

## Question 2: What is the Biology Underlying Autism Spectrum Disorder?

**Aspirational Goal:** Discover how alterations in brain development and the function of physiological systems lead to ASD in order to enable the development of effective, targeted interventions and societal accommodations that improve quality of life for people on the autism spectrum.

Current scientific evidence demonstrates that ASD results from subtle alterations during brain development that affect brain structure, function, and connectivity. However, our knowledge remains incomplete and significant gaps in science have stymied attempts to develop therapies to improve quality of life (QOL) for individuals with ASD. Over the course of the last decades, several studies have elucidated the role of genetic contributions to the risk of developing ASD, possibly acting through perturbations in early brain development. The biologic mechanisms by which known gene mutations cause syndromic ASD (i.e., the subtypes of ASD that are usually caused by a single genetic abnormality) by altering the underlying neural circuitry of the brain are under intense investigation. These genetic variants are associated with remodeling of genetic material, changes to ion channels (which are the basis for cellular function and communication), and proteins that regulate cell-to-cell communication. Taken together, this research suggests there may be shared features of the underlying biology across the spectrum of autism. However, we currently know very little about the precise pathways that cause the circuit abnormalities that drive the core behavioral features in ASD, and new tools promise to accelerate this area of investigation. Moreover, while there have been recent gains in understanding the nature and prevalence of comorbidities in persons with ASD, more work is needed to understand their biological basis and strategies to alleviate their symptoms.

### **Molecular Mechanisms Implicated by Gene Mutations that cause ASD or Common Variants that influence the risk of ASD**

#### **Genetic Mutations that Cause Syndromic ASD**

Genetic studies have identified more than 70 high risk genes and estimate that several hundred additional genes of this type will be identified in the future [1]. It seems likely that more than a thousand genes that confer lower degrees of autism susceptibility will be identified in the future [2, 3, 4, 5, 6, 7]. At present, the known functions of these genes converge on biologic processes important for neuronal communication and that regulate the expression of genes and proteins. The discoveries of gene mutations that cause syndromic ASD (e.g., tuberous sclerosis complex (TSC), Rett syndrome, Fragile X syndrome, Phelan McDermid Syndrome), and the dozens of rare *de novo* (new) mutations that disrupt gene function in ASD [8] have enabled scientists to explore the biological effects of the various involved genes in cellular and animal experiments. This has led to an explosion of research stretching from how these mutations alter the biology of cells to their effects on neural circuitry and rodent behavior. In some cases, the research has identified potential therapeutic targets to counteract the adverse biologic effects of a gene mutation and even reverse symptoms in animal models. The challenge remains to understand how the hundreds of implicated gene effects converge to cause ASD's common features. And conversely, how different genes and their interactions with early life events explain the biologic basis of the heterogeneity of ASD symptoms, which range from severe intellectual disability and absence of verbal language, to mild social deficits with normal cognitive function. A few examples of the clues and therapeutic implications of this work are highlighted here.

**Tuberous Sclerosis Complex:** Approximately 40-50% of persons with TSC are diagnosed with ASD. TSC mutations negatively regulate the mTOR protein complex (a protein complex that functions as a nutrient/energy/redox sensor and controls protein synthesis) and affect cell growth and synapse formation. In animal models, treatments with mTOR inhibitors reverse behavioral abnormalities [9]. These and other

therapies to treat epilepsy and brain tumors in TSC patients are currently in clinical trials, which are also examining possible beneficial effects on ASD symptoms (e.g., ClinicalTrials.gov ID: NCT02849457).

Rett Syndrome: Rett syndrome is a rare disorder caused by a mutation in the gene for MeCP2 protein that predominantly affects females and leads to developmental regression and autism-like behaviors, such as stereotypic hand movements, poor socialization and communication skills, as well as profound cognitive impairment. The MeCP2 protein regulates gene expression by affecting DNA remodeling, transcription (copying the genetic message into RNA), microRNA processing and splicing (RNA editing). Importantly, in animal models of Rett syndrome, re-expression of the normal gene in later life reversed many behavioral abnormalities [10] motivating a search for potential treatments. The MeCP2 mutation, along with other gene mutations associated with autism (Fragile X, Shank3, Gabrb3, Shank 3) caused hypersensitivity to gentle touch in mouse models, a symptom that is common in persons with ASD [11]. Deletion of MeCP2 and Gabrb3 (a protein that mediates inhibitory signaling in the central nervous system) in peripheral sensory nerves during development, but not in adulthood, led to a lifelong ASD-like behavioral phenotype. These studies support the idea that genetic impact resulting in sensory overload in infants or young children could lead to long lasting changes in social behavior. Interestingly, macaque monkeys transgenic for the *MECP2* gene were found to exhibit some behavioral features reminiscent of those in autism [12].

Fragile X Syndrome: About 50% of males with Fragile X Syndrome are diagnosed with autism. FMRP is an RNA-binding protein that affects the production of many proteins involved in synaptic plasticity, including some that have been shown to influence ASD risk in large genetic association studies (Shank3, PTEN, neuroligins and neurexins, and TSC2). In animal models of Fragile X, researchers have discovered impairments in brain circuitry and function. For example, an imbalance of excitatory and inhibitory neurotransmission in cortical circuits may contribute to some of the clinical symptoms associated with Fragile X (hyperarousal, hypersensitivity, anxiety, cognitive and language impairments). The resulting effects on synaptic function in Fragile X mice led to clinical trials of a novel drug, AFQ056 (an mGluR5 negative modulator), designed to improve neural plasticity and language learning in very young children with Fragile X syndrome (ClinicalTrials.gov ID: NCT02920892).

