of genetic risk with candidate environmental exposures. The construction of a "polyenvironment" score, analogous to a polygenic risk score, could be explored to summarize information from several exposures thought to be acting through common mechanisms for use in genetic/genomic studies. Other approaches might include measures of genomic instability such as global copy number burden, used in two different gene-environment interaction studies [97, 101].

Mechanisms of Environmental Risk and Gene-Environment Interaction
Increasing knowledge of genetics has led scientists to understand gene pathways that affect neural circuits rather than single genes acting in isolation. Early studies have demonstrated the convergence of genetic influences and environmental factors in the activity of these different gene pathways [102], providing evidence that genes and the environment might work synergistically, rather than additively. Studies that move beyond identification of genetic and environmental risk factors to reveal functional biological consequences associated with these risk factors are a priority. Epigenomics, metabolomics, transcriptomics, and proteomics can provide useful functional readouts for this purpose.

Model systems provide an attractive means for supporting causality and understanding biological mechanisms that underlie associations observed in human studies. Human induced pluripotent stem cells (hiPSCs) generated from individuals affected by ASD with a known genetic background are being used increasingly to study ASD [103]; these provide a unique opportunity to assess susceptibility of early developmental processes to environmental chemicals in the context of defined genetic risk [104]. There are a few reports of screening or computational approaches used to identify possible environmental exposures that could be priorities for pursuit in human studies [102, 105]. The Collaborative Cross and Diversity Outbred mouse populations [106] represent important mouse resources that could be harnessed to dissect the contribution of environment to complex disorders such as ASD. Additional efforts that bring together interdisciplinary teams to facilitate integrative analyses and bidirectional flow of clues from human observational studies to laboratory-based experiments in model systems are warranted.

In addition to the utility of epigenomics (the study of the complete set of epigenetic modifications – such as methylation – on the genetic material of a cell) as easily attainable exposure biomarkers, many researchers recognize the potential for mechanistic roles in ASD. Epigenomics is a leading candidate for mediating effects of exposures on regulation of transcription (the first step in gene expression) [107, 108, 109] and could provide a point of convergence for genes and environment in autism risk. Multiple lines of evidence implicate altered epigenetic marks in ASD etiology. Several known genetic disorders with ASD-related presentation, such as Fragile X and Angelman's syndrome, have established epigenetic mechanisms. Further, results from rare-variant ASD genetic discoveries point to remodeling of genetic material as a shared pathway in ASD genetic risk. Few studies have directly examined chromatin marks or

DNA methylation for association with ASD, but some small studies have observed associations [92, 110, 111].

A significant body of work demonstrates that environmental chemicals can alter DNA methylation, and these alterations have been linked to changes in gene expression and a range of behavioral phenotypes [112, 113, 114]. ASD studies that integrate methylation, exposure, and phenotype data in the same population are a priority. Research to establish whether epigenetic marks measured in peripheral tissues are predictive of changes in target tissues is especially important for interpretation of human studies [115]. Also needed are studies that identify exposure-induced impacts on a full range of epigenomic mechanisms and that determine their relevance to ASD. Finally, research to understand how exposure-induced epigenomic changes may transmit autism risk across generations is warranted.

Multivariate Risk across Complex Systems
There is a need to capitalize on findings emerging from existing studies to examine how genetic and environmental factors interact to contribute to phenotype, not only at the molecular and cellular level, but also in the broader physiological context. For example, a substantial body of work implicates immune dysregulation in ASD, including the association of ASD with maternal infections and autoantibodies, cytokine and other immune biomarker signatures, functional alterations in immune cell subsets [116], and differential expression of innate immune and inflammatory genes [117]. These findings together have motivated studies exploring how a range of environmental exposures may contribute to the immune alterations observed in ASD, some of which have been detectable at birth [118].

The endocrine system is another promising area of inquiry. The established role of hormonal systems in brain development, the marked male bias in ASD, and a growing recognition that many environmental chemicals act as endocrine disrupting chemicals (EDCs) sets the stage for investigations exploring possible links between ASD and EDCs [119]. Some research studies have suggested that factors that protect females from autism risk may be found on hormone-sensitive genes [120] and these could be targets for EDCs. Further work elucidating connections across metabolic, hormonal, and central nervous systems in the context of EDCs is needed.

The microbiome (the combined genetic material of the microorganisms in the body) represents a third priority area of inquiry, with increasing evidence for links between the gut microbiome, brain, and behavioral phenotypes relevant to ASD [124, 125]. The microbiome is also emerging as an important component of response to environmental exposure. Studies have demonstrated persistent changes in the function of the microbiome after exposure to immune activation and environmental chemicals [126, 127], particularly during early life when the microbiome is being colonized. A role for the microbiome in metabolism of environmental chemicals is now established [128, 129]; this means that differences among individuals in microbiome composition can affect the internal dose and biotransformation of toxicants and act as susceptibility factors. Small clinical studies using antibiotics or microbiome transplant support a potential role for microbial imbalance in contributing to core ASD behaviors. Taken together, these data suggest possible linkages among exposures, microbiome function, and ASD phenotypes.

