

ReMIND AWARDS

8/29/24

\$67,524,203 GWG RECOMMENDED

\$20,675,797 REMAINING

\$88,200,000 AMOUNT AVAILABLE

Number of GWG
Votes

APP #	TITLE	BUDGET REQ	FUND?	SCORE	1	2	3
DISC4-16295	Translational epigenomics: dissecting cell type-specific function of neuropsychiatric risk genes in vivo	\$11,376,314	Y	1	14	0	0
DISC4-16285	Deep phenotyping of human brain organoid models of autism spectrum disorder to unravel disease heterogeneity and develop biomarkers and treatments	\$12,297,272	Y	1	14	1	0
DISC4-16377	Modeling the genetic basis of psychopathology in schizophrenia and autism	\$12,703,708	Y	1	14	1	0
DISC4-16322	CIRM Center for Neuropsychiatric Stem Cell Proteomics	\$13,781,522	Y	1	13	2	0
DISC4-16292	Multiomic Studies of Idiopathic Intellectual Disability and Autism Spectrum Disorder (ID/ASD)	\$17,365,387	Y	1	12	3	0
DISC4-16337	Defining Neurovascular Metabolism in Neurodevelopmental and Neuropsychiatric Disorders	\$12,000,482	REVISE	2	7	7	0
DISC4-16360	Patient-derived organoids for early diagnosis and personalized prognosis of intellectual disability	\$12,556,739	REVISE	2	2	13	0

APP #	TITLE	BUDGET REQ	FUND?	SCORE	1	2	3
DISC4-16507	From genes to circuits: leveraging neural assembloids to decipher multi-level mechanisms in neurodevelopmental disorders	\$15,261,984	REVISE	2	2	13	0
DISC4-16283	Prenatal Marijuana Exposure and Neuropsychiatric Predispositions: A Single Cell Perspective	\$10,658,194	REVISE	2	1	12	2
DISC4-16336	Human neural organoid models for opioid, cocaine and alcohol substance use disorder to identify pathomechanisms in addiction	\$12,608,943	REVISE	2	0	14	1
DISC4-16338	A Framework for Enhancing Clinical Utility of Precision Genomics in Psychotic Disorders	\$12,520,839	REVISE	2	0	10	5
DISC4-16378	Mechanistic understanding of neuronal maturational timing	\$12,180,898	REVISE	2	0	15	0
DISC4-16400	High throughput, multi-modal analyses of neuropsychiatric disorder risk genes in a diverse cohort	\$14,247,871	REVISE	2	0	14	1
DISC4-16345	Development of novel therapies to treat CNS-associated microdeletion syndromes	\$10,172,372	REVISE	2	0	13	1
DISC4-16369	Exploring mechanisms of substance use disorder and its psychiatric comorbidities using diverse patient-specific iPSCs	\$10,505,476	N	3	0	1	14
DISC4-16399	Schizophrenia: genetic, molecular and neurophysiological convergence at the synapse	\$13,674,600	N	3	0	1	14
DISC4-16437	A functional genomics approach to dissect the regulatory mechanisms of genetic variants associated with bipolar disorder	\$12,628,200	N	3	0	0	15

APP #	TITLE	BUDGET REQ	FUND?	SCORE	1	2	3
DISC4-16461	Discovery of Neuroimmune Mechanisms in Schizophrenia From Genes, to Proteins, to Circuits to in vivo Neuroimaging	\$12,307,472	N	3	0	0	15
DISC4-16273	Multidimensional investigation of neuropsychiatric disability: Aggressive behavior challenges (The 'MIND-ABC' Study)	\$11,610,067	N	3	0	0	14
DISC4-16468	Epigenetic & infectious pathways affecting autism spectrum disorder & developmental disability in children exposed to maternal COVID-19 in pregnancy.	\$10,853,604	N	3	0	1	13



Application #	DISC4-16295
Title (as written by the applicant)	Translational epigenomics: Dissecting cell type-specific function of neuropsychiatric risk genes in vivo
Research Objective (as written by the applicant)	Our objective is to enable scalable genetic screening to study how different cell types and epigenetic networks are impacted by risk genes implicated in human psychiatric disorders.
Impact (as written by the applicant)	We will develop and apply state-of-the-art genomic analysis to seek mechanisms and to design and test therapeutic agents for disorder modifying solutions.
Statement of Benefit to California (as written by the applicant)	Mental health disorders are one of the most common health conditions faced by Californian citizens: 1 in 6 California adults have experienced some form of mental illness, and 1 in 24 have a serious condition that makes it challenging to carry out major life activities. Our work is to approach both the basic mechanisms involved in these disorder and the therapeutic development using antisense oligos to help with these devastating conditions.
Funds Requested	\$11,376,314
GWG Recommendation	Tier 1: warrants funding
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 1

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	1
Count	14
Votes for Tier 1	14
Votes for Tier 2	0
Votes for Tier 3	0

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14	<ul style="list-style-type: none"> • This project aims to assess the effects of loss of function mutations within over 70 autism spectrum disorder (ASD)-related genes + UBE3A. The project will assess this comprehensively in both mouse and human cortical model systems. Effects will be



<p>No: 0</p>	<p>measured on cell type proportions, gene expression, chromatin accessibility, methylation, and chromatin interactions. A model will then be built describing the effects of ASD-related gene mutations on multiple aspects of gene regulation.</p> <ul style="list-style-type: none"> • The project aims to understand how rare and de novo variants in ASD-associated genes affect gene regulation and brain development. The project addresses a major gap in understanding ASD etiology and pathogenesis: the cell type-specific and epigenetic mechanisms of ASD risk genes. The project also has the potential to develop novel and targeted therapies for ASD using ASO technology. • The overarching goal is to bridge the gap from fundamental ASD gene research to therapeutic development by leveraging human organoid models, integrated multi-omics data, and AI-driven analysis to identify and validate potential interventions for ASD. • The key knowledge gap is the function of ASD-related risk genes. The major innovation is use of a pooled CRISPR screening approach that allows many genes to be tested simultaneously. • This proposal could have a broad impact on understanding the convergence of ASD risk genes into similar or different cellular/molecular effects. If convergence exists, they plan to develop and test ASO therapeutics targeting upstream regulators of ASD networks. This is an exciting idea for broad disorder therapies, instead of bespoke therapies differing based on each rare mutation (which would be difficult to scale). • The major outcome measures are data describing the effect of each ASD risk gene and a model integrating these measures to predict the effects of modulating each of these genes. The dataset generated in Aims 1 and 2, with appropriate controls, incorporates a causal manipulation that is an important complement to iPSC-derived organoids from patients with these mutations or mouse models. • The project will generate valuable datasets and will help understanding the molecular underpinnings of loss-of-function of ASD risk genes. • The project has good integration of gene regulatory networks (GRNs) for guiding the investigation.
<p>GWG Votes</p>	<p>Is the proposal innovative?</p>
<p>Yes: 14 No: 0</p>	<ul style="list-style-type: none"> • Several new techniques will be incorporated, including highly multiplexed CRISPR-screens that allow the manipulation of multiple genes, epigenomic measurements of methylation and chromatin interaction, ASOs for treatment, and human organoids implanted into mice in order to improve the maturation of iPSC-derived neurons within these organoids. While these technologies have all been used previously in the team members' labs, they will be integrated here in a novel way. • The critical novel technology is highly multiplexed CRISPR screens, wherein multiple mutations can be screened in different cells from a single animal or organoid. • The applicants have developed improved CRISPR screens that allow more perturbations to be assessed. This is an important increase in efficiency needed for the approach. • The project will develop and apply two types of models: ASD gene regulatory networks (GRNs), standard machine learning models for GRNs, and ChatACTG, advanced AI multimodal cross-attention-based foundation model for GRNs affected by ASD risk genes. • The proposed computational models are highly innovative and should have a significant impact on the field. • Yes. The project incorporates AI approaches and single-cell multiomics, as well as organoids. • This proposal will cut across disciplines of human genetics, CRISPR screens, AI, and genome-targeted therapeutics. Expertise is acquired from both clinician scientists and basic scientists. Each member of the team has been a pioneer in their field. • The project team comprises experts with diverse backgrounds in neuroscience, genomics, bioinformatics, and clinical medicine. • The conceptual framework and hypotheses are not new, but established and sound. The question of whether ASD mutations converge on a specific biological pathway, cell type, or time period has been pursued for many years.



	<ul style="list-style-type: none"> • Similarly, the idea that ASD-associated mutations impact gene regulation has been established over 10 years ago (by investigators including the applicants). The advantage of this study is that all ASD-associated mutations discovered through de novo mutations and whole exome sequencing (WES) will be studied simultaneously so can be compared. • UBE3A ASOs are already being developed and tested in clinical trials, so this aspect is not novel, but the outcome measures to be evaluated are novel and will give some validation to the approach.
GWG Votes	Is the rationale sound?
Yes: 14 No: 0	<ul style="list-style-type: none"> • Yes. The project uses statistically significant ASD-associated mutations, many replicated in previous studies, suggesting that they are bona fide ASD risk factors. The methods have all been previously validated. • Preliminary data are highly compelling showing success of previous CRISPR screens in vivo embryonic brain as well as vector optimization for rapid expression. Implanting organoids into mouse cortex has resulted in higher maturity of neural cells in the organoids. Previous work generating gene regulatory networks from large datasets, including datasets generated by the authors, is also demonstrated. • Yes, the applicants have generated preliminary data using various single-cell omics techniques to profile the effects of ASD risk gene perturbations on gene expression, chromatin accessibility, DNA methylation, and chromatin conformation in mouse and human brain cells. The applicants have also developed CRISPR-edited iPSC lines and cerebral organoids. • Yes, the rationale is sound with strong preliminary data supporting the proposed work. • Both human iPSC and mouse models are proposed to be used in this project. Mouse models are higher throughput so most CRISPR screens are planned to be conducted in this model system, whereas human organoids implanted into mouse cortex have lower throughput so only a few genes will be evaluated in human cells. This complementary evidence is useful because human organoids are an imperfect model system that does not contain all cell types or the organization of the human brain. • Yes, the research approach is detailed and multi-pronged, highlighting the strengths of integrating multimodal data. The results of the project could have substantial impact for therapeutic treatment of ASD by identifying GRNs affected by ASD risk genes. • Yes, the project is relevant and impactful because it addresses a major gap in understanding ASD etiology and pathogenesis, namely the cell type-specific and epigenetic mechanisms of ASD risk genes. • Examining gene expression profiles across human brain development and focusing on specific time points in mouse brain development captures the effects of risk genes during neurodevelopmental stages, including neurogenesis, gliogenesis, and synaptogenesis. • The project is highly relevant to ASD because the selection of genes is based on ASD risk.
GWG Votes	Is the project well planned and designed?
Yes: 14 No: 0	<ul style="list-style-type: none"> • The project aims to develop and apply physiologically relevant in vivo mouse and human neuron-based models for high-content, high-resolution functional assessment of ASD risk genes. They will do this by focusing on cell type diversity and epigenetic signatures in the developing cortex, a brain region strongly implicated in ASD, across developmental time windows. The proposal aims to illuminate the regulatory disruptions in ASD and develop targeted therapies using antisense oligonucleotide (ASO). • This is a well planned program with cohesive aims and structure. The aims are interconnected and clear. • The research approach is detailed and multi-pronged, The subprojects inform each other but are independent of the outcome of one another. • Data sharing of all datasets and models are described, and will be a valuable resource for the psychiatric genetics community. • In general, potential pitfalls are well described and given the applicants extensive familiarity with each method. The applicants are highly likely to succeed in their goals.



	<ul style="list-style-type: none"> • The major undescribed limitation is that all variants are heterozygous in humans with ASD but the CRISPR screens cannot separate homozygous vs. heterozygous mutations and are likely predominantly homozygous (given the approach). The applicants are thus not modeling the ASD-relevant mutations (most relevant to disease) but are modeling the ASD-relevant genes. Understanding gene effects are important but may be different in heterozygous versus homozygous mutations. This is somewhat alleviated by the two genes that will be modeled using heterozygous mutations in iPSCs, but this is only two genes out of more than 70. • Similarly, the heterozygous iPSCs and CRISPR screens are not able to select a maternal vs paternal deletion. UBE3A is an imprinted locus in neurons, so the relevant mutation would be to delete the maternal allele. While deleting both alleles in the CRISPR screens is probably fine, maternal deletion was not described for the heterozygous iPSCs. • Also, by injecting perturbations in early development and observing impacts later on, gene regulatory results could be due to either changes in fate decisions of early progenitor cells or current modulation of genes, and the inability to disambiguate these two could lead to difficulties in building models in Aim 2. • Although the expected results appear realistic and achievable, potential limitations around modeling assumptions and cross-species translation should be carefully considered. The analysis plan incorporates reasonable hypotheses and leverages appropriate computational tools but also faces significant analytical hurdles due to the dataset's unprecedented scale and multi-dimensional nature. • They should consider the possible off-target effects, incomplete knockdown from CRISPR perturbations, and the challenges in generating consistent, high-quality organoids across replicates. • All the proposed parts of the project are highly complementary. • It would be helpful to introduce patient-relevant mutations. • One challenge will be in translating findings between mouse to human.
<p>GWG Votes</p>	<p>Is the project feasible?</p>
<p>Yes: 14 No: 0</p>	<ul style="list-style-type: none"> • Yes. Many of the techniques have been performed in the project team's labs. Given the dataset's unprecedented scale and multidimensional nature, the project team has the appropriate computational power to complete the work. • The project has a low technical risk, as the in vivo Perturb-seq methodology is already developed. Contingency plans include adding or training team members, data backup, and addressing unanticipated results. • Yes, the collaboration-wide meetings will be the main forum for discussing and agreeing on any substantial changes to the proposed scope of work. Specific Aims are handled by the overseeing Investigator. A dedicated Conflict Resolution Committee composed of representatives from each group will serve as a neutral forum for addressing disputes. The process will involve open communication, active listening, and mediation. A structured escalation process will be in place for more complex issues. • Yes, the proposed team is highly qualified and has the diverse expertise necessary to manage this large project. • The team has reasonable plans for managing the large collaboration, with each investigator leading the aspect of their own expertise. A project manager will organize across the groups. Many members of the team have already worked together. • Yes, the proposed team is impressive with significant expertise in neuroscience, genomics, bioinformatics, and clinical medicine. • The investigators developed several of the methods to be utilized in the project. • The aims are led by the investigators who developed the methods. • This is a very strong applicant team. • The scale and use of filtering of genes to be included in the project makes this more feasible. • The project is highly feasible. Timeline and milestones are clear.
<p>GWG Votes</p>	<p>Does the project uphold the principles of diversity, equity and inclusion (DEI)?</p>
	<ul style="list-style-type: none"> • Diverse groups are considered in this project.



<p>Yes: 13 No: 1</p>	<ul style="list-style-type: none">• The mutations to be studied were identified in multiple ancestries and both sexes so the work here is likely broadly applicable.• Since the mutations were identified in different ancestries, the work likely will extend to diverse ancestries and underserved populations.• Yes, the researchers will ensure sex balance and ethnic diversity in selecting cell lines and use cryopreserved iPSC lines from more than 10 neurological conditions, including ASD, and over 60 lines from healthy subjects aged 10 to 70.• CRISPR screens will be performed in at least two independent cell lines, and the authors have established diverse collections across sex and ancestry. However, two cell lines cannot be used to sample multiple sexes within multiple ancestries. They do say that they will perform additional validations with additional lines if time and budget permit.• The project team will replicate and test the results in at least two independent cell lines to account for different genetic backgrounds, sex, epigenetic memories, and ancestry information.• The proposal highlights the various programs and initiatives the team members have implemented or participated in to recruit, mentor, and support trainees from underrepresented backgrounds across different institutions.• The investigators have an extensive history of participating in and starting programs designed to increase representation in research.• This is unclear. There is not a clear plan to actively include multiple ancestries in the work. The applicant's plan is to perform validation studies in additional cell lines (representing greater diversity) "if time and budget permit." This is not sufficient for upholding DEI principles.
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Application #	DISC4-16285
Title (as written by the applicant)	Deep phenotyping of human brain organoid models of autism spectrum disorder (ASD) to unravel disease heterogeneity and develop biomarkers and treatments
Research Objective (as written by the applicant)	We will uncover pathways through which ASD mutations cause disease and close the gap from disease research to therapeutic testing using organoids, primary human neurons, machine learning, and AAVs.
Impact (as written by the applicant)	Our studies are impactful because outcomes will lead to therapeutic avenues to pursue for ASD treatment.
Statement of Benefit to California (as written by the applicant)	1 in 22 children in California is diagnosed with autism, up from a previous estimates of 1 in 44 children in December 2022. Our study will identify the pathways through which ASD genetic mutations cause disease and will uncover new therapeutic targets. With our interdisciplinary team, we are able to close the gap from disease research to therapeutic testing using the most human relevant disease models, machine learning, CRISPR screening and AAVs.
Funds Requested	\$12,297,272
GWG Recommendation	Tier 1: warrants funding
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

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KEY QUESTIONS AND COMMENTS

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GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13	<ul style="list-style-type: none"> • The successful completion of this project will significantly advance the field of neuropsychiatric disorders by filling crucial knowledge gaps and overcoming research



<p>No: 0</p>	<p>bottlenecks. The project provides a comprehensive understanding of the interplay between genetics and environment, identifies novel genetic modifiers and biomarkers, develops targeted therapeutic strategies, and embraces genetic diversity in research.</p> <ul style="list-style-type: none"> • This well thought-out and synergistic proposal will address a research and development bottleneck - there are likely causal subtypes of autism spectrum disorder (ASD) that require different interventions. However, subtyping based on existing datasets has been unsuccessful. The datasets and analytic platform outlined here have the potential to address this key challenge. • The project's success will significantly advance the understanding and treatment of neuropsychiatric disorders by uncovering the intricate mechanisms of ASD, developing new diagnostic and therapeutic tools, and ensuring the inclusivity and applicability of the findings. • The applicants' multifaceted approach will enhance both the scientific understanding and clinical management of ASD, and potentially other neuropsychiatric disorders. • The synergistic subprojects proposed in this application have high potential for identification of new pathways and genes for targeted therapies. • The combination of the iPSC and postmortem cortical brain cell bank can provide new insights into ASD. • The project outcomes will include ample data, resources, and cell lines that will enable the research community to formulate and test novel hypotheses in the study of neuropsychiatric disorders. Both the transcriptomic and phenotypic data will be made publicly available. • Screening of AAVs across cell types and genetic backgrounds will fill a knowledge gap for gene cell therapies targeting ASD-related pathways identified herein. • Identification of AAV serotypes for efficient gene therapy across genotypes will be beneficial for many future efforts. • Support for possible AAV-delivered therapies would be of significant impact to the field. • The identification of biomarkers for ASD would have a big impact on the field. • The clinical characterization of the subjects from whom iPSC will be derived is useful. This characterization will aid interpretation of results from the cell-based experiments, and guide application of findings to the <i>in vivo</i> situation. • Identification of potential subtypes of ASD may be critical for future success in clinical trials and, in turn, for treating ASD subtypes.
<p>GWG Votes</p>	<p>Is the proposal innovative?</p>
<p>Yes: 12 No: 1</p>	<ul style="list-style-type: none"> • Investigation into the role of genetic background in ASD, and what genes might be protective, is innovative and highly relevant. • This project will use a combination of patient-derived iPSC and a bank of human primary postmortem brain cell lines to attempt to better understand ASD. These two cell sources are very complementary. • Proposed integration of cross-platform data analyses tools and machine learning approaches are innovative. • The applicants' novel automated cell culture feeding platform to enhance reproducibility is technically innovative. • Overall this is not highly innovative. But, the project combines human brain organoids, CRISPR/Cas9 and CRISPRi screening, advanced machine learning algorithms, cell-type specific AAVs, and high-throughput phenotyping platforms which together can bring new insights into neuropsychiatric disorders. • The proposal is a good example of cutting across technical silos and engaging different disciplines to address complex questions in neuropsychiatric disorders. By integrating clinical insights, genetic engineering, neurobiology, data science, and therapeutic development, the project fosters a comprehensive and interdisciplinary approach. • The applicants are testing new conceptual frameworks and hypotheses regarding neuropsychiatric disease mechanisms. These include the integration of genetic and environmental factors, identification of genetic modifiers, multi-modal data integration for biomarker discovery, cell-type specific therapeutic approaches, and the embrace of genetic diversity in disease modeling.



