Chapter 2: Biology

What Is the Biology Underlying Autism?

Aspirational Goal: Discover the roles of brain development, cognition, and physiological function in autism and its co-occurring conditions to enable the development of effective, targeted interventions and societal accommodations that promote positive outcomes across the lifespan.

Introduction

Current scientific evidence suggests that autism arises during early development and results in differences in brain structure, function, and connectivity. Those brain differences may lead to challenges in areas such as social behavior, learning, communication, sensory perception, motor function, and intellectual ability. Over the course of the last decades, research has revealed that genes and environmental influences in early development are contributing factors. The biological mechanisms by which known gene variations may lead to autism by altering the underlying neural circuitry of the brain are areas of active investigation. These genetic variants are associated with several general functions in neurons (brain cells), including regulating when and how genes are expressed and the function of synapses, which are the points of connection and communication between neurons.

However, much remains to be learned about the specific biological mechanisms that lead to autism and differences in cognition (including facial processing and emotion regulation), language development and communication, motor development, and sensory processing. Research is needed to understand how biological differences lead to observed behaviors and how autistic individuals experience the world. More research is also needed to better understand how sex and gender impact the presentation of autism. Additionally, studies on the relationship of common co-occurring mental and physical health conditions, intellectual disability, and autism are also needed, as are longitudinal studies to better understand the developmental trajectories of autism and co-occurring conditions. Greater insight and understanding of these molecular, neurological, and developmental differences will allow for personalized, targeted interventions that can promote positive outcomes for all individuals on the autism spectrum across the lifespan.

Molecular Mechanisms and Genes Implicated in Autism

Genetic studies over the past 20 years have identified over 100 genes that contribute to the development of autism, though many of these genes also are implicated in other mental health and developmental conditions.[1-3] This number is growing rapidly, and it is likely that over 1,000 genes that make smaller contributions to the development of autism will also be identified in the future.[2-14] At present, the known functions of some of these genes converge on biological processes that are important for neurogenesis (the development of neurons) and synapse formation and function. However, much more remains to be learned about the molecular mechanisms that may lead to observable characteristics of autism.

The discovery of genetic variants that cause single-gene disorders that often have autism as one component (such as tuberous sclerosis complex, Rett syndrome, Fragile X syndrome, and Phelan

McDermid syndrome) and the large number of rare spontaneous or *de novo* mutations that contribute to autism have enabled scientists to explore the biological effects of specific molecular pathways in cellular and animal model experiments. This has led to rapid growth in research toward understanding how these variants alter the biology of cells and impact neural circuitry and behavior. One of the challenges of cellular and animal model research, however, is that sometimes model systems cannot fully replicate the complexity of cognitive or behavioral characteristics observed in humans.

The ability to take skin or blood cells from people on the autism spectrum, create induced pluripotent stem cells (iPSCs), and differentiate these cells into neurons has enabled advances in the study of neural function at the cellular level in various health conditions, including autism. This technology allows scientists to compare how iPSCs derived from autistic individuals differ from those derived from neurotypical individuals on the cellular and molecular level [15, 16], as well as to compare differences between cells derived from animal models with introduced autism-related genes (transgenic animal models) and those without any genetic alterations.[17] As CRISPR gene editing technology continues to advance, more high-throughput pooled CRISPR screens[18, 19] will also be possible as a means of identifying and validating the relevance of genetic variants to autism. While patient-derived iPSCs are creating new opportunities in autism genetic research, small sample sizes, and variability and genetic heterogeneity of derived cells remain challenges in this research. To date, it has been easier to interpret results in iPSCs derived from patients with autism related to known genetic causes. Identifying cellular phenotypes among iPSCs derived from individuals whose autism does not have a known cause has been more challenging. Larger sample sizes and better powered analyses may be helpful in overcoming these limitations.

iPSCs also make it possible to grow brain organoids, which are clumps of brain tissue partially organized to have some features of the human brain. These partially matured "mini-brains" can be grown in a laboratory and can be used to enable the study of the early development of brain structures that occurs *in utero*, as well as the cellular and neural circuit abnormalities related to autism-linked variants.[20-24] However, careful attention needs to be given to ensuring that variables related to cell-culture conditions do not affect the reproducibility of results.