Resource Needs for Accelerating Research in Studies of Environmental Risks and Gene Environment Interaction

Broad Data and Resource Sharing
As the number of studies focusing on environmental risks for ASD increase, attention to broad data access and sharing becomes critical for enabling reuse and extracting the maximal value from the data that have been collected. Consideration of privacy and consent issues in environmental health data is needed to ensure the development and implementation of policies that protect privacy while ensuring the value of shared data. Combining data across observational studies can yield increased power and strengthen generalizability, yet heterogeneity of the types of exposure measures used creates challenges for both meta-analyses and pooled analyses of primary data. On the other hand, when different types of measures of exposure in different studies all lead to consistent findings, that consistency alone increases confidence in the conclusions.

The development of consensus data standards will make it possible for investigators to consider, at the outset of a study, inclusion of common environmental measures/standards [130]. Use of low burden exposure measures, such as those available through PhenX [131] or the Early Life Exposure Assessment Tool (ELEAT), enable genetics researchers to enrich their analyses to account for environmental contributions to risk. Increased sharing of study-specific exposure instruments and methods is another area of need. The National Database for ASD Research (NDAR) currently provides a robust platform for making data – particularly data in standard formats – easy-to-find and accessible. Implementing common data standards for exposures could facilitate the incorporation of these type of data in NDAR.

In addition, genetic databases including MSSNG and the Autism Sequencing Consortium (ASC) are anticipated to provide mechanisms for expanded data libraries to include key environmental variables, allowing for assessment of gene/environment interactions. Recent findings [4, 97] illustrate the benefits of incorporating environmental information in large data resources. With regards to mechanistic tools, new models of ASD, especially those with distinguishing genetic mutations of interest, should be made widely accessible to researchers. This can include, but is not limited to, sharing breeding pairs of animal models with commercial vendors for their widespread distribution. Finally, to realize the potential impact of data sharing, efforts must be put into the analytic approaches needed to make gene-environment discoveries from the aggregation or collective analysis of large heterogeneous data sources. Efforts that encourage methodological development as well as bioinformatics implementation and secondary data analysis funding will be necessary.

Interdisciplinary Training and Career Development
The workforce needs related to environmental research in ASD align with an increasing recognition that solving complex questions will require team science approaches. Programs and opportunities that train scientists and support research and networking programs in ways that encourage crosstalk and coordination of efforts spanning cellular and molecular neurobiology, toxicology, genetics, epidemiology, and exposure science are needed. Training opportunities should be created around novel statistical and big data approaches geared toward complex exposure data, with goal of accelerating analyses that address multivariate risk.

Public Health Implications

Communication and Dissemination Activities for Environmental Risk Findings
The multivariate risk structure of ASD, with many factors contributing modest risks, and different combinations of risks likely to operate in different individuals with ASD, presents challenges for communicating findings to affected families and the broader public. Epidemiologic studies that report associations of specific exposures with ASD at the population level can lead to serious misinterpretation if extrapolated to individual cases, and a focus on individual risks can mask the importance of exposures whose modification could have substantive impact when measured across the population. Moreover, the limitations inherent to observational studies means that results of a single study require additional independent studies for replication and assessment of generalizability. Conflicting findings among studies are common, and may reflect spurious results or an unappreciated dependency of the association on other factors. Additionally, it is particularly difficult to separate the effects of some exposures from other factors, due to inherent collinearity – for example, distinguishing true medication effects from effects due to the underlying health condition for which medication was required. For these reasons, communicating environmental and genetic findings in ASD requires careful attention to context, including providing information about the strength of any newly reported finding on the scale most appropriate for the audience, the difference between cause and association, the specific potential limitations of any individual study including the possibility of unmeasured confounding, the population attributable risk, and the need for additional studies to confirm the association.

In many cases, risk factors for ASD are shared by other disorders, and corresponding public health efforts will have broader utility for protecting health than just the implications for autism. The hope of identifying environmental risk factors is that they can be mitigated is to reduce ASD-related disability.

Objectives

Objective 1: Strengthen understanding of genetic risk and resilience factors for ASD across the full diversity and heterogeneity of those with ASD, enabling development of strategies for reducing disability and comorbidities in ASD.

- Understand the contribution of regulatory and other genomic regions to ASD risk. Whole genome sequencing will begin to illuminate the role of non-gene coding regions of the genome.
- Identify additional autism risk genes but also contribute to an understanding of the common variant patterns that enable expression of the mutations.
- Understand the causal relationship between identified ASD risk genes and clinical outcomes so that guidelines for genetic counseling can be illuminated. Understand parental concerns and attitudes when communicating complex genetic information.

Objective 2: Understand the effects on ASD risk and resilience of individual and multiple exposures in early development, enabling development of strategies for reducing disability and comorbidities in ASD.
- Understand the timing of exposures relative to the cascade of events that unfold during brain development to identify and understand the molecular basis of exposure-associated ASD risk.
- Conduct multiple studies in different populations and settings, with high quality measures of exposure and adequate controls, to reconcile disparate findings and establish robust linkages of environmental exposure to ASD risk.

- Refine more targeted, conventional exposure assessment tools to characterize the exposome.

Objective 3: Expand knowledge about how multiple environmental and genetic risk and resilience factors interact through specific biological mechanisms to manifest in ASD phenotypes.

- Develop low-burden exposure measures that can be incorporated in large-scale genetic studies, perhaps leveraging innovations in exposomics or epigenomics.
- Move beyond identification of genetic and environmental risk factors to reveal functional biological consequences associated with these risk factors.
- Integrate methylation, exposure, and phenotype data in the same population.

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