<p>GWG Votes</p> <p>Yes: 13</p> <p>No: 0</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • A combination of published findings and the investigators' own data support the ASD risk genes selected. A strength is that a number of patients with these risk genes have already been identified and some iPSC have already been produced. • The preliminary data are a strength of this application. The data support the experiments planned and provide proof of concept behind the ability to successfully obtain the necessary data. • The scientific rationale underlying the project is robust, supported by evidence from previous research and the strategic use of advanced technologies. The integration of human brain organoids, high-throughput genomic screening, and machine learning-based data analysis ensures that the project is well-grounded in current scientific understanding and methodologies. • The interdisciplinary team and their commitment to diversity further strengthen the project, making it well-equipped to achieve its goals and contribute significantly to the understanding and treatment of ASD. • The overall project is well thought-out and presented. There is clear synergy between groups and projects that all contribute to the end goal of identifying new therapeutic strategies for ASD. • The rationale for Subproject 1 is sound, and the genes chosen for analysis are well supported. The proposed approach is well-worn, but an important component of the overall goals of the project. • The rationale for Subproject 2 also is sound. Interrogating the contribution of diverse genetic backgrounds could be incredibly informative. That said, it also is risky and there is not evidence presented that clinically genetic background influence expressivity for the genes to be studied (minor concern as this will be interrogated under this award). • The rationale for Subproject 3 is sound and exciting. As noted in the text, the data to be acquired present a novel opportunity to potentially identify ASD subtypes. • The rationale for specific approaches would be strengthened by rigorously presenting support for reproducibility of organoid generation and cell type distribution across organoids within multiple genetic backgrounds. Fig. 2 is cited for reproducibility but it does not address this point.
<p>GWG Votes</p> <p>Yes: 13</p> <p>No: 0</p>	<p>Is the project well planned and designed?</p> <ul style="list-style-type: none"> • This is a remarkably well designed and thoroughly planned proposal designed to accomplish the outlined specific aims. The three projects complement one another well, while also addressing specific goals that build cell-based and data resources relevant to the study of ASD. • A strength of the proposal is the comprehensive and complementary way these aims fit together. The proposal is an excellent mix of mechanism discovery and work towards potential therapies. • The overall project and its subprojects are strategically designed to accomplish the specific aims through a comprehensive and integrative approach that leverages advanced technologies, diverse expertise, and a broad understanding of autism spectrum disorder (ASD). The project is structured around key objectives that collectively aim to enhance understanding, diagnose, and treat ASD. These project components and subprojects are planned to meet the specific aims. • The components and subprojects of the proposed project are designed to offer substantial scientific synergies through their integrated, interdisciplinary approach and the use of advanced technologies. • Each component of the project is meticulously planned to ensure that the outputs are not only scientifically robust but also clinically relevant. • Potential pitfalls and alternative approaches are discussed but in addition, each of the aims stands alone such that unanticipated issues will not derail the overall project. • Potential pitfalls are recognized and addressed for each aim within each project and reasonable alternative approaches are presented. The team takes strides to carefully consider the most likely roadblocks, which they are well placed to identify given their previous collective works. Exceptions are the following:



	<ul style="list-style-type: none"> ○ Choice of differentiation protocols: Comparison of organoid differentiation protocols to be used would strengthen the rationale for using multiple protocols. Further, incorporation of microglia and enhancement of the inhibitory neuron contribution to these models would strengthen the rationale as well. ○ The electrophysiology approach is perhaps the best available currently, however, the preliminary data presented in Figures 5 and 6 as supportive of reproducibility falls short of convincingly showing the robustness and reproducibility that may be necessary for identifying more subtle network phenotypes expected with these mutations. ○ The potential role of other cell types in ASD pathogenesis (microglia, higher numbers and diversity of inhibitory neurons) is only recognized sufficiently in the third subproject. ○ The challenge in reproducibility in MEA readouts is recognized, but further development prior to deployment of the technology is not sufficiently addressed. <ul style="list-style-type: none"> ● The team mentions power calculations briefly in subproject 1 (noting they will be done). However, it would be helpful to present power calculations in the proposal for those phenotypes for which preliminary data exist. Of particular importance is the measure of cell type distributions of organoids, which is a major phenotypic readout across multiple aims and projects.
GWG Votes	Is the project feasible?
<p>Yes: 13 No: 0</p>	<ul style="list-style-type: none"> ● The team is qualified. A strength of the application is the clear delineation of which Co-Investigators will be responsible for each aim. ● The Co-Investigator responsible for each group of experiments has the necessary experience to carry out the proposal as evidenced by the preliminary data in the application. ● The team's qualifications and staffing are well-suited to the multifaceted approach required for such a complex and interdisciplinary field of research. ● Yes, the proposed team is well qualified to execute this technically challenging research plan. ● The budget is appropriate and the investigators' percent efforts are sufficient to ensure project success.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 13 No: 0</p>	<ul style="list-style-type: none"> ● The proposal lays out plans to ensure underrepresented groups feel comfortable in joining the cohort, including translators and multi-language documentation. ● The investigators will generate iPSC from an existing cohort which is diverse, though not at the same ratios as the CA population. The brain tissue to be used in Aim 2 is more broadly diverse. ● Many of the co-Investigators are involved in DEI initiatives at their institutions and they have already built a network of educators that they work with to advance STEM education. ● Yes, the project plan and design seem to adequately address and account for the influence of race, ethnicity, sex, gender, and age diversity in several ways: <ul style="list-style-type: none"> ● Diverse Genetic Backgrounds: The proposal outlines the use of a library of nearly 200 human postmortem cortical progenitor cell lines from individuals of diverse ancestry. ● Gender Representation: The use of CRISPR knock-out cell lines for ASD variants specifically includes both male and female hPSC lines, providing balanced sex representation. ● Age Diversity: The project includes samples from individuals across various age groups, including infants, children, and adolescents. ● The team plans to use translators and multi-language documentation to increase ethnic diversity among participants, particularly by including Spanish to better engage Hispanic communities. The research team is actively involved in DEI programs at their institutions. The applicant has established connections with universities in locations far from major educational hubs and have close relationships with autism patient groups.



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| | <ul style="list-style-type: none">• Yes, this proposal in part aims to identify the influence of ancestry on expressivity of phenotypes with genetic manipulations. This will allow for deeper study of pathways altered across diverse genetic backgrounds leading to ASD. |
|--|---|



Application #	DISC4-16377
Title (as written by the applicant)	Modeling the genetic basis of psychopathology in schizophrenia and autism
Research Objective (as written by the applicant)	We propose to apply scalable platforms for the characterization of copy number variations (CNVs) and genes coupled with detailed characterizations of neurodevelopment of the four major CNV loci in model systems and patients.
Impact (as written by the applicant)	A unified understanding of how genes influence neurodevelopmental processes, circuitry & how these processes influence specific psychiatric disorders will achieve new advances in psychiatric medicine.
Statement of Benefit to California (as written by the applicant)	A deeper knowledge of mechanisms of neuropathology in schizophrenia and autism could advance the field of psychiatric medicine and could directly benefit patients. Another major aim of this study is to extend the key findings to underserved populations. We will specifically recruit and generate 30 new patient iPSC lines from subjects in our clinical cohort that are from underrepresented populations of African, Asian and LatinX ancestry, many of which will be from Southern California.
Funds Requested	\$12,703,708
GWG Recommendation	<i>Tier 1: warrants funding</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

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- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRP for clarity.



<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • A higher throughput approach to efficiently evaluate effects of knockdown of neuronally-expressed putative or known neurodevelopmental disorder (NDD) risk genes would be invaluable. • This proposal addresses one of the most pressing gaps in psychiatry: moving from the genes that have been discovered to illuminating causal neurological mechanisms. The proposal uses a combination of state-of-the-art data approaches and experimental technologies to understand mechanisms. The investigators have a clear plan to move from a couple of copy number variations (CNVs) of interest to a larger number of implicated genes. This could not just help us better understand schizophrenia (SCZ) and autism spectrum disorder (ASD) but lead the way in showing how we can move from gene discovery to mechanism. • The field needs a systematic approach to investigating functional and phenotypic convergence across the >100 risk genes linked to SCZ/ASD.
<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The project is not exactly innovative but cutting edge technically and innovative conceptually. • The proposal brings together researchers across domains to advance our understanding of SCZ and ASD. The investigators are testing a novel conceptual framework for modeling how genetic effects are transmitted through regulatory networks. • This is a strong functional genomics proposal firmly grounded in the newest genetic findings. Exploration of convergence of CNV and loss-of-function (LOF) SZ/ASD risk and interaction between CNV and polygenic risk score (PRS). • This is a novel conceptual framework for modeling how genetic effects are transmitted through regulatory networks, whereby nodes represent perturbations rather than genes, intended to better model how genetic effects propagate through networks.
<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • There is high enthusiasm for rationale for 193-gene screen, but lower enthusiasm for studying the CNVs due to the variable penetrance of phenotypes. If they could make 10 isogenic pairs of lines of each CNV, they would likely find clusters with distinct gene expression and electrophysiological properties. This supposition is based on published studies, particularly with 22q, showing different phenotypes in stem cell-based models and human study participants with or without schizophrenia. If there are genetic background-based differences in CNV phenotypes, results from studying these CNVs will be difficult to interpret. • That said, Aim 4 will assess an aspect of this issue if they are able to study whether a disease PRS correlates with in vitro phenotypes in iPSC-based models from individuals with CNVs. Either way, interpretation of data in Aim 3 will need to consider this penetrance/background effect and not assume any result is suggestive of some "ground state" for a given CNV. • Strong preliminary data demonstrating that clinical outcome in CNV carriers is influenced by genetic modifiers, and that ASD / SCZ share common genes but differ in how gain of function (GOF) and loss of function (LOF) are distributed in pathways and brain regions. • The investigators provide pilot results from two CNVs demonstrating the feasibility of the proposal and potential for novel insights.
<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Is the project well planned and designed?</p> <ul style="list-style-type: none"> • Yes. The project is largely in vitro stem cell-based studies to test the convergent impact of ASD/SCZ CNVs and gene mutations on transcriptome, networks, and phenotypes. • Yes, with the following caveats: <ul style="list-style-type: none"> ○ What concentration of glucose will be used? This greatly influences neuronal usage of OXPHOS in vitro, and the absence of mention in the context of mitochondria measures is concerning. ○ The media being used for these experiments is not mentioned despite its crucial relevance.



	<ul style="list-style-type: none"> ○ Synaptic marker analysis would be more reliable if including apposition of Syn1 with PSD95 colabeled on a MAP2+ dendrite. ○ In Aim 4 what are the phenotypes of the individuals with CNVs from whom iPSCs will be generated? SCZ PRS is used as an independent variable but what about clinical phenotype? <ul style="list-style-type: none"> ● The investigators demonstrate a clear plan and the breadth of this work and pilot data make a very strong case that risks can be addressed.
GWG Votes	Is the project feasible?
<p>Yes: 14</p> <p>No: 0</p>	<ul style="list-style-type: none"> ● The project is highly feasible for this outstanding team. ● The applicants are leading researchers and have significant experience delivering large, high impact programs. ● Aim 1-3: The proposal considers pitfalls, but alternative approaches are sometimes lacking (for example, lack of isogenic 16p11.2 and 22q11.2 Del and Dup isogenic hiPSCs). It's not clear the extent to which studies of convergence between CNVs and LOF genes will be confounded if isogenic studies are not feasible. ● Aim 4: The proposal seeks to compare 16p11.2 and 22q11.2 hiPSCs derived from diverse donors (ancestry and PRS), but it's unclear whether the clinical cohort is large enough to allow identification of biologically meaningful differences in PRS and/or how PRS will be accurately calculated in diverse/admixed donors, since PRS is most accurately computed in European ancestry donors at present. The proposal doesn't consider how variable CNV size and boundaries (particularly of 22q11.2) could confound. ● The project is rooted in expertise of team: psychiatric genetics, systems biology, use of 2D and 3D models, and recruitment of patients. ● Detailed aims, milestones, and timelines are described.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 14</p> <p>No: 0</p>	<ul style="list-style-type: none"> ● Aim 4 is all about this goal. ● Notably, the proposal will include samples from individuals belonging to groups that are currently underrepresented in research studies. This is unusual and highly commendable. The ethical and scientific justifications both support this approach. ● The proposal demonstrates team commitment to underserved trainees, patient organizations and diverse patient cohorts. ● A major aim is to recruit 30 new hiPSCs from 16p11.2 and 22q11.2 carriers of African, Asian and Latinx ancestry.



Application #	DISC4-16322
Title (as written by the applicant)	CIRM Center for Neuropsychiatric Stem Cell Proteomics
Research Objective (as written by the applicant)	This project will interrogate interactions, distribution, and function of high-confidence neuropsychiatric disorder risk proteins and identify convergent pathobiology of patient genetic variants.
Impact (as written by the applicant)	Datasets and stem cell resources in this project will establish a molecularly informed genotype to phenotype discovery platform for defining druggable pathways in neuropsychiatric disorders.
Statement of Benefit to California (as written by the applicant)	Californians have voted to support stem cell research towards treatments for brain disorders by earmarking over 25% of the funds for brain and CNS projects. Neuropsychiatric disorders represent major fraction of CNS disorders, and are characterized by phenotypic heterogeneity, and complex etiologies. This project will create data and resources to provide biologically informed estimate of disease risk and identify molecular pathways that can serve as molecular targets for drug development.
Funds Requested	\$13,781,522
GWG Recommendation	<i>Tier 1: warrants funding</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 1

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	2
Count	15
Votes for Tier 1	13
Votes for Tier 2	2
Votes for Tier 3	0

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • The project will produce foundational datasets describing protein-protein interactions (PPIs) relevant to schizophrenia (SCZ) and will create valuable reagents for the field including: a reference collection of stem cell lines carrying tagged alleles of hcSCZ genes; PPI networks of proteins encoded by hcSCZ genes identifying protein interaction and localization changes linked to SCZ variants; phenotypic datasets based on organoid models that should reveal common phenotypes associated with rare variant and idiopathic cases of SCZ. • Yes – I’ve often wondered why there is less investigation of protein levels in neuropsychiatric disorders (NPDs) and its probably just the ease of use of sequencing. By looking at molecular and tissue-level consequences of protein changes in NPDs you provide high confidence and also a mechanistic explanation for disease. • One of the best things about this innovative pipeline is you could simply clone the project in the context of other NPDs and it would deliver equally valuable results. • Yes – the project includes investigation and production of coherent disease mechanisms, and unique protein binding information to be generated that would be of broad interest. • The study will fill some key gaps in NPDs and reveal specific details regarding protein-protein interactions, structure and function in SCZ. • Scored '1' based especially on the generalizability of mechanisms to be identified combined with the robustness of the design and methods. • The PPI networks defined, and how these change with gene risk variants will be of high value. • This is a broad scale approach with the potential for generating seminal new data. • A large array of tagged iPSC lines and data will be produced. • Results could possibly lead to therapeutic interventions. • The path to transformative results is well outlined. • The mechanistic focus of this proposal is a significant strength. • Will provide mechanistic insight into major psychiatric disorders. • Scored '1' based on the mechanistic and coherent approach. • Unique datasets will be produced.
<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • Yes - the detailed protein binding experiments (which are the centerpiece of the proposal) and extensive systems biology and structural predictions are a first for NPDs. • The exploration of proteomics in mental health diseases is innovative since much of the previous work has been done on the genetics of these diseases. • The proposals main innovation is around the use of tagged patient hcSCZ variants for high-resolution and high-throughput characterization. • A huge range of technologies is applied, from cell biology to molecular approaches. • The hypothesis is not entirely novel or surprising - the innovation is in the approach. • Qualified yes – they take the perspective that rare protein-coding mutations will converge on core disease mechanisms. • For the most part, the key experimental pipelines are established, which is a strength that speaks to the feasibility of the project and experience of the team. The major innovation lies in the unique datasets that will be produced, along with information produced by their analysis. • Yes. Protein-protein interactions are promising and under-researched in psychiatry research. • The unbiased proteomics approach is a strength.
<p>GWG Votes</p> <p>Yes: 14</p> <p>No: 0</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • The rationale is technically sound. However, the justification for this massive effort on a rather narrow (candidate based) aspect of the disease is a possible limitation. • Yes. The project manages to transition plausibly from rare coding mechanisms to more general disease mechanisms that are extensively characterized. This approach mitigates the challenges related to understanding the actions of hundreds of weak variants that plague most studies in this field.



	<ul style="list-style-type: none"> • The rationale is supported by fantastic preliminary data. Also, the network diagrams well illustrate conceptual points with a network structure. • The rationale is highly sound. It can be applied to SCZ and most diseases. • The overall project/subprojects nicely leverage the expertise of the team, and the mapping of PPIs to discover new mechanisms of disease in SCZ is well justified. • It would be helpful and important to compare 22q11.2 deletion lines from people with SCZ to those without SCZ. • The proposal is strong on mechanism, in a very real sense - especially in terms of generalizability to other conditions. • There may be a problem with the company source of cell lines. Phenotyping SCZ in 22q can be challenging, and their study design does not include non-SCZ 22q patients. Thus, it will be difficult to know which 22q effects might generalize to the wider 22q patient population. • At the small scale both cell studies seem feasible, though the scaling may present a challenge. • The team has unique expertise to enable successful completion. • The unbiased proteomics approach is solid.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 14 No: 0</p>	<ul style="list-style-type: none"> • Each aim contains experimental detail and outlines the approach sufficiently. • The applicant notes synergies, but for the most part these appear complementary rather than additive. • The aims are absolutely coherent and linear. • Many alternatives and combinations of recent methodologies are planned. • The project offers great synergies: deep learning to expand the set of variants, systems biology to find convergences, plus electrophysiology and organoid characterization. • The project is well designed. The proteomics network mapping will provide foundational information that will synergize with other aims. • The project plan would be improved by inclusion of psychiatric and other assessments of the 22q11.2 study participants. Since there is no 22q group that does not have psychosis, how will the applicant interpret findings as psychosis, related versus 22q but not psychosis-related? 22q subjects without SCZ generally have multiple neuropsychiatric phenotypes, such as intellectual and attentional disability and anxiety. • The computational analysis will be ongoing throughout the project, and results may change experimental priorities and approaches. • The excellent datasets produced will be useful to the field. This project needs a strong plan for sharing these data with the community in a useable format. • The project has strong technical features but parts of it seem somewhat disconnected from the overall plan. • The data analysis plan was not very specific or convincing. • This project will be catalytic in moving the field forward. • This is a strong team.
<p>GWG Votes</p>	<p>Is the project feasible?</p>
<p>Yes: 13 No: 1</p>	<ul style="list-style-type: none"> • Scale and final interpretations will be a key challenge. Taking this project towards actionable targets rather than stopping at the foundational atlas and large reference sets will be a future challenge. Very little is outlined in terms of creating actual patient impact. • Yes - Application from one of the premier protein and systems biology groups, which really lives up to their reputation in terms of the comprehensiveness and innovation of the plan. • Yes – excellent facilities for experimental and computational work. • The team expertise nicely fulfills the ReMIND goal of assembling interdisciplinary teams, that should include clinical and computational expertise. It could be argued that this team is uniquely positioned to undertake the ambitious proteomics projects described in the proposal. • The applicant has a track record of technical innovation. • The team has unique expertise to enable successful completion.



	<ul style="list-style-type: none"> • This is a massive endeavor. Will they accomplish everything? • Can the applicant adequately analyze everything? Is this broad research without depth?
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 13</p> <p>No: 1</p>	<ul style="list-style-type: none"> • Adequate. • No concerns. • The team members have unusually strong track records of DEI-oriented efforts. • With regard to sex, yes. Arguably the findings will extend to various ethnicities because the genetic variants of interest have been found in underserved communities. However, the applicant will not directly incorporate samples from diverse backgrounds into the project. • The proposal does not sufficiently address DEI. For example, the question of donor characteristics and diversity is mentioned but not appropriately addressed. The cell lines are sourced from a company, so the applicant has little control. • It is unclear whether the stem cell lines to be used in this project are from diverse backgrounds. • The applicant includes a broad comment on trying to use diversified lines.



Application #	DISC4-16292
Title (as written by the applicant)	Multiomic Studies of Idiopathic Intellectual Disability and Autism Spectrum Disorder (ID/ASD)
Research Objective (as written by the applicant)	We provide mechanistic insight into idiopathic Intellectual Disability (ID) and Autism Spectrum Disorder (ASD) based on aberrant redox-mediated posttranslational modifications related to air pollution
Impact (as written by the applicant)	How air pollution contributes to ASD/ID should provide impetus for environmental control of such pollution. With our insights into idiopathic ID and ASD, new treatment approaches will become possible.
Statement of Benefit to California (as written by the applicant)	By discovering the mechanisms whereby air pollution and other environmental factors contribute to the development of idiopathic ID/ASD, this study will help develop new ways to prevent and treat this important cause of ID/ASD.
Funds Requested	\$17,365,387
GWG Recommendation	Tier 1: warrants funding
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 1

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	2
Count	15
Votes for Tier 1	12
Votes for Tier 2	3
Votes for Tier 3	0

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12	<ul style="list-style-type: none"> • The successful completion of the project will address key knowledge gaps and research bottlenecks in the study of neuropsychiatric disorders, particularly autism spectrum disorder and intellectual disability (ASD/ID), through several critical advancements: <ul style="list-style-type: none"> • Understanding Environmental Contributions: By focusing on air pollution as a significant environmental factor and elucidating its role in the etiology of
No: 1	



	<p>ASD/ID, the project addresses a significant gap in our understanding of how non-genetic factors contribute to these disorders. This is particularly important for cases where genetic causes are not clear, helping to explain the rising prevalence of ASD/ID in populations exposed to increasing levels of environmental pollution.</p> <ul style="list-style-type: none"> ● Mechanisms of Nitroxidative Stress: The project aims to detail the mechanisms through which reactive nitrogen species (NOx) and particulate matter (PM2.5) contribute to neurodevelopmental disorders. This involves studying the pathways of nitroxidative stress and protein modifications such as S-nitrosylation, which have been less explored in the context of environmental factors compared to other stress-related pathologies. Uncovering these mechanisms could lead to novel therapeutic targets. ● Novel Biochemical Pathways: By applying advanced mass spectrometry techniques and innovative probes, the project will identify and characterize aberrantly S-nitrosylated proteins that potentially drive ASD/ID phenotypes. This contributes to a broader understanding of the molecular dysfunctions at play, providing a link between environmental exposure and specific biochemical pathways that may result in disease. ● Multiomic Integration: The integration of proteomics, transcriptomics, and metabolomics offers a comprehensive approach to understanding the multifaceted impact of environmental pollutants on the human body, particularly the brain. This multiomic strategy not only helps to identify biomarkers but also enables a deeper understanding of how these markers interact within the larger network of cellular processes, thereby improving our grasp of complex disease etiologies. ● Bridging Research and Clinical Practice: The project's findings could have direct implications for public health policies and clinical practices by providing scientific evidence of the links between air pollution and neuropsychiatric disorders. This could lead to improved screening, prevention strategies, and targeted therapies based on environmental risk factors. <ul style="list-style-type: none"> ● The project is set to generate several valuable outcomes that will empower the research community to formulate and test novel hypotheses about neuropsychiatric disorders, particularly in the context of environmental influences. ● The role of air pollution in GxE interactions in neurodevelopmental disorders is an important area of study. S-nitrosylation has been shown by the PI to be a likely key mediator of pollution effects on brain disease, and if true in NDDs this would be highly impactful ● The project addresses the impact of pollution (particularly nitric oxide) on the development of phenotypes associated with ASD/ID – GxE effects are complex and clearly involved in ASD and other neurological disorders. Successful completion of the project will address significant gaps in our understanding of GxE effects, which remain poorly understood at a mechanistic level. ● Potentially yes but unclear with the current data. ● The applicant overweighs on the hypothesis that environmental factors cause oxidative stress.
<p>GWG Votes</p>	<p>Is the proposal innovative?</p>
<p>Yes: 11 No: 2</p>	<ul style="list-style-type: none"> ● Yes, the proposal introduces new technologies to the study of neuropsychiatric disorders, specifically through its innovative use of advanced mass spectrometry techniques and the development of novel chemical probes. These technologies are aimed at identifying and analyzing protein S-nitrosylation—a type of post-translational modification that may be influenced by environmental factors like air pollution. ● The application of these technologies allows for a more precise understanding of the molecular changes associated with ASD/ID under environmental stressors, potentially leading to the discovery of new therapeutic targets and biomarkers. This represents a significant advancement in the field, as it combines biochemical, molecular, and environmental health perspectives to study the etiology of neuropsychiatric disorders.



