# Summary and Recommendations of the CIRM Autism Workshop
Parc 55 Hotel, San Francisco, CA
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# EXECUTIVE SUMMARY

The goal of this workshop was to bring clinicians, scientists and patient advocates together in order to review the current understanding of the pathophysiology of autism spectrum disorder (ASD) and to identify the key scientific challenges in developing therapeutic approaches for this condition. In addition, CIRM sought to determine whether stem cells or related regenerative medicine technologies might play a role in unraveling the complex biological phenomena that underlie ASD. Finally, the participants were asked to propose a research agenda that would ideally lead to the highest impact, most efficient route to diagnosis, prevention, or intervention for autistic patients. The resulting recommendations will allow CIRM to determine the nature and the extent to which the agency could make an impact.

**Introduction**

The term autism more precisely refers to a spectrum of neurodevelopmental disorders that share a common set of features, yet vary enormously in their overall manifestations. Due to tremendous phenotypic heterogeneity, it has been extremely challenging to elucidate the biological basis of this condition. To put this in perspective, workshop participants likened the study of ASD to the field of cancer biology, where it is now clear that there are multiple diseases and routes to pathology, but they ultimately converge on a single diagnosis of cancer.

## The Enigma of Autism: Challenges

**Need for Standardized ASD Definition and Phenotypic Categorization**

Currently, ASD is diagnosed based on behavioral assessment of children in social interactions, cognitive tests, and language patterns relative to developmentally appropriate controls. Because these descriptions are rather general, it has been difficult to determine from clinical and administrative data whether ASD represents a few, a dozen, or even hundreds of separate disorders, each with potentially distinctive biological underpinnings. It is also difficult to assess whether the prevalence of ASD is increasing due to evolving methods by which diagnostic criteria are gathered, measured and defined. Despite these caveats, it is generally accepted that the overall prevalence of autism is 6-7 per 1000 with some estimates higher and some evidence for geographic variation. While ASD is diagnosed primarily by behavioral characteristics, a significant percentage of ASD patients display other pathologies including gastrointestinal disease, epilepsy, various psychopathologies, significant intellectual impairment, and mitochondrial cytopathies. The timing and pattern of onset, as well as the severity of symptoms can vary significantly, even among affected members of the same family. Despite this enormous complexity, there is currently no standardized set of definitions that can be used to document and quantify this heterogeneity.

**Elucidating the Genetic Basis of ASD**

While the nature of ASD is poorly understood, there is a growing body of evidence to suggest a significant role for an underlying genetic contribution. Monozygotic twins are known to display as much as 90% concordance for ASD, and family members of affected individuals are more likely to display ASD or related symptoms than the general population. More recently, however, high throughput and genomic analyses have revealed that the risk of developing ASD cannot be easily predicted. To date, only a small percentage of ASD diagnoses have been associated with specific genetic or chromosomal abnormalities, yet not all individuals who inherit these alleles will

become autistic. These observations suggest that other factors, importantly epigenetic controls or exposure to environmental triggers, are playing a role in the development of ASD. As with other complex traits, the genetic transmission of ASD is likely to involve multiple genes. Efforts to distill the complex outcomes of these studies have been thwarted by poor communication between investigators, an absence of compatible and/or accessible formats for data sharing, and a lack of systematic phenotyping to provide context for comparison.

**Understanding the Biological Origins of ASD**

Perhaps the most fundamental challenge in the study of autism is to determine the cellular and molecular basis of the disorder and its developmental timeline of onset. With few clues in hand, scientists have focused much of their attention on neurological development and studies of the brain. These studies are fraught with difficulty, however, due to the unique challenges posed by the ASD population. It is simply not feasible to perform extensive, invasive diagnostic techniques on very young and sensitive children. While brain samples can occasionally be obtained from deceased individuals, these tissues can be difficult to acquire and are often of poor quality. Adding further difficulty, the high incidence and diversity of ASD-associated co-morbidities suggest that there may be multiple biological pathways that are perturbed in ASD patients, and thus confining studies to the nervous system might result in critical observations being overlooked.

**Modeling ASD**

Our incomplete understanding of the etiologies of ASD leads to an additional set of challenges when attempting to model disease processes in the laboratory. Although certain known ASD-related mutations are associated with autism-like phenotypes in model systems and have proven quite useful for targeted investigations, it is clear that the entire range of cognitive functions and human behaviors cannot be adequately reflected in nonhuman models, making translational research particularly difficult.

