AUTISM: What we know. What is next?

This document begins a conversation concerning what we know and what we need to learn about autism and related developmental disorders. It is intended to provide an outline of recent research advances and suggestions about next steps. It discusses different levels of analysis, ranging from behavior to molecular biology, with the aim of building bridges between them. For simplicity, throughout we use ‘autism’ as shorthand for ‘autism spectrum disorder,’ as defined below.

Neither the claims about what we know nor the questions raised here are comprehensive. The field is moving rapidly. We invite you to suggest additions, deletions, corrections or wholesale rearrangements.

Phenotype
According to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), the core features of autism spectrum disorder (ASD) are:

“Deficits in social cognition and communication,” which manifest as difficulties in reciprocal relationships such as joint attention, eye contact, empathy and understanding the thoughts and intentions of others.

“Restricted interests and repetitive behaviors,” which manifest as inflexible adherence to purposeless routines.

Skilled clinicians can agree on quantitative measures of such behaviors. These measures are the best ‘biomarkers’ we have at present to chart progress over time and the efficacy of treatments. With current instruments, an autism diagnosis is usually made in the third year of life.

What we know
- Beyond the core features, individuals on the autism spectrum may exhibit one or more associated deficits that add to the heterogeneity of the clinical picture:
  - A low nonverbal intelligence quotient (nvIQ), which is significantly correlated with autism severity.
  - Language impairment, which may be evident in acquisition of words, semantic processing, echolalia and prosody.
  - Motor deficits, usually in the form of poor control of skilled movements.
  - Hypo- or hypersensitivities to sensory stimulation.
  - Macrocephaly, evident in about 20 percent of individuals with autism.
  - Epilepsy (recurrent seizures), which occurs at some time between birth and adolescence in about 30 percent of individuals with autism.
  - Abnormal EEG discharges during overnight electroencephalograms, exhibited by more than 70 percent of individuals with autism.
  - Sleep disorders, including short rapid-eye-movement sleep and short total sleep time.
- Regression, defined as a loss of previously acquired skills, occurs in approximately 20 percent of individuals with autism.
Nearly 20 percent of Simons Simplex Collection families report reduction in autism-related behaviors when their children experience a fever.

What is next
- We need to stratify the broad autism phenotype by correlating autism traits with the following:
  - Quantitative measures of social cognition, working memory, attention and language.
  - Autism risk genes, insertion and deletion mutations and copy number variants.
  - Gene expression profiles or epigenetic marks.
  - Anatomical or functional neural correlates.
  - Trajectories of individuals with autism with low and high intelligence quotients.
  - Measures of autonomic functions (such as pupil diameter, pulse rate and blood pressure) and patterns of motor activity.
- Do sensory abnormalities arise in the periphery or in the central nervous system?
- Are the beneficial effects of fever due to a sensitive, temperature-dependent neural process, or are they a different consequence of inflammation?

Epidemiology
There has been a remarkable increase in the reported prevalence of autism, but the evidence does not bear out that we are in the midst of an autism ‘epidemic.’

What we know
- The latest survey from the U.S. Centers for Disease Control and Prevention (CDC) indicates that the prevalence of autism is 1 in 68 children in the U.S.
- The distribution in the U.S. is uneven, with a threefold variation among states.
- According to the CDC, prevalence more than doubled between 2002 and 2008 and increased tenfold over the past 20 years.
- Much of the reported increase in prevalence reflects the application of broader diagnostic criteria, greater availability of services and reduction in stigma associated with the diagnosis.
- Autism, across the entire spectrum, is four to five times more common in boys than in girls. The gender ratio is close to 1:1 in individuals with low intelligence quotients and 8:1 for high-functioning individuals.
- The incidence of autism increases with paternal age.
- Current evidence for specific environmental triggers is weak.

What is next
- Do pockets of increased prevalence exist in certain communities?
- Is the prevalence of autism the same worldwide?
- Is autism underdiagnosed in girls?
- What accounts for the gender gap? Genetics? Environment?
- What social determinants influence the prevalence of autism?
- Do environmental risk factors influence the expression of autism risk genes?
Genetics
Genes play a major role in autism. First, the concordance among identical twins is 90 percent in some studies. Second, several monogenic syndromes, such as fragile X syndrome, are associated with traits of autism. Third, the burden of loss-of-function mutations is greater in idiopathic autism than in unaffected individuals.