Brain organoids and iPSCs cannot replace careful studies of postmortem tissues, which have been used to successfully identify differences in structure and gene expression in brains of autistic individuals compared to neurotypical brains.[25-27] Future advances in single-cell RNA sequencing technology will allow for better characterization of these altered patterns of gene expression in specific brain cell types, offering the opportunity to precisely associate gene expression differences at a cellular level.[28] However, the number of available postmortem samples is limited, with a heterogeneous mix of characteristics, including sex, age, and medical history, making high-powered statistical analyses difficult. Therefore, efforts are still needed to increase the accessibility and diversity of brain tissue for autism research. Collaborations such as the Autism BrainNet, the Hispano-American Brain Bank of Neurodevelopmental Disorders (CENE), and the National Institutes of Health (NIH) NeuroBioBank facilitates the distribution of high-quality, well-characterized human postmortem brain tissue for the research community. Enhancing efforts to increase public awareness about the value of tissue donation for understanding autism will most effectively advance research on the biological mechanisms of autism. Additionally, performing

analyses in addition to sequencing and storing samples to be used in the future as better technology becomes available can also advance our understanding of autism biology.

Another remaining challenge is to understand how the effects of hundreds of implicated genes converge to create the common features of autism. In addition to examining rare variants that lead to high likelihood of developing autism, studying more common gene variants that contribute to the development of autism to a lesser degree may highlight previously undetected gene networks and molecular pathways. Studies of multiple genes in parallel and more complete data about relationships between genes and gene function can also help identify additional connections. Conversely, the autism genes identified so far often play multiple roles within a cell and organism, and this presents a major challenge in translating the discovery of an autism-related gene to viable intervention options, as medications targeting such genes can result in unwanted side effects. A better understanding of human brain development will provide valuable information on where and when to look for autism-related biological changes, which can advance needed research to determine how individual genes and their interactions in early life events explain the biological basis of the heterogeneity of autism features.

Structure and Function of Brain Circuits in Autism

Autism is characterized by atypical patterns in physical brain connections (structure) and how regions communicate with each other (function). Brain structure in individuals with autism can be compared to typically developing children using advanced magnetic resonance imaging (MRI) techniques to measure the size and shape of brain regions over time, as well as diffusion tensor imaging (DTI) to examine the structures of the major connections between brain regions. Brain circuit function can be investigated using non-invasive technologies, such as functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), electroencephalography (EEG), and functional near-infrared spectroscopy (fNIRS) to image the brain or detect changes in brain activity. These techniques have revealed neurological differences in cognition, executive functioning, sensory processing, social communication, and language development in autism.[29-31]

Despite a large body of work, challenges remain in fully understanding the neurobiology of autism. Current brain imaging studies often report conflicting results and suffer from reproducibility issues. The heterogeneous nature of autism and technical limitations of the techniques used means large sample sizes and careful statistical analyses are often necessary to obtain truly representative and accurate results. Large collaborations and consortiums can help to increase sample size and improve rigor and reproducibility.[32-35] In addition, females on the autism spectrum, individuals of racial and ethnic minorities, minimally speaking individuals, and individuals with higher support needs are often underrepresented in brain imaging studies. Increasing the diversity of study participants will improve the data quality of brain imaging studies and produce results that are relevant to a broader cross-section of the autism community.

New technologies can also help to overcome some of the logistical issues associated with many brain imaging techniques. For example, MRI scans require the patient to be completely still in a confined and noisy environment, which may present sensory or other issues for many on the autism spectrum and is not representative of real-world environments. Alternatives such as high-density diffuse optical

tomography (HD-DOT) are better tolerated, portable, and allow for high-density measurements in more naturalistic settings.[36, 37] Use of these techniques and developing advances to overcome tolerance and sensory issues can improve data quality in brain imaging studies.

Imaging and electrophysiology studies in model animals such as mice have provided invaluable information on the neurobiological and molecular basis of autism. As discussed in the previous section, however, because autism impacts uniquely human aspects of social-communicative behavior (such as spoken language), developing and measuring analogous phenotypes in animals has proven difficult. In addition, because autism impacts brain regions not developed in some animal species, some neural circuitry is not readily amenable to study in model organisms. Therefore, human neuroimaging studies remain critical to understanding how autism impacts brain function and structure.

Lastly, in addition to characterizing how autistic brains differ from neurotypical brains in terms of structure and function, it is important to also ask which of these differences contribute to autism phenotypes and characteristics and which are simply correlations. For example, a recent review suggested that despite a correlation of between differences in functional connectivity in regions of the brain that support complex social interactions and the severity of social symptoms, more data are needed to determine whether connectivity differences cause the observed social challenges.[38] Teasing out these causal relationships by examining brain dynamics in the context of different tasks and situations can be helpful in developing interventions to reduce the cognitive and social difficulties associated with autism.