As a complementary approach for modeling the human condition, scientists hope to develop *in vitro* systems for probing the underlying ASD defects at a cellular level. With recent advances in stem cell technology, it will be feasible to generate induced pluripotent stem cells (iPSC) from ASD patients, which could be rigorously characterized as they undergo differentiation into a variety of tissues and cell types. Such cells could also be subjected to environmental insults or other perturbations to in order to elicit a relevant phenotype or exacerbate an existing one. While the potential impact of these studies may be high, there are many challenges to putting such models into practice. First, it is not known whether ASD phenotypes will correlate with *in vitro*, cell autonomous defects, although evidence from analogous mouse studies suggests that this will be possible in at least a subset of ASD. Other issues include uncertainties as to the number of independent cell lines that would be needed per patient, the phenotypic range to be targeted, the manner in which the lines should be grouped, and the purity of the cell populations that would be required. It is also not clear which assays would offer the most precision and relevance for identifying or quantifying putative defects. As technology for reprogramming cells is changing rapidly, it remains unknown how a particular methodology would impact the ability of iPSC or their derivatives to accurately mirror the cellular behavior and function of their endogenous counterparts. It is also unclear which cell types would offer the most promise for *in vitro* modeling. As many specific cell lineages have yet to be recapitulated *in vitro*, it may not be possible to capture every relevant phenotype that could relate to ASD. The diversity of synapses in the central nervous system, some of which may be affected in ASD patients, may also prove difficult to model in cell culture. Despite these difficulties in capturing the full array of manifestations of ASD,

scientists remain optimistic that iPSC models can now begin to provide significant insights into the condition.

## Pathways Forward

The primary obstacle to progress in the ASD field relates to a fundamental lack of knowledge as to the underlying biology that gives rise to ASD. To overcome this roadblock, workshop participants were asked to devise strategies that could pave new pathways to the clinic. While several different agendas were proposed, a set of common elements were identified and highlighted as the most promising. First, it was clear that a unifying, standardized set of phenotypic measurements and categories to which ASD could be assigned would be enormously useful. Clearer phenotyping has potential to not only shed new light on the results of previous investigations, but will enable a better understanding of what is being modeled with *in vitro* or *in vivo* assays for ASD. Second, the workshop participants were unanimously enthusiastic that stem cells, particularly induced pluripotent lines derived from patients, offer unprecedented opportunities for correlating disease with cellular phenotypes through comparative studies of gene expression, epigenetics and a host of cellular functions and signaling pathways during *in vitro* differentiation. Once such a correlation has been identified, high throughput screens with targeted readouts could be implemented to allow identification of candidate molecules for further therapeutic research and development. Workshop participants were universally optimistic about the potential for iPSC studies to contribute significant insights into the pathophysiology of ASD and that this work can take place in parallel with the work needed to standardize clinical phenotyping.

**Summary of Recommendations and Outcomes**

- Multiple parallel scientific, epidemiologic, and clinical approaches are needed to rapidly improve our understanding of ASD, its origins and its prevalence. In particular, standardization of clinical phenotyping and iPSC modeling should be pursued simultaneously and inform each other as they progress.
- The clinical phenotyping of ASD needs to be standardized. Phenotypic categories need to be quantifiable to account for and capture heterogeneity of symptoms as well as patterns of onset. Additional diagnostic criteria must be included to capture differences in etiology, including a battery of tests to determine the extent and correlation of physiological co-morbidities. Such criteria could include assessment of mitochondrial and immune function, MEGs and EEGs to detect electrophysiological abnormalities, functional MRI to detect neural activity, and detailed psychological assessment to qualify specific behaviors.
- Efforts need to be made at a national level to coordinate the criteria by which ASD are diagnosed and reported. Only when records are standardized can meaningful conclusions be drawn from meta-analysis of clinical and administrative data to address questions of autism incidence and prevalence, and association with factors such as pesticide use and other environmental exposures.
- Scientists need improved access to brains and other tissues from autistic individuals in the appropriate developmental windows. A public health monitoring system for flagging ASD patients that have died or are undergoing surgical procedures should facilitate the distribution of such materials.
- Efforts to sequence the genomes of ASD patients will continue to be useful, and could be maximized if standardized definitions of ASD phenotypes can be used to identify meaningful patterns in the data.