	<ul style="list-style-type: none"> • The applicants are testing new conceptual frameworks and hypotheses regarding neuropsychiatric disease mechanisms. They are exploring the hypothesis that environmental pollutants such as PM2.5 and NOx contribute to autism spectrum disorder and intellectual disability (ASD/ID) through mechanisms involving oxidative and nitroxidative stress leading to protein modifications like S-nitrosylation. • This approach is innovative because it connects environmental exposure directly with molecular changes in the brain that could mimic or influence the effects of genetic mutations known to cause ASD/ID. The use of a novel chemical probe for identifying S-nitrosothiols, along with cutting-edge mass spectrometry, to analyze the S-nitrosoproteome in brain tissues and cells is a significant advancement. • These technologies are applied to examine the hypothesis that redox-mediated posttranslational modifications, primarily S-nitrosylation, play a critical role in the pathogenesis of neuropsychiatric disorders, potentially triggered by environmental factors. • The PI's lab is the main group focusing on S-nitrosylation in brain disease, and has modified or created many reagents and approaches for these studies. • Technology development will include refinement of the applicant's novel probe with MS analysis to examine the S-nitrosoproteome in an unbiased manner (already applied to brain samples, human ASD plasma) – proximity labeling (APEX) has also been incorporated into the assay, enabling detection of associated proteins and S-nitrosylation. • In depth molecular analysis of the GxE effects in neuropsychiatric disease is novel, and the project will generate many interesting hypotheses for follow-up • The PI of this proposal has been studying the same mechanisms for other neurodegenerative diseases and stroke for many years. It is unclear if the same hypothesis and mechanism can be applied to intellectual disability and ASD. So it's a high risk project. • This is the same hypothesis that the same group has promoted for many other neurological diseases. It has not been tested in autism.
<p>GWG Votes</p> <p>Yes: 12</p> <p>No: 1</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • Yes, the overall project and its subprojects are based on sound scientific rationale. The proposal is grounded and builds upon a substantial body of prior research linking environmental factors, specifically air pollution, with neuropsychiatric disorders. • Very sound much of it based on PIs own outstanding contributions. • The overall project rationale is sound - the project will exploit functional genomics/proteomics pipelines established by the group, and previously used to study other neurological diseases (Alzheimer's, Parkinson's). Much previous work from team members and others has shown that elevated NO (including from air pollution) triggers aberrant protein S-nitrosylation (SNO-proteins) with consequent pathological effects on proteins that can contribute to neurological diseases. • The link between environmental factors and the proposed mechanism is not well-established.
<p>GWG Votes</p> <p>Yes: 13</p> <p>No: 0</p>	<p>Is the project well planned and designed?</p> <ul style="list-style-type: none"> • The overall project and its subprojects are meticulously designed to accomplish the specific aims of elucidating the molecular mechanisms through which environmental pollutants contribute to neuropsychiatric disorders like ASD/ID. • Potential pitfalls and alternative strategies are identified and detailed. • Overall, this is an outstanding design. There are some weakness in the lack of detail on xenotransplantation. The microglia and astrocyte part is OK (but could use methods for astrocyte generation and transplant) but the neuronal part does not specify what neurons, what part of brain, why that neuron added to that brain part would affect behavior. The impact of mouse immune suppression on outcome measures is not addressed.



	<ul style="list-style-type: none"> The general goal is to study GxE interactions in ASD/ID. The team proposes a logical initial focus on MEF2C, and will choose additional SNO proteins to study as the project progresses - this is a reasonable approach.
GWG Votes	Is the project feasible?
Yes: 12 No: 1	<ul style="list-style-type: none"> Yes. This is a team of specialists in the proposed research areas. The project is highly feasible except perhaps the neuronal transplantation. Preliminary data included in the proposal, along with published work, provide strong support for the feasibility of the approach – specifically, preliminary work with the MEF2C transcription factor, which when mutated causes severe ASD/ID, shows that S-nitrosylation of MEF2C can ‘mimic’, the effects of genetic mutations, and the mechanistic basis of this observation will be further explored in the project. The team members have strong track records of collaboration. Reasonable plans to manage the project are described. No. The team does not have the necessary stem cell expertise and establishing validated models could be risky.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
Yes: 13 No: 0	<ul style="list-style-type: none"> Yes. The applicants will use hiPSCs that are obtained from diverse populations, including genders, and minority and other populations, that are representative of the population of California. A plan to use hiPSCs obtained from diverse populations is described. It is noted that the effect of air pollution on ASD affects mainly minority populations living near highways, etc. The proposed study uses iPSCs from a diverse group of donors. However, the DEI plan is limited - it is mostly a list of general institutional resources.



Application #	DISC4-16337
Title (as written by the applicant)	Defining Neurovascular Metabolism in Neurodevelopmental and Neuropsychiatric Disorders
Research Objective (as written by the applicant)	Neuropsychiatric disorders correlate to impaired metabolism, though understudied. We will describe how metabolism impacts disorders etiology and identify new, tractable therapeutic strategies.
Impact (as written by the applicant)	We will uncover metabolic drivers of neuropsychiatric disorders, resulting in new therapeutic targets including rigorously investigated dietary interventions and novel metabolic drug targets.
Statement of Benefit to California (as written by the applicant)	Strong data suggests metabolism is involved in the emergence of neuropsychiatric disorders, impacting a large fraction of Californians. Metabolism as studied in this project opens an entirely new horizon for drug development; members of our team have already brought metabolism targeting drugs to clinic for other indications and these data may also suggest ways in which diet can be therapeutically leveraged. These new strategies will positively impact patient and family well being.
Funds Requested	\$12,000,482
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	2
Count	14
Votes for Tier 1	7
Votes for Tier 2	7
Votes for Tier 3	0

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

Minority Report

Under Prop 14, if an application is not recommended by the GWG but 35% or more of the GWG scored '1', the Review Summary must include a Minority Report. The Minority Report summarizes and contextualizes final comments from those GWG panelists that recommended funding.

Application DISC4-16337 was scored by 14 panelists on the GWG and received a split vote of 7 scores of '1', 7 scores of '2'. In overview, final comments from supportive GWG panelists indicate that the innovative hypothesis and strength of the applicant team drove their recommendation.

Reviewers noted a range of potential impact including mechanistic insight to both neurovascular coupling and disease etiology, new therapeutic avenues, validation of a new model system, introduction of "cutting-edge



methodologies" with potential for use in other diseases, and provision of foundational data. They noted published genetic, postmortem, and neuroimaging findings from ASD, SCZ, and other conditions that support a metabolic and/or neurovascular origin of disease. They were enthusiastic about the prospect that "altered metabolism - via dietary interventions - can impact development in neuropsychiatric diseases" as it represents a potential non-invasive treatment. They were impressed by the project team's expertise and accomplishments in the field, and confident of the project's feasibility based on preliminary work.

Like reviewers who scored '2', the supportive reviewers recorded critical questions for the applicant about the study design - e.g., on how underlying mitochondrial function would be addressed experimentally and how cell lines for the study were chosen - and described ways in which study findings may be difficult to interpret.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the project hold the necessary significance and potential for impact?
<p>Yes: 11</p> <p>No: 1</p>	<ul style="list-style-type: none"> • Neurovascular interactions are important for development and probably for neurodevelopmental disease but approaches to study them with human stem cell models are lacking. • The fact that evaluation of cell type expression of genes affected by high confidence risk variants highlights neuronal genes generally, and genes encoding synaptic proteins in particular, strongly suggests that the 'vascular hypothesis' is unlikely to be relevant to most individuals with schizophrenia (SCZ), although of course reduced synaptic density/efficacy in SCZ would alter/dysregulate blood flow. • That said, there is tremendous enthusiasm for this application using the systems proposed to study neurovascular interactions, including those involving synaptic-like interactions as mentioned. But there would be more enthusiasm for validating the core hypotheses with mostly "normal" sourced tissue, in replicates adequate to ensure broad ancestry relevance. To that end, there is high enthusiasm for Aim 4. • But to allow scope for more normative studies, perhaps focusing on either autism spectrum disorders (ASD) or SCZ, with at least 3 different lines of each non-isogenic mutant/control pairs, would have greater likelihood of impactful interpretations of findings. • The project would specifically highlight whether new avenues of treatment or prevention of schizophrenia and ASD can be accomplished via dietary treatments or via drugs affecting metabolism and neurovascular coupling. The project could additionally give single-cell data on brain development in relation to these disorders. • The detailed studies of mechanisms related to metabolism and neurovascular coupling to schizophrenia and ASD could give new insight into treatments and prevention of these conditions. • The project would validate the use of organoids that are matured and subsequent studies in vivo in mice. • The project would add new data on brain development and its relation to metabolism and possibly vascularization on a number of specific mutations related to schizophrenia and ASD as well as healthy controls. • The proposal will provide a substantial advance in understanding how maternal metabolism can drive cell fate specification in the CNS, in particular, in the developing neocortex. • The proposal will address the important non-neuronal contribution, i.e., the vascular counterpart, to human brain development and how metabolic perturbations might have profound consequences on brain function.



	<ul style="list-style-type: none"> • The project will decipher how neurovascular coupling develops and how, when dysregulated, contributes to the etiology of neuropsychiatric disorders (SZ and ASD). • The proposal will provide innovative technology in the field, such as combined LCMS-based metabolomics, and single cell-capture and transcriptomic analysis leading to the generation of metabolic tracer studies. • The multidisciplinary approach will generate foundational knowledge, accelerate science, and drive the development of new metabolic and non-invasive interventions (systemic treatments through diet). • Unclear because of the very early phase in research on organoid-brain interface.
<p>GWG Votes</p>	<p>Is the proposal innovative?</p>
<p>Yes: 11 No: 1</p>	<ul style="list-style-type: none"> • Metabolic syndrome and other cardiovascular risk factors are highly prevalent in people with SCZ. Metabolic profiling has indicated risk factors for children who will eventually be diagnosed with ASD (https://www.nature.com/articles/s42003-024-06102-y) or SCZ (https://doi.org/10.1038/s41537-022-00282-4). However, the mechanisms underpinning the risk for SCZ and ASD during human cortical development remain unclear and hence justify the proposal. • Yes. The proposal combines the latest organoid technologies, in vivo studies, and OMICS evaluation. The approaches have potential for use in wider studies of the same or other neuropsychiatric disorders. • Yes. The proposal spans from clinical practices to advanced stem cell models, in vivo biology, and OMICS, as well as functional analysis. • Yes. The applicant is testing the hypothesis that altered metabolism - via dietary interventions - can impact development in neuropsychiatric diseases. This concept is established for epilepsy and certain inborn errors of metabolism but not for ASD or SCZ. • This proposal will result in the generation of novel data sets, methodological tools, and conceptual advances in the field. By addressing the role of metabolism and vasculature in SCZ and ASD it will generate new information (data quantity, quality, availability) and research venues. Measurements of the functional impact of data-driven specific dietary interventions in SCZ and ASD animal models (either reducing or exacerbating neural phenotypes) will provide novel therapeutic approaches. • The project plan includes data deposition of different data types in proper repositories and integration of the generated data with existing published data (e.g., mouse models, human stem cell-based models, postmortem human brain studies). • The team has proven records and expertise (metabolism, cell fate specification with single-cell transcriptomic, in vitro physiology, etc.) that will expand our understanding of metabolism's impact on human brain development and neuropsychiatric disorders onset at key developmental stages leading to the identification of metabolic pathways controlling cell fate specification in the developing cortex. • A strength is the plan to evaluate the role of metabolism in schizophrenia and human neural development. • The innovation in this proposal was score-driving towards a good score - it uses very innovative approaches. • An interesting initiative is the inclusion of a patient focus group. • The study includes cutting-edge methodologies.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>
<p>Yes: 9 No: 3</p>	<ul style="list-style-type: none"> • The overall rationale is based on accumulated evidence from genetic, postmortem, and neuroimaging studies indicating that metabolic and microvascular abnormalities might be associated with neuropsychiatric disorders. This has not been well studied. • Overall, the ASD/SCZ neurovascular coupling hypothesis is a fascinating and not unreasonable hypothesis. However, the proposed studies may not be well justified by existing literature or preliminary data. • Some statements in the application contain inaccuracies or unclear bases. Careful, accurate phrasing and proper citation would reassure reviewers that the applicant team recognizes their embedded assumptions. • Overall, the methods proposed to evaluate the hypothesis are sound. Two aspects are major risk factors a) the selection of the patients for studying the metabolic aspects of



	<p>the diseases are not representative for the conditions, several mutations are included and thus the number of patients for each mutation is low; in addition, isogenic controls are not available for all lines, and b) a major piece of the project is dependent on organoid transplant into mice.</p> <ul style="list-style-type: none"> • Many of the proposed studies are based on human patient-derived cells. The relevance of these systems to humans in vivo is, however, not fully validated. • The use of rodents is justified as they are mostly used to mature organoids and study dietary interventions, which is very difficult to do in fully in vitro systems. • The PTEN studies are highly relevant to metabolism, but the focus on lipid metabolism per se is hard to understand relative to the many other aspects of metabolism that will be altered in this system, for example mitophagy. • The proposal is focused on providing a better understanding of the mechanisms in ASD, using a genetic model of the disease, hiPSC cells with a deletion, which are known to have alterations in the replication of mitochondrial DNA and mitophagy. How is this underlying mitochondrial dysfunction taken into account? Is there any evidence that women with mitochondrial defects have more children with ASD? • There are clear and compelling data for both methods and the use of some of the specific patient-derived materials. • Explanation of the cell lines being used and why they have been chosen would strengthen the proposal.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 10 No: 2</p>	<ul style="list-style-type: none"> • The research team defines 4 Aims, each with several activities and milestones, to be accomplished in parallel throughout the project, to study their main hypothesis: Metabolism can play a role in the etiology of SCZ and ASD and diets can modify the disease phenotype. The work plan is clear and includes expected results and pitfalls. The team is building upon existing collaborations, has shared publications, and presents complementary approaches to tackle the different aims. • There is very high enthusiasm for this design, but it has drawbacks that the applicant needs to address. <ul style="list-style-type: none"> ○ The proposed studies, for the most part, will not have adequate sample sizes for differences between groups to be detected. Relatedly, statistical analysis plans are not well-presented. ○ The project will use healthy controls rather the mutant individuals with SCZ, which is sound and feasible but will limit interpretation of results. ○ Electrophysiology experiments seem beyond capacity of the staff and available equipment. ○ The diet experiments in Aim 2 are amazing. However, the applicant does not address the attendant complexities of interpretation. The effects observed may be autonomous to the human cells in the organoid and/or host neurovasculature, microglia/brain macrophages, and perhaps oligodendrocytes that have invaded the graft. ○ Isolating a metabolic effect versus other mechanisms (e.g., maturation) will be similarly difficult. The proposal reflects an unfounded assumption that any benefit of transplanted versus cultured organoids will be due to metabolics per se (unless metabolics is so broadly defined as to essentially include cell health, since of course unhealthy cells will surely have altered metabolics). • The applicant's (outstanding) 2019 paper showed that cortical pyramidal neuron-like cells in cerebral organoids harbor metabolic stresses not apparently present in vivo, and harbor co-expression of markers of laminar and cortical areal fate not present in vivo. The paper did not demonstrate that there is a causal relationship between the metabolic and fate disruptions, relative to the obvious issues that organoid media cannot perfectly recapitulate the in vivo signaling environment. Transplantation of organoids into mouse brain alleviated both the metabolic stress and the mixed expression of fate markers, but other aspects of the transplantation are likely to be influencing outcomes apart from vascularization per se. One of the most obvious differences is the in vivo versus in vitro is oxygen tension. Another obvious difference, as above, is the delivery of agents via the invading mouse vasculature not secreted by the neurovasculature itself. Together with



	<p>the above this raises major concerns that the applicant is at risk for misinterpreting findings of what is indeed a fascinating experimental design.</p> <ul style="list-style-type: none"> • Overall, the project is very well planned and designed both in terms of studies and team members. One weakness is the number of mutations included and number of patients for each mutation. • Pitfalls and alternative strategies are partly addressed, including the possibility that no converging data between the individual lines can be seen - in this case the applicant's suggestion is to increase the number of studied donors. • Overall it is a bit unclear how all mutations should be combined. In addition, the discrimination between mouse and human impact in the transplanted organoid approaches are not addressed. • There are several parts of the project that are dependent on organoid generation. • The preliminary results are promising and are consistent with the proposed research. <ul style="list-style-type: none"> ○ The research team has shown that vascular cells of the prenatal human brain express neurotransmitter receptors and transporters, and maturation of neurovascular coupling. ○ The research team has generated a metabolic atlas of the developing human brain ○ Metabolism has been measured in organoids, and metabolomics in organoids and primary fetal tissue shows similarity, validating the model system • Even though the proposal mentions the importance of the neuro-glia-vascular unit, and bulk metabolomic analysis will be performed, the focus seems to be on measuring neuronal activity and morphology. For instance, aim 2A proposes 7-8 week-old organoids dissociation and labeling for P4 injections, and Figure 5 shows that there is limited presence of glia at this time. • The underrepresentation of functional glia in the organoids could be a major challenge. The research team could refer more explicitly to the presence of glial cell components of the NVU (at the three selected time points) for a robust understanding of brain structure and function. Consideration of the NVU niche in the organoids, and once within the host mouse cortex (taking into account interspecies differences in the niche), is pivotal for the proper interpretation of experiments. • To represent the spectrum of SZ and ASD, the research team decided to study 10 selected lines from a variety of mutational backgrounds. Nevertheless, the selection of mutations should be further discussed and isogenic controls clearly indicated when available. Adding a table would be helpful. • Regarding the xenotransplant experiments: What is the impact of host mouse vasculature on organoid vasculature? Mouse metabolism is different from human metabolism. The research team assumes that host-mediated vascularization is healthy and can deliver properly to the organoid (ASD or SZ). • Regarding the use of a mild maternal inflammation response murine model of ASD to enhance mTOR signaling: How does the research team include inflammation per se as a variable? • The organoid-mouse interface is complex, and not very well justified or characterized in the proposal. • The project investigates acute changes, but disease phenotypes are chronic-developmental.
<p>GWG Votes</p> <p>Yes: 11</p> <p>No: 1</p>	<p>Is the project feasible?</p> <ul style="list-style-type: none"> • Team qualifications are outstanding. However, the electrophysiology studies may require unrealistic throughput for the number of personnel and equipment proposed. • This is an excellent investigative team with evidence of feasibility. • The team is experienced in the proposed techniques. • Yes, the applicants have strong experience with the suggested techniques. The feasibility is possibly lowered slightly because the PI does not seem to have many senior author publications. Another possible weakness is that there seem to be too few researchers at the postdoc and PhD level for the number of experiments proposed. • Yes. Plans are well outlined and several of the investigators have collaborated before.



	<ul style="list-style-type: none"> • Yes; both in terms of lab and IT infrastructure resources the team seems to have relevant and sufficient access. • Yes, the budget is overall appropriate, but possibly with too low a number of involved lab scientists. Overall some of the tasks have too low running costs. • State-of-the-art infrastructure and core facilities are available at the main hosting institution, and all the necessary logistics and expertise have been recruited or are included in the proposal revealing excellent collaborations with other center members (e.g., assuring access to patient cell lines and databases, electrophysiology). • The team comprises researchers with different academic backgrounds and expertise, at different stages of their careers (senior investigators and young investigators), balance between developmental neuroscientists and clinical experts, and adequate formation of human resources (graduate students and postdocs). • There is coherence between what is requested in resources and what is available. The budget is well-organized and justified • Coordinated efforts assure interdisciplinary collaboration: adequate distribution of workload among institutions, Key Personnel assigned for each aim led by one of the core research team members, and a variety of experimental approaches covered by several Investigators (multiple labs can generate cortical organoids, metabolomics). • An excellent initiative is the Collaboration manual. • The project is way too ambitious.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 12 No: 0</p>	<ul style="list-style-type: none"> • DEI is appropriately addressed. • Yes, in experimental outline, outreach, and the formation of a focus group, DEI aspects are very well addressed. • Yes. Both outreach activities and formation of a patient focus group are described in the proposal. • The research team plans to utilize cell lines from several sources that represent gender, and diverse racial backgrounds. As for age diversity, the information is not provided. • The project includes lines derived from underrepresented minorities. • As stated by the research team, DEI is central to the accomplishment of their mission. Currently, investigators are engaged in stem cell and neuroscience education programs for community college and high school students, and creating opportunities (e.g., training sessions and laboratory mentorships) for students from underrepresented backgrounds and ethnicities. • Educational development in STEM is another initiative to be developed within the research plan; headed by a team member with extensive experience in this regard.