Sensory and Motor Differences

Historically, cognitive, social, and behavioral differences have been characterized as the core features of autism. Recent studies have suggested, however, that differences in the sensory and motor nervous systems may play a less recognized but significant role in autism and may represent important opportunities for intervention and improvement of quality of life. The vast majority of individuals on the autism spectrum experience hypo- or hyper-sensory abnormalities, which may have negative impacts on cognitive performance,[39] social interactions and communication,[40, 41] and stress.[42, 43] The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) includes sensory characteristics as a diagnostic criterion for autism, highlighting the frequency with which autistic individuals experience sensory difficulties. These sensory difficulties occur across the core sensory systems, including visual, auditory, gustatory, olfactory, tactile (touch and pain), vestibular, and proprioceptive (sense of self-movement and body position) systems, and the severity and impact of these differences vary from person to person.[44, 45] Extensive research in this area in recent years has led to a better understanding of the neurobiological differences within the central nervous system that lead to hypo- or hyper-sensitivity.[46-51]

However, more work can be done to explore how differences in peripheral nerves and organs such as the eye, nose, mouth, ear, and skin can contribute to sensory differences. Additionally, multiple stimuli can activate different sensory systems simultaneously and may lead to cognitive, social, and behavioral challenges. Research in mice has provided some information on the neurobiological mechanisms that lead to multisensory dysfunction in autism.[52] It will be important for future research to determine

whether these findings are translatable to humans and develop interventions to improve the sensory experiences of individuals on the autism spectrum. Additionally, sensory challenges occur in a heterogeneous manner across the autism spectrum and across the lifespan.[53-57] Future research should continue to investigate differences in sensory processing in different subgroups within the autism community and how these change with development and across the lifespan; this will enable the development of more customizable interventions that can meet the sensory needs of individuals of all ages across the autism spectrum.

Differences in motor development and function are also common in individuals on the autism spectrum, though not currently recognized among the core features of autism.[58] Motor skills that have been reported to show differences in autism include both fine and gross motor skills such as gait, manual dexterity, postural control, motor learning, and motor imitation. Motor differences in infancy, such as delayed postural motor skills, can often be detected prior to and often correlate with later development of differences in social skills.[59-63] Studies have also identified motor differences in autism in adulthood[64]. Currently, it is not clear what neurobiological mechanisms drive atypical motor development and how that may manifest across the lifespan and contribute to other aspects of autism. Research is needed to gain a better understanding of differences in motor development and expand the overall clinical understanding of autism. This has the potential to serve as the basis for new strategies to identify autism in early infancy or in adulthood, as well as develop interventions to improve motor skills at all ages and thus improve quality of life.

Cognition, Intellectual Disability, and Communication Challenges

According to the CDC's ADDM study, approximately 1/3 of autistic children have an intellectual disability, as defined by an IQ score below 70. This population is among those who have the most intensive support needs within the autism community and often need long term services and supports[65]. Autistic individuals with ID are at increased risk for co-occurring conditions such as aggression and self-injurious behaviors. Many autistic individuals with intellectual disabilities also have a language disability, creating additional challenges. In spite of the significant needs of this population, this population is underrepresented in autism research due to traditional research exclusions created to prevent confounding variables in studies and the challenges that may be associated with working with subjects with difficulties communicating or complying with research protocols[66]. More research is needed to develop improved methods to assess intellectual disabilities in individuals with autism, and better understand the causes of intellectual disability within autism, the intersection of symptoms of autism and intellectual disability, and the similarities and differences between intellectual disability in autism and intellectual disability in other populations[67-69]. It will also be critical to explore the potential of and optimal modalities for individuals with autism and intellectual disabilities to learn and acquire new skills across the lifespan[70], and how to best support the needs of this population. To make strides in this area, efforts to include this population in research studies are needed, as well as studies focused on this population specifically, and efforts to learn from research done in the broader intellectual disability community.