However, the genetic landscape of autism is far from clear.

What we know
- Highly penetrant, loss-of-function de novo mutations, including copy number variants (CNVs), single nucleotide variants and insertion and deletion mutations, have been identified in trio and quad families (a quad family consists of two biological parents, the child with autism and one unaffected sibling) in the Simons Simplex Collection and other cohorts.
- Recurrence is the principal criterion for accepting a de novo variant as a true autism risk factor.
- De novo risk variants are individually rare (each accounting for less than 1 percent of cases), but together they may account for more than 25 percent of autism cases. It is likely that this proportion will rise as researchers examine larger cohorts and implicate missense mutations in the disorder.
- Girls with autism tend to have larger CNVs than boys do, suggesting that females have compensatory mechanisms that may relate to more robust social behaviors.
- To date, most de novo variants have been found in individuals with low intelligence quotients, but the causal relationship between intelligence and autism is unclear.
- About 30 percent of children with idiopathic intellectual disability or monogenic syndromes, such as fragile X, tuberous sclerosis, neurofibromatosis, Rett syndrome and Timothy syndrome (all of which are associated with intellectual disability), exhibit traits of autism.
- There is significant overlap between de novo variants found in the Simons Simplex Collection and the 849 genes regulated by fragile X mental retardation protein.
- Dose-dependent effects are evident when comparing deletions and duplications at chromosomal regions 16p11.2, 7q11.23 and 22q11.2.
- Analyses of gene networks in autism point to variants in genes involved in synaptic function and plasticity, neuronal development, voltage-gated ion channels, neuroimmunology and the regulation of chromatin structure.
- Younger siblings of children with autism have a relatively high probability of developing autism (20 percent in some studies), consistent with inherited risk factors or with germ-line mosaicism.

What is next
- Can additional de novo variants be discovered and suspect variants validated by targeted DNA sequencing or by whole-genome sequencing in large cohorts (5,000-10,000 individuals)?
- Can telephone interviews be validated to acquire detailed phenotypic data in the same cohorts as are used for genetic analyses?
- Do normal- and high-intelligence individuals with autism have novel or less severe genetic variants?
- Are there correlations between autism-risk genes and particular autism traits?
- Can somatic mutations in brain cells be identified using autopsy material or re-differentiated pluripotent stem cells?
- Can sex-specific factors or patterns of gene expression be identified?
- Are there genes and epigenetic mechanisms that modify the expression of known risk variants (including allele-specific inactivation)? Recent studies of CNVs are consistent with a multi-hit etiology of autism.
Can analyses of gene networks be refined to take into account changes in gene expression over time in various regions of the brain?

What is the clinical spectrum of individuals who bear the same autism genetic risk variant (for instance, 16p11.2 microdeletion)?

Can high-throughput functional assays be devised to screen several hundred candidate genes?

Nongenetic Factors

It is likely that exposure to environmental factors and altered immune function can precipitate or exacerbate autism. Research into the interaction of genes and environment must proceed in parallel with the search for genetic risk factors.

What we know

- Nearly 20 percent of Simons Simplex Collection families report significant improvement in autism symptoms when affected children experience a fever. Convincing anecdotal reports have accumulated from many other sources.
- Maternal infection during the first trimester of pregnancy has been reported to increase the risk of autism.
- Elevated levels of pro-inflammatory cytokines have been found in the plasma of children with autism.
- About 10 percent of mothers with autism have circulating anti-brain antibodies.
- Rodent and primate models of maternal infection produce offspring that have deficits associated with autism in humans, including impairments in communication and social interaction.
- Mice and rhesus monkeys exposed in utero to antibodies derived from mothers of children with autism later display autism-associated behaviors. Exposed monkeys also exhibit abnormal brain growth.
- Glia, the non-neuronal cells that reside in the brain, influence neurogenesis and synaptic function in the normal brain. Studies of microglia provide evidence for cerebral immune attack in autism.
  - Positron emission tomography has detected relatively large numbers of microglia in the brains of young males with autism.
  - Bone-marrow transplantation, allowing the infiltration of healthy myeloid cells into the brain, in radiation-conditioned mice that lack the MeCP2 gene (a rodent model of Rett syndrome) alleviates classic symptoms of Rett syndrome.
- Exposure during pregnancy to valproic acid, a therapy for epilepsy and bipolar disorder, among other disorders, increases the risk of autism in the child.

