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 (De Rubeis & Buxbaum, 2015; Geschwind & State, 2015) and very reasonable predictions are that 1,000 or more autism risk genes may ultimately be identified (De Rubeis & Buxbaum, 2015). A plethora of potential environmental challenges have also been associated with autism, although studies in this area lag behind 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. Nearly 64 percent of women surveyed in the US have experienced an infection during their pregnancies (Collier, Rasmussen, Feldkamp, Honein, & National Birth Defects Prevention, 2009). And maternal infection has been suggested as a risk factor for autism. Yet, obviously, infection during pregnancy does not lead to autism or any other neurodevelopmental disorder in most cases. The current thinking is that additional factors are at play to determine the risk of autism. For example, Mazina et al., (2015) reported that, among mothers who experience infection during pregnancy, the risk of autism depends on whether the mothers and fetuses also have a specific genetic predisposition. 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 what are typically referred to as comorbid symptoms. Comorbid symptoms range from sleep disorders to gastrointestinal disorders to anxiety, all of which affect large segments of the autistic population. There is a renewed appreciation that these symptoms are often the most debilitating to the individual and most problematic to the families. Given the complexities of ASD, it is unlikely that there will be a magic bullet that solves all of them. In many cases, progress on the causes of these co-morbid symptoms are ahead of those for the core symptoms of autism. While there are currently no pharmacological treatments for the core autism symptoms, there are many established drugs for the treatment of anxiety or sleep problems or gastrointestinal problems. It is further appreciated that because of differences in the severity of autism and co-morbid symptoms, some autistic individuals may require several treatments or intensive interventions, and others may not need or desire behavioral or medical help.

There is a consensus that the medical problems associated with autism 1) need to be better detected in autistic individuals - particularly those who are nonverbal or have intellectual disability and 2) available treatments should be assessed and refined for use with autistic patients. Convincing evidence has been developed that the lifespan of individuals with autism is shorter than typical and that they suffer from a
whole host of medical problems (Croen et al., 2015; Hirvikoski et al., 2016). There is a growing appreciation that the causes of these medical problems need to be urgently 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 autism has many positive and beneficial features and thus autism, per se, is not the target for prevention. As discussed above, it is the various 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 hence the emphasis in this chapter is on targeting its associated disabilities.

**Genetic Risk Factors**

**Introduction**
The application of whole exome, genome, microarray, and targeted sequencing studies has significantly advanced the understanding of the genetic etiology of autism spectrum disorder (ASD). Dozens of new ASD susceptibility genes have been identified in the past 5 years and genome-wide technologies have facilitated a molecular diagnosis in ~5-40% of ASD cases. The variation detected depends on the cohort examined, for example, idiopathic or syndromic, and the technology used.

Previous chromosomal microarray studies comparing individuals with ASD to typically developing controls have indicated that as much as 7-8% of the individuals with ASD carry either a large (>100 kbp) pathogenic deletion or duplication (Pinto et al., 2010; Sanders et al., 2011; Sebat et al., 2007), and more recent studies corroborate these findings (Pinto et al., 2014). Earlier results from exome sequencing studies have further confirmed the role of gene-disruptive mutations to ASD (O’Roak, Vives, Fu, et al., 2012; O’Roak, Vives, Girirajan, et al., 2012); (Neale et al., 2012) (Sanders et al., 2012) and more recent data also support and add to these findings (De Rubeis et al., 2014; Iossifov et al., 2014; Tammimies et al., 2015). The rates of de novo likely gene-disruptive (LGD) and missense mutations 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) (Iossifov et al, 2014). As much as 21% of autism diagnoses may be accounted for by de novo single-nucleotide variant (SNV) and indel mutations (Ronemus, Iossifov, Levy, & Wigler, 2014). 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 (Krumm et al., 2015). Recent whole genome sequencing studies of a large heterogeneous ASD collection revealed a molecular basis in 11.2% of participants, including the finding that 7.2% carried CNVs or chromosomal abnormalities (RK et al., 2017), which further emphasize the need to use comprehensive genomic technologies (Talkowski et al., 2012). Overall, a consistent message emerging from genomic studies is the vast genetic heterogeneity involved in ASD (Leppa et al., 2016; Sanders et al., 2015; Yuen et al., 2015).
Follow up evaluation of individuals with ASD who harbor disruptive mutations to the same gene or genomic region, using a genetics first approach, 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. Distinct phenotypic patterns and symptomatology have been associated with disruptive mutations to CHD8 (Bernier et al., 2014); DYRK1A (van Bon et al., 2016); ADNP (Vandeweyer et al., 2014); and with copy number variations including 16p11.2 (D'Angelo et al., 2016) and 1q21.1 (Bernier et al., 2016). 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 microarrays and sequencing, 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**