Autism diagnosis is characterized by restricted, repetitive behavior and social and communication challenges. Therefore, cognitive studies of psychological and mental processes, including memory,

language production and comprehension, executive function, problem solving, decision making, and social communication, are vitally important for understanding the biology of autism. As discussed in the previous section, research has recently highlighted the role of sensory processing in cognitive function and social communication, with multiple studies exploring how eye gaze and movement impacts facial recognition and social interactions.[71-73] In addition, researchers are now beginning to understand the neuronal activities that occur following verbal stimuli and conversation and during speech processing.[74-76] The role of the cerebellum and other areas of the "social brain" (including the prefrontal cortex, amygdala, hippocampus, and limbic system) in cognition and social function has also been intensely studied.[77, 78] An understanding of how differences in executive functioning affect restricted, repetitive behavior and other cognitive difficulties is beginning to emerge, with an imbalance in neural excitation and inhibition being a leading hypothesis.[79-81]

Some individuals on the autism spectrum have difficulties producing verbal language; these can occur in the presence or absence of intellectual disability. A recent study has demonstrated that differences in vocalizations can be detected as early as 12 months[82]. While some individuals with delayed language eventually catch up to their neurotypical peers, up to 30% remain non-speaking or minimally speaking[83]. Difficulty with spoken language may hinder ASD diagnostic tests and other evaluations of abilities[84], which may in turn hinder qualification for and provision of services. In addition, individuals with limited spoken language often experience more challenges in education, daily living, relationships, and community integration. Thus, advances that can lead toward improvement of either spoken language or alternative forms of communication have a high potential for increasing positive outcomes. Researchers have identified several differences in the structure and connectivity of language-controlling regions in the brains of autistic individuals[85, 86]. Identifying biomarkers that predict language development may be a strategy to direct autistic children to appropriate interventions during critical developmental windows[87]. In particular, quantity and quality of parental language, as well as frequency of parental gestures, are both positively associated with language outcomes in autistic children[88, 89]. In many cases, minimally speaking autistic individuals have motor difficulties that limit speech, rather than cognitive difficulties[90]. Use of alternative and augmentative communication (AAC) methods may be especially beneficial in providing a means for these individuals to communicate effectively with others (discussed further in Chapter 4). It is important to identify biomarkers and develop evaluation tools that can effectively characterize the level of communicative ability in all autistic individuals, including those who are minimally speaking.

Much remains to be explored about the neurobiology underlying differences in cognition and social communication. While some regions of the brain have been definitively shown to be altered and play a role in autism, the heterogeneity of cognitive and social phenotypes make it difficult to pinpoint the neuronal and molecular changes that cause cognitive impairments. More studies, potentially in model systems, are needed to better understand the exact nature of neurobiological differences that occur in autism. This knowledge will allow for the development of biomarkers for cognitive processes such as memory and problem solving to better identify how and what interventions may help individuals on the autism spectrum. Additionally, complex cognitive processes likely require communication between multiple brain regions, and studies of the brain at the systems level can lead to a more holistic

understanding of brain networks and connections. Information at the connectome-level (pertaining to the wiring and connectivity of the nervous system) may allow for better predictions of cognitive and social communication outcomes in autistic individuals. It will also be important to study how cognition, social communication, and behaviors may change over time to better support individuals on the autism spectrum throughout the lifespan. In particular, research on cognitive and social adaptations of individuals on the autism spectrum can help lead to interventions that promote resilience and maximize positive outcomes[91].

Immune System and Autism Development

Current evidence suggests immune differences and neuroinflammation are implicated in the severity and pathogenesis of the autism phenotype.[92] For example, recent meta-analyses found higher concentrations of pro-inflammatory cytokine molecules in individuals on the autism spectrum compared to the control group[93] and a subset of immune-related genes were significantly associated with autistic traits.[94] Despite many studies demonstrating altered levels of immune biomarkers and abnormal immune function in both the peripheral and central nervous system in autism, it is still not clear whether the immune system plays a direct role in autism development via alteration of neurodevelopmental processes.

Several recent studies suggest that maternal immunological factors may play a role in the development of autism during gestation. Maternal infection and fever, autoimmune disease, asthma, and obesity are all associated with autism development in the offspring.[92, 95] However, these results are largely based on data from peripheral blood, which may not be representative of changes that occur in the brain. Therefore, future research will need to answer whether the immunological changes observed cause differences during neurodevelopment or whether differences in neurodevelopment change the function and activity of the immune system.

Microglia are immune cells that reside in the central nervous system and are activated as part of the defensive response to infection or inflammation. Even in their so-called resting state, they are essential for maintaining neuron function the central nervous system.[96] Microglial activation has been linked to altered brain connectivity in children on the autism spectrum, and pro-inflammatory cytokines and microglial phenotypes are also seen in autistic individuals.[92] Further investigation of the role of microglia are warranted based on our emerging understanding of their role in typical neurodevelopment and potential contribution to autism phenotypes. In addition, more studies are needed to identify the roles of molecules secreted by immune cells on brain development and function.