Phelan McDermid Syndrome: Phelan McDermid syndrome is caused by a spontaneous deletion on chromosome 22 (22q13.3). The gene *SHANK3* is deleted in almost all cases, and is a recognized cause of a small proportion of ASD cases. There is a family of Shank proteins, each of which helps form the scaffold for excitatory synapses. Shank3 transgenic animals display reduced connectivity between cortex and striatum and impaired social behaviors [13]. Multiple Shank genes as well as a number of other genes related to synapse formation, size, and function have been associated with ASD [14].

### **Genetic Variants Linked to ASD**

Hundreds of rare mutations including deletions, copy number variants, missense, and gene disruptive mutations that substantially alter neural function have been identified in large ASD genetic studies. However, the large number of different biologic abnormalities that exert their effects via distinct pathways also raises the possibility that different genetic variants may require different treatments.

Variations in genes that occur commonly in the population are thought to account for roughly 40% of ASD risk. Studies of post-mortem brain tissue from persons with ASD have demonstrated decreased expression of sets of genes related to synaptic function including many of the ASD risk genes. Surprisingly these changes in gene expression were not more evident in males than females. However, there was an observed upregulation of genes related to microglial and astrocytic function (brain cells that provide support for

neurons) [15]. These gene expression differences were more pronounced in males and may help explain why males are more affected by ASD.

## **Structure and Function of Brain Circuits in ASD**

### **Structure & Function**

Autism is characterized by atypical patterns in physical brain connections (structure) and in how regions cohere or communicate with each other (function). Brain structure in individuals with ASD can be compared to typically developing children using advanced MRI techniques to measure size and shape of brain regions over time, as well as diffusion tensor imaging to examine the structures of the major connections between brain regions. Brain circuit function can be investigated using noninvasive markers. Examples include using functional Magnetic Resonance Imaging (fMRI) to examine signals produced in the sensory cortex of youth with ASD who have extreme negative reactions to sensory stimuli [16], abnormal patterns of connectivity measured by magnetoencephalography (MEG) to touch stimulation [17], the utility of electroencephalography (EEG)-based evoked potentials and functional near-infrared spectroscopy (fNIRS) as biomarkers of altered circuit function [18, 19]. These studies have shown differences in activation patterns in individuals with ASD in response to sensory processing of visual, tactile, auditory and verbal stimuli.

Although non-invasive measures of brain connectivity have demonstrated differences between brain regions in persons with ASD compared to controls, it is unclear how these differences account for the heterogeneity in ASD symptoms. Earlier findings from fMRI, diffusion tensor MRI, and pathological studies highlighted a pattern of reduced long-distance connectivity (particularly in frontal and parietal brain regions) and increased local connectivity [20]. While these principles still largely hold, newer research has revealed greater nuance and specificity [21, 22]. For example, using studies with higher temporal resolution such as MEG and EEG have revealed *reduced* local and long-distance connectivity during a face processing task in individuals diagnosed with ASD compared to typically developing individuals [23]. Functional MRI research on large-scale brain networks has highlighted several key networks as consistently atypical in autism including the salience network (associated with detection of novel or personally-salient information), default mode network (associated with social processing and communication), and fronto-parietal networks (associated with executive function and attention) [24, 25]. Additionally, atypical connectivity is seen in regions associated with reward and social processing [26, 27]. In general, over-connectivity in autism is seen when looking at the connection strength *between* networks associated with social cognition [28] whereas connections *within* networks show varied patterns of under- or over-connectivity depending on the network examined, age of the participants, and methods used [29]. Some report that under-connectivity dominates in connections within the cortex or between brain hemispheres and over-connectivity dominates in connections between the cortex and sub-cortex [21].

On the gross anatomical level, the brains of autistic individuals appear normal, but brain weight may be increased. Current evidence suggests that early brain overgrowth is present in approximately 15% of two-year-old boys with ASD, but is much less common in girls [30, 31]. Brain enlargement relative to body size in this subset of boys persists at least through 5 years of age [32] and is associated with lower language ability at age 3 and reduced intellectual ability at age 5 [33]. Currently, the prevailing theory is that early brain overgrowth normalizes during adolescence and adulthood [34]. One investigation using Induced Pluripotent Stem Cells (iPSCs) generated from fibroblasts of individuals with autism and early brain overgrowth, suggests that neural precursor cells display increased proliferation and neurons display abnormal neurogenesis and reduced synaptogenesis. Microscopic studies have reported disordered cell organization with smaller, more densely packed neurons in many regions of the limbic system, which is known to play an important role in learning,

memory and emotions. One of the more reproducible findings is a reduction in the number of Purkinje cells in the cerebellum.

Interestingly, recent studies have identified significant inter- and intra-individual variability in neural functioning, in ASD during a resting state [35, 36] and in evoked responses, such as during naturalistic movie viewing [37]. Heterogeneity in the ASD phenotype also contributes to greater functional variability within ASD groups. To address this heterogeneity, a greater number of studies are examining dimensional traits in large samples of ASD and neurotypical groups. For example, patterns of functional connectivity in toddlers at high and low risk for ASD were related to joint attention, a pivotal social ability [38]. Interestingly, reactivity of the amygdala in a face-viewing task was shown to vary with the subject's level of anxiety.