Autism spectrum disorder is highly familial, so that siblings of children with autism are 10-20x more likely to receive an ASD diagnosis themselves than non-siblings (Constantino et al., 2013; Risch et al., 2014; Sandin et al., 2014). Twin studies beginning in 1977 (Folstein & Rutter, 1977) provide strong evidence that autism spectrum disorder (ASD) is strongly associated with genetic factors. There have been a total of 13 twin studies focused on autism to date. All find genetic and environmental contributions to autism, although the proportions of the two factors and interpretations have varied substantially. One research team, (Hallmayer et al., 2011) 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 percent. This somewhat surprising finding—that environmental factors contribute more substantially than genetics—has been challenged by a more recent, large-scale twin study, (Colvert et al., 2015) which found that the largest contribution to autism liability comes from additive genetic effects. A recent meta-analysis (Tick et al., 2016) 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. A recent survey of autism twin studies (Bourgeron, 2016) finds that concordance for monozygotic twins is roughly 45 percent, versus 16 percent for dizygotic twins. 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 (Geschwind & Flint, 2015), and rare and common forms of genetic variation have been robustly linked to ASD risk, including common polygenic variation, de novo variation, copy number variation, and inherited rare variation (B. Bulik-Sullivan et al., 2015; B. K. Bulik-Sullivan et al., 2015; De Rubeis et al., 2014; Gaugler et al., 2014; Iossifov et al., 2014; Krumm et al., 2015; Sanders et al., 2015; Stein, Parikshak, & Geschwind, 2013). 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 (Gaugler et al., 2014; Iossifov et al., 2014). 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 at an individual level. For example,
it is likely that even genetic events of large impact, such as some CNVs or de novo SNVs, are modified by common variation and underlie risk in individuals with ASD (Robinson et al., 2014).

**Sex Differences**
The overrepresentation of males among those diagnosed with ASDs has been observed for decades (Autism, Developmental Disabilities Monitoring Network Surveillance Year Principal, Centers for Disease, & Prevention, 2012; Duvekot et al., 2016; Werling & Geschwind, 2013). Overall, the male to female (m:f) ratio is approximately 4:1, but that ratio varies substantially based on proband IQ and other features of ascertainment (Autism et al., 2012; Van Wijngaarden-Cremers et al., 2014; Werling & Geschwind, 2015). Specifically, in individuals with ASD and very low IQ, the m:f 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 (Werling & Geschwind, 2013). The extreme male brain hypothesis posits that the male brain is predisposed to many features that are on the ASD spectrum (Baron-Cohen, 2002; Baron-Cohen, Knickmeyer, & Belmonte, 2005). The ‘female protective effect’ (FPE) hypothesis against ASD is amongst the most commonly interrogated in recent genomics studies (Robinson, Lichtenstein, Anckarsater, Happe, & Ronald, 2013). The FPE hypothesis suggests that females are ‘protected’ from ASD (for unspecified reasons) such that, on average, a greater aggregation of genetic and environmental 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 (Iossifov et al., 2014; Jacquemont et al., 2014; Krumm et al., 2015; Pinto et al., 2014). Deleterious CNVs are three times more likely to be identified in autistic females when compared to males (Jacquemont et al., 2014) and private loss-of-function mutations show a maternal transmission disequilibrium suggesting mothers are more likely to be carriers of such mutations than fathers (Krumm et al., 2015). 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 (Werling, Parikshak, & Geschwind, 2016). This suggests that naturally occurring sexually dimorphic processes, seemingly involving glial biology may modulate the impact of risk variants and contribute to the sex-skewed prevalence of ASD. These data are consistent with the upregulation of astroglial and microglial pathways in postmortem ASD brain (Parikshak et al., 2016; Voineagu et al., 2011), which represent a consistent genome-wide finding at the level of RNA.