What is next

- Are the beneficial effects of fever due to temperature-dependent neural processes or to pro-inflammatory cytokines (for instance, interleukin-6)?
- How do trajectories and sex differences affect therapies and placebo responses?
- What roles do microglia and astrocytes play in the evolution of autism-associated behaviors?
- Are the distributions of activated microglia and astrocytes useful biomarkers for regions of the brain that are at risk in autism?
- Can a cell culture system be developed to study the interactions among various combinations of wild-type and genetically modified neurons and glia?
- Can effects of microglia and astrocytes be examined early in development, both prenatally and perinatally?
- What antigens do maternal antibodies recognize?
- How do maternal anti-brain antibodies affect the development of the fetal brain?
- Can modulators of immune function alter the course of autism?
Cells and Synapses

Several autism-risk genes affect the function of chemical synapses. The relevant literature is vast. Here we begin with studies of the balance between excitation and inhibition, synaptic plasticity, neurogenesis and neuromodulators.

**Excitatory/inhibitory (E/I) balance:** This balance is determined in part by the number and function of excitatory and inhibitory synapses, the excitability of input and output neurons and the ability of microcircuits to compensate for synaptic alterations, known as synaptic homeostasis).

**What we know**

- Genes encoding proteins that affect neurotransmitter release, receptor function and inactivation have been implicated in autism.
- Evidence for increased excitatory drive is based largely on the frequency and amplitude of spontaneous and action-potential-evoked excitatory synaptic potentials. Most studies have focused on isolated brain slices, and most slices have been prepared from the hippocampus.
  - Increased frequencies and amplitudes of excitatory synaptic potentials have been observed in mouse models bearing human mutations in genes such as NLGN3, SHANK3, TSC1 and TSC2.
  - Mouse models of fragile X syndrome exhibit a higher density of dendritic spines in basal dendrites of cortical pyramidal neurons.
  - Reduction in glutamate-receptor activity by genetic or pharmacological means reverses cellular and behavioral deficits in fragile X mouse models.
  - Perturbation of E/I balance using optogenetic techniques leads to changes in social behavior in mice.
  - Genes encoding voltage-gated calcium, sodium and potassium channels that likely contribute to E/I balance have been implicated in autism.
- Evidence for reduced synaptic inhibition is based on assays of function mediated by gamma-aminobutyric acid (GABA).
  - Mouse models of Rett syndrome and others bearing human autism risk variants show a decrease in parvalbumin-stained GABAergic interneurons.
  - Postmortem tissue samples from the cerebella of individuals with autism show reductions in glutamic acid decarboxylase, the rate-limiting enzyme in GABA synthesis, and in the number of Purkinje cells.
  - GABA levels are low in several cortical areas of individuals with autism as measured by magnetic resonance spectroscopy.

**What is next**

- Is E/I imbalance evident in local circuits in vivo in response to natural stimuli?
- When do E/I imbalances first appear?
- How do compensatory changes in synaptic function alter the initial perturbation in E/I balance?
- Does the shift in the role of GABA from excitatory neurotransmission to inhibitory neurotransmission during early development play a role in autism?
- What are the proximate causes of E/I imbalance?
- How do E/I imbalances affect local microcircuits and long-distance connections?
- Can E/I balance be restored pharmacologically (see “Therapeutics”) or genetically?