Ongoing work is linking these functional and structural differences to core features of ASD including studies on social communication, language, and restricted and repetitive behaviors. For example, hyper-connectivity in children within and between social brain networks is related to social symptom severity [28, 39]. Difficulties in social communication are related to functional connectivity between a region important for voice and social perception and regions involved in reward, affective processing, and memory in children with autism [40]. Further, activation within language areas of the brain in toddlers and young children before an ASD diagnosis predicted good or poor language outcomes [41]. Finally, restricted and repetitive behaviors in children with ASD are predicted by the extent to which a "brain state" flexibly changes between periods of rest and periods of task [42]. Recent work has identified neurobiological correlates of sensory processing in autism [43, 44, 45] including findings of reduced modulation of connectivity between the thalamus (a brain structure responsible for relaying sensory and motor signals to the cerebral cortex as well as regulating consciousness, sleep, and alertness) and cortical regions in response to sound or touch. The extent of this reduced connectivity was related to parent reports of sensory over-reactivity [44]. In the domain of touch, reduced response was seen in ASD children in social-emotional brain regions to a soft caress compared to typically developing children, while increased activation was seen in response to non-caress-like touch in the brain's primary sensory cortex. This over-activation may be related to the hyper-sensitivity to touch seen in some ASD individuals. Longitudinal and cross-sectional studies also reveal developmental and age related changes in structure and function. There is evidence for altered trajectories of cortical thinning in adolescence and adulthood [46, 47]. A shift from hyper- to hypo-functional connectivity within and between multiple large-scale brain networks is seen in ASD compared to neurotypical peers [48, 25, 39]. Consistent with this shift, studies of infants and toddlers with autism suggest over-connectivity in the microstructure of frontal white matter pathways at younger ages that reduces with age [49].

### **Circuit Activity in ASD**

As is true of almost all brain disorders, the symptoms experienced by persons with ASD are linked to alterations in brain circuit function. Epilepsy, a dramatic manifestation of brain circuit dysfunction in which millions of neurons fire in an abnormal synchronous pattern, occurs in almost 20% of persons with ASD. Abnormalities in the formation of brain circuits that occur *in utero*, during infancy, and in childhood can have long lasting impact on circuit function in adulthood. During these early critical time periods connections between brain regions are dramatically molded by brain activity and may be altered by injury or inflammation.

Identification of genes involved in syndromic autism enable the study of the impact of the same genes in other humans with the same mutation as well as in genetic animal models. The abnormalities in these models vary depending upon the particular gene mutation and region of the brain examined. Decreased numbers of inhibitory interneurons and evidence of decreased inhibitory neurotransmission have been described with multiple mutations as well as evidence of increased or decreased excitatory

neurotransmission; decreased long term potentiation (a mark of neuroplasticity), and increased long term depression of synaptic activity [50].

A number of studies demonstrate hyperexcitability during what is known as a critical period of development for experience-dependent plasticity in Fragile X mouse models [51] and deletion of the Fragile X syndrome gene in mice disrupts the normal process of pruning synapses during development [52]. The majority of information flow occurs at synapses between neurons that are located on spiny outgrowths on neuron's dendrites. Increased spine density has been described in a number of genetic mouse models of ASD. Rett syndrome mutant mice were discovered to have abnormal plasticity in auditory cortex mediated by specific inhibitory neurons which impaired their response to the distress calls from their pups [53]. Environmental insults, for example those due to infection *in utero*, premature delivery, or perinatal cerebellar hemorrhage also alter the construction of brain circuits eventuating in ASD. Recent animal models study the link between genetic and environmental factors such as the three-way interaction of genetics, inflammatory stress and sex [54].

### **Role of Immune System in Brain Development and ASD**

Increasing evidence suggests immune dysregulation and neuroinflammation may be implicated in the severity and pathogenesis of the autism phenotype [55, 56, 57]. One recent meta-analysis of 17 studies identified significantly altered concentrations of immune regulators known as cytokines in ASD patients compared to healthy controls, adding to the evidence of increased inflammatory signals in ASD [58]. Despite many studies demonstrating altered levels of immune biomarkers and abnormal immune function in both the peripheral and central nervous system in ASD, it is not clear whether the immune system plays a direct role in the development of the disorder via an impairment of neurodevelopmental processes. Several recent studies suggest that maternal immunological factors may play a role in the pathogenesis of ASD during prenatal development [59, 60, 61, 62, 63, 64, 65].

Microglia are innate immune cells that reside in the central nervous system that are activated in response to infection or inflammation. Even in their so-called resting state, they perform critical functions, including regulating the number of neural precursor cells [66], synaptic organization and pruning [67, 68]. Analyses of autism brain tissue reveal alterations in genes that control microglial activation states and an association between microglia dysregulation and neuronal activity [69]. Evidence from human postmortem studies have found increased microglia activation, density, or size in various brain regions, including cerebellum [70], dorsolateral prefrontal cortex [71], and visual and frontoinsula cortex [72]. Animal studies have also shown that microglia-mediated synaptic remodeling is abnormal in a mouse model of autism [73]. In addition, an increase in activated microglia in the amygdala, a structure that plays a primary role in the processing of memory, decision-making and emotional reactions, was observed in a subset of cases (2 out of 8) [74]. More recently, an investigation in temporal cortex revealed decreases in ramified microglia in white and gray matter, and increases in primed microglia in gray matter [75].