**Overlap with Other Disorders**
Neuropsychiatric and developmental disorders share many genetic risk factors, and this varies depending on the specific disorders being compared (B. Bulik-Sullivan et al., 2015; Cross-Disorder Group of the Psychiatric Genomics, 2013; Cross-Disorder Group of the Psychiatric Genomics et al., 2013). The common, polygenic influences on ASD risk are similarly associated with multiple phenotypic outcomes. In several recent studies, and in contrast to the de novo findings, common polygenic risk for ASD has been associated with higher IQ and greater educational attainment in the general population (Clarke et al., 2016). It is also associated with risk for several other neuropsychiatric disorders, particularly schizophrenia. On average, ASD shares most risk with schizophrenia in the population, followed by bipolar disorder, but very little with substance abuse or major depression. But, 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. For example, it is currently estimated that ASD and schizophrenia share approximately 25% of their genome-wide

Rare Mendelian genetic syndromes and de novo influences on ASD risk are also strongly associated with intellectual disability, epilepsy, and global developmental delay (Iossifov et al., 2014; Moreno-De-Luca et al., 2013) and neurological comorbidities are identified in the majority of children with ASD (Geschwind, 2009; Jeste & Geschwind, 2014). 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. A particularly salient example is the neulolin 4 mutation, which was one of the earliest ASD associated mutations (Laumonnier et al., 2004). Similarly, several CNV that increase risk for schizophrenia, also increase risk for ASD, such as the 22q11-13 deletion and CNV in neurexin 1 (Bucan et al., 2009; Hoeffding et al., 2017; Kim et al., 2008; Marshall et al., 2008; Rujescu et al., 2009). This is consistent with these mutations having major effects on brain development, which are subsequently pleiotropic when measured with respect to clinical outcomes. However, there are many intellectual disability genes that do not appear to increase risk for ASD (Stessman et al., 2017), 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 (Parikshak et al., 2013).

**Genetic Testing and Communication of Risk**

Genetic testing has a high yield and is recommended by the ACGME (Schaefer, Mendelsohn, Professional, & Guidelines, 2008, 2013). This includes chromosomal microarray (CMA) followed by fragile X testing and other specific tests depending on the phenotype. Several studies review current recommendations for genetic testing in ASD (Jeste & Geschwind, 2014). In addition to its yield in research studies cited above, more recent 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 (Fogel, Lee, Strom, Deignan, & Nelson, 2016; Lee et al., 2014).

Given the incomplete penetrance of many large effect or Mendelian genetic risk factors, care must be taken in pre-symptomatic or prospective risk counseling. Further understanding of genotype phenotype relations within ASD and 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 impact on family planning is also important (Reiff et al., 2017; Xu, Mitchell, Richman, & Clawson, 2016).

**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 five years, several large-scale projects have been initiated. Recent large-scale efforts include MSSNG funded by Autism Speaks with the goal of carrying out WGS on 10,000 individuals affected by ASD and the SPARK study, funded by the Simons Foundation, that has the goal of carrying out whole exome sequencing of 50,000 families affected by autism. These studies will note only identify additional autism risk genes but will 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 lead to increases in health care disparities, we must be vigilant to avoid this and support policy that provides access to all. Because of differences in population histories, understanding of genetic risk in one population may not be informative in others. So, 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 to interpretation of pathogenicity. When predictive testing is performed, care must be taken to insure accurate prospective/predictive testing and communication of probabilities etc. rather than absolutes in many cases. 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**

**A Broad Definition of Environment**

The growing number of studies that are exploring environmental etiologies reflect an emerging consensus that non-genetic avenues of research are likely to bear fruit. We believe it is advantageous to adopt a broad definition of “environment” as encompassing all potentially non-heritable etiologic influences. This includes both exogenous exposures (e.g., industrial chemicals, pharmaceuticals, 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 Disability in ASD**

As noted in the introductory section, the update of this Chapter reflects an important shift in focus from primary prevention of ASD to prevention or preemption of its disabling aspects. 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 germane to studies of environmental risks in ASD. Many lines of evidence point to prenatal origins of ASD. These include early reports of ASD linked to maternal exposure to thalidomide and rubella (Chess 1971, 1977; Stromland et al., 1994), as well as more recent findings from transcriptomics and pathology studies in postmortem brain (Willsen et al., 2013; Stoner et al., 2014; Voineagu et al., 2011). 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 (Atladottir et al, 2010).

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 (Schmidt et al., 2014). For example, the preconception/periconception period may be critical for the observed association of ASD risk with maternal folate intake (Suren et al., 2013; Schmidt et al., 2012). A few of the studies on air pollution exposure suggest an enhanced risk in the later part of pregnancy (Kalkbrenner et al., 2015, Raz et al., 2015). While animal studies linking maternal immune activation to autism phenotypes have focused on early prenatal exposure windows (e.g., Malkova et al., 2012), other studies making use of the maternal immune activation (MIA) model reveal varied manifestations of
behavioral abnormalities and neuropathologies depending on the gestational timing of exposure (Meyer et al., 2006, 2008).