**Synaptic plasticity:** Another hypothesis, not entirely independent of the first, suggests that autism-related changes in synaptic transmission depend on neuronal activity, or experience. Short-term (seconds to minutes) and long-term (hours to days) mechanisms have been implicated.
What we know

- Genes that regulate activity-dependent synaptic plasticity, including ARC, have been implicated in autism.
- Variants of the UBE3A gene are associated with reduced activity-dependent synaptic plasticity.
- The critical period in the visual system (plasticity of ocular dominance columns) is exaggerated in mice that model fragile X syndrome.
- Fragile X mice show reduced levels of long-term depression and also reduced plasticity of the visual cortex following monocular occlusion. Antagonists of mGluR5 can reverse the deficit in long-term depression and in cortical plasticity.
- NLGN mouse models show altered long-term potentiation.
- Synaptic plasticity in the hippocampus is altered in mice that lack SHANK2.
- Short-term synaptic plasticity (facilitation and depression) is altered in mice bearing 22q11.2 deletions and other mouse models.
- BDNF has been implicated in deficits in activity-dependent plasticity in models of autism.

What is next

- What is the influence of neuronal activity on the expression of autism-risk genes?
- What molecular pathways are involved in changes in synaptic plasticity and their reversal?
- Do reported variants in genes that regulate autophagy, or cellular self-ingestion, play a role in synapse elimination?
- Can the relative influence of short-term synaptic plasticity (facilitation, depression) be distinguished from long-term synaptic plasticity in maintaining local circuit function and relevant behaviors?

Neurogenesis: Hypotheses about altered brain development in individuals with autism involve changes in the birth and programmed cell death of neurons and glia, altered fate determination and aberrant cell migration.

What we know

- Brain size, measured by head circumference and more directly by magnetic resonance imaging, is increased in about 20 percent of pre-teenage children with autism.
- The number and size of neurons in gray matter, as well as the number of axons and glia in the underlying white matter, is increased in autopsy specimens from individuals with autism.
- Subcortical nuclei, including those in the amygdala and cerebellum, are also enlarged.
- Head size is large in mouse models of autism (PTEN and others), and large displaced neurons are observed.

What is next

- Are changes in brain size due to excess proliferation of neuronal precursors or the failure of normal regressive events such as synapse elimination, axon retraction and programmed nerve-cell death?
- Are changes in fate determination evident in animal models of autism?
- What roles do glia (astrocytes, oligodendrocytes and microglia) play in the axonal and synaptic deficits associated with autism?
- Are changes in neuron number and brain volume associated with changes in circuit function?
- Do other animal models of autism, including zebrafish, Drosophila melanogaster, and Caenorhabditis elegans, offer unique advantages in studies of synaptic plasticity?

Neuromodulators and neurohormones: Certain transmitters and hormones that modulate synaptic transmission have been implicated in autism.
What we know

1. Serotonin is increased in the serum of individuals with autism. Positron emission tomography studies suggest that serotonin synthesis is impaired in children with autism, particularly in the frontal cortex, thalamus and cerebellum.
2. Acetylcholine binding to nicotinic and muscarinic receptors is reduced in the parietal and frontal cortices of postmortem brains from individuals with autism. The alpha-7 acetylcholine nicotinic receptor gene is included in the 15q13.3 microdeletion.
3. Melatonin secretion is reduced in many individuals with autism, and the deficit may be due to a deficiency in ASMT, the gene that codes for the rate-limiting enzyme in melatonin synthesis.
4. Children with autism experience reduced rapid-eye-movement sleep. Melatonin treatment improves their sleep latency and total sleep time.
5. Genetic variants of the oxytocin receptor and of CD38, a protein that modulates the secretion of oxytocin, have been noted in individuals with autism.

What is next

1. Do any of the modulators listed above suggest targets for drug therapy?

Neural Circuits

Autism is described as a 'disconnection' syndrome, and researchers have been attempting to distinguish local circuits from long-range connections. However, the terms ‘local’ and ‘long-range’ are poorly defined, and mechanisms of disconnection are not clear in either case.