### **Development, Natural History, and Variability in ASD**

#### **Brain Development and Developmental Trajectories**

ASD is a developmental disorder, yet most studies of brain structure and function have focused on single time points and cross-sectional differences. More studies are needed that will enhance our understanding of brain development, through longitudinal imaging studies (using methods such as structural and functional MRI and electroencephalography), with standardized acquisition parameters to enable comparability across studies, and

with robust data sharing policies to enable expert analysis of the data by a variety of computational scientists [76, 77].

Although defined behaviorally, the neurobiological basis of ASD has begun to be elucidated based on the identification of causative genetic variations, many of which converge on basic processes in early brain development, such as cortical organization, synapse formation and function, excitation/inhibition balance and the development of robust, functional cortical and subcortical networks that may impact early perceptual and cognitive processes [50, 78]. These processes may be measured through functional and structural neuroimaging methods well before behavioral signs of atypical development emerge. Historically, infant siblings of children with ASD have constituted the primary focus of research in early markers, not only because they are at heightened risk for ASD and other developmental delays, with prevalence estimates of ASD up to 20%, but also because they are identified prenatally and can be followed from birth [79, 80, 81, 82, 83, 84]. This body of research has led to the identification of atypical behaviors, particularly in the social domain, within the second year of life [85] with some evidence of motor delays [86] and altered patterns of social attention [87] within the first year.

With regard to brain imaging, promising results have emerged from the Infant Brain Imaging Study (IBIS), in which low and high risk (sibling) infants are being examined longitudinally with structural MRI at age 6, 12 and 24 months. Differences in white matter tract development from 6 to 24 months, particularly a slower change in a measure of fiber density, have been reported in infants who develop ASD [88]. More recently, the IBIS network has identified hyperexpansion of the cortical surface area between ages 6 and 12 months in those infants who developed ASD, with brain volume overgrowth related to autism severity [89]. Using EEG as an assay of brain function, studies have also found differences in developmental trajectories between high and low risk infants, from intrahemispheric coherence changes between 6 and 12 months [90], linear coherence changes during language processing between 6 and 12 months [91], and trajectories of alpha symmetry and resting state oscillations across the first two years of life [92, 93]. Notably, in these studies, it was *changes* in brain function or structure across development that related to and predicted ASD outcomes.

The brain connectivity changes that underlie autism are not static, indeed their manifestations appear during the dramatically dynamic period of brain development and continue to change over the lifespan of the individual. Therefore, understanding the biology of autism requires large longitudinal studies to chart the trajectory of neural circuits over time, including how they adapt to inborn wiring errors and environmental exposure. Studies are needed that include pregnancy and follow maternal exposures and response, fetal development, and brain response to events that occur *in utero* and perinatally. The genetic and phenotypic heterogeneity of ASD are daunting, making generalization of findings dependent upon large numbers of subjects. The measures in these studies are often complex, subject to variability in their acquisition or analysis. This makes them difficult to reproduce and diminishes their value. To compare among individuals requires standardization; variability needs to be minimized and then measured to include in the analysis. The Lifespan Connectome Project (<https://www.humanconnectome.org/>) is one example of a sophisticated brain imaging study of a large sample of typically developing individuals across the lifespan.

### **Phenotypes, Subtypes, and Natural History of ASD**

Even within genetically determined syndromic ASD there is considerable variability in the range and severity of symptoms. ASD most likely occurs due to a complex genetic architecture composed of multiple interacting biologic pathways which mix to cause the phenotypic richness even between siblings [94]. In preliminary studies that require replication, genetic associations have been found to segregate to some extent with specific phenotypes such as ASD with and without intellectual impairment [95], ASD with motor speech disorder [96, 97], and other subphenotypes [98, 7]. As an example, one study has identified *FoxP1*, a gene

associated with autism, as necessary for vocalization in the mouse [99]. As described above, certain functional and structural changes in brain circuits are associated with specific phenotypes. The basic underlying biology of circuits underlying aggression, anxiety, theory of mind (ability to understand and reason about the thoughts of others), language development, attention and social cognition is still incomplete but fundamental advances would enable search for disturbances in persons with ASD to eventually understand phenotypic variability. To further understand the basis of phenotypic variation it is necessary to standardize the classification criteria and include greater sample size in linking phenotypes to genetics, brain imaging, and brain tissue examination.

### **Biomarkers and Prediction of ASD**

The past few years has seen an increase in large-scale and longitudinal datasets. This combined with increasingly sophisticated analytical techniques has allowed for a more refined search for potential biomarkers of ASD. For example, baseline functional connectivity measures within key networks described above (frontal-parietal, default mode, and salience networks) predicted changes in autistic traits and adaptive functioning approximately two years later in a group of teenagers and young adults with ASD [100]. Pre-diagnosis fMRI response to speech combined with clinical behavioral measures in toddlers and young children predicted ASD prognosis [41]. Brain response to social stimuli (biological motion) accurately predicts whether boys have autism, but not girls [101] -- again highlighting the need for a greater understanding of the neurobiology of females with ASD. Potential biomarkers are also being developed to predict treatment outcome success [102, 103]. There have also been advancements in the identification of biomarkers that can predict autism risk in infants. Utilizing an infant sibling design, two promising biomarkers have been identified in infants as young as 6 months of age, including surface area expansion in specific cortical regions from 6-12 months of age [89] and the presence of excess cerebrospinal fluid surrounding the brain at 6-24 months [104, 105]. Ongoing work is using data-driven classification and prediction methods. Increasing use of these methods combined with validation and replication samples is needed to further refine and develop these biomarkers.