Application #	DISC4-16360
Title (as written by the applicant)	Patient-derived organoids for early diagnosis and personalized prognosis of intellectual disability (ID)
Research Objective (as written by the applicant)	We aim to identify biomarkers in organoids derived from patients with intellectual disability (ID) and potential correlations with disease mechanism and clinical electroencephalograms (EEG) data.
Impact (as written by the applicant)	Identifying biomarkers reflecting the severity of ID, and correlating patient EEGs with organoid signatures, may transform clinical practice by aiding diagnosis and treatment.
Statement of Benefit to California (as written by the applicant)	This study introduces new technologies and frameworks for early diagnosis and personalized prognosis of ID, impacting California's population and healthcare system. In partnership with a large children's hospital, we will generate organoids from ethnically and racially diverse ID patients, reflecting the affected communities across California. Our advances in mental health research through collaborative research and data sharing may change the treatment regimen of ID patients.
Funds Requested	\$12,556,739
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	2
Count	15
Votes for Tier 1	2
Votes for Tier 2	13
Votes for Tier 3	0

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG's review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 13</p> <p>No: 0</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • This proposal outlines a plan to make important strides towards improved biomarkers of ID progression and severity. • This proposal addresses technical bottlenecks for measuring electrophysiological outcomes in vitro in a robust and reliable manner (and in a higher throughput manner). • This proposal will make strides towards addressing key knowledge gaps regarding molecular subtyping of ID. • This project develops experimental and analytic platforms, as well as robust data sets directly relevant to elucidating mechanisms underlying ID that will be highly valuable for the field. • If organoid electrophysiology correlates with intellectual disability (ID) severity and/or EEG in ID, the project could deliver a method for pathway analysis and intervention development. • The general idea to potentially identify electrophysiological biomarkers for ID using patient-derived organoids (cortical and ganglionic eminence) is of relevance. • The title overreaches. In the future, perhaps organoids may help with the diagnosis of certain types of ID but making predictions with regard to 'personalized prognosis' based on organoids is speculative.
<p>GWG Votes</p> <p>Yes: 13</p> <p>No: 0</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The project has high innovation and also technology development - organ on chip and RNA-based lineage tracing. • Strengths: <ul style="list-style-type: none"> ○ The approach to better understand intellectual disability by focusing on one genetic subset of ID patients is reasonable and within scope. ○ Establishment of new iPSC lines and use of diverse analytical methods (molecular, cellular, computation, functional) is relevant. • This proposal is highly innovative, both technically and conceptually, as specifically outlined in the bullets below. <ul style="list-style-type: none"> ○ Development of new microfluidic platforms for longitudinal electrophysiological recording of advanced, 'next-gen' organoids is innovative. ○ Development of RNA exporters that report longitudinally on proliferation and cell death in organoids (and potentially for cell type distribution) would establish a unique approach. ○ Establishment of platforms for developing and testing neuromodulation strategies in vitro would be novel. ○ The project will develop novel methods for integrating in vitro data with in vivo data (organoid readouts with EEG readouts from the same individuals, integrated ML approaches). • The idea of identifying 'hidden' features of EEG data or electrophysiology-related measurements as biomarkers is novel. If successful, this could have important implications for disease subtyping and ultimately for person-specific interventions. In addition, if validated, the data generated also would provide novel strategies for interventions, and a platform for testing those interventions.
<p>GWG Votes</p> <p>Yes: 10</p> <p>No: 3</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • A reviewer has high enthusiasm for attempting to demonstrate an organoid activity - human EEG link in the relatively low hanging fruit context of this ID subtype. • In general, the overall projects (organized under 6 Aims) are based upon sound scientific rationale, and the approaches are based upon robust preliminary data generated by the team. • While the team provides evidence that EEG and electrophysiology measurements in vitro in organoids have associations with clinical phenotypes, the data presented are for a very limited population. The focus on these outcomes here is high risk based on limited data sets, but high reward and worthy of testing due to the impactful outcomes, if successful.



	<ul style="list-style-type: none"> • Even if not successful in the primary goals of subtyping and having predictive readouts in organoids for disease severity, the datasets and tools generated will be of high value to the field and will inform future efforts to reach this goal. • Correlation of clinical EEG with organoid electrophysiology is highly risky. Based on published literature, only 25% of patients with ID show abnormal EEG. In addition, a large fraction of patients with the ID that is the focus of this proposal suffer from epilepsy. Hence, it will be very difficult to establish distinct electrophysiological markers for ID versus epilepsy.
GWG Votes	Is the project well planned and designed?
<p>Yes: 10 No: 3</p>	<ul style="list-style-type: none"> • The proposal is highly well written; Aims are synergistic and interactive. A panelist agrees with the applicant's enthusiasm for the possibility that organoid based models may be better for therapeutic screening than quicker and more accessible carpet cultures (neurons+astrocytes and maybe microglia). But the investigators should acknowledge in the proposal that for many disorders, for example single genes with major effects on synaptic transmission, a reduced system may be just as effective for Rx screening as the more complex organoid. • Experiments to compare the utility of organoid and carpet cultures (with imaging of synaptic transmission under various levels of stimulation) for finding in vitro-in vivo associations would be even better, rather than overstatements that organoids are necessarily better, especially for studying mutants affecting synaptic transmission. • Preliminary data and publications supporting the algorithms and pipelines relevant to machine learning appear to make logical sense. • Thorough planning of human study participant selection among mutation carriers is compelling. Further description for participant selection for idiopathic ID is warranted. • Overall, the projects are thoroughly planned and carefully designed to accomplish the Aims. Detailed and clear descriptions of approaches for each of the six Aims are provided, and key features of the approaches rigorously considered. In instances where potential technical issues may hinder progress, the team proposes specific efforts for addressing these (Aims 2T and 3T). Below are some additional considerations that in their current form are weaknesses. <ul style="list-style-type: none"> ○ Descriptions of the number of differentiations and number of organoids per differentiation for each phenotypic readout would strengthen the research plan. On a related note, the team has excellent preliminary data for each approach, but in spite of this, no power calculations are performed to provide context to the likelihood of success for each approach for phenotyping of organoids. ○ The degree of discrimination required for "success" between mild-severe disease from EEG or electrophysiology data in vitro for individuals is unclear. ○ Milestone success criteria should be more specific, especially for 2T and 3T. • The team has developed robust methods for generating complex cortex plus ganglionic eminence (Cx-GE) organoids, which will be used in this project. • Matching EEG and in vitro findings for each patient is a strength. However, the use of only 100 minutes of recording, when 24 hours is available, is questionable. It is unclear which 100 minutes would be chosen, i.e., what metrics will determine the cutoffs. • In general, potential pitfalls are deeply considered and solid alternative approaches are presented. These careful considerations are a strength of this excellent application. • For activity 2.2 (developing machine learning (ML) approaches to identify biomarkers of ID), there are several alternatives proposed if there isn't initial success. However, if the data aren't robust enough, using alternative ML approaches will not be of benefit. There need to be clear 'no go' outcomes that involve return to optimization of the experimental system (for example, altering cell type compositions, increase 'n', etc). this is critical for moving on to activity 2.3. Similar comment for activities 5.2 and 5.3. • Cell type composition of organoids is considered carefully in aim 3. However, this data may be critical for success in Aim 2, and the activities will be simultaneous. A clear plan for integration of these cell type distribution data (for each individual organoid) early in the process of identifying electrophysiology-based biomarkers may be critical for success.



	<ul style="list-style-type: none"> • It is not recognized that the lack of microglia and other cell types/brain areas/peripheral factors may confound the ability of the team to readily identify subtypes. An external stressor may be necessary to illuminate subtle phenotypes. This is not weighed heavily in scoring here, but it is a potential pitfall that should be recognized when interpreting data and planning future studies. • The range of technical and biological variability that is inherent to organoid models is not addressed and could compromise the identification of true disease phenotypes. This is particularly important when clinical data is compared to in vitro experiments.
GWG Votes	Is the project feasible?
Yes: 12 No: 1	<ul style="list-style-type: none"> • The project is highly feasible for this team. No feasibility concerns except that Aim 6 may be underpowered. The team appreciates this, and it is a reasonable place to start. • Feasibility for developing RNA exporter reporters is supported by previous publications by the team. • Progress on the microfluidic device development is evidenced by robust preliminary data in Figure 7. • Data in Figures 3 and 4 show feasibility for measuring network activity within Cx-GE organoids, but there is a disappointing lack of quantification or statistical analyses of the phenotypes. This makes it impossible to determine the reproducibility of the findings (nor is there mention of 'n' of organoids, differentiations or lines - only example data panels). • Preliminary data of in vitro phenotypes from a single ID donor have been published, and the data are supportive of expansion to a larger dataset. • The key hypothesis is risky, and identification of network-level biomarkers is speculative. Which neuronal networks are expected to change in an organoid model that is based on random self-organization of pre- and postsynaptic partners and formation of random projections and synaptic connectivity? • It is unclear what methods will be used for quality control of long-term cultured organoids.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
Yes: 13 No: 0	<ul style="list-style-type: none"> • The proposal is outstandingly focused on this specific ID subtype, which is prominent in underserved groups. • The project will address underrepresented minority groups (Hispanic and African American), and the team works closely with a research fund for the disease. • The team clearly and specifically outlines efforts to capture diverse populations and considers these variables in their modeling. • The humans to be studied herein represent the breadth of diversity present within the greater patient population. • Yes, the applicant clearly and specifically describes a track record of efforts for outreach, collaboration and training relevant to DEI efforts.



Application #	DISC4-16507
Title (as written by the applicant)	From genes to circuits: leveraging neural assembloids to decipher multi-level mechanisms in neurodevelopmental disorders
Research Objective (as written by the applicant)	Identifying neurodevelopmental pathomechanisms at the molecular, cellular, and circuit level in human neural assembloids will lead to tailored therapeutic approaches.
Impact (as written by the applicant)	Identifying neurodevelopmental pathomechanisms at the molecular, cellular, and circuit level in human neural assembloids will lead to tailored therapeutic approaches.
Statement of Benefit to California (as written by the applicant)	An estimated 2.8% of Californian children are diagnosed with autism spectrum disorder, 1.6% with intellectual disability, and 1% of adults with schizophrenia. We will determine the role of neural circuits in neurodevelopmental disorders and integrate findings with data about molecular and cellular abnormalities as well as validate our findings across diverse cell lines. A better understanding of how human brain circuits are dysfunctional will point us toward better ways to protect, improve, and treat those symptoms.
Funds Requested	\$15,261,984
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	2
Count	15
Votes for Tier 1	2
Votes for Tier 2	13
Votes for Tier 3	0

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12	<ul style="list-style-type: none"> • Successful completion of this project would provide an answer to whether there are shared nodes that could be targeted across various neurodegenerative diseases (NDDs).



<p>No: 1</p>	<p>The ability to treat different NDDs with a single drug targeting a common affected circuit would greatly benefit patients.</p> <ul style="list-style-type: none"> • Project deliverables include lots of heterozygous knockout (het-KO) iPSC lines in three diverse backgrounds, and large datasets generated and used for AI and machine learning. • Establishing rigorous in vitro models to investigate genes' functions in neuropsychiatric diseases is of great relevance. However, the proposed approach appears to be unrealistic in terms of timelines (e.g., gene editing of large numbers of iPSC lines) and some speculative assumptions that assembloids faithfully recapitulate local and long-ranging neural networks that are present in the human brain. • The proposal uses a combination of state-of-the-art data approaches and experimental technologies to understand mechanisms. • The project addresses the field's critical need for a systematic yet flexible, multi-level infrastructure for comparing many NDD genes in a human-specific experimental platform. • The field is overwhelmed with findings drawn from studies limited to a small number of genes, platforms, and biological levels of organizations. This project addresses this bottleneck by creating a scaled, comprehensive, and rigorous testing framework. • The project will likely elucidate which biological phenotypes are shared or distinct among three neuropsychiatric conditions. Importantly, it will likely provide insight into the type of circuitry most affected and the developmental sequelae from gene expression to emergent circuit properties. • As ambitious as the current project is, one can easily imagine how it can be used in future studies to screen or test small molecule or gene-targeting therapies as a preclinical platform. • The project will generate an extensive and highly valuable suite of histological, sequencing, live imaging, electrophysiology, metabolomics, and animal behavioral data. • The application indicates data will be deposited in a named, open access data repository. The Milestones and Timelines table includes data sharing activity through much of the project period, beginning in Q4 of Y1. That said, given the potential value of the project's outcomes, CIRM staff should be encouraged to work closely with the investigators to ensure that FAIR principles are upheld during and after the project period.
<p>GWG Votes</p>	<p>Is the proposal innovative?</p>
<p>Yes: 11 No: 2</p>	<ul style="list-style-type: none"> • The proposal integrates a range of cutting-edge techniques, including Kirigami bioelectronics and patch-seq, which have not yet been applied to brain organoids. Additionally, advanced methods like assembloid production, typically restricted to specialized labs, are also featured. This underscores a major strength of the proposal, supported by team members' expertise in diverse fields such as machine learning, electrochemical bioengineering, and neuroscience. • The proposal is highly innovative. Highlights include the circuit phenotyping and plans to validate in vivo. • This proposal would be the continuation of similar work that this group has published. Despite the promise, many questions related to the enormous technical and biological variability that is inherent to organoids and assembloids remain unaddressed. • Yes, the proposal brings in an impressive array of technologies. The first two aims integrate and scale the technology in a way that has not been done before. The last two aims then pushes the frontier of organoid circuit technologies. • Aim 1&2 are absolutely cutting-edge technology. Given the PI and team's track record, there is strong confidence that robust and replicable neural circuit phenotypes will be identified in these aims. In particular, the innovation is the scaled growth of organoids (i.e., semi-automated media changer) and the integration of -omics data. These innovations will serve as a vital technology resource for the California research enterprise. • In contrast, Aim 3&4 have a lot more risk and unknowns, but they pursue exactly the kind of exploratory circuit and bioinformatic neuroscience that should be developed for conceptual layering onto human cellular model systems. In particular, the incorporation



	<p>of Kirigami electrodes may be a transformational step in circuit analysis of organoids. Their current development of Neurostrings into the Kirigami electrodes is also extremely exciting.</p> <ul style="list-style-type: none"> The proposed hypotheses are fairly generic, representing the majority viewpoint in the field. However, this is not criticism of the project itself. These hypotheses are also the same ones that desperately need scaled and rigorous experimental testing. And that is where the project shines – its development of tractable, experimental platform to examine human mechanisms across psychiatric conditions and across scales of analysis.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>
<p>Yes: 11 No: 2</p>	<ul style="list-style-type: none"> The preliminary data are promising but given that the entire project hinges on generating 90 gene-edited iPSCs, additional preliminary data demonstrating the team's capability to accomplish this within the proposed timeline would have been beneficial. A schematic outlining the iPSC genome editing pipeline, including timelines for editing, screening, and quality control of the lines, would be informative. It would be good to include more rationale for which 10 genes per disease are going to be edited. The project could use some comparison of hypothesis testing with simpler 2d+ models, for example neurons+ astrocytes, for a given mechanistic hypothesis versus the far more complex multi-oids here. The applicants seek to advance our understanding of the mechanisms from genes via circuitry to disease processes. The proposal is overreaching in terms of goals and data interpretation using organoid models that are difficult to control with regard to standardization and reproducibility. In addition, assembloids have the tendency to fuse, cells constantly migrate in all directions, and extend axonal projections randomly in all directions. Without accurate spatio-temporal organization of neurons and their projections the usefulness of organoids is of limited value to study neurocircuitries. Hence, large amounts of data could be generated with unknown significance for neuropsychiatric diseases. The potential to identify meta-phenotypes is speculative considering that many of the NDD genes have small effects and it is unclear if such phenotypes exist and can be modeled ex vivo using highly variable organoids. Yes, the overall scientific rationale is strong, particularly in the early aims. The preliminary data are strong for Aims 1&2 but become increasingly conceptual in Aims 3&4. For example, one technique proposed in Aim 3 is still very much under development. In addition, the computational methods to create valid meta-phenotypes have not been developed yet. They have a model for the starting point, but the model will likely be increasingly biased toward spurious correlations rather than biological signals as they move toward increasing levels of biological complexities. Yes, the project is relevant because it uses human cellular systems. However, the extrapolation to the dynamic environment in living humans will always be a concept leap to some degree. They will transplant NDD cortical organoids into immunodeficient newborn rats to examine in vivo circuit relevance as they demonstrated previously with another NDD. This is an important technical and conceptual proof-of-concept of the functional relevance of their in vitro system.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 7 No: 6</p>	<ul style="list-style-type: none"> The overall project and subprojects follow a reasonable and logical progression. The project shows strong scientific synergies, with results from early aims directly informing the selection of testing conditions in later aims. The strongest scientific synergies are between the cellular and systems approaches, which addresses a key knowledge gap in the field. Identified pitfalls for Aim 1 are lacking. An option would be to outsource cell line creation.



	<ul style="list-style-type: none"> • The proposal has a convincing project plan to test 30 genes, and high-level technological expertise. However, all depends on the success of Aim 1, so the project is high risk. • Aim 2-4 are dependent on a successful and on time completion of Aim 1. The strategy for generating het-KO is not well designed. Adding 3 gRNAs to a nucleofection to create het-KO clones will not work well. It is highly likely to give complete KO, but not heterozygous clones with intact WT alleles. Consider adding a blocking single stranded oligodeoxynucleotide (ssODN) donor to trap the WT allele or dead Cas9 to prevent the WT alleles from being chewed up. Also, they only put in one gRNA to reduce off-targets and increase the chance of not KO both alleles. • Using microarray to detect off-target editing is confusing and not done in the field. Why use microarray when proposing WGS for each clone? • WGS is likely overkill and quite expensive. If making 90 clones and QC'ing only 2 clones, it would cost \$63,000 at a \$350 genome cost. This will also generate a lot of data that will need to be analyzed, but there is no plan for this in the proposal. • A large portion of the work will be done at an institutional stem cell core facility with \$1.4M in fees. It would be helpful to have more details about the staff and expertise in that group. Will the core need additional FTEs to support this large project in addition to providing services to the larger institutional community? • What is the quality control for using long-term cultured organoids or assembloids before they are subjected to an experiment? For instance, how are organoids qualified after culturing them for several months and then deciding to use them for a recording? • Pitfalls and alternative approaches are identified but increasingly vague as the proposal progresses through the aims. For example, they mention that their model may overfit, but the alternative proposed approach is vague. • Plans for final processing across all data types are unclear. • The genome editing strategy is a bit weak.
<p>GWG Votes</p> <p>Yes: 7</p> <p>No: 6</p>	<p>Is the project feasible?</p> <ul style="list-style-type: none"> • This is an ambitious proposal, but if any team can do it, they can. The applicant needs to clean up the genome editing piece. • The proposed PI and team are outstanding and represent leading investigators in their respective fields. However, the personnel for the circuit and computational approaches in later aims seems rather understaffed. • The team has access to outstanding resources and is well-suited to carry out the proposed activities. • Yes, the budget is appropriate for such an ambitious project. • The project is too ambitious in terms of resources, timelines, anticipated results and data interpretation. • There are some concerns about feasibility. • Much of the research plan is dependent upon successful high throughput CRISPR targeting of a large number of genes. • A large amount of work raises some concerns that this is overambitious overall. • They mention that the team will meet monthly, but this seems inadequate for the complexity of the project. • More thought and personnel time should be allocated to project management. The current descriptions are fairly generic and vague.
<p>GWG Votes</p> <p>Yes: 13</p> <p>No: 0</p>	<p>Does the project uphold the principles of diversity, equity and inclusion (DEI)?</p> <ul style="list-style-type: none"> • The investigators will use or generate hiPSC lines from diverse racial, ethnic, and gender backgrounds and test findings in both sexes in rodent models. • New lines will be deposited with CIRM and could increase diversity of hiPSC available to community. • The team will attend a DEI forum and symposium. • The team shows a history of training across the education spectrum regardless of background, and one member of the team is on three DEI promotion committees at their institution. They plan science talks to the local community about findings.



	<ul style="list-style-type: none">• The project does a reasonable job of accounting for demographic variability. This is performed at two key junctures in the application, in the validation of connectivity phenotypes in Aim 2 and the validation of meta-phenotypes in Aim 4.• Insufficient consideration is given to the initial cell lines. The investigators plan to expand to diverse genomic context.• The project proposes reasonable validation studies in populations of diverse ancestries, but follow-up studies should focus on further exploration of larger sample sizes.• The outreach plans seem underdeveloped. They mention that one investigator will reach out to advocacy groups to explain the results and help them understand the research, but no specific activities are described.• The investigators should be encouraged to provide more detail on specific communities will be engaged and what activities will be conducted.
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Application #	DISC4-16283
Title (as written by the applicant)	Prenatal Marijuana Exposure and Neuropsychiatric Predispositions: A Single Cell Perspective
Research Objective (as written by the applicant)	The study will investigate the underlying mechanisms where maternal marijuana use during pregnancy could predispose individuals to neuropsychiatric diseases later in life.
Impact (as written by the applicant)	The study will provide a more definitive answer to the ongoing debate about the safety of marijuana use during pregnancy. Study findings will inform community support and public policy making.
Statement of Benefit to California (as written by the applicant)	In California, ~19% of young women use cannabis products during pregnancy (toxicology tests) and marijuana use is perceived as safe by the public. However, population studies have consistently reported increased neuropsychiatric risk among the offspring of the mothers who regularly use marijuana during pregnancy. By identifying the mechanisms, this study will facilitate evidence-based policy making and will call for support for the underserved communities to achieve better birth outcomes.
Funds Requested	\$10,658,194
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	1
Lowest	3
Count	15
Votes for Tier 1	1
Votes for Tier 2	12
Votes for Tier 3	2

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the project hold the necessary significance and potential for impact?
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<p>Yes: 11 No: 3</p>	<ul style="list-style-type: none"> • It's very important to understand the impact of cannabis during pregnancy. The proposal presents a unique design to study this. • Potential harms of cannabis exposure are understudied, particularly with prenatal exposure. This knowledge gap may disproportionately affect underrepresented groups. • Yes, the project aims to study the effects of prenatal marijuana exposure on human brain development and neuropsychiatric predispositions, motivated by the high prevalence and perceived safety of cannabis use during pregnancy, despite epidemiological evidence of adverse outcomes. • If successful, the result will arguably represent the first rigorous foray into the mechanistic consequences of high marijuana exposure during pregnancy. • The stepwise fashion the research plan is presented in may point to increasing specificity of how to conduct the search for mechanistic action, from cell-type prioritization to temporal actions and effects on neuronal excitability. • The ability to do deep single cell omics in developing brains will be a good resource for other researchers. • The single cell data will likely produce a valuable atlas that can be explored in many directions by the wider community. • The results of this project will provide some of the first rigorous experimental evidence linking risk factors identified through epidemiological studies and specific cellular mechanisms of high marijuana exposure in utero. • The proposal does an excellent job of clearly organizing all critical aspects of the data sharing. The application had a clear and strong data-sharing plan. • The project aims to communicate potential findings to the community, which could be valuable, though this is often done as a matter of course in normal dissemination of research. A reviewer is not 100% convinced of the value this adds, and requests more quantification of the outcomes from this Aim. • Yes, the program aims to identify communication approaches and tools to build trust, support, and reduce stigma and punishment related to pregnant people's cannabis use and to develop and disseminate accurate and trustworthy information about the effects of cannabis use during pregnancy. The program also plans to hold an annual symposium to increase awareness and discuss interventions. • The project's approach is to leverage ex vivo models (Aim 1) and brain samples (Aim 2) to identify vulnerable cell types and developmental stages and dissect dysregulated cellular and molecular components underlying neuropsychiatric predispositions, focusing on the maturation of excitatory neurons and caudal ganglionic eminence (CGE)-derived interneurons expressing cannabinoid receptor CNR1. • The study will provide a wealth of information at ultra-high (single cell) resolution. Data will undoubtedly be valuable. The analysis, which is a key part is mostly described at the technical level. and how the detailed information of differential expression across millions of cells is used to answer the high-level question is less clear. • Once completed the project will provide a rich atlas of single cell data across stages and different exposures. Differential gene expression will be identified, and it is likely to suggest that exposure to THC/CBD triggers a cell type specific response. While Aim 3 will explore some developmental progression and effects and aim 4 will target the community, it is still not clear how the detailed molecular data would now turn around the high use among pregnant women. • Aim 1 and 3 offer a controlled environment but may only partially represent the in vivo brain response. Aim 2 will be highly variable due to the lack of details on exposure. • There is a risk of poor control of psychiatric histories.
<p>GWG Votes Yes: 8 No: 6</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • Yes, the project brings together computational biology, stem cell research, clinical genetics, public health epidemiology, and community engagement. • This is an outstanding team of diverse expertise and perspectives ranging from developmental neurobiology, high-throughput technology, computational biology, psychiatric genetics, and health policy. • The applicant presents excellent plans for integrating single spatial technologies at transcriptomic, proteomic, and metabolic levels.