What we know

1. Several long axon tracts, including interhemispheric, corticostriatal, frontotemporal and cerebellar connections, measured by magnetic resonance imaging or in autopsy material, are reduced in size in some but not all cases of autism.
2. Connectivity differences can be detected as early as 6 months of age.
3. The diameter of cortical mini-columns in human autopsy material is reduced in autism brains, and the packing density of the mini-columns is increased.
4. Functional magnetic resonance imaging studies show decreased synchrony between frontal and parietal areas in adults with autism who are performing social, language and executive tasks.
5. The temporoparietal junction and regions of the medial prefrontal cortex are hypoactive in individuals with autism who are experiencing socially relevant stimuli such as biological motion and theory-of-mind tasks.
6. Individuals with autism show an increased variance of cortical responses to repeated sensory stimuli, although mean responses are not altered.
7. The autism-risk genes TAOK2 and CNTNAP2 expressed in mice are associated with changes in axon outgrowth and connectivity.

What is next

1. Are the alterations in white matter detected by diffusion tensor imaging due to a decrease in the number of axons, to altered myelination of axons or to more complex interactions within axon bundles?
2. Can methods for electrophysiological or optical recording from large populations of neurons with single-cell resolution reveal 'neural signatures' of internal states that are characteristic of autism?
3. What computational or modeling approaches are needed to manage such large amounts of data?
Can principles of circuit function, such as divisive normalization, be defined that might serve as biomarkers for autism?

Is there a resting-state neural signature (for instance, neural correlates of social cognition) for autism that functional magnetic resonance imaging, electroencephalography or magnetoencephalography can detect?

Are feedback connections, perhaps involving the basal ganglia and thalamus, altered in autism?

What cellular mechanisms (for example, the excitatory/inhibitory balance) might account for local or long-range functional disconnection?

Is the function of the mirror neuron system altered in individuals with autism?

**Therapeutics**

**What we know**

**Medical Interventions**

- Improvement in autism-related behaviors during fever offers hope that the syndrome can be reversed rapidly.
- Genetic or pharmacological intervention can reverse signs of autism in mouse models of fragile X and Rett syndrome. Adult as well as developing animals have shown this reversal, offering hope for gene therapy and pharmacological intervention.
- Oxytocin inhalation (or intravenous infusion) has produced modest and inconsistent results in measures of trust and social interaction. Several oxytocin trials in children, adolescents and adults with autism are underway.
- Risperidone and other antipsychotics reduce repetitive behaviors, hyperactivity, aggression and self-injury in some individuals with autism.
- In a double-blind placebo-controlled trial, the GABA mimetic bumetanide reduced autism traits in children.
- Arbaclofen, a GABA mimetic, showed some improvement in social cognition in a subset of individuals.
- Melatonin reduces sleep latency and increases total sleep time in about 60 percent of children with autism with reported chronic sleep difficulties.
- Carnitine replacement therapy may improve communication and sleep efficiency.

**Behavioral Interventions**

Behavioral interventions fall into two categories: those that provide a holistic developmental framework, such as the Early Start Denver Model, and those based on the principles of applied behavioral analysis, such as UCLA/Lovaas approach.

- The Early Start Denver Model offers significant improvement in verbal intelligence after one year of intensive (40 hours per week) intervention. At two years, the improvement in intelligence persists, and researchers noted other improvements in language skills, activities of daily living and motor skills. They saw no change in the severity of autism, according to the Autism Diagnostic Observation Schedule, or degree of socialization even after two years of intensive therapy.
- The UCLA Model of Early Intensive Behavioral Intervention offers significant gains in adaptive behaviors, as shown through four years of follow-up testing.
Devices
- Transcranial magnetic stimulation at low frequency over the dorsolateral prefrontal cortex may reduce repetitive behaviors in high-functioning individuals with autism.

What is next
- Can efficient clinical trial networks be developed that will standardize methodologies, patient populations and informed consent?
- Which biomarkers are most useful to monitor changes in the core symptoms of autism?
- What is the potential of GABA mimetics, oxytocin mimetics and other candidates, as they arise, in children and adults with autism?
- Can agents that emerge from genetic screens, such as carnitine and branched-chain amino acids, prove beneficial?
- Does transcranial magnetic stimulation improve social cognition in autism? If so, do the effects last beyond the period of stimulation?
- Can useful outcomes in early, ‘proof-of-concept’ clinical trials be defined?
- Can induced pluripotent stem cells be used to assay candidate drug therapies? Can appropriate assays in flies, worms, fish and other genetic-model organisms be developed?