- Efforts must be made to assimilate clinical and genomic data from multiple sources that are currently inaccessible or incompatibly formatted.
- Efforts must be made to foster collaboration and communication between basic scientists and psychiatrists, neurologists, psychologists, educators and other clinicians.
- Targeted research should be pursued on genetic, cellular, and molecular pathways that show evidence for association with ASD, and on related disorders for which the genetics is better defined (for example Fragile X, Rett Syndrome and tuberous sclerosis). Insights gleaned from these studies will lead to new avenues of research for investigating ASD of unknown genetic origin.
- The development of ASD model systems will be essential for elucidating biological mechanisms of ASD, identifying diagnostic criteria, and ultimately for translating findings to the clinic. Efforts must be made to identify relevant models that are predictive and reflect ASD phenomenology. For some studies, it may be adequate to recapitulate only a subset of ASD symptoms. For others, it will be necessary to develop readouts that are indicative of more complex cognitive defects.
- iPS cells should be generated from ASD patients of defined phenotypes and subjected to a battery of tests in order to:
    - Identify *in vitro*, cell autonomous phenotypes that correlate with ASD by:
        - Examining the effects of ASD on various cellular, molecular (genetic and epigenetic), and developmental pathways.
        - Examining the effects of ASD on relevant cell function (i.e. electrophysiological behavior of neurons, timing and specificity of subtype differentiation).
- Consent forms for patient contact should be implemented so that the natural history and course of ASD progression can be followed and correlated with cell studies.
- iPSC should be generated from well-defined murine models of autism-like disease. Molecular, cellular, genetic and functional analysis of these cells should be compared and correlated with those from human iPSC to validate the presumptive ASD-specific parameters. Standardized mouse behavior assays should also be incorporated into this analysis.
- Identification of validated ASD-specific parameters from autistic iPSC should enable:
    - Assessing the response of ASD lines to environmental insults and other challenges (threshold effects).
    - Screening for candidate therapeutics
    - Identification of potential diagnostics for early detection of ASD risk, or ability to predict severity of particular symptoms.

**Conclusion**

Much like cancer biology, the study of ASD will rely on knowledge drawn from a diversity of cellular and molecular phenomena that converge on a heterogeneous but definable set of outcomes. While still in the basic stages, the combination of standardized diagnostic definitions and the availability of powerful new tools should enable novel insights to be made from old data as well as open the door to new avenues of research, including the design of better and more comprehensive model systems for developing therapeutic interventions for ASD. The mechanistic studies that are needed to understand ASD biology will inform our understanding of differentiation and development, thereby potentially impacting a number of diseases and conditions that extend well beyond the spectrum of autism.

## Appendix:
## Outcomes of Breakout Sessions: Pathways to Translation

Group 1 Slides

- Goal: Rapidly define in vitro phenotype to enable:
  - Compound testing
  - Definition of molecular phenotype for diagnosis
  - Correlative models
- Generate iPSC lines (multiple labs) from patients strateified for phenotype ( use existing databases, include defined single gene mutations, and single gene disease eg fragile X with and without autism) :
- Characterize: molecularly, epigenetically, functionally (excitability, dendritic spine density) during and after differentiation. Can define a cellular phenotype?
  - Verify phenotype in mice
  - Line up multiple labs with specific characterization expertises, common database, communication tools (website?)

- Corroborative animal models
- Choose iPSC lines from mutantmice with same single gene mutations to generate IPSC
  - Use same assays for functional outcome
- Well validated mutant mouse models can then be used to test treatments

# Group 2 Slides

Discovery-Preclinical

Disease Modifying Therapies (towards)

- Tools for Discovery **iPS and hESCs**
  - Validate the cells
  - Work out reliable in vitro differentiation regiments for neural populations
  - FIND cell surface markers for sorting
  - Develop end point phenotyping ASSAYS

- **Sequence Genomes** of Autistic Patients and make iPS from same patients… this will provide genotype/phenotype association.

Discovery-Pre-Clinical:

- Preventive

-Biomarkers (fetal protein in mother's blood, id?)
-Diagnostics

- Research

Tissue:
Public health systems to ID autistic patients -- brain sample obtention (epilepsy, death)

Group 3 Slides

## Top down Approach: the patients

- Extensive biological characterization in the clinical setting: MRI, EEG, MEG: functional, descriptive, physiological, cytological (mitochondrial, immune)
- Profiling patients' genetic profiles, looking for biomarkers, pathways
- Access to patient tissues for analysis, epigenetic profiling, quantifying neuron types and subtypes, etc.
- Targeted extensive study of TS, FRAX and other select syndromes

## Bottom up Approach: Analysis of iPS cells from ASD patients

- Extensive phenotypic characterization
  - Morphological characterization of every step in neural differentiation pathways, quantify and characterize, classify by subtypes and subclasses)
    - Cytological (IIF)
    - Ultrastructural (EM)
    - Neurite characterization
  - Functional (electrophysiology, Ca flux, etc.)
  - Look at mitochondrial function

Group 4 Slides

## Autism Spectrum Disorder iPS Cell Bank

- Patients with genetically defined mutations as well as unknown etiologies
- Clinically phenotyped patients – consent for followup
- Collect and bank fibroblasts, generate and bank iPSCs
- Genetically profile iPSCs
- RFP rather than an RFA (contract rather than a grant) – guarantee funding, ensure oversight, remove pressure to publish
- Funds for distribution and data curation



## Transformative RFAs for new approaches to understand molecular etiology and treatment of ASDs

- Comparing iPSCs generated from ASD patients to identify common cellular phenotypes and dysregulated molecular pathways
- Developing assays for high-throughput analysis
- Developing screening assays for non-genetic components
- Integrating stem cell approaches into animal models
- Immune and non-neural components