### **Co-occurring Conditions in ASD**

ASD is associated with a wide-range of co-morbidities that can cause an increased financial and psychological burden on families and caregivers as well as decreased quality of life for persons with ASD. Since 2013, much progress has been made in understanding the prevalence and underlying biology of conditions that commonly co-occur with ASD, including gastrointestinal (GI) disturbances, epilepsy, sleep disorders, psychiatric disorders, and immune/metabolic comorbidities.

### **GI Disturbances**

GI symptoms and an inflammatory mucosal pathology have been demonstrated in several studies of ASD, and it has been estimated that up to 50% of ASD patients have feeding and GI problems [106, 107, 108, 109]. A recent rigorous meta-analysis of 15 studies in 2215 children with ASD indicated a greater than twofold elevated risk of general GI symptoms among children with ASD than in those without ASD, and that children with ASD are more prone to specific symptoms of abdominal pain, constipation, and diarrhea [110]. Importantly, many of the genes implicated in autism are also expressed in the neurons present within the GI system. Thus the same genetic changes may impact function of both the brain and GI system.

Additionally, alterations in the composition of the gut microbiome have been implicated as playing a causal role in the ASD pathophysiology. Studies of fecal DNA have found certain bacterial clusters are overrepresented in children with ASD and GI complaints compared to neurotypical children with similar GI complaints, and demonstrate an altered microbial community both with respect to bacteria and fungi in ASD [111, 112, 113].

Current research suggests that disturbances within the microbiota-gut-brain axis may contribute to the occurrence and development of ASD and that the application of modulators such as probiotics, helminthes and certain special diets may prove useful for the treatment of ASD [114].

### **Epilepsy**

While the relationship between ASD and epilepsy remains unclear, studies have shown that many of the risk genes for epilepsy and autism overlap. Several studies demonstrate an increased prevalence of epilepsy in individuals with ASD well above the general population risk, and many further suggest that there is an increased risk of epilepsy in females with ASD when compared to males with ASD [115, 116, 117]. The largest study to date comparing the autism phenotype in children with ASD with and without epilepsy found that children with ASD and epilepsy had significantly more autism symptoms and maladaptive behaviors than children without epilepsy [118]. Research in animal models suggests that early life seizures may result in altered function of neurotransmitter systems and intrinsic neuronal properties during neurodevelopment that lead to the disrupted cortical connectivity that is characteristic of ASD [119]. One recent study in rats demonstrates that early-life seizures alter the development of oscillations and the excitatory/inhibitory balance in the cortex, interrupting brain connectivity and function during maturation and resulting in long-standing autistic-like behaviors [120]. The PREVeNT trial (Preventing Epilepsy Using Vigabatrin in Infants with Tuberous Sclerosis Complex) is an NIH-funded phase 2 clinical trial that began in 2017 and will assess whether anti-epileptic treatment can prevent development of epilepsy in infants with TSC who display EEG biomarkers of abnormal brain activity prior to onset of seizures (NCT02849457).

### **Sleep and Sensory Disorders**

ASD is frequently accompanied by a variety of sleep problems that worsen daytime behaviors and core symptoms such as stereotypic, self-injurious, and repetitive behaviors. Studies indicate the prevalence of sleep problems in ASD are as high as 50-80% and that children with ASD have higher prevalence of sleep disorders than children with other neurodevelopmental disorders. The types of sleep disorders in ASD include insomnias, parasomnias, sleep-related breathing disorders, and sleep-related movement disorders [121]. The most common sleep problems reported in ASD are sleep-onset insomnia, or difficulty initiating sleep, and sleep-maintenance insomnia, or decreased sleep duration. Several neurotransmitters, including serotonin, melatonin, and gamma-aminobutyric acid (GABA) play a vital role in the maintenance of sleep-wake cycles, and abnormal levels of these neurotransmitters have been described in ASD. Hyper- and hypo-sensory abnormalities are frequently observed in individuals with autism and may have negative impacts on cognitive performance, social interactions, and stress. Recent work in animal models suggests that peripheral disorders have widespread impact on behavior in experimental animals [11].

### **Psychiatric Disorders**

It has been estimated that 69% of patients with ASD suffer from co-occurring psychiatric disorders and symptoms [122]. As with the general population, age appears to be a relevant factor for psychopathology in patients with ASD. One study of adults with Asperger's Syndrome showed that the most frequent comorbidities were depression and anxiety disorder, and that obsessive-compulsive disorder and alcohol abuse/dependence were also observed [123]. Recent studies reveal that consistently high levels of psychological symptoms and distress occur across the adult lifespan in ASD, where individuals with more severe depression and anxiety disorders demonstrated more severe ASD symptoms [124, 125]. At the molecular level, one study suggests that a functional polymorphism in the serotonin 2A receptor gene may modulate the severity of depression symptoms in children with ASD [126].