When considered together, many existing ASD studies suggest that preconception and early gestation are vulnerable periods for environmental exposures. Evidence from the broader neurotoxicology literature (see Bennett et al., 2016), however, 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 systems combine to offer vulnerability and provide biologic plausibility for environmental impact on risk for ASD 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 corresponding body of evidence generated from those studies, is growing, most potential environmental risk factors have not been investigated sufficiently to draw firm conclusions (reviewed in Lyall et al., 2016). The limitations inherent to observational studies mean that multiple studies in different populations and settings, with high quality measures of exposure and adequate control for known and plausible confounders, 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 (Gee et al., 2002; Evans et al., 2004; Adamkiewicz et al., 2011); additional attention is needed to ensure that these populations are represented in ASD research. 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 ASD and Developmental Disabilities Monitoring (ADDM) system, indicate that non-Hispanic black (NHB) children are significantly less likely than non-Hispanic white (NHW) children to receive an early (age 36 months or younger) comprehensive developmental evaluation (Christensen et al., 2016), 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.

One of the more consistent epidemiological findings in ASD is the higher observed prevalence in males than females. The CDC reports that about four times as many boys are diagnosed compared to girls (MMWR), although this can be variable based on method of identification. Some studies report that girls with ASD are more likely than boys to have comorbid intellectual disability and more severe autism symptoms (Lai et al., 2012; Frazier et al., 2014), while genetic findings point to a higher burden of disorder-associated genetic mutations in females with ASD (e.g., Sebat et al., 2007; Sanders et al., 2012; Jacquement et al., 2014). Taken together, these findings have been interpreted as a female protective effect (Werling and Geschwind, 2013), with either a genetic or environmental factor or both conferring protection or resiliency to autism symptoms or diagnosis. One prediction of the female protective effect
model is that siblings of autistic females should be at increased risk for ASD or ASD symptomatology compared to siblings of males with autism. Recent investigations have provided mixed support for this prediction (Constantino et al., 2016; Messinger et al., 2015; Robinson et al., 2013; Park et al., 2017) and some studies have failed to observe sex differences in cognitive performance or symptom severity (Reinhardt et al., 2015; Messinger et al., 2015). 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 (such as over-sampling) 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 (Cui et al., 2016; Stingone et al., 2017) represents a key advance that could accelerate progress in identifying environmental risks for ASD. The concept of the exposome, developed as a complement to the genome, calls attention to the totality of exposures across an individual's lifespan (Wild, 2012; Rappaport, 2012). In addition to the universe of external environmental factors, the exposome concept can be extended to include endogenous biomarkers of exposure response as well as 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 times. Recognizing that no single approach or tool likely will suffice, the field is embracing a multi-faceted strategy, with exploitation of 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, from social and location data to physical activity and diet as well as chemicals in ambient air. 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 (e.g., Ladd-Acosta et al., 2016; Houten et al., 2016; Marsillach et al., 2016) 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 biologic 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 when non-targeted analyses are conducted.

**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 have reported an interaction of exposures with common or rare structural genetic variation (Schmidt et al., 2011, 2012; Volk et al., 2014; Mazina et al., 2015; Webb et al., 2017), but each focused on different combinations of exposures and genes and needs independent replication, and two of these studies addressed severity of symptoms rather than impacts on incidence (Mazina et al., 2015, Webb et al., 2017). 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. A recent genetic study of schizophrenia (Borglum, 2014) demonstrates the potential benefits of including environmental factors in risk analyses. That study revealed a novel schizophrenia genetic locus that was not evident without information about maternal infection. Another example is provided by Traglia et al., 2017, who found that both maternal and fetal genetic background contribute to circulating levels of environmental chemicals through differences in xenobiotic metabolism. 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 ASD have not emerged in any of the dozens of genetic studies using genome-wide screening; only in the presence of mothers who had low folic acid intake do those one-carbon metabolizing genes appear to play an etiologic role (Schmidt et al., 2011). Similarly, ozone has not on its own shown an association with ASD risk, however, in combination with a high global copy number burden, an association was observed (NEED REF). Replication of these findings is awaited. At this stage, it appears that 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 large scale human population research can be applied (reviewed in Gauderman et al., 2017). 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 (Webb et al 2017, Kim et al, Autism Res, in press).