	<ul style="list-style-type: none"> • They will follow up on some intriguing and potentially impactful preliminary results suggesting a disruption in the central ganglionic eminence intraneuron (CGE-IN) system. • Yes, though in some ways it seems iterative on preliminary data. In practice providing a new resource, similar to what has been produced for other traits but deployed for cannabis, would be innovative and valuable. • Potential public health impact is high due to 20% of women using marijuana during pregnancy. • The work seems focused on a particular neuroscience approach in single cell. There is an integration of omics data and approaches with the neuroscience in separate aims. • The innovation is rather limited. The proposal will use well-established (mostly commercially available) single cell technologies and pipelines. The access to samples seems a strength and these are clearly adding value. • The main approach here is to use single cell transcriptomics (and some proteomics) to explore molecular responses to exposures in various ex vivo, in vivo and in vitro contexts. • Fairly standard experimental techniques are proposed. • The different sites are well integrated with specialties and specialists at each site, but it doesn't necessarily seem especially 'cross-cutting'. It seems like a fairly typically collaboration of recruitment centers, data production core, and analysts. • No, the project uses organotypic slices from brain samples across developmental stages and exposes them to THC or CBD to study cellular and molecular responses. It also leverages spatial transcriptomics to provide spatial context for cellular responses and evaluates dysregulated genes for their involvement in neuropsychiatric diseases. The question is important, but the technologies are not new. • No, studies have found a significantly increased risk of psychopathology (social problems, impulsivity, ADHD, schizophrenia) in offspring with prenatal marijuana exposure. Birth registry data shows a robust association between maternal cannabis use and autism spectrum disorder in offspring. Converging evidence suggests prenatal marijuana exposure predisposes developing fetuses to neuropsychiatric disorders by perturbing brain development.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>
<p>Yes: 11 No: 3</p>	<ul style="list-style-type: none"> • Yes, the applicant presents very strong and sound rationale. • Yes, results from Figures 10-12 are particularly striking and give confidence that the results are worthy of extensive follow-up. • Yes, it is highly relevant to human disease and health policy. The development of a single-cell developing brain atlas modeling prenatal marijuana exposure will likely have a significant hypothesis-generating impact. • Non-human models are not proposed. • Rationale is sound, and preliminary data imply the feasibility of accomplishing aims. • The project will use human tissue and should be relevant to human biology. • Yes, the preliminary data show pervasive cellular responses to both THC and CBD exposure, significant overlap with dosage-sensitive genes, and reduction in inferior parietal cortex excitatory neuron-derived population. • Preliminary data makes the approach seem somewhat less novel but provides confidence in the possibility of success. • Yes, the project focuses on the cortex due to its role in neuropsychiatric disorders, high cannabinoid receptor expression, and reported effects of prenatal exposure on cortical thickness. The project also investigates both THC and CBD, the major cannabinoids in marijuana, to understand their differential effects on neurodevelopment. • From a molecular biology perspective, the idea certainly makes sense. Whether or not this in-depth dissection is needed for the purposes of reducing consumption in pregnant women is not fully convincing. • No, the project's goals are to establish a multidisciplinary research paradigm that combines single-cell multi-omics technologies, human brain samples, and organoid models to achieve high resolution in studying prenatal marijuana exposure effects and to engage with local communities to disseminate scientific knowledge and support. But



	there are flaws in the design that could make interpretation of results difficult (see below under plan and design).
GWG Votes	Is the project well planned and designed?
Yes: 8 No: 6	<ul style="list-style-type: none"> The overall project follows a sound logic with an appropriate balance of risk. The first two aims depend mostly on the cohort of samples and data generation. The proposed plan and also exclusion criteria seem reasonable. The biggest concern regards the outcome, not on the actual experimental execution. The application describes multiple points of conceptual and technical synergies, both for hypothesis-generating and validating purposes. More detail on other exposures in Aim 2 is needed. Yes, the plan is well explained in text and figures. Sample plan was generic, seems like it could be applied to any trait. Some of the terminology in the plan seemed confusing. For example the applications references '144 experimental conditions' in the text and '144 samples' in Table 1. They plan to use RNA-seq to identify expression of transmembrane proteins that the primary proteomic assay are less sensitive to find. How stable is the protein/RNA relationship? Could protein not be present in the absence of detectable RNA? Immunohistochemistry is proposed to verify a variety of cell types. Organoid models will be used to investigate tissue with earlier gestational week than can be acquired. How analogous are organoid models to matched gestational week samples? Aims seem somewhat dependent on each other in a sequential fashion. Yes, the project has several strengths, such as the comprehensive multi-omics approach at single-cell resolution, the use of organotypic slices that preserve tissue architecture and biological relevance, the examination of effects across diverse developmental stages and gestational windows, and the building upon preliminary data implicating specific cell types and pathways. Yes, the project also has weaknesses. The experimental setup is highly complex, requiring precise execution and optimization at multiple stages for fresh tissue. Obtaining sufficient high-quality brain samples across all developmental stages could be challenging. Addressing sample loss and ensuring consistent data quality across large-scale profiling is critical for success. Potential confounding effects from other substances, co-morbidities, or environmental factors are not accounted for. The project leverages ex vivo models (Aim 1) and developing brain samples (Aim 2) to identify vulnerable cell types and developmental stages, and it dissects dysregulated cellular and molecular components underlying neuropsychiatric predispositions. The work utilizes human cortical and CGE organoid models (Aim 3) to study exposure effects on these cell types. The project establishes a Strategic Advisory Group (SAG) comprised of community members, clinicians, researchers, public health professionals, and policymakers. The purpose of the SAG is to engage in a co-learning process to share knowledge on science, values, historical and policy context, and public communication strategies. There is a problem about potential confounding. Cannabis consumption maybe confounded with other risky behaviors. This is only partially countered with the ex vivo work. In general, yes, it is well planned and designed, but deeper phenotyping of cases and controls is necessary.
GWG Votes	Is the project feasible?
Yes: 11 No: 3	<ul style="list-style-type: none"> Yes, the data clearly show the team is capable of executing the experimental plan. Yes, the project team has expertise in stem cell research, genomics, medical geneticist and public health epidemiology. The applicant team is a talented group of investigators with strong track record of productivity. Yes, this is an outstanding multidisciplinary team.



	<ul style="list-style-type: none"> • Yes, their project management plans are clear and detailed. • Plans seem to be in place; several figures and tables explain how this is organized. • They appear to have necessary access to samples, expertise, and equipment. • Conflicts will be resolved by face-to-face discussion or by relying on a neutral senior third party. • Yes, the project leads for the aims have the resources to complete the project. • Yes, the budget and justifications are appropriate. • The PI would take the lead on the analysis, and that part is a bit too generic. • This is highly feasible but not particularly innovative. • There is concern about the team's ability to analyze the data.
<p>GWG Votes</p>	<p>Does the project uphold the principles of diversity, equity and inclusion (DEI)?</p>
<p>Yes: 14 No: 0</p>	<ul style="list-style-type: none"> • DEI plans are adequate, but of course largely driven by sample availability. Three iPSC lines are proposed for the studies in for Aim 3. • While the proposed samples are limited in demographic variability, the team's deep commitment to community engagement increases one's confidence that future studies will incorporate broader demographics once the initial platform is created. • The immediate outcomes of this project are unlikely to extend or validate to diverse populations. However, we can imagine how their community plans will create a pipeline to do so in subsequent phases of the project. • Yes, several members of the investigators have strong ties with the DEI efforts in the local community. A Strategic Advisory group made up of community members, researchers, clinicians, and public health officials is proposed. • An entire Aim is dedicated to outreach. Strong consideration of DEI. • They effectively describe in the introduction how the problem they seek to address may disproportionately impact underserved populations and groups. • Yes, the project's diversity plan outlines several key components to ensure representation and address potential biases, such as sample diversity, genomic analysis, covariate regression, and community engagement. The study will collect brain samples from a diverse population center, representing the inpatient population's diversity. • The study used monopogen software to call genomic variants from single cell sequencing and ADMIXTURE software to perform population admixture analysis. The study will consider population membership coefficients, maternal age, and sex as potential confounding factors and regressed them out as co-variates in the downstream analysis. The aim of the study is to identify exposure effects on brain development that are not biased by sex.



Application #	DISC4-16336
Title (as written by the applicant)	Human neural organoid models for opioid, cocaine and alcohol substance use disorder to identify pathomechanisms in addiction
Research Objective (as written by the applicant)	We will establish in vitro human neural organoid models for substance use disorder (SUD) that recapitulate human substance SUD subjects and rodent addiction model that the field is lacking.
Impact (as written by the applicant)	We anticipate finding novel therapeutic targets for SUD. Preliminary results indicate Nicotinamide adenine dinucleotide (NAD+) could be a potential therapy for withdrawal symptoms. We anticipate finding additional gene candidates.
Statement of Benefit to California (as written by the applicant)	Substance abuse disorder is a condition whose medical outcomes are associated with large racial and ethnic disparities, with individuals of African American and Native American descent disproportionately affected. Our study aims to clarify the molecular mechanisms that are common and specific to different substance abuse disorders to identify novel therapeutic targets, thus directly addressing an urgent unmet medical that disproportionately affects California's underserved populations.
Funds Requested	\$12,608,943
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	2
Lowest	3
Count	15
Votes for Tier 1	0
Votes for Tier 2	14
Votes for Tier 3	1

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG's review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRP for clarity.



<p>GWG Votes</p> <p>Yes: 10</p> <p>No: 3</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • The primary focus of the proposed analyses will be on advancing techniques to study how psychoactive substances affect brain biology through various experimental models. • CRISPRi screens could uncover new mechanisms and potential targets for drug development. Given the ongoing opioid crisis in the US, largely driven by overprescription of pain medications, this project's successful completion could directly contribute to saving lives. • The immediate impact on enhancing our understanding and treatment of substance use disorders is expected to be limited. • Strengths: <ul style="list-style-type: none"> • The project is very relevant due to the opioid crisis in the U.S. • It establishes human organoid models (cortex and striatal) for substance use disorder. • Single cell analysis and generation of large datasets (transcriptome, translome, epigenome) after treatment with 3 substances (morphine, cocaine, alcohol) are proposed. • The applicant will correlate data from a rat model of oxycodone self-administration to data from human cells. • A panelist's score was driven towards positive by the important and understudied question and general approach, but it was weakened by aspects of the approach. • The main impact of the proposed analyses will be related to the technical advancements to investigate the effect of psychoactive substances on brain biology using different experimental models. However, the direct effect of the expected findings on understanding and treating substance use disorders will be moderate.
<p>GWG Votes</p> <p>Yes: 11</p> <p>No: 2</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The proposal demonstrates high innovation through its application of a novel technology for single cell RNA binding protein and translome analysis. The strategic combination of human organoid models, a rat model, and diverse technologies enhances its robustness. • Some parts of the project are innovative (e.g. the translome analysis method). • The proposal is highly innovative in its application of translome analysis technology. • Based on the published literature, the proposed mechanism based on NAD+/nicotinamide phosphoribosyltransferase (NAMPT) activation is not novel. • The proposal is extremely focused on the integration of human and non-human models. However, there are no efforts to directly relate them to molecular changes observed in living individuals. This is a major limitation.
<p>GWG Votes</p> <p>Yes: 9</p> <p>No: 4</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • The overall rationale is solid and systematic generation of comparative data is of relevance. However, modeling withdrawal and SUD using organoids is highly speculative. • Applicants propose to test the effects of NAD+ and NAMPT activators to reverse some of the phenotypes that are associated with addiction/withdrawal. This is interesting but very difficult to dissect how oxidative phosphorylation, oxidative stress, astrogliosis, and blood brain barrier leakage are interconnected with regard to cause, consequence, or compensation. • The majority of the analyses proposed are based on strong scientific rationale supported by previous studies and preliminary data previously generated by the investigators. • Criteria for selection of differentiated cell types is not consistent throughout the proposal and needs more justification. • How will the investigators relate their findings to living people?
<p>GWG Votes</p> <p>Yes: 9</p> <p>No: 4</p>	<p>Is the project well planned and designed?</p> <ul style="list-style-type: none"> • Combined use of in vivo and in vitro models and various analytical methods are proposal strengths. • Weaknesses include:



	<ul style="list-style-type: none"> ○ Variability of organoid models could impede with reproducibility and data interpretation. ○ Altered energy metabolism and increased ATP demand due to addictive substances is a plausible mechanism that could perhaps be modulated by NAD+/NAMPT activation. However, modulating this pathway is only one approach. ● How will they model withdrawal in organoids? ● The proposed NAMPT activator is commercially available. Furthermore, testing only one NAMPT activator would be a weakness, considering that small molecules have different target selectivity profiles and potency. Hence, it is unclear what value this aspect would bring to this project. ● The overall proposal structure and the design of the subprojects support the feasibility of the analyses described. Detailed alternative strategies are presented for Aims 2 and 3. Conversely, those related to Aims 1 and 4 appear to be rather generic and do not explore adequately the pitfalls that may occur in the analyses proposed. ● Description of pitfalls and alternative approaches are lacking for Aims 1 and 4.
GWG Votes	Is the project feasible?
<p>Yes: 13</p> <p>No: 0</p>	<ul style="list-style-type: none"> ● The proposal needs additional support to show that the applicant team have the necessary screening expertise. They mention testing dozens of gRNAs in the proposal, but they do not say if they tested these in pooled format, how they were selected or the results. If they are going to mention this point, it would be good to include this information,. ● The project is feasible but has risky parts and could generate data of unknown significance. ● Based on the data shown, it remains unclear how robust the generation of striatal organoids is. No quantitative data were provided. ● The investigators have the expertise needed to complete the proposed study. A detailed collaboration plan is included. Facilities and resources are state-of-the-art for the proposed analyses.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 11</p> <p>No: 2</p>	<ul style="list-style-type: none"> ● In the DEI statement, the investigators mention that non-European descent representation is lacking in SUD studies, but they do not explicitly define how they will ameliorate this issue in this project. ● In some analyses, the investigators mention that age- and sex-matched controls will be used. However, there is no information regarding whether sex differences will be tested and how human population diversity will be modeled. This is particularly surprising because the investigators mention these aspects in their DEI statement. ● Population diversity is not well-developed in this project.



Application #	DISC4-16338
Title (as written by the applicant)	A Framework for Enhancing Clinical Utility of Precision Genomics in Psychotic Disorders
Research Objective (as written by the applicant)	Multiple genetic variants have been linked to psychotic disorders (PDs). Yet, the exact variants and mechanisms leading to phenotypes, remain largely unknown in most cases, limiting precision medicine
Impact (as written by the applicant)	We aim to deeply characterize causal variants and their effects at a subset of genomic loci strongly linked to psychotic disorders (PDs) with the goal of enabling improved precision medicine for PDs.
Statement of Benefit to California (as written by the applicant)	Our project has the potential to result in improved precision medicine for psychotic disorders (PDs). We focus on induced pluripotent stem cells (iPSCs) and their neural derivatives, aiming to advance the understanding of physiology and disease by using samples obtained from individuals from various genetic backgrounds. We also use genomic data from multiple ancestry groups. Thus, our work has the potential to benefit the State of California and its highly diverse citizens.
Funds Requested	\$12,520,839
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	2
Lowest	3
Count	15
Votes for Tier 1	0
Votes for Tier 2	10
Votes for Tier 3	5

- 1- The application has exceptional merit and warrants funding.
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- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 10</p> <p>No: 3</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • This is a powerful hypothesis generating proposal that could bridge the gap between gene discovery and our understanding of their effects. • The study will use computational and experimental approaches and multiple data types to understand the molecular mechanisms underlying loci associated with psychotic disorders. The breadth and depth of the analyses proposed are likely to provide new insights to resolve known questions and generate new hypotheses regarding psychosis pathogenesis. • The application does not articulate a vision for what happens if all proceeds as planned. They also do not cover the more likely case of a dozen SNP's contributing to phenotypes and how those will be knit into a coherent disease strategy. The authors claim "We envision that successful completion of the proposed aims will reveal the molecular mechanisms driving some of the strongest known genetic association signals with PDs" however they also say they expect to capture many weakly acting variants, and have no plans for covering trans effects. In terms of establishing a mechanism there is some electrophysiological phenotyping but there are no plans for understanding convergence of many variants on the phenotypes measured. Thus, there is very little chance of this actually defining a mechanism. Rather, it's largely a reductionist approach focused on drilling down to single nucleotide resolution, with insufficient thought applied to what happens after that – in particular what to do in the best case scenario of unearthing 100 causal variants. • The rationale that psychosis is more severe and therefore more likely to be detected in organoids doesn't seem correct as the expectation is that psychosis, with its intermittent nature, is likely to have more functional neuroimaging correlates, although there could be underlying structural changes. • The demonstration of the scBEseq and MPRA work will help to narrow down the loci involved in these diseases (within already known larger loci). The isogenic lines will not be useful unless they work, in which case others could attempt rescues. • This is an ambitious proposal on a subject that has been repeatedly frustrated in the literature, although it been around for six decades. The investigators make a strong argument that previous literature has been limited by several methodological/technical problems, for example, focusing on SNPs and CNVs, while tandem repeats and structural variants have been less studied-- and they offer the credible argument that this is the predominant risk in PDs and human disease in general. • The investigators make the argument of the wrong focus on molecular phenotypes rather than cellular or more systemic (neurodevelopmental) mechanisms. This argument is not well-addressed in the application. • A potentially large early-onset psychosis cohort is a very valuable asset that could be used by many others. However, doubling the size of the existent cohort in a very complex syndromic group like PD, and with little clinical support, may not be feasible. • Generation of iPSCs harboring disease-risk mutations of interest may also be a great tool for the general research community. So is the deep phenotyping of cortical organoids generated from 3D cultures of iPSC-derived neural cells and microglia. • In view of the complex nature of psychotic disorders, as well as interaction between genetics and environment, it is difficult to know how broad the impact of this project will be. • The project is linked to the idea of gene-specific treatments of psychotic disorders. This bottom-up therapeutics approach is encouraged by select neurological diseases like SMA and ASO, with mediocre results. The genetic complexity of psychotic disorders would make this approach less plausible. However, building pathogenic mechanisms bottom-up may help identify intermediate steps that may allow for more specific therapeutic targeting and allow the construction of more complex models in the future. • The proposed cohort is the most impactful part of the application.
<p>GWG Votes</p> <p>Yes: 9</p> <p>No: 4</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • There is a need to understand tandem repeats since these are understudied, hence this is novel.