### **Research Policy Issues**



A major challenge for the biological sciences is to utilize the most sophisticated technologies that produce ever enlarging data sets while still ensuring the rigor and quality of research [127]. Moving forward, the field should embrace policies that enhance the replicability of findings and promote transparent reporting of experimental methods, use of common data elements, and sharing of data and analysis tools. Follow-up validation studies are a necessary part of this process, and data sharing should be integrated into the design of studies from the beginning. The NIMH NDAR platform is a valuable repository for high quality ASD data, tools, and methodologies that researchers should leverage to enable re-analysis of data and facilitate collaboration to accelerate research progress. Larger longitudinal studies require coordination among the major research centers and a shift in focus toward team science across multiple disciplines. The coordinated collection and analysis of valuable imaging, behavioral, genetic, phenotypic, and iPSC data can be enhanced by the recruitment of a more diverse workforce that includes not only neuroscientists, immunologists, and psychiatrists but also experts in bioinformatics, machine learning, and monitoring device engineers.

Though new iPSC and brain organoid technologies allow study of human cells and circuits derived from persons with ASD, there is no substitute for careful structural and transcriptomic studies in post-mortem tissue which remains exceptionally rare. Efforts need to be redoubled to increase the accessibility of brain tissue from well-characterized ASD cases. The NIH Neurobiobank and Autism BrainNet facilitate the distribution of high-quality, well-characterized human post-mortem brain tissue for the research community, and enhancing efforts to increase public awareness about the value of tissue donation for understanding brain disorders like autism will most effectively advance the science toward better unraveling the causes and identifying new treatments for ASD. Finally, the inclusion of persons on the autism spectrum in research plans and messaging is crucial to identifying practical applications for improving the quality of life for ASD patients and their families. Standard methods for behavioral measurements and tracking QOL across the lifespan are essential for addressing prescient issues supporting individuals with ASD in their daily lives.

### **What Are Some of the Opportunities to Advance this Science?**

#### **Gene Expression Mapping**

New technologies enable characterization of altered patterns of gene expression in specific brain cell types, offering the opportunity to precisely associate gene expression differences at a cellular level.

#### **Induced Pluripotent Stem Cells (iPSCs) and Organoids**

A major advance over the last few years is the ability to take skin or blood cells from persons with ASD, create stem cells (Induced Pluripotent Stem Cells) and differentiate these cells into neurons, permitting studies on the impact of autism as manifested by different individuals on neural function at the cellular level. This new technology allows scientists to study the effects of ASD mutations in human brain cells in addition to commonly used transgenic animal models. Drug screens can be used to identify agents that improve or reverse the abnormal cellular phenotypes using these human cells and drugs identified through these screens can be further tested for efficacy in human populations.

Another area of great interest is new research that makes possible growth of “brain organoids”, partially matured mini-brains in a culture dish from iPSCs. These organoids enable the study of the early development of brain structures that occurs *in utero*, as well as the cellular and circuit abnormalities related to ASD-linked mutations [128, 129].

#### **Molecular Phenotype Identification and Validation**

iPSCs with isogenic controls are attractive models to search for molecular phenotypes linked to syndromic ASD, but higher throughput means of identifying and then validating their relevance to ASD are needed. A

strategy for identifying relevant molecular phenotypes in iPSCs from the much more common idiopathic ASD remains a daunting task.

### **Role of the Immune System**

More studies are needed to identify the roles of molecules secreted by immune cells on brain development and function. Studies are also needed to identify environmental and epidemiological factors that interact with immunological factors to elevate the risk of autism. Of special interest are cells known as microglia that are resident immune cells in the brain that have been shown to mediate sculpting of dendrites and synapses. Further investigation of the role of microglia in animal models of ASD are warranted based on our emerging understanding of their role in normal development and potential contribution to ASD phenotypes.

### **Developmental Processes and Potential for Reversibility**

Methods to examine brain development are now more powerful, and normative data in typical development are needed to inform the studies in atypical development. One such collaborative effort includes the ‘baby connectome’ project (BCP), which is a longitudinal study intended to provide a better understanding of how the brain develops from infancy through early childhood and the factors that contribute to healthy brain development. Variables of interest include patterns of structural and functional connectivity and their relationship to core behavioral skills from infancy to early childhood. Additional biological (e.g., genetic markers) and environmental measures (e.g., family demographics) are being collected and examined to provide a more comprehensive picture of the factors that affect brain development. Study data will be made available to the scientific community as it is measured. This knowledge will be tremendously useful in understanding brain function and how early interventions may shape our brain throughout our lifespan. Such coordinated efforts, with standardization of data acquisition and analysis, are needed in other imaging methods, such as EEG or MEG, and in the integration of multiple modes of imaging (structural and functional), particularly as they relate to later behavior. Ultimately, these studies will lead to the development of more scalable imaging tools that can be applied to large, more representative cohorts of infants and children. Additionally, as ASD manifests during development, it will be important to understand the critical windows during which circuit abnormalities may be reversible.

### **Sensory Processing and Development**

Another question that remains for future research is whether hard-wired abnormalities that disturb sensory processing secondarily contribute to alterations in brain circuits involved in behavioral and social functions. This may lead to novel ways to therapeutically alter the developing child’s sensory environment in order to improve later developing social skills. Further, it will be important to explore whether and how impairments in sensory processing during infancy may alter early brain development and contribute to the development of social and cognitive impairments later in life.