Sex and Gender Differences

The most recent epidemiological data in the U.S. suggests that autism is about 4 times as prevalent in boys and men as compared to girls and women.[97, 98] Additionally, autism is more prevalent in gender-diverse individuals compared to cis-gendered individuals.[99, 100] While it has been a topic of recent research studies, relatively little is known about the biological mechanisms that contribute to sex and gender differences. Further work is needed to understand the phenotypic differences between males and females and gender diverse individuals on the autism spectrum and how these differences should

inform the development of screening and diagnostic tools, interventions, and services that meet the needs of all autistic individuals, regardless of sex and gender.

One hypothesis for the male to female autism diagnosis ratio is the existence of the female protective effect (FPE), which posits that females would require a greater accumulation of autism-related genetic differences to reach the diagnostic threshold. Sequencing and genetic data appear to support this hypothesis, with autistic females carrying higher average numbers of rare *de novo* mutations compared to their male counterparts,[1, 2, 101] and common and rare variants of autism genes are enriched in mothers and unaffected sisters of autistic individuals.[1, 2, 102] However, results from family- and inheritance-based studies are more conflicting. Some studies show that siblings of autistic females were more likely to receive an autism diagnosis compared to siblings of males on the autism spectrum,[103] while other results show that the relative risk of autism between maternal and paternal lineage is similar, indicating that FPE is likely not the primary mechanism for the sex differences found in autism prevalence.[104] Additional studies are needed to determine whether the FPE exists and the extent to which such an effect contributes to the sex differences seen in autism diagnosis.

Finally, more studies are needed to investigate the neurological sex differences that occur downstream of the genetic and molecular variances. Recent functional connectivity studies found sex differences in several brain regions which play key roles in sensorimotor, cognitive, and socio-affective processes.[105, 106] Additionally, brain structure and anatomy also differ between males and females.[107, 108] A recent neurogenetic analysis found that autistic females had a larger number of genetic variants in autism-implicated genes than autistic males[109]. However, these studies investigating sex- and gender-influenced differences in autism neuroanatomy and genetics of remain rare and are often limited by small sample sizes. More research is needed to better understand how differences in genetics, brain functioning, and brain structure can lead to the sex differences seen in autism presentation and outcomes.

In addition to biological theories of sex differences, ascertainment bias is another theory about why more boys are diagnosed with autism (discussed in more detail in the Chapter 1). Girls and women with autism are also often diagnosed later than autistic males and not well-represented in research studies, and research to explore the relationship between gender identity and autism development and outcomes is just beginning. Better understanding of the genetic, molecular, and neurobiological differences between males and females and cis-gendered and gender-diverse individuals on the autism spectrum can lead to the development of more accurate biomarkers and diagnostic tools, as well as more appropriate interventions and services to maximize the quality of life for individuals of all genders and sexes on the autism spectrum.

Longitudinal Studies

Autism is a developmental disorder, and life outcomes are extremely heterogeneous. While longitudinal studies conducted so far have shown that autism diagnoses at 14 months of age are stable,[110] the strengths, challenges, and support needs associated with autism change over time.[56, 57, 111-113] Longitudinal studies have shown correlations between motor skills and later development of autism and language,[114, 115] as well as associations between autism symptoms and sleep problems.[116] Studies have also shed light on how differences and changes in the brain over time correlate with different subgroups

and mental and physical health outcomes.[117-120] However, much remains to be learned about differences in developmental trajectory and what biological factors and interventions determine better life outcomes for individuals on the autism spectrum.

The brain connectivity changes that underlie autism are not static; they are dynamic during early 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 differences in wiring and environmental exposures. Studies are needed that include pregnancy and follow maternal exposures and response, fetal development, and brain response to events that occur during pregnancy and perinatally. More longitudinal studies that gather brain imaging data from the same set of subjects repeated over an extended study period are also needed to enhance our understanding of brain development. Furthermore, advances in human imaging technology and longitudinal study designs may provide an opportunity to better distinguish true causes from consequences of specific findings by making it possible to image brain tissue in live subjects throughout the lifespan. These kinds of studies will require standardized acquisition parameters to enable comparability across studies, and robust data sharing policies should be in place to enable expert analysis of the data by a variety of scientists. Finally, large, organized longitudinal studies across the lifespan are needed to better understand the biology, developmental trajectories, and natural history of autism, from prenatal development to early childhood and through adulthood to older adulthood. In addition, longitudinal studies of adults that extend into older adulthood will be needed to better understand the health and service needs of older autistic adults. These studies are critical to identify the biological variables that can help autistic individuals maximize their strengths and receive the interventions, services, and supports they need to overcome challenges throughout the lifespan.