	<ul style="list-style-type: none"> • Innovation stems more from the smart combination of existing technologies than development of new ones. • The construction of an expanded cohort of early-onset psychosis patients with genomic and deep clinical characterization for large-effect size is a great strength. • This is an impressive, though not too novel, combination of deep functional characterization of genetic variants for genomic regions known to be linked to psychotic disorders not only with statistical, clinical, and molecular approaches that abound in the literature, but also more extended use of cellular and functional essays for phenotypes with the use of two and especially three-dimensional humanized patient-derived neural systems. • While no new technologies will be developed during the study, the investigators will use a range of computational and experimental approaches that in some cases have been developed by them previously. • The massive perturbation tests to identify effects of non-coding variants are new enough in this context to qualify, and the scBESeq would also be novel. • There are a variety of methods employed, however they generally aren't synergizing (early aims aren't substantially redirecting later ones). • No. Everything is very mainstream (and logical, just not a new conceptualization). Sadly, there's not anything that looks to bring us rapidly out of the sad situation of knowing hundreds of causal variants (albeit with low resolution) but not understanding how they collectively act.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>
<p>Yes: 8 No: 5</p>	<ul style="list-style-type: none"> • The overall design and the analyses proposed are based on strong scientific rationale. • A qualified yes. The general concept of identifying variants and perturbing them in cellular and organoid models is something that's almost universally embraced for complex disease. However, when it comes to the particular program proposed under this banner there are significant limitations. • Despite the use of hCOs, which are a great endophenotype, there's an unjustified assumption in this application - if we drill down to a single base pair(s) for disease, we will understand it. Moreover, this reductionist genetic approach lacks clear justification in the application, as well as a clear plan for following up on findings. • There are key areas of the study that are not justified - the gain in explanatory power from combining diseases vs focusing on a single one with late vs early psychosis is never made, there's no preliminary data on the ability of cellular or organoid models to classify disease origin, there's no false positive rate established on VUS, and more globally there's not demonstration of the synergy of the set of proposed approaches. • It's not clear if the applicant can generate co-cultures. Also, at this time experiments should be run in a triple co-culture. • A substantial amount of preliminary data has been beautifully presented. However, illustrations on methods and techniques are overwhelming and too didactic. These could be avoided for the sake of better expounding on concepts and methods. • The hypothesis of a bottom-up mechanism from genes to mechanisms is not justified in the application. There is quite a bit of neurobiological literature, especially in schizophrenia, to be examined. • It is difficult to see novel concepts or hypotheses. • The work is very descriptive.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 5 No: 8</p>	<ul style="list-style-type: none"> • Significantly increasing the number of participants for LRS if feasible. • The overall projects and subprojects are well-planned and designed. One minor weakness is related to the limited representation of population diversity in the datasets used to investigate molecular phenotypes (e.g., GTEx and PsychENCODE). It would have been nice to have the investigators acknowledge this and explain how this will affect their results. Similarly, there may be challenges in investigating VUS in diverse population groups. • The whole project is based on these several loci, and there are any number of criterion that could be deployed. The key point is to find ones related to early psychosis, but



	<p>there's actually nothing in these criteria that particularly relate to psychosis or its age of onset and they don't clarify which loci pass which criteria(on).</p> <ul style="list-style-type: none"> • In the justification for further recruitment there is mention of VUS for loci of interest, but there's no provision of any false positive rate for these, so it's not clear to what extent larger cohorts are going to help. There's never been a justification for gathering across two or three diseases vs one from the perspectives of a genetics study mainly interested in psychosis. • Hundreds of thousands of samples might render findings of commonalities (i.e. EOP), but not the small sample size here. • The investigators might subtype the individuals coming in via neuroimaging, because right now everything is treated as a single phenotype. • The recruiting capacity is unclear. There is no guarantee that a sibling will participate. While that would be clearly superior, it also calls into question recruitment capabilities. • Yes, pitfalls are presented, but they do not address the deep issues around constructing coherent disease mechanisms out of a set of variants, no matter how well defined they might (optimistically) be. • Lack of synergy among the aims is a major problem with this project. All aims focus on identifying variants or their effects which fall in 6 known multi-gene regions for these PD's. Since the investigators are applying scBEseq to understand exactly which loci have effects, it's not clear what benefit the fine mapping and other efforts of the first aims will have. • The VUS from the additional individuals they gather will all be in these same regions, so ultimately they may pick up some more specific base pairs, but in the same overall regions. It's not clear that they will describe additional mechanisms. In fact the seBEseq is focused on genes which will be determined by simple KO and expression analysis, so why would you not skip right to doing this, as it's really the novel part of the proposal? The prior analysis won't change anything about it (regions considered). Similarly the MPRA targets 100 variants in each region, pulling from existing resources in addition to the one proposed, so it's not clear the first part of the grant is needed as the number of known variants may be more than can be targeted. • There might be some interesting findings from the first few aims, but these may not be novel or feed into the prioritization of variants by scBEseq or the MPRA. • The investigators use CRISPR genome editing to generate isogenic iPSC lines for SNP and TR variants strongly associated with increased risk of schizophrenia and bipolar disorder. They think they will have about two isogenic lines for each variant, which is ambitious. • They use iPSCs to generate mutant cortical organoids on which they perform more or less standard characterization with respect to cell composition/phenotyping and cytoarchitecture, the first with scRNA-seq. A strength, they will also explore network development by calcium imaging and multi-electron array recordings. • The clinical complexity of early-onset psychotic disorders is underestimated, differential diagnosis is not discussed, and comorbidities are not dealt with. The application conveys limited input by clinicians specializing in psychotic disorders.
<p>GWG Votes</p> <p>Yes: 8</p> <p>No: 5</p>	<p>Is the project feasible?</p> <ul style="list-style-type: none"> • Defining "understanding" in biology is tricky, but that's the nominal goal. A list of base pairs related to organoid development will not equate understanding. The teams needs to bring on a systems biologist or perhaps a deep learning expert to assist with data integration and variant identification by integration of larger resources. • There are no concerns regarding the team qualifications regarding the feasibility of the proposed study. • The number of edited lines proposed is ambitious. • It is problematic that there is dearth of clinical psychiatrist effort, which raises some questions as to the ability of this group to expand the cohort. • There is only one early-career child and adolescent psychiatrist. Are there proper recruitment networks in the community? On whom and what are the diagnoses and differential diagnoses of these syndromes based?



	<ul style="list-style-type: none"> • Where are the psychotic disorders clinical experts? Who would characterize clinical populations in the extremely heterogeneous group of PDs so that the investigators achieve the deep phenotyping necessary to make some of the unique features of the project (cohorts, patient-derived iPSCs) worth funding? • Reviewers noted a lack of clinical partners to help diagnose the clinical disorders. • No evident display and explanation of interactions, meetings, administrative structure and management. Not clear what the management expertise of PI is.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 13</p> <p>No: 0</p>	<ul style="list-style-type: none"> • Population origins and age are adequately accounted for in the study. However, biological sex will be included only as a covariate. It would be important to conduct sex-stratified exploratory analyses to investigate possible differences between females and males. • The application is focused on early psychosis for scientifically justified reasons, so there are no older individuals. Other covariate coverage is acceptable. • Based on the makeup of their pilot cohort it seems likely that results will generalize to African, Hispanic and Asian populations, although there is no commitment to targets for these groups in the proposed study. • Sex and race seem to be appropriately addressed by performing multi-ancestry analysis of large genetics data sets on East Asians, Europeans, Hispanic, and African-American patients in the EOP cohort. Human iPSCs will be derived from individuals of what they call "any ancestry" and both sexes, although it's unclear how this is going to play out in creating the appropriate number of isogenic lines, etc. • It appears that the investigators have a strong track record contributing to DEI via research, mentoring and service. The proposal lays out such efforts in detail and great specificity. • Each of the scientists have notably above average voluntary engagement with underrepresented groups.



Application #	DISC4-16378
Title (as written by the applicant)	Mechanistic understanding of neuronal maturational timing
Research Objective (as written by the applicant)	We explore the overlap between genes involved in the timing of neuronal maturation and psychiatric disease and explain mechanistically the origins of psychiatric disease.
Impact (as written by the applicant)	We will have solved the problem of neuronal timing and shown that the pathogenesis of severe mental illness has its roots in this mechanism for establishing brain structure and function.
Statement of Benefit to California (as written by the applicant)	Current treatments for serious psychiatric disease are relatively ineffective and none cure disease. By identifying the mechanistic basis of psychiatric disease, we will be in a position to develop novel, effective treatments.
Funds Requested	\$12,180,898
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

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KEY QUESTIONS AND COMMENTS

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GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 11	<ul style="list-style-type: none"> • The overarching goal of this project is to find genetic elements (genes or regulatory sequences) that govern neuronal maturity. This is an important goal as humans exhibit slower maturation, called neoteny. Whether neoteny is an important part of psychiatric disorder etiology is unknown, mainly because we do not have a good understanding of this biological process. This proposal addresses that gap.
No: 2	



	<ul style="list-style-type: none"> • This proposal will have an impact on understanding the biological processes associated with human neurogenesis and/or maturity. Whether those processes are involved in psychiatric disorders is unknown. It is possible that the investigators will identify maturity associated genes/regulatory elements that are not involved in psychiatric disorders. • This proposal will also generate ~50 new cell lines from individuals with high/low polygenic risk scores (PRS), genome-edited non-human primate and mouse iPSC lines, genome-edited human iPSCs containing maturation-associated psychiatric disorder risk variants, genome-edited human iPSCs containing fluorophores marking important developmental switches, as well as screen and transcriptomic data. • The data and cell lines will be shared with the research community through standard databases. • The project addresses our fundamental knowledge gap in the mechanisms underlying neuronal maturation and how these factors might contribute to the cellular disruptions observed in psychiatric disease. • Studying brain maturation as relevant to neurodevelopment disorders (NDDs) is reasonable, and the proposed approach (in overview) is excellent. • The project will likely improve our understanding of psychiatric disease. However, the likelihood of identifying possible therapeutic strategies is much lower. • The proposal will generate a list of genes and human regulatory elements that are involved in neurogenesis and/or maturation. • Several data types and deliverables will be generated: Single-cell sequence data from iPSC-derived neuronal stem cells, engineered stem cell lines with maturational markers, engineered lines with mutations introduced into maturational genes, and imaging from xenotransplantation studies. • Strengths: <ul style="list-style-type: none"> • This is an ambitious genetic mapping project in human iPSC-derived neurons. • Xenotransplantation will allow for mechanistic insight. • Weakness: Low 'n' will be studied for polygenic risk score (PRS) calculation. • There is unclear impact to neuropsychiatric diseases, so alignment with programmatic priorities is unclear. • It's not clear that the selected genes overlap with disease risk.
<p>GWG Votes</p>	<p>Is the proposal innovative?</p>
<p>Yes: 12 No: 0</p>	<ul style="list-style-type: none"> • Looking at mechanisms of neuronal maturation as a source of psychiatric risk is innovative. • The techniques and goals are unique. • There are several new technologies to be used - mainly CRISPR screens in organoids derived from multiple species to assess maturation/neurogenesis associated outcomes. • There are multiple disciplines involved including psychiatric genetics, organoid differentiation, neural development, electrophysiology, and bioinformatics. • The idea that neuronal maturation is an essential part of psychiatric illness etiology has not been evaluated before because the genes involved in neuronal maturation are not well defined. • While the proposed technologies are all cutting-edge, they are not themselves novel, per se. The project's innovation comes from the combination of sound technologies in an innovative conceptual framework. • The project proposes a very intriguing hypothesis, proposing an orthogonal approach to the more standard way of starting with known psychiatric genes and following the biology. The hypothesis is very clever and elegant. There is no doubt we will learn a lot about the mechanisms underlying human-specific neoteny, but whether this will lead to insights on the nature of psychiatric disease is much more speculative. • The interdisciplinary nature of the project is modest to moderate. • It would be great to go back to patient data to corroborate study findings.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>
<p>Yes: 10</p>	<ul style="list-style-type: none"> • A clear role for neuronal maturation in psychiatric disease development has been established. • The field needs better ideas about maturation of human neural cells.



<p>No: 2</p>	<ul style="list-style-type: none"> • The overarching idea of finding genes and regulatory elements associated with neuronal maturity, and learning why it is slower in humans, is an important scientific question. Solving this question will facilitate development of more mature iPSC-derived model systems of human cells. Whether this will extend to a greater understanding of psychiatric disorders is unknown, but possible. • More discussion about how maturation genes might be expected to overlap with disease risk would be useful. • There are some specifics about the scientific rationale of the project that are questionable. <ul style="list-style-type: none"> • Finding genes enhancing expression of <i>[named gene]</i> may not address functional maturation of neurons, but neurogenesis. As a result, the screen may yield hits associated with neurogenesis (rather than maturation). • This concern is ameliorated somewhat with the use of the planned CRISPR screens using fluorophore reporters expressed by elements known to be involved in developmental switches. However, this proposed CRISPR approach may be too difficult and slow to be completed during the project. • Aim 3 specifically is highly dependent on the other 2 Aims and rests on assumptions that psychiatric risk variants influence maturity. While the experiments proposed are exciting, they are also extremely ambitious (electrophysiology, generation of multiple edited cell lines, xenotransplantation, and generation of ~50 new iPSC lines from high/low PRS). This aim is risky. • Strong preliminary data are shown for cortical organoid generation, fluorophore expression, and electrophysiology in organoids. CRISPR additions into organoids are shown for a single gRNA and control gRNA, though not for a whole screen. • The cell lines expressing a fluorophore with known developmental switches have not yet been generated, but other edited lines have been produced by the core facility to be used here. • The project proposes a very intriguing hypothesis and takes an orthogonal approach to the standard of starting with known psychiatric genes and following the biology. The hypothesis is very clever and elegant. There is no doubt we will learn a lot about the mechanisms underlying human-specific neoteny, but whether this will lead to insights on the nature of psychiatric disease per se is much more speculative. • Yes, the preliminary data are generally supportive of the feasibility of all the experiments. However, how strong the results will be at each step remains unclear. Since the project relies on a funneling logic, whereby the strongest signals from early experiments are used to direct subsequent ones, it's unclear how solid the foundation will be for the experiments once they reach Aim 3. • The project focuses on human accelerated regions and patient samples with high/low PRS. • Mainly human iPSC-based organoid model systems will be used. All the proposed uses of non-human model systems are very well justified. • Engineered mouse cortical cell lines are proposed as proof of concept, with non-human primate organoids further used for validation. Mouse xenotransplantations are also proposed. The proposed experiments are reasonable forays into the functional impact and generalizability of their results. • The project is highly relevant to human-specific biology. Whether it is relevant to disease will only be known after completion of the project. • No. Linkage between development and neuronal maturation to later-stage (often adult) diseases has unclear validity.
<p>GWG Votes</p> <p>Yes: 8 No: 4</p>	<p>Is the project well planned and designed?</p> <ul style="list-style-type: none"> • The Aims follow a logical progression and appropriately utilize the strengths of each experimental method both individually and in combination. • There are multiple, highly complementary techniques proposed to screen and assess maturity. • The project creates elegant conceptual synergies by intersecting studies focused on fundamental timing mechanisms in neuronal development with the current knowledge of psychiatric risk and unique genetic signatures in the human lineage.



	<ul style="list-style-type: none"> • The proposal needs more preliminary data, and the description of pitfalls and alternative strategies is weak. • If Aim 1 is unsuccessful, Aims 2 and 3 won't be possible. • Aim 3 in particular is entirely dependent on the the success of the prior work, and this risk is not well-addressed. • Yes, overall, but use of a single assay readout is a weakness. An additional level of filtering would be helpful prior to CRISPR targeting of hits. • These caveats should be addressed: <ul style="list-style-type: none"> ○ Will they really pick up maturation or will they find neurogenesis associated genes? ○ N~50 may be an underpowered sample for calculating polygenic risk scores (PRS). • This proposal uses 3 separate viruses inserted into each cell. In order for each experiment to work, all 3 viruses need to integrate into each cell. For the project to succeed, this has to happen for multiple gRNAs for every gene. Selection is proposed after each transduction, but this still might be difficult to achieve. Also, preliminary data showing success of this method are not provided. • The second screen (using a reporter for known developmental switches) is much more exciting but requires generation of CRISPR edited iPSCs expressing fluorophores at the marker gene locations. This will be difficult. • Non-coding regulatory elements will be targeted in Aim 2. This is an interesting evolutionary angle, though the design is subject to similar limitations. • The genome elements proposed to be studied have previously been examined using massively parallel reporter assay (MPRA) frameworks, so the novelty here is low. It's not clear what value is gained by those experiments. • Aim 3 is highly ambitious - covering electrophysiology, human genetics, and cross-species maturation. The electrophysiology/calcium imaging alone may be sufficient to demonstrate maturity. The xenotransplantation, animal model lines, and new iPSCs generated from ~50 individuals seem hard to accomplish within the time limits of the project, though each is incredibly interesting. • The project has methodological limitations, and the team has unclear experience with CRISPR screens. • Screen throughput will be limited. • Potential pitfalls are identified, mainly dependencies between Aims. Their argument is that by screening so many elements/genes they are likely to find some elements that influence maturity. However, whether those maturation elements are psychiatric disorder-associated is unknown until completion of the experiment.
<p>GWG Votes</p>	<p>Is the project feasible?</p>
<p>Yes: 9 No: 3</p>	<ul style="list-style-type: none"> • There is a logical flow to the sequence of experiments. • This is an outstanding team of investigators, all well-placed to conduct and interpret the studies. • The team is at one institution and has a history of collaboration. • The PI is a clinician scientist who has led many previous large, successful genetic association studies for psychiatric disorders in both human and mouse. • An expert in systems neuroscience who has previously conducted xenotransplantation experiments of human neurons into mouse will lead part of Aim 3. • Other Key Personnel have (i) expertise in PSCs, (ii) expertise in organoid differentiation, (iii) experience developing statistical tools for analysis of CRISPR screens, and (iv) experience with MPRA. • Appropriate plans are in place to manage the collaboration. • Overall, the project management plans and personnel seem solid, but given the history of collaboration, there may be less room for incorporating fresh and diverse perspectives. • The team has all the necessary resources to conduct the proposed activities. • The team seems to have access to all necessary resources.



	<ul style="list-style-type: none"> • Description of pitfalls and alternative approaches are not adequate for the project. • The project is high risk. It is not clear if the general approach to start with neuronal maturation will indeed offer insights about ASD or other neuropsychiatric conditions. • The budget is reasonable for the proposed project. However, given the novelty of the overall hypotheses, it seems that CIRM may want to consider first supporting this at a smaller scale. • The investigators need to make the iPSC lines for key experiments to increase evidence of feasibility. • The budget is high.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 12</p> <p>No: 0</p>	<ul style="list-style-type: none"> • The team includes a plan to sample historically underrepresented racial and ethnic communities. However, this may also introduce ancestry-related variability that is problematic for calculating PRS. • Multiple ancestries are proposed to be used in the iPSC generation in Aim 3.5, but exact numbers are not given. Because of the low power for schizophrenia GWAS/PRS in all but European and East Asian populations, it's unclear how this recruitment will work in other populations. • The screen will be conducted in both male and female iPSC lines. Ancestry is unlikely to play a major role in neoteny, a feature common across all humans, so the results will likely be broadly applicable. • The genes/regulatory elements involved in neoteny are likely common across all human populations. • The applicants state that they will leverage institutional programs dedicated to diversity, but do not list specific programs. • The proposed DEI-oriented experiments are quite modest. If the project were supported at a small scope, this would be fine, but at this budget more intentional effort should be made to consider additional demographic factors. • There are no explicit DEI outreach activities. • DEI efforts are very modest.



Application #	DISC4-16400
Title (as written by the applicant)	High throughput, multi-modal analyses of neuropsychiatric disorder risk genes in a diverse cohort
Research Objective (as written by the applicant)	Drug development for autism and schizophrenia is hampered by disease mechanism knowledge gaps and minimal inclusion of ancestral diversity. We will address both bottlenecks to improve drug discovery.
Impact (as written by the applicant)	We will identify disease phenotypes in stem cell models that are shared between autism and schizophrenia, which will enable future drug screens to help find treatments for these conditions.
Statement of Benefit to California (as written by the applicant)	Autism and schizophrenia are neuropsychiatric disorders that collectively affect 3-4% of people. Thousands of Californian families are impacted, and the estimated annual economic costs are in the tens of billions of dollars. Here, we will use stem cell-derived models of the developing human brain to identify disease mechanisms and nominate drug targets. We will conduct these investigations using human cell lines from ancestral backgrounds that represent the diversity of California residents.
Funds Requested	\$14,247,871
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

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Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 8</p> <p>No: 4</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • We do not understand how different non-coding variants contribute to changes in cellular phenotype including gene expression. This project will generate data that will bring us closer to closing this knowledge gap. • The project will provide phenotyping on what would be the largest collection of African American cell lines from individuals with autism or schizophrenia. As such, it contributes to a more stable basis for inferring the actions and identity of key genes and molecular systems towards these diseases. • Yes, potential for impact is high; although, there are insufficient preliminary data to tell if the project will be successful. Electrophysiological phenotyping of human organoids has strong face validity for disease, especially for developmental disorders. • This proposal will generate a large functional genomics data set from iPSCs from non-European backgrounds that will be a valuable resource for the community. • This proposal addresses one of the most pressing gaps in psychiatry: moving from the genes that have been discovered to illuminating causal neurological mechanisms. • The proposal uses a combination of state-of-the-art data approaches and experimental technologies to understand mechanisms. This could help us better understand schizophrenia (SCZ) and autism spectrum disorder (ASD) and lead the way in showing how we can move from gene discovery to mechanism. Of note are the high throughput approaches that will enable investigation of a large number of genes and their effects as well as the novel analytical techniques to connect the different types of data. • The application presents a highly innovative approach to gain important new insights into disease mechanisms relevant to psychiatric diseases. Specifically, the use of iPSC cell models for follow up of genes identified in human genetics studies is innovative. • The proposed experiments will likely provide novel insights into molecular and cellular changes underlying ASD and SCZ, in particular for genes associated with these diseases. • Genetic insights can lead to the development of novel therapies. The applicants propose innovative data analysis methods and utilize relevant databases with the goal to identify novel treatment options. • The large number of lines and data to be generated will be impactful in the field. • The data generated through this proposal are likely to be useful for the scientific community. • The project will generate relevant iPSC cell lines. • This project will be helpful for hypothesis generation only. No data are presented to convey that the number of cell lines will be sufficient to describe disease, let alone disease in the context of genetic ancestry. The project is likely to yield false positive results due to the small number of cell lines versus what is generally required for complex/sporadic disease. • There are major doubts about all downstream analysis, lack of meaningful power analysis, and the use of precious samples/lines. • Giving the weakness in the AI component and the drug-discovery pipelines, this will not put us closer to a treatment. • Deliverables are (i) a large dataset and iPSC lines from diverse backgrounds. There is a question about the feasibility of conducting meaningful analysis without an additional collaborator. • If the project is not successful, the most we can expect is a collection of iPSC lines. • An analysis plan is lacking.
<p>GWG Votes</p> <p>Yes: 8</p> <p>No: 4</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The project incorporates advanced state-of-the-art technologies including iPSC-derived cell models, 3D cerebral organoids, and cell villages. • The overarching framework reflects the concept that iPSCs model phenotypes which reflect underlying disease mechanisms for SCZ and ASD. This is further guided by the results of human genetic studies where risk variants and genes have been identified. • Many Aims propose highly innovative technologies and data analysis methods. For example, Aim 4 is innovative, and the data generated will both demonstrate the power of this approach and generate data from a large number of cell lines in a cost effective way.