### **New Technologies to Explore Brain Circuits**

New powerful technologies for interrogating and modulating brain circuits are revolutionizing neuroscience. The BRAIN Initiative is a multi-Federal agency, public-private partnership in the U.S. to advance these brain circuit neurotechnologies and also engage multiple international efforts. These technologies promise to expand the ability to understand brain circuit differences due to genetic and environmental influences that contribute to many diseases, including autism. Greater knowledge of the brain circuit abnormalities implicated in ASD in animal models can now be explored in detail using these new technologies to map neural connections over large expanses of brain [130], record from a large number of neurons during a behavioral task, precisely turn on or off specific types of neurons to understand the nature of brain circuit abnormalities caused by biologic mechanisms tied to ASD, i.e. genes, immune challenge, gender, premature birth, etc. Future studies can also begin investigating cellular mechanisms of brain overgrowth in ASD.

## **Exploration of Peripheral and Enteric Nervous System Alterations that Reduce the Quality of Life and Result in Impaired Cognition, Behavior, and Quality of Life**

Even though many known autism risk genes are expressed in neurons outside of the CNS, including sensory, autonomic and enteric neurons, comparatively little is known about their functions in sensory perception, motor function and the digestive tract. Recent work has shown that disruption of these genes can have strong impact on sensory perception [11].

### **Non-Human Primates**

On the horizon are genetic tools to introduce mutations into non-human primates [131, 132], which possess a much greater behavioral repertoire and a more human-like brain structure as compared to rodents. This is especially valuable in investigating frontal lobe circuits, which are rudimentary in rodents as compared to primates. The national Japan Brain/MINDS project ([www.brainminds.jp/en/](http://www.brainminds.jp/en/)) focuses on developing transgenic marmoset monkey lines for brain disease modeling as well as a multiscale marmoset brain map to enable identification of disease-specific changes [133].

### **Brain Function During Rest and Task Periods**

There is a need for a greater understanding of the relation between intrinsic functional brain organization during rest and functional connectivity dynamics during task states. Additionally, there is a need for a better understanding of brain function and connectivity during tasks that better capture the complexity of real-world interactions [37, 134].

### **Behavioral Monitoring Technologies**

New technologies are becoming available to monitor and quantify behavior over long time periods which should aid autism research in its integration of behavior with genetics, neuropsychological tests, neuroimaging and other measures of biologic function.

### ***In Utero* Imaging Studies**

New imaging techniques enable safe study of the developing brain during prenatal development.

### **What are the Barriers to Progress?**

Despite significant progress in understanding the biological basis of autism, considerable challenges remain. Many of these challenges represent enduring difficulties for autism research and have been commented on in prior reports from the IACC.

### **Separating Causes from Consequences**

One challenge in many studies of the causes of autism is that because of their timing (i.e., postmortem), it is unclear which factors are the cause of autism or the effect of a remote insult related to the cause of death. Advances in human imaging technology and longitudinal study designs may provide an opportunity to better distinguish true causes from consequences of specific pathological findings by making it possible to image brain tissue *in vivo* throughout the lifespan.

### **Designing Therapeutic Interventions**

Translation of biological understanding to clinical benefit has been limited by several factors. Even within typical development, there is little knowledge of techniques to design therapeutic interventions to target specific brain systems. Second, the impact of mutations, such as Fragile X, are uneven across the brain, so treatment that corrects an abnormality in structure or function in one region may impact negatively the

functioning of other brain regions and behaviors. Though there is a desire to demonstrate the impact of treatment on brain function, the practical import of alterations in brain function on development is not yet understood. Further, there is not yet a frame of reference to apply such insights in a predictive fashion to guide treatment selection or anticipate treatment response. These are key translational objectives. To exert meaningful public health impact, there must be movement towards methods of measuring quantitatively biological and behavioral traits that are sufficiently cost-effective and accessible to support deployment at a large scale.

### **Large Scale Longitudinal, Lifespan Studies**

Large organized longitudinal studies across the lifespan are needed to better understand developmental trajectories and natural history of ASD. Early results underscore the need to track and better understand longitudinal changes in brain development before autism symptoms emerge. Such measures can help us to understand the underlying mechanisms of atypical development and to elucidate the ideal timing and target of early interventions. These measures can also link back to genetic mechanisms implicated in neurodevelopmental disorders more directly than behavioral assays. Relatively unexplored remain the earliest behavioral and biological markers of risk, the unfolding of ASD in early infancy, and the comparison of these developmental processes in these defined genetic syndromes with those found in familial risk groups. Other key questions that can be answered through longitudinal studies of brain development include identification of adaptive brain changes in response to a developmental disturbance, changes that may be either beneficial or harmful, adaptive changes in brain function and structure that predict response to interventions, and developmental changes that inform core developmental features, such as language or nonverbal cognition.

### **Brain Tissue Access**

Studies of human gene expression, malformed cellular and structural circuitry, and the role of microglia in ASD are severely hampered by limited accessibility to post-mortem autism brain tissue. Through the establishment of collaborations like the NIH Neurobiobank (<https://neurobiobank.nih.gov/>) and Autism Brain-Net (<https://autismbrainnet.org/>), individuals with ASD and their families are able to donate their brain tissue to tissue repositories around the country that provide precious tissue to scientists for research.