Co-occurring Conditions

Autism is associated with a wide range of co-occurring conditions that decrease quality of life for autistic individuals. Progress has been made in recent years to better understand the prevalence and underlying biology of conditions that commonly co-occur with autism, including gastrointestinal (GI) conditions, epilepsy, sleep disorders, and psychiatric disorders. However, more research is needed on the underlying causes of these co-occurring conditions and the biological interactions with autism to facilitate interventions that can improve the health and well-being of individuals on the autism spectrum.

Gastrointestinal Conditions

GI symptoms and an inflammatory mucosal pathology has been demonstrated in several studies of autism. Children with autism are more likely to experience GI symptoms compared to typically developing children or children with other developmental disabilities.[121] GI symptoms lower quality of life and can cause discomfort, distress, and pain. The presence of GI symptoms has been associated with loss of skills and language and communication ability, self-injurious behavior and aggression, sleep problems, and sensory issues.[121] It is common for individuals on the autism spectrum to eat a restricted diet, and studies have shown that the dietary patterns of autistic children differ from that of typically developing children.[122] It is unclear the extent to which this contributes to the development of GI symptoms or if the GI symptoms themselves lead to a more restricted diet. Additionally, while GI

symptoms were not found to vary with age and were stable over time,[121] it is unclear if gender and sex play a role in number and severity of GI symptoms experienced and what roles sex hormones may play in the process. Most surveys also rely on parents to report symptoms, which may lead to inaccuracies. Improvement and standardization of study tools such as questionnaires and surveys are needed to obtain more accurate results that can be compared across studies.

The role of the gut-brain axis and the microbiome in neurophysiology has been under intense study in recent years. The microbiome within the gut plays important roles not only in gut health and metabolism but also in immune activation and neuromodulation.[123] Given the prevalence of GI co-morbidities in autistic individuals, it is hypothesized that the microbiome may play a role in autism development, and studies of fecal DNA have found certain bacterial types overrepresented in children on the autism spectrum compared to neurotypical children.[123] Additionally, some studies have found interventions designed to normalize the gut microbiome improved both GI and autism symptoms.[124-126] However, a recent metagenomics study did not find any association between autism and the gut microbiome,[127] instead positing that the differences in microbiome may be due to dietary preferences. Other recent work has suggested the possibility that gut issues in autism may be linked to differences in the gut neurons[128]. Therefore, more work is still needed to determine the role of the gut-brain axis in autism development.

Epilepsy

Epilepsy occurs in 5-46% of individuals on the autism spectrum, and approximately 30% of children with epilepsy also have autism.[129] Studies have shown that many of the genetic factors for epilepsy and autism overlap,[129, 130] suggesting that autism and epilepsy may share a common etiology. In addition, differences in EEG patterns and perturbations in the neuronal excitatory/inhibitory equilibrium are seen in both epilepsy and autism.[131] Despite these connections, it is unclear what the causal relationship is between autism and epilepsy. For example, epilepsy could be a causal factor in autism development, or the neurodevelopmental differences that lead to autism could contribute to the occurrence of epileptic seizures. Alternatively, epilepsy and autism may occur on a developmental spectrum in which the specific outcome depends upon other factors. A better understanding of the biological underpinnings of both epilepsy and autism and the relationship between the two will lead to more targeted and effective medical interventions that improve the health and well-being of individuals on the autism spectrum who have co-occurring epilepsy.

Sleep Disorders

Autism is frequently accompanied by a variety of sleep problems that cause worsened challenging and self-injurious behaviors, anxiety, hyperactivity, and inattention during the daytime.[132] Studies indicate the prevalence of sleep problems in autism are as high as 50-80%, with initiating and maintaining sleep being one of the most common co-occurring clinical disorders.[133, 134] Several neurotransmitters, including serotonin, melatonin, and gamma-aminobutyric acid (GABA) play vital roles in the maintenance of sleep-wake cycles, and abnormal levels of these neurotransmitters have been described in autism. Model animals have improved our understanding of how genetic changes that lead to autism may also cause sleep disturbances.[135] In the future, it will be important to explore if genes that regulate sleep may also contribute to autism development. Additionally, more research is needed on how

confounding variables such as potential sensory issues and GI symptoms may be contributing to disrupted sleep to better design interventions that improve sleep quality.