	<ul style="list-style-type: none"> • The proposal brings together investigators with diverse expertise. A broad spectrum of methods and approaches are utilized. The data analysis methods are well described and innovative. • The highlight of this proposal is the extensive electrophysiological phenotyping of the cell lines. Some of those methods have never been brought to bear on human data in these diseases. • While the proposal is comprehensive in terms of applying recent phenotyping methods, there is a major missed opportunity which is in line with the electrophysiological emphasis of the grant. This would be to scale up the level of neuroimaging from these same individuals or from disease cohorts. Essentially generic brain models of cortical columns or neural mass models could be tweaked to instantiate the observed disease changes. The proposal already talks about finding convergent phenotypes at the level of neuronal activity, and based on the structure of the brain, there is much more chance of convergence at the level of electroencephalogram (EEG)-based analyses (such as power spectra) and other whole-brain disease markers. There's even discussion of finding excitation/inhibition balance which is a major parameter addressed in large-scale brain models. There is a lot coherence in terms of what could be done. • Overall, the proposal is solid, but lacks anything surprising or highly motivating. This extends to the analysis side of the proposal which either (i) uses older methods or (ii) uses newer methods without the appropriate degree of support or expertise. • Most modern biology is interdisciplinary in nature and this application is no exception. • The subject explored is highly mainstream and likely useful, but not new. • The technologies in terms of data analysis are standard in the field and the AI component is not convincingly presented. • Producing these cell lines would be valuable, but the approaches are not innovative.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>
<p>Yes: 10 No: 2</p>	<ul style="list-style-type: none"> • The justification is mainstream and logical at a high level, though there are major technical issues. • There are insufficient preliminary data to provide confidence that 25 lines will be sufficient for modeling a complex genetic disease. The rationale for this project needs a plausible justification for the use of 25 lines. <ul style="list-style-type: none"> • The data needed to test this are available, as researchers say they already have 100+ lines. The investigators could take pairs of 25 of those (chosen at random) and test their ability to correctly classify their origin, based on whatever phenotypes are available. • The investigators should search the literature for electrophysiological phenotypes, finding the variability associated with them and proxies for them in adult data. • If preliminary investigations don't indicate the 25 lines will suffice for generation of meaningful results, the investigators might first devote more project funds and resources to line generation. Less intensive phenotyping can occur later in the project. • Possibly 25 lines are sufficient, but there's no direct or indirect evidence of that in the proposal. • Power calculation is not adequately employed in the proposal. Moreover, the applicants refer to power calculation in a quoted effect size, related to a monogenic disease, that is not applicable. Moreover, estimates from sporadic autism neuroimaging studies, which should be a reasonable proxy, put the effect size statistic well below 1. • The investigators must describe and justify how the lines will be selected. What is the ideal amount of admixture and how much diversity do you want in this collection? Do you want to select for individuals with variation around known loci? • Highly relevant to human biology and disease – there's arguably not a better combination of throughput and face validity for modeling autism. • The preliminary data are compelling and supportive of the proposed activities: ~100 hiPSC lines representing 8 ASD associated mutations. The investigators also contributed to discovery building up to this proposal. The team has excellent technological expertise.



	<ul style="list-style-type: none"> • The proposed experiments are likely to recapitulate human disease and are therefore relevant to human disease. • Some aims propose to follow up on genes identified in genetic studies. The focus is particularly on African Americans (AA), for whom there is limited knowledge related to genetics. The focus on a minority is justified. • The proposed experiments are well justified. The overall rationale that differentiated iPSCs are likely to exhibit disease relevant phenotypes is well supported. • As the proposal focuses on AA, plans for additional recruitment and establishment of iPSC lines from patients and respective controls is sound and well justified. • The list of genes to be studied is provided but requires more detail. Specifically, it's not clear in what cohorts the association signal was detected. In addition, the applicants state that the proposal will focus on elucidating the risk in AA. Most likely the proposed genes have been identified mainly in other races. • Aim 1 proposes knockdown in a few unaffected AA iPSC lines. Since the genes are likely identified in non AA studies, the rationale is not well presented. Do the applicants postulate that genetic background effects specific to race will contribute significantly to the disease risk? What is the evidence that the proposed genes contribute to disease risk in AA? This aspect is underdeveloped. • The preliminary data support in large part the proposed aims. • The applicants provide extensive preliminary data on their ability to conduct the experiments using iPSCs and various differentiated cell types. • There are sufficient preliminary data related to the proposed data analyses. • The project will require more cell lines to yield conclusions. Power calculation is missing. • The proposal lacks power analysis.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 5 No: 7</p>	<ul style="list-style-type: none"> • The lack of power calculations make the feasibility somewhat unclear. • Yes, but there are areas outlined by the reviewers that should be addressed, • The various projects, aims, and data to be generated are well integrated. The project benefits from synergies. • The technical aspects of the proposed experiments are described in detail. The projects and respective aims are well developed and integrated. The projects are complementary and necessary synergies are well described. • Potential issues are discussed, and the alternative approaches are well described and appear appropriate. • The proposal is weak in terms of the systems biology – which doesn't address the core issue of how known or novel variants (especially trans variants) interact with an AA background. Deep neural network (DNN) approaches are key to synthesizing all the data into actionable predictions, and the DNN section (5c) in this proposal is very poorly developed. The current personnel may not have the expertise for this topic. • There's an overall theme (and necessity) of relatively high throughput technologies which are well aligned for line generation, knock-ins, electrophysiology, and single cell omics. • The analysis plan is underdeveloped. • Aim 5 is underdeveloped. • The project is well planned mainly in terms of valuable data sets. • The experimental variability of organoids and assembloids should be better defined. Otherwise, meaningful comparisons will be difficult and of unknown significance. • The authors should also address how they will identify disease phenotypes and signatures if genes effects are small. • The in vitro approaches are well designed. This includes Aims 1-4. One key concern is the lack of power calculations. • Aim 5 covers data integration and development of therapeutic leads, but with insufficient detail. The specific methods described do not provide significant innovation. The program does not include the necessary analytical innovation to ensure impact from the data that are generated



	<ul style="list-style-type: none"> The proposal does not include a plan on architecture of models and the ability to revise them going forward.
GWG Votes	Is the project feasible?
<p>Yes: 8</p> <p>No: 4</p>	<ul style="list-style-type: none"> All aspects of the project are supported by preliminary data; the investigators are highly experienced, and the staff seems appropriate. All resources appear supportive. In part, this project also benefits from a similar NIH funded project. The team offers leading technological expertise to deliver the in vitro work. The collaboration structure is well described. The budget appears appropriate. The team has fantastic facilities, especially for a high throughput project like this. The lack of power calculations make the feasibility somewhat unclear. There is no doubt of the global expert level qualification of the PI in ASD, and one co-investigator in genomics. However, there is insufficient deep learning expertise on the team for this important project. It's unclear if there is needed expertise in the group for a variety of methods. Designing and tuning neural topologies is a distinct skill set from much of machine learning (ML). A collaborator with a track record of class-leading results is needed. Building networks that will predict the effects of multiple omics on expression and phenotyping is one of the hottest challenges in ML now, with entire, large teams devoted to it and making minimal progress. The project offers a large data set and cell lines but is underdeveloped in terms of data analysis. Aim 5 lacks supporting evidence of feasibility and/or necessary expertise. The project is feasible with the exception of Aim 5.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 12</p> <p>No: 0</p>	<ul style="list-style-type: none"> The proposal addresses and accounts for the influence of race, ethnicity, sex, gender diversity. The outcomes will benefit African Americans in particular. DEI is one of the strengths of the proposal. The project includes iPSCs from African American participants. The purpose of the proposal is to uphold principles of DEI. Outreach and partnership is addressed. The team has excellent long-standing partnerships. There should be more thought on how already-acquired genetics data and admixture will influence selection of cell lines.



Application #	DISC4-16345
Title (as written by the applicant)	Development of novel therapies to treat CNS-associated microdeletion syndromes
Research Objective (as written by the applicant)	Chromosomal microdeletion syndromes result in severe neuropsychiatric syndromes and lack therapy. This proposal will define critical genomic regions needed to generate new tools for functional rescue.
Impact (as written by the applicant)	This work offers a new approach to restore gene function in 16p and 22q deletion syndromes. Success will create a model for treatment of >200 microdeletion syndromes with neuropsychiatric symptoms.
Statement of Benefit to California (as written by the applicant)	Microdeletion syndromes have neuropsychiatric manifestations and few therapeutic options. Given the cognitive deficits and need for lifelong care, these conditions affect the resources and well-being of patient caregivers and providers. Our studies will generate novel therapeutic approaches using 16p and 22q deletion syndromes as exemplars. We will also use clinical outreach and advocacy panels to understand the patient experience and ensure patient-driven goals are part of the research process.
Funds Requested	\$10,172,372
GWG Recommendation	<i>Tier 2: needs improvement, could be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 2

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	2
Lowest	3
Count	14
Votes for Tier 1	0
Votes for Tier 2	13
Votes for Tier 3	1

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 11</p> <p>No: 2</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • Genomic copy number variations, such as microdeletions, often lead to reduced expression of many genes and many result in deficits in development and psychiatric problems. There are over 200 microdeletion syndromes. • Restoring gene expression of multiple genes simultaneously remains a major technical challenge. The major goal of the proposal is to restore gene expression using CRISPR-based activation of multiple genes from the intact allele. • The potential impact is twofold: (i) new tools for CRISPRa restoration of microdeletions and (ii) development of in vitro human cell based and xenograft mouse assays for assessing therapeutic function and selecting therapeutic candidates. • The proposed work will identify functional elements, mainly promoters and enhancers, that regulate expression of the genes within each microdeletion. This work will generate a list of sgRNAs for gene expression activation, which could be useful for the field. This work will also generate edited iPSC lines with known microdeletions. • If successful, we will have a better understanding of the cellular and circuit dysfunction in microdeletion syndromes (95% of which are neurodevelopmental). • If successful, the project will generate new tools for studying diseases caused by microdeletions. • New methods for development and preclinical testing of gene editing approaches for microdeletion-associated diseases would become available and greatly accelerate progress in this area. • There is a potential for high impact on patients with this novel approach. • This depends on the extent to which cell-based systems and xenograft models are predictive of what occurs in intact human brain. If their results do generate preclinical models which are predictive, the impact would be huge. • The project does not seem technically feasible. Many claims are overstated. • The proposal is overly ambitious; the applicants should narrow the research question. • Excellent idea but not feasible or adequately planned. • The project seems too ambitious.
<p>GWG Votes</p> <p>Yes: 12</p> <p>No: 1</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • Yes, the group is bringing together several cutting-edge techniques from a group of researchers with appropriate expertise. • The proposal cuts across technical silos and engages different disciplines. All six PIs have a broad range of skills, expertise, and technologies that will de-risk this project; they have great preliminary data for each component of the project. It's the synergy of bringing them together that makes this project work. • The project is highly innovative and makes great use of 2D and 3D in vitro models as well as xenotransplant system for ex vivo studies. • Except for the application of electrophysiology to measure functional effects, almost all components of the proposal would be at the cutting edge. • Yes, the project is innovative. The proposal involves a very wide range of expertise and technologies that, to my knowledge, have never before been combined in the manner proposed. • The conceptual framework is certainly new, but it relates more to developing treatments for a known genetic defect rather than generating new hypotheses. • Gene activation via CRISPRa technology targeting individual genes has been demonstrated. The proposed work aims to develop the CRISPRa to target multiple genes for microdeletion syndromes and explore its efficacy using iPSC-based cellular models in vitro and after transplantation. Both 16p11 and 22q11 are associated with risk for neuropsychiatric disorders. • The proposal includes promising technology.
<p>GWG Votes</p> <p>Yes: 8</p> <p>No: 5</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • The rationale is theoretically sound. • The overall rationale is simple - restore gene expression due to DNA microdeletion. The preliminary data only demonstrate the efficacy of CRISPRa for a few genes individually.



	<p>No data provided show that simultaneously manipulating more than one gene will have benefit.</p> <ul style="list-style-type: none"> • There is concern that manipulating only a few of the enhancers rather than the whole group won't be sufficient. • The proposal does not address the critical issue of stoichiometry for the different genes that need to be upregulated. • The scientific rationale for using the human cell-based xenograft mouse makes very good sense. The risk of "off target" effects of gene editing requires testing in some in vivo animal assay prior to going to humans. • Patient-derived iPSCs and/or edited iPSC lines for 16p11 and 22q11 will be used. Mice will be used for transplantation studies, which provide a platform for long-term monitoring of human cells after transplantation. • The proposal emphasizes a potential therapy too much, given the many potential pitfalls of trying to 1) determine which of ~40 genes in a microdeletion need to be upregulated, and 2) upregulate those genes to therapeutically relevant levels. • The proposal would be stronger if it focused on understanding which genes need to upregulate to address different disease phenotypes. It seems like an overreach to use this strategy for a therapy at this time. • The absence of certain caveats in the discussion makes the applicant's interpretation of the preliminary data less compelling.
GWG Votes	Is the project well planned and designed?
<p>Yes: 8 No: 5</p>	<ul style="list-style-type: none"> • The overall project and subprojects are well thought out and in a logical order. The magic with this project is in the collaboration of these six investigators with complementary expertise that has a high likelihood of success if the goal was to understand the underlying circuits of the disease instead of developing a potential therapeutic. • The proposal is very nicely designed. It builds from a human genetic defect, a proposed novel intervention involving increasing function of several genes, and a functional "brain like" in vitro system for testing. The creation of mouse xenograft assays adds value. This is very elegant in terms of building a case for the therapeutic approach. • The project depends on the success of Aim 1 to generate efficient AAV constructs for gene activation with CRISPRa. There are major concerns about the proposed technology regarding specificity and efficacy. • Each aim would provide valuable resources for the community. • There is a lot of potential, but the project needs to be more focused. • The project greatly depends on the success of the CRISPR approach to increase the function of multiple genes. • It's unclear the team will be able to generate iPSC cell or organoid systems with the properties needed to evaluate the effects of the gene editing. • Some project pitfalls are touched upon, but the proposal does not adequately address the performance characteristics required for assays to inform go/no go decisions for treatment development. Issues as to variable degrees of upregulating function of several genes, the stoichiometric complexity, and lack of means to control this are not addressed. • Both 16p11 and 22q11 microdeletions are large and contain many genes. The expression of these genes in different cell types is likely temporally dynamic and cell type-specific. A one-fits-all strategy may not work. Enhancers often regulate the expression of multiple genes, and multiple enhancers can be used for the regulation of a single gene.
GWG Votes	Is the project feasible?
<p>Yes: 4 No: 9</p>	<ul style="list-style-type: none"> • Overall, yes, the project is feasible in terms of planning and executing components of the proposal. However, given the many issues that are likely to arise, the applicant may be underestimating the person power required to execute the needed studies. • Yes, it is feasible based upon their optimistic predictions of what can be achieved within the time frame.



	<ul style="list-style-type: none"> • This project is probably not feasible, given the multiple issues which one anticipates with the ambitious and optimistic goal of simultaneously increasing function of several genes. • Aims 2b and 3b are dependent on the success of Aim 1. It would be great to have a bit more preliminary data to de-risk Aims 2b and 3b. With that said, Aim 2a and 3a have high likelihood of success and generation of impactful data. • Technical feasibility of co-expression of many target genes is not entirely clear. • The proposed work will be carried out by a team of investigators with complementary expertise in the areas of molecular and cellular biology, stem cell biology and electrophysiology. However, there is a clear dependence upon the success of Aim 1. • A description of each investigator is provided, but the management plan appears to be missing. • Yes. The management plan looks sound. • A narrower focus is needed.
<p>GWG Votes</p>	<p>Does the project uphold the principles of diversity, equity and inclusion (DEI)?</p>
<p>Yes: 13 No: 0</p>	<ul style="list-style-type: none"> • Yes, the project includes proposed work to understand how to diversify patient cohorts. • The team has a history of DEI efforts. • They have a large number of iPSC lines for the 16p microdeletion (a pretty even split between genders) and are being intentional about which lines they will use in terms of the diversity of donors. • Aim 4 includes patient outreach and education. This is a nice addition that will also likely increase diversity of patient recruitment and trust among different patient groups. • The proposal upholds DEI principles to the extent that the advocacy groups for the microdeletion syndromes try to involve everyone. There is the issue that underprivileged groups are less likely to have had the kind of evaluations needed to recognize the genetic issues in question. • DEI principles are upheld with the reality of what is possible given current means of identifying relevant subjects. • Yes, given that microdeletion syndromes affect all populations. If powerful treatments became available this information would be brought to underserved populations since there would be a rationale for providing the needed genetic screening. • The extensive interactions with the advocacy groups can be trusted to include attention to DEI goals. • A detailed description of DEI effort is provided for each of the investigators. The occurrence of copy number variants, either inherited or de novo acquired, is quite high across the whole population. Development of treatment for microdeletion syndromes will benefit all patients with different backgrounds.



Application #	DISC4-16369
Title (as written by the applicant)	Exploring mechanisms of substance use disorder and its psychiatric comorbidities using diverse patient-specific iPSCs
Research Objective (as written by the applicant)	We seek to understand how small changes in a gene known to be involved in brain reward circuitry increases a person's risk of developing dependence on opioids.
Impact (as written by the applicant)	Knowledge gained will identify new targets for therapies that will ultimately help those suffering from substance use disorders (SUDs) regain control of their lives.
Statement of Benefit to California (as written by the applicant)	The opioid epidemic is exerting an intolerable societal burden in California, affecting all demographics, ethnicities and communities. It impacts State income due to workforce loss, and expenditure due to costs associated with homelessness and law enforcement. Deaths from opioid overdose exceed those from auto accidents annually. This research will help explain why some individuals are genetically predisposed to develop opioid addiction and identify new strategies for therapeutic intervention.
Funds Requested	\$10,505,476
GWG Recommendation	<i>Tier 3: sufficiently flawed, cannot be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

SCORING DATA

Final Score: 3

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	2
Lowest	3
Count	15
Votes for Tier 1	0
Votes for Tier 2	1
Votes for Tier 3	14

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG's review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 5</p> <p>No: 8</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • There is no doubt that substance use disorders (SUDs) plague our health system and society and any research into meaningful therapies is important. • The specific gene selected for study has ample evidence of involvement in addiction. • If the proposal results in optical sensor readouts for relevant neurotransmitter release, these will be very useful for the scientific community. However, the feasibility of these experiments and the investigators' experience with these assays are not well documented. • The generation of the CRISPR-corrected lines will help determine whether or not the selected gene is a main factor in opioid use disorder (OUD) or if there is a genetic background that is more responsible. • If successful, the project may shed mechanistic insight into the basis of OUD, a current knowledge gap. • The ability to look at both acute and chronic exposure is scientifically important. • The questions to be addressed are important and understudied at the molecular level. • The proposal is strong in elucidating functional implications of a specific genetic variant and opioid exposure and the underlying biological mechanisms. Broader impact of the findings is unclear. • The project is underpowered and overly focused on one gene variant. • The study lacks power due to the small number of cell lines. • This is an important area of work, but the scientific plan falls short.
<p>GWG Votes</p> <p>Yes: 8</p> <p>No: 5</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The use of the optical sensors to measure the DA and GABA release would be more compelling if there was better preliminary data showing the investigators' use of this technique. • The proposal is cross-discipline, but due to this it becomes too ambitious. Since the team has a great track record in 3D culture, maybe the 2D experiments can be reduced or done only if the 3D results are not interpretable. • The study of strong genetic risk factors for substance abuse disorders is important and there aren't many strong gene variants identified in the field. The study of this variant is interesting and solid. • The proposal builds on the DA-GABA midbrain iPSC model recently developed by the group; in general, existing approaches will be applied to create iPSC lines from patients for their genetic engineering and their phenotypic characterization. • Focus on one gene, looking broadly at many readouts but how does that translate into broader treatment? • Yes, the proposal is conceptually innovative.
<p>GWG Votes</p> <p>Yes: 4</p> <p>No: 9</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • The proposal provides considerable preliminary data describing the establishment of the human iPSC-derived DA-GABA neuron culture model, which is the foundation for the proposed projects. • Yes, the rationale is sound. • The idea of looking at strong genetic variants associated with OUD is exciting and it is rational to use iPSC models for this purpose. • There is excellent preliminary data on the 2D and 3D neuronal model systems and on quantitating DA release by HPLC. • As stated above, the proposal lacks preliminary data for optical sensors to measure DA and GABA release. • The project has strong biological mechanisms; there is insufficient attention paid to SNP-based variance. • This plan is too ambitious. The plan is not sufficiently well structured. The aims are too broad. • Some parts of the proposal are scientifically sound, but others are lacking. • Too much of the preliminary work is unpublished. • Underpowered sample size. Single SNP tested. • Too broad and lacking in preliminary data.