### **MRI Studies in Individuals with Intellectual Impairment**

MRI studies have been difficult in ASD individuals with intellectual impairment and more have been done on high-functioning individuals. However, new methodologies have been developed that may enable high-quality imaging in ASD children with intellectual impairment [135].

### **Heterogeneity – Study Design Issues**

A broad challenge to clinical studies is heterogeneity in the diagnostic entity of autism itself. Rather than a singular diagnostic construct, autism represents a common behaviorally-defined developmental pathway reflecting numerous etiologies and an unknown number of involved mechanisms. For this reason, it is difficult to ascertain the generalizability of biological underpinnings from sample to sample. Historically small samples provide limited information to understand this variability and conflate true variability in pathophysiological mechanisms with variance attributable to underpowered studies, inconsistent approaches, and diverse methodologies for measuring biological processes. A key step forward will involve undertaking large studies involving thousands of individuals with autism with structured and consistent measurement according to rigorous methodological standards. For example, the Autism Biomarkers Consortium for Clinical Trials (ABC-CT) project, which is a collaboration among three NIH Institutes, the Foundation for the NIH, Food and Drug Administration, Simons Foundation, Yale University, Janssen Research & Development, and the European Autism Interventions (EU-AIMS), is focused on developing biomarkers for

ASD subgroups based on evaluations of brain function, visual attention, as well as behavior and speech in children aged 6-11. Such studies will enable investigators to determine the presence of mechanistically meaningful subgroups and, in the absence of true subgroups, to understand continuous relationships among biological processes and quantifiable aspects of the phenotype. It is critical that studies are designed with longitudinal components that can offer insight into changes associated with human development across the lifespan. The National Database for Autism Research (NDAR) provides a medium for making datasets publicly available to benefit from the universe of analytic resources beyond those maintained at individual laboratories carrying out the research.

### **Heterogeneity – Measurement Issues**

Characterizing relevant aspects of heterogeneity is complex. Some, such as biological sex, are readily operationalizable and developmentally stable. This consistency is also true of genetic contributions. These factors represent viable starting points for constraining and characterizing heterogeneity. However, within these categories, there is nested heterogeneity that has not yet been characterized in a consistent, reliable, or universal fashion. For example, distinct biological processes may be associated with cognition, language, social motivation, repetitive behaviors, or other factors that are challenging to quantify and are variable across development. Great progress has been made in developing nuanced and reliable measures of these constructs, and their integration into large studies of biology is necessary to elucidate distinct contributors to varying manifestations of autism. A notable challenge to autism research is the poor understanding of many of these factors in typical development; rigorous and longitudinal approaches to characterization and biological measurement in control samples are essential steps to developing a meaningful frame of reference for understanding atypical development in autism.

### **Environmental Exposures**

Though it is suspected that gene-environment interactions underpin the development of autism, little is understood about specific environmental factors [136]. Critical examination of the potential impact of a wider variety of environmental exposures during prenatal developmental, infancy, and early childhood is needed, as well as understanding the pathways through which they interact with known or suspected genetic factors.

### **Animal Models**

In addition to these considerations germane to research in humans, there are continuing challenges in applying animal models to understand the biology of autism. Described circuit abnormalities in ASD models vary considerably and the contribution of different methods among laboratories studying like processes is not clear. Addition of a more standardized and systematized approach to the circuit abnormalities in ASD models would be valuable to the field.

Because, in many cases, autism impacts uniquely human aspects of social-communicative behavior (e.g., spoken language), developing and measuring analogous phenotypes in animals has proven difficult. When face valid analogues are available (e.g., ultrasonic vocalizations in mice), there remains uncertainty about functional equivalence to symptoms observed in humans. Likewise, because autism impacts brain regions not developed in some animal species, some neural circuitry is not readily amenable to study in some animal models. As more is understood about genetic and biological pathways in humans, research can focus on these mechanisms without regard to phenotypic similarity or dissimilarity. A second strategy for advancement of animal models research will be increased use of species more comparable to humans in both biology and behavior (e.g., genetic tools previously limited in application to mice can now be applied in rats and even primates, such as macaques and marmosets).

## Objectives

**Objective 1: Foster research to better understand the processes of early development, molecular and neurodevelopmental mechanisms, and brain circuitry that contribute to the structural and functional basis of ASD.**

**Example 1.** Identify one or more neural circuits that are impaired in significant groups of ASD individuals.

**Example 2.** Understand some of the mechanisms that render females more resistant to autism than males and understand the mechanisms of the fever effect

**Example 3.** Identify quantitative and reproducible biomarkers or behavioral monitors for ASD of use for assessing effectiveness of future therapeutic or behavioral intervention trials.

**Objective 2: Support research to understand the underlying biology of co-occurring conditions in ASD and to understand the relationship of these conditions to ASD.**

**Example 1.** Determine whether seizure is causal for ASD

**Example 2.** What is impact of GI dysfunction on ASD related behaviors and cognitive performance?

**Example 3.** What is the impact of sleep disorders on ASD related behaviors and cognitive performance?

**Objective 3: Support large scale longitudinal studies that can answer questions about the development of ASD from pregnancy through adulthood and the natural history of ASD across the lifespan.**

**Example 1.** Support creation of large, characterized both phenotypically and genetically with complete health records from early embryogenesis through adulthood.

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