Self-Injurious and Challenging Behaviors

Several studies have estimated that up to half of all individuals on the autism spectrum display self-injurious behavior (SIB) and or challenging behaviors such as aggressive behavior at some point in their lifetime; these behaviors are more common in individuals with intellectual disability and/or communication challenges[136-141]. These behaviors often cause substantial difficulties for autistic individuals, their families, and other caregivers, including significant physical injury, stress and trauma, and property damage. The triggers for these behaviors in any given individual are not always well-understood or observable to caregivers, and their presentation and duration are often unpredictable. Aggressive behavior and SIB may be due to emotional dysregulation, responses to sensory hyper- or hypostimulation, frustration with limited communication or other adaptive skills, or other co-occurring conditions such as GI distress or sleep problems[139, 142, 143]. The exact mechanisms for these behaviors in autism are not fully understood, but a recent study has indicated that common brain circuitry involved in emotion, motivation, cognitive processing, and decision making may be involved.[144] Anti-psychotic medications are the most common treatments, but continuing research to better understand the underlying causes so that next generation approaches can be developed is needed[145, 146]. The inherent challenges presented by SIB and other challenging behaviors have traditionally made it difficult to include individuals presenting these behaviors in research studies. However, it is critical to increase understanding of these behaviors so that underlying issues can be appropriately addressed and behaviors that are potentially harmful to autistic people and their families and caregivers can be alleviated.

Mental Health and Psychiatric Conditions

It has been estimated that approximately 70% of people on the autism spectrum have one or more co-occurring psychiatric disorder.[147] The most common of these conditions are anxiety and mood disorders, obsessive compulsive disorder (OCD), ADHD, and oppositional defiant disorder. In addition, depression, bipolar disorder, schizophrenia, eating disorders, and suicidal ideation and death by suicide have been reported at higher rates in individuals on the autism spectrum compared to the general population. Recent studies have also noted that autism is associated with an increased vulnerability for substance use disorders.[148-151] Many autistic individuals may simultaneously experience multiple co-occurring conditions. Current research indicates that these co-occurring conditions may share common genetic, epigenetic, and neurological variations.[152-155] However, additional research is needed to establish clear causal links. In addition, research is needed to better characterize the manifestation of these mental health conditions in the context of autism and vice versa to develop improved diagnostic tools and interventions to treat co-occurring conditions, in particular depression and suicide. Studies on co-occurring mental health conditions should also explore the role of sex and gender, intellectual ability, and age to determine if these factors mediate the severity of symptoms and provide more information on what interventions may be most appropriate for people of all genders and abilities across the lifespan.

Individual, intergenerational, and historical trauma also needs to be considered in mental health research of autistic individuals. Current data indicates that adverse childhood experiences (ACEs) are associated with autism;[156] caregivers of autistic youth also experienced higher rates of ACEs compared to caregivers of non-autistic youth.[157] Brain imaging studies indicate that ACEs may lead to changes in neurological functioning, some of which are already known to be associated with autism.[158-160] ACEs and other traumatic experiences greatly impact mental and emotional well-being, leading to suicidal ideation and emotional distress and impacting development into adulthood.[161] More research is needed with larger studies to understand how different traumas may differently affect diverse individuals and the differences in how acute versus chronic trauma impacts life outcomes for individuals on the autism spectrum. Research is also needed on resilience factors, which may include places of refuge and identity,[161] that may protect individuals from the effects of adverse experiences to better develop interventions that can improve health and well-being.

Ehlers-Danlos Syndromes and Dysautonomia

Ehlers-Danlos syndromes (EDS) are a group of disorders characterized by hypermobility of the joints, skin hyperflexibility, and tissue fragility. EDS patients report chronic pain, fatigue, social withdrawal, and anxiety in their daily life.[162] Co-occurrence of EDS and autism has been described in the literature since the 1980s.[163] More recent studies have shown that children on the autism spectrum have greater joint flexibility compared to age- and gender-matched peers[164] and generalized joint hypermobility and EDS are associated with both autism and attention-deficit/hyperactivity disorder (ADHD).[165-167] Many co-occurring conditions are also shared in both autism and EDS.[168] However, it is unclear what the exact connection is between EDS and autism and if they share common genetic factors. More research is needed on the biological mechanisms and interactions of EDS and autism to facilitate interventions for both conditions that can improve health and well-being.

Dysautonomia is caused by dysfunction of the autonomic nervous system, which regulates nonvoluntary bodily functions such as heart rate, blood pressure, breathing, digestion, and perspiration. Symptoms of dysautonomia are varied and can include nausea and vomiting, balance problems, dizziness, weakness, and visual disturbances. Postural orthostatic tachycardia syndrome or POTS is a form of dysautonomia and is characterized by reduced blood volume upon standing leading to lightheadedness and fainting. Recent research indicates higher prevalence of dysautonomia in autistic and neurodivergent populations and strong associations with EDS.[169, 170] However, like with EDS, it is still unclear what the biological connection is between autism and dysautonomia. Research is needed to understand the mechanisms of dysautonomia and how the disorder may manifest differently in individuals on the autism spectrum to develop interventions that can alleviate symptoms and maximize quality of life.

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. Moving forward, the field should embrace policies that promote collaborations and enhance the reproducibility of findings and promote transparent reporting of experimental methods, use of common data elements, and sharing of data and analysis. 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 National Institute

of Mental Health Data Archive (NDA) platform is a valuable repository for high-quality autism data, tools, and methodologies that researchers should leverage to enable re-analysis of data and facilitate collaboration to accelerate research progress.

Larger sequencing, brain imaging, and longitudinal studies require coordination among research centers and a shift toward team science across multiple disciplines. Large-scale science initiatives and collaborations such as the Autism Biomarkers Consortium for Clinical Trials (ABC-CT), SPARK, the All of Us Research Program, and ENIGMA can contribute greatly to our understanding of the biology underlying autism. The coordinated collection and analysis of valuable imaging, behavioral, genetic, and phenotypic data can also be enhanced by the recruitment of a research workforce that includes not only neuroscientists, immunologists, and psychiatrists, but also experts in bioinformatics, machine learning, and biomedical engineers. Planning of such initiatives, however, requires thoughtful consideration of the needs of autistic individuals and their families, particularly in terms of ensuring meaningful use of data and benefits for participants, and effort to make materials and protocols fully accessible and minimally burdensome.

Community-based participatory research and the inclusion of people across the autism spectrum and across the lifespan in research development, implementation, and dissemination is crucial to identifying practical applications for promoting positive outcomes for autistic individuals and their families. The inclusion of autistic people in research validates the unique lived experiences of individuals on the autism spectrum and empowers them to contribute to important research on autism. When planning studies with human subjects, researchers must ensure that the privacy of participants are safeguarded, and that the data is stored and shared in a secure manner. In addition, researchers must also make certain that participants know what data is being collected and how that data will be used. In longitudinal studies with participants who start as children, informed consent needs to be established as the participants age into adulthood. The results of the study should also be made available to all participants in an accessible and timely manner. The dignity, rights, and welfare of the participants should be kept in mind throughout the research process. Lastly, the inclusion of individuals of underrepresented minority groups as both researchers and study participants will lead to study findings that are more accurate, applicable, and representative of the entire autism community.

Summary

Significant progress in understanding the biological basis of autism has been made, but considerable challenges remain. Though there is a desire to demonstrate the impact of interventions on brain function, fundamental research that will allow us to fully understand the importance of alterations in brain function and development is still needed. Basic science research on the underlying biology of autism continues to be critical to provide the foundation for translational advances that will lead to effective interventions to maximize positive outcomes for autistic individuals across the spectrum and across the lifespan.

Recommendations

RECOMMENDATION 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 autism.

Examples:

- Identify neurological differences that occur in significant groups of individuals on the autism spectrum.
- Understand the differences in cognitive development and communication in individuals on the autism spectrum.
- Understand the role of the immune system and metabolic processes in autism.
- Understand how atypical sensory and motor functions arise and the role they play in autism.

RECOMMENDATION 2: Support research to understand the underlying biology of co-occurring conditions in autism and to understand the relationship of these conditions to autism.

Examples:

- Determine the molecular basis of epilepsy in autism.
- Determine how GI dysfunction impacts autism-related characteristics.
- Determine how sleep disorders impacts autism-related characteristics.
- Determine the relationship of co-occurring psychiatric conditions to autism and their impact on the health and well-being of people on the autism spectrum.
- Elucidate the underlying causes of self-injurious and other challenging behaviors in autism.

RECOMMENDATION 3: Support large-scale longitudinal studies to answer questions about the development and natural history of autism across the lifespan, from pregnancy through childhood, adolescence, adulthood, and older adulthood.

Examples:

- Support the creation of large and diverse cohorts, characterized both phenotypically and genetically through the collection of autism-relevant exposure data and medical data on the autistic person and family members from the prenatal period through childhood, adolescence, adulthood, and older adulthood.
- Support research on how the neurobiology of autistic individuals change throughout the lifespan and into older adulthood.

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