	<ul style="list-style-type: none"> Lack of preliminary data.
GWG Votes	Is the project well planned and designed?
Yes: 2 No: 11	<ul style="list-style-type: none"> A strength of this proposal is the depth in which it proposes to investigate one mutation. Using multiple model systems, the investigators will look at many aspects of this mutation in OUD. The weakness is that there is not enough info on who will be doing which experiments and the timeline in which they will be done. The project is too ambitious, and this detracts from some of the valuable work that they propose. There are good preliminary data regarding their iPSC and CRISPR efforts as well as the differentiation (both 2D and 3D) but the preliminary data for the optical work are lacking. There seems to be some inconsistency in the numbers of iPSC cell lines they propose generating. Much of the text discusses 10 lines from each of the 3 groups, but the cell lines proposed in milestones 3-5 do not add up to 30. Potential pitfalls are discussed; rather than providing some experimental details, it may have been better to discuss anticipated mechanistic insights, and plans for exploring other genes involved in OUD, should the culture system not prove as useful as anticipated. There are some issues with disorganization of ideas and experimental plans in the proposal. There are too many aims and it's not clear what each co-I's role is in each of the aims. The proposal design is not well organized and seems too broad. Not sure who is doing what and if the necessary expertise is there. It's not clear which investigator will be doing what.
GWG Votes	Is the project feasible?
Yes: 4 No: 9	<ul style="list-style-type: none"> The team is strong, and members bring complementary expertise to the project. Perhaps, but more preliminary data would be helpful for evaluation of feasibility. A detailed timeline is not included, so it's unclear if the proposed work is feasible in the time frame required. The proposed team has a lot of experience, but the grant is very ambitious. A weakness is that it is not clear how much experience they have with the optical system proposed in Aim 5. It is not clear which members of the team will be responsible for the various parts of the project. The justification for the requested budget would be much easier to evaluate if all the PIs budget justification format was organized in the same manner. While each aim in the research plan section of the proposal clearly states rationale and experimental design, alternative approaches and potential problems are not always well identified. In Aim 1, for instance, how many subjects need to be collected to get 10 in each of the 3 desired groups? There are also no alternatives proposed for Aims 2 and 3. Aim 4.1 discusses concern about the specificity of existing MOR antibodies and mentions that this will be tested in their mouse models, but does not provide any alternatives. Weaknesses included the potential impact of interacting factors on addiction, the hypothesis-free experimental design, and disorganization of the design. It's unclear if the team has sufficiently broad expertise. This study is underpowered.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
Yes: 9 No: 4	<ul style="list-style-type: none"> The project has a clear plan and track record in working with diverse patients. Recent clinical study resulted in 45% African American and 15% Hispanic enrollment. Recruitment plan for study participants includes covering travel and other costs. The proposal does not consider genetically determined ancestral groups. Focused on one genetic variant. Just one gene.



Application #	DISC4-16399
Title (as written by the applicant)	Schizophrenia: genetic, molecular and neurophysiological convergence at the synapse
Research Objective (as written by the applicant)	We know that schizophrenia is driven by changes at the synapse, where neurons connect, and that there is a strong genetic component underlying this. But we do not understand this well enough.
Impact (as written by the applicant)	Understanding the changes at the synapse in schizophrenia and the underlying genetic factors will present powerful new options for disease prediction and developing treatments.
Statement of Benefit to California (as written by the applicant)	Schizophrenia is a common and devastating neuropsychiatric disorder, in California alone it strongly affects an estimated 1.5M people, and indirectly, but often severely, also their families and communities. The cost from this to California is in the many billions of dollars while the human cost in pain and suffering caused is incalculable. Profoundly more effective treatments than what is available currently are needed and are what our project aims to make possible.
Funds Requested	\$13,674,600
GWG Recommendation	<i>Tier 3: sufficiently flawed, cannot be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 3

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	2
Lowest	3
Count	15
Votes for Tier 1	0
Votes for Tier 2	1
Votes for Tier 3	14

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



GWG Votes	Does the project hold the necessary significance and potential for impact?
<p>Yes: 7</p> <p>No: 6</p>	<ul style="list-style-type: none"> • The project will investigate a possible convergence point between distinct large-effect size mutations increasing the risk for schizophrenia (SCZ). Finding where the convergence occurs is a key question in explicating SCZ biology. • Understanding the biology behind SCZ is one of the largest outstanding clinically relevant biological questions. It will not only open up avenues for possible treatments but has the additional value of drastically de-risking the disease area, facilitating re-entry of large-scale pharmaceutical companies. • Main project outcomes beyond hypothesis testing are cell lines and computational tools. • Successful completion would further illuminate the genes and pathways altered downstream of known genetic risk factors for schizophrenia. Synaptic phenotypes, if present, will be identified and mechanistic insights underlying these synaptic phenotypes are likely to be acquired. • The team proposes that this research plan will address the 'bottleneck' in the field that the synaptic phenotype in schizophrenia is not well defined or understood at the mechanistic level. However, this is a very broadly defined goal that would not be sufficiently addressed with this proposal, which solely relies on iPSC-modeling of specific genetic variants using a limited number of approaches. • Six of the seven aims of this application are fairly stand-alone. While of value for understanding the molecular consequences of particular copy number variations (CNVs) and one deletion, there is little synergy across groups, even in the seventh aim which is to integrate datasets generated across aims. Overall, this reads similarly to 6 separate R01 studies. • This is a very large project aimed at understanding synaptic deficits in human stem cell models carrying two separate schizophrenia associated mutations. • The key knowledge gap that will be addressed by the many (7) aims of this proposal is not really clear to me. Instead, the application attempts to generally acquire many phenotypes from iPSC-derived neural cell culture without a strong unified question to answer. • There is a possibility that different schizophrenia associated mutations converge on similar pathways, which could be targeted together. • Aim 1 alone will generate data from ~100 iPS cell lines, in duplicate, for 3 time points, across at least 4 omic measures and optical electrophysiology. • Other datasets will include structural variant analysis, cell type prioritization via GWAS, tripartite synapse omics and electrophysiology datasets, generation of 30 iPSCs from idiopathic SCZ, and isogenic iPSC lines for the genetic deletion under study. • Sequencing data and metadata will be stored at dbGaP. Cell lines will be shared to requestors rather than in a public repository, which often makes reagents more difficult to acquire. • The proposal is for a massive amount of work in the absence of a coherent hypothesis. • Convergence was flagged as a major strength.
GWG Votes	Is the proposal innovative?
<p>Yes: 6</p> <p>No: 7</p>	<ul style="list-style-type: none"> • Establishing a large set of cell lines is imperative to the field. • The proposal brings together neuroscience, genetics and computational biology in a well-thought-out way. • While the larger conceptual framework is not entirely novel, this is an early attempt at the necessary scale. The large scale will give the investigators a chance to test the hypotheses. • These are excellent investigators, but due to overlapping expertise there is a lack of synergies on the team. • The proposal gathers together expertise in multiple domains. • Yes. New technologies proposed include study of the tripartite synapse, multi-omics, and assembloid cultures. • The proposed approaches are of value for studying gene function, but they are fairly standard for uncovering pathways downstream of genetic variants using human iPSC technology.



	<ul style="list-style-type: none"> • There is no new conceptual framework presented. The hypothesis is quite broad and widely postulated, i.e., that there is one synaptic molecular pathway commonly dysregulated in SCZ. • It's unclear what new conceptual frameworks or hypotheses are being tested. Many experiments and large-scale data acquisition is proposed, but the unified question is not clear. • This is innovative in some, but not all, parts.
GWG Votes	Is the rationale sound?
Yes: 8 No: 5	<ul style="list-style-type: none"> • Yes, the rationale is, overall, sound and well-developed. There are some issues in terms of what type of phenotype(s) the investigators expect (i.e., molecular vs. functional). • The rationale for the CNV studies (astrocytic involvement) is better developed than that for the deletion. • Is the hypothesis that synapses will have morphological, synaptic, or molecular changes? • Given the large limitations of the interpretability of iPSC systems for patients (who are adolescent/young adult) when the symptoms emerge, it is surprising the applicants have not included an aim where they investigate whether any findings of molecular synaptic phenotype replicate in patients. • Plenty of preliminary data show that the project is feasible. The lack of a molecular synaptic phenotype in <i>[name gene]</i> cultures makes the rationale a bit weaker. • The rationale for studying these CNVs is solid, but it is unclear how the derivation method of iPSC lines will be controlled for, and if the sample numbers represent unique patients or multiple clones from the same person. Controls lines are not defined. • While many subprojects are based on sound scientific rationale and studying specific mutations that are strongly associated with schizophrenia risk is well motivated, it is not clear how all these projects will combine together to give a better understanding of schizophrenia pathobiology. • Preliminary data establishing feasibility for each method are given and convincing. Preliminary data addressing the scale of what is proposed here are not given, and the project as proposed does not seem feasible with the time, budget, personnel, or expertise of the team. • Yes, the project is relevant to two mutations associated with schizophrenia. • Non-human models are only proposed in Aim 6.2, 6.4 to validate the findings in human induced neuron cultures. • There are too many disconnected aims. • The proposal lacks power calculations.
GWG Votes	Is the project well planned and designed?
Yes: 2 No: 11	<ul style="list-style-type: none"> • This is a well-integrated effort - the different parts fit well together, except Aim 3. • There seems to be a lack of synergy of each subcomponent contributing to the overall goals. • There is concern that the control cell lines are not well described, and it is not clear how they will be used. • The aims are too diverse and do not fit together well. • Seems like 6 R01 written independently, and then brought together in Aim 7. • Aim 1 is over ambitious for the funding provided – it is just too much work. • The analytic pipeline for extracting meaningful insights from the data in Aim 1 is missing. • Aim 2: There is no discussion of power to detect significant modifiers using their approach. • Aim 2: Even with 1000 individuals, it will likely be quite difficult to find statistically significant rare variant loci that modulate rare variant penetrance. Similarly for fine mapping experiments proposed, what criteria will be used to demonstrate that an SV versus a SNP is the causal variant? There are not enough details given to be confident that this Aim will identify causal SVs or just SVs existing in these genomes. • Aim 3: In the absence of additional protein-level and experimental validation, these analyses will have minimal yield.



- Aim 3 will use existing transcriptomic atlases to map GWAS loci to cell type, by making assumptions that variants near genes expressed in specific cell types affect those cell types and using these genes to prioritize existing drugs for treatment of schizophrenia by matching transcriptomic profiles of cell types. This type of analysis has been previously done (<https://pubmed.ncbi.nlm.nih.gov/29785013/>) and the novelty here is just using higher cell type classifications. It is unclear why the applicants expect ancestry specific effects on brain cell types.
- Aim 3: It is unclear how the information will be used in the continued effort since most experiments in other aims will not be done in "real" cell types but rather artificial neurons.
- Aim 3 is not well-described and has many potential pitfalls.
- Aim 4 will yield valuable data, but it is not well integrated with the rest of the aims. Further, Aim 4 proposes to rescue phenotypes to interrogate mechanisms which have not yet been shown in the model to be used.
- Aim 4 will switch over to studying *[name gene]*-heterozygous deletions effects on synapses in a tripartite culture system, as well as bulk, single cell, and RNA-seq and proteomics from subcellular fractions. An interesting approach is to evaluate the effects of *[name gene]*-heterozygous deletion on synapses of different excitatory to inhibitory combinations.
- Then *[named deletion]* will be assessed on synaptic transporter currents in human astrocytes and combinations of deletions in neurons or astrocytes will be conducted to determine if there is a synergistic effect. Finally, phenotypes will attempt to be rescued using overexpression constructs. This work seems complementary to what was previously done in mouse and well-motivated.
- Aim 5 will likely yield interesting results, but the protocol for triple culture is unvalidated (an unpublished protocol is cited) and there are no data presented that relays reproducibility across lines or within line.
- Aim 5: Creating isogenic lines for the full 1.5 Mb deletion region in 2 lines has questionable feasibility.
- Rescue attempts will be made using overexpression of individual genes within the deletion region. This work also seems well motivated, but the generation of 1.5 Mb deletion isogenic lines undermines the feasibility of this proposal despite preliminary data given for generation of iPSC line for a much smaller region.
- Aim 6 will interrogate homeostatic synaptic plasticity in excitatory and inhibitory neurons using both an RA-treatment protocol and a chronic synaptic blockade protocol. This is a reasonable approach with feasibility established, but the second half of the aim is to interrogate the mechanisms of defects that have not yet been established and which may not exist in this system.
- Aim 7 may be the strongest aim, which may have the highest impact if successful. Aim 7 interrogates potential shared synaptic mechanisms in the two genetic models. The main existing preliminary data supporting the concept is that the chosen deletion affects *[named gene]* levels in one set of lines. A CRISPR screen also may be fruitful, but there is little description of the methods to be used, and no consideration relayed of potential pitfalls for any aspects of the approach.
- In general, there are very few experimental details for each aim describing analysis approaches or design making the number of aims difficult to evaluate, because there are so many aims.
- Non-specific overall power analysis and statistical approaches are proposed. It is unclear if group sizes can be increased to achieve 80% power when the expected effect size is not given, and the iPSC group sizes have already been established.
- The scale of this, the budget given, the personnel assigned, do not seem feasible. What are the expected outcomes other than a list of DEGs?
- There is no evidence presented that the team can do all these things in time.
- The project lacks clarity on how to do all the work and then analyze it.
- The proposal lacks discussion of pitfalls.



	<ul style="list-style-type: none"> The proposal is difficult to comprehend and poorly structured.
GWG Votes	Is the project feasible?
Yes: 2 No: 11	<ul style="list-style-type: none"> The team is divided into two groups: genetics and neurobiology. The team is world-renowned with expertise in all the proposed methods. Many of the team members have successfully collaborated in the past. The proposed team investigators have expertise in all of the many methods proposed. The staff does not seem sufficient to complete the amount of work proposed. 1.5 postdocs are proposed for the differentiation of 100 iPSC lines in 4 years, which does not seem feasible. The team has plans for meeting consistently to manage the large collaboration. All the necessary components are in place. Plans to manage the collaboration are well worked-out. Some feasibility aspects were not well described; pitfalls and alternatives could be better described and considered. Synergy between aims could have been more clearly described. There are issues of feasibility for each aim which are unrecognized in the proposal, with no discussion of potential pitfalls and alternative approaches. The project is too ambitious for the purpose of addressing the stated goal/scope/aims. The budget does not seem sufficient to tackle the scale of the samples proposed here, including the iPSC differentiation, multiome, additional scATAC-seq, etc for the many samples in duplicate, even with the matching funds. The applicant does not provide data on reproducibility of their methods. No pitfalls are addressed where they would be many. There is too much to accomplish. The project is too broad and difficult to achieve with the staff listed. This is a consortium with strong applicants with the suitable background. Aim 1 is too ambitious for the proposed staffing. This is feasible except Aim 1. There is too much proposed and there are some necessary preliminary data missing. Feasibility was flagged as a major weakness.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
Yes: 10 No: 3	<ul style="list-style-type: none"> The ancestry/sex of the large number of iPSC lines in Aim 1 was not given. It is not clear how well these distributions match the population. The applicants use existing genetic association studies from non-European samples to increase applicability to all populations, as well as using a small number of iPSC lines and a small number of post-mortem human tissues from non-European individuals. The applicants are participants in several existing DEI programs that aim to address the health needs of a diverse patient population. The project proposes to utilize iPSC models from non-European donors. Details relating to this point are sparse. The description relating to prior DEI efforts is weak and limited to broad institution-wide wording. Limited details are provided.



Application #	DISC4-16437
Title (as written by the applicant)	A functional genomics approach to dissect the regulatory mechanisms of genetic variants associated with bipolar disorder
Research Objective (as written by the applicant)	The genetic variants associated with many hereditary neuropsychiatric disorders are difficult to study because they are often non-coding and likely affect gene regulation during brain development.
Impact (as written by the applicant)	This study will yield a novel way of studying how non-coding genetic variant affect genes during organismal development and identify causal variants involved in bipolar disorder and treatment failure.
Statement of Benefit to California (as written by the applicant)	Neuropsychiatric diseases carry immense societal and monetary costs. These can be reduced by improved treatments and reduced treatment failure. Further, better prediction of the propensity to develop neuropsychiatric disorders may allow early intervention to reduce the likelihood of manifestation of the disorders. The goal of the proposed study is to generate knowledge that will contribute to said improvements and predictions and thereby benefit the citizens of the State of California.
Funds Requested	\$12,628,200
GWG Recommendation	<i>Tier 3: sufficiently flawed, cannot be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 3

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	3
Lowest	3
Count	15
Votes for Tier 1	0
Votes for Tier 2	0
Votes for Tier 3	15

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 6</p> <p>No: 8</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • The project is ambitious, seeking to elucidate the gene regulatory networks that distinguish healthy and bipolar disorder (BD) patient-derived neurons, and to dissect the effects of BD risk variants on neuronal differentiation and lithium response. • A major strength of the application is the development and application of a dual massively parallel reporter assay (MPRA) to investigate bipolar disorder (BD) GWAS-implicated potential cis regulatory elements (CREs). • All of the cell lines to be developed are from European American donors. This lack of diversity is an issue. Could some cell lines be derived from people of other ancestral groups? • It is well accepted that there is a genetic component to BD based on family studies, GWAS, and more. Loci have been identified as conferring a small risk for BD, but many are located in non-coding regions, making interpretation extremely challenging. The Dual-TSS-MPRA strategy in Aim 3 of this application to test a plasmid pool could provide valuable new insights about the functional impact of these loci. • The Dual-TSS-MPRA strategy in Aim 3 of this application to test a plasmid pool including CREs with BD GWAS SNV could provide valuable new insights about the functional impact of these loci. If successful, the Dual-TSS-MPRA strategy would apply to other neuropsychiatric disorders for which SNVs in non-coding regions have also been found. • Unlikely to change existing solutions to diagnosis and treatment of bipolar illness. • Is study powered to see differences/responses?
<p>GWG Votes</p> <p>Yes: 6</p> <p>No: 8</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The technologies proposed and questions to be addressed are innovative. • The dual MPRA assay method is highly innovative. • The most innovative part of this proposal is the Dual-TSS-MPRA strategy to determine the effects of BD-associated SNVs on CRE function at base resolution. Performing this assay in neurons derived from healthy control iPSC lines could provide valuable information about sequence variations in enhancer regions. That said, it is difficult to understand the benefit of doing this experiment in BD-LiR and BD-NR lines, as proposed in Aim 4C. • This proposed project is highly multidisciplinary, combining the preparation of new iPSC lines, multi-omics of patient-derived stem cell organoid models, the development of a new approach to study variants associated with BD (Dual-TSS-MPRA), integration of those data to identify top variants and prioritize them for further investigation, and a lot more. With so much proposed, it is impossible not to cut across technical silos. • This is a multifaceted proposal that combines stem cell modeling (brain organoids and 2D monolayer differentiation), high-throughput sequencing methods (RNA-seq and ATAC-seq), a novel assay (Dual-TSS-MPRA) to elucidate the impact of BD-associated SNV in non-coding regions, and a variety of bioinformatic analyses. • The main goal mentioned at the beginning of the Research Plan is to establish a framework to study the molecular mechanisms underlying heritability of BD and lithium responses. Overall, this is a discovery project that does not explicitly test a new mechanism for the pathogenesis or pathophysiology of BD. • The main innovation is combining case/control design and MPRA approaches to resolve Li response.
<p>GWG Votes</p> <p>Yes: 8</p> <p>No: 6</p>	<p>Is the rationale sound?</p> <ul style="list-style-type: none"> • Overall, the rationale is sound. • The rationale for the dual MPRA assay experiments is sound. Do these experiments need to be performed in BD patient-derived cell lines? • The rationale for developing ~20 cell lines from European American subjects was not clear. • Major concern: Is the study powered to resolve inter-donor differences in genetic regulation between NR and R? • No, based on concerns related to composition and maturity of organoids, the complexity of neurologic diseases, and lack of connection from organoid to disease.



	<ul style="list-style-type: none"> • Aim 2: The reasoning presented in the Preliminary Data-Human Organoids plan is confusing. The applicant states that brain development continues well beyond the prenatal stage and that conditions like BD have onset during early teen years, yet brain organoids to be tested in this project will be matured for up to 6 months, which is more representative of the prenatal stage. • A more robust rationale is needed to justify the value of completing the extensive single nuclei multiomic (Aim 2, Milestone 2B) with brain organoid models that will be matured for the suggested time points. Also, including iPSCs in this analysis seems unnecessary to me. The critical comparisons are between the organoids' time points. • Aim 2, Milestone 2D: The rationale for comparing organoid multiomics results to PsychENCODE data is weak. The average donor age of the BD dIPFC samples analyzed in the PsychENCODE appears to be more than 50 years, while the organoids data set would correspond more to a prenatal stage of development. • Aim 4, Milestone 4C: What is the relevance of testing the library of BD-associated SNVs on CRE function with Dual-TSS-MPRA in BD-LiR and BD-NR lines? Furthermore, adding lithium as a condition in this extra should be better justified. The applicant should propose a mechanism by which lithium would be acting on the BD-associated SNVs.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 4 No: 10</p>	<ul style="list-style-type: none"> • The project aims to use a wide variety of techniques to understand the role of gene expression changes in bipolar disorder. The project includes use of data from GWAS studies to attempt to functionally implicate those in disease risk/progression. • The focus on non-coding SNPs is important. • Aim 1: No issue. The reprogramming of ~20 new iPSC lines, ~10 of which are from BD patients, would benefit research on the molecular and cellular underpinnings of this neuropsychiatric disorder. • Aim 2: Preparing brain organoids using the novel iPSC lines and completing the proposed multiomic analysis is well-designed, but interpreting those results may have critical limits. It is most important to remember that brain organoids represent, for the most part, a model of prenatal development. How molecular changes between control and BD specimens can be interpreted for a condition where the onset typically occurs in the early teen years is not straightforward. This is even more challenging for changes that may be identified between the BD-LiR and BD-NR conditions. Finally, see comment above about comparing datasets from this multiomic analysis to the PsychENCODE dataset. • Aim 3: This is this proposal's most original and exciting part. Overall, it is well-planned. • Aim 4: Again, this is a multiomic screen (scRNA-seq and ATAC-seq) to be performed with iPSC differentiated in a monolayer as glutamatergic and inhibitor neurons. This could provide interesting results. Notably, the characterization of the differentiation protocol will include comparison to previously published results (i.e., the circadian rhythm analyses and MEA experiment). As part of this aim, the applicant proposes to perform the Dual-TSS-MPRA with iPSC from BD-LiR and BD-NR individuals differentiated as neurons in a monolayer fashion (critique made in other points). The rationale for this is not clear. Finally, key enhancer regions in neuronal cells are well-known to be regulated by activity (see doi: 10.1038/nature09033). This has not been considered in the proposal. • There are aspects that are well designed and planned but other aspects that require modification. • No, based on concerns about variance and not properly addressing pitfalls and alternatives. • The pitfall discussion was too simplistic. • The proposal includes evidence of feasibility that the team can generate organoids, but no evidence of case/control differences of Li response. Specifically, no consideration of inter- or intra-donor variability. Unclear the extent to which inter- and intra-organoid variability (between donors, differentiations, and within organoids