**Mechanisms of Environmental Risk and Gene-Environment Interaction**

Increasing knowledge of genetics (reviewed in Chapter 2) 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 (Pearson et al., 2016), 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 elucidate functional biologic consequences associated with these risk factors are a priority. Epigenomics, metabolomics, transcriptomics, and proteomics, previously highlighted in the context of exposomics, can provide useful functional readouts for this purpose.

Model systems provide an attractive means for supporting causality and understanding biologic mechanisms that underlie associations observed in human studies. For example, epidemiology findings of increased ASD risk from air pollution prompted controlled inhalation studies in rodents (see Klocke et al., 2017) to assess behavioral, molecular, and cellular sequelae. Human induced pluripotent stem cells (hiPSCs) generated from individuals affected by ASD with a known genetic background are being used increasingly to study the pathogenesis of ASD (Ben-Reuven and Reiner, 2016); these provide a unique opportunity to assess susceptibility of early developmental processes to environmental chemicals in the context of defined genetic risk (Hogberg et al., 2013). There are a few reports of screening or in silico approaches used to identify possible environmental exposures that could be priorities for pursuit in human studies (Pearson et al., 2016; Carter and Blizzard, 2016). The Collaborative Cross and Diversity Outbred mouse populations (Chessler, 2014) 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 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 (Ladd-Acosta and Fallin, 2016; Keil and Lein, 2016; Vogel and Lasalle, 2016) 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 chromatin remodeling as a shared pathway in ASD genetic risk. For example specific genes associated with epigenetic mechanisms, like CHD8, have been linked with ASD. Brain tissue from ASD-affected individuals also reveals changes in epigenetic expression, including histone modifications and noncoding RNAs. Few studies have yet directly examined chromatin marks or DNA methylation for association with ASD, but some small studies have observed associations (Ladd-Acosta et al, 2014). Recently, a small study comparing methylation patterns in placental tissue from newborns later diagnosed with ASD and those with typical development suggested highly methylated and partially methylated domains may have
prognostic utility (Schroeder et al 2016). Likewise, Feinberg et al. (2015) reported regions of differential methylation in paternal sperm that were associated with early indicators of ASD in the offspring.

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 (see reviews by Hou et al., 2012; Feil and Fraga, 2012; Bakulski & Fallin, 2014). ASD studies that integrate methylome, 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 (Bakulski et al., 2016). Also needed are studies that identify exposure-induced impacts on a full range of epigenomic mechanisms (e.g., histone modifications, small and noncoding RNAs) 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 physiologic 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 (reviewed in Meltzer and van de Water, 2017) and differential expression of innate immune and inflammatory genes (Tylee et al., 2016). These findings together motivate 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 (Krakowiak et al., 2017).

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 (reviewed by Schug et al., 2015). Some research studies have suggested that factors that protect females from autism risk may be found on hormone – sensitive genes (Park et al., 2017) and these could be targets for EDCs. One of the most replicated findings in the literature is related to insulin regulation. A meta-analysis of twelve studies examining maternal gestational diabetes or type 2 diabetes during pregnancy demonstrated increased risks for ASD with high precision (Xu et al., 2014); this result has been consistently replicated in multiple studies, including two exceptionally large ones based on electronic medical records from HMOs (Xiang et al., 2015; Connolly et al., 2016). Related conditions of pre-eclampsia (Walker et al., 2015), and obesity have generated similar results, suggesting disruption of the complex interplay among maternal metabolism, inflammation, and fetal energy needs. Further work elucidating connections across metabolic, hormonal, and central nervous systems in the context of EDCs is needed.

The microbiome represents a third priority area of inquiry, with increasing evidence for links between the gut microbiome, brain, and behavioral phenotypes relevant to ASD (Fung et al., 2017; Vuong et al., 2017). 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 (Gao et al., 2017; Lu et al., 2014), particularly during early life when the microbiome is being colonized. A role for the microbiome in metabolism of environmental chemicals is now established (Alava et al., 2015; Rubin et al., 2014); 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 dysbiosis 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 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 (Mattingly et al., 2016). Use of low burden exposure measures, such as those available through PhenX (Hamilton et al., 2011) 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, findable 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 ASC should provide mechanisms including expanded data libraries to include key environmental variables to allow for assessment of gene/environment interactions. New findings reported by Webb et al., (2016) and Mazina et al. (2016) 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 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 medication indication. 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. 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 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 (e.g., avoiding infection and reducing exposure to pesticides during pregnancy, taking prenatal vitamins in the periconception period). 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 a large population representing the full diversity and heterogeneity of those with ASD, enabling development of strategies for reducing disability and comorbidities in ASD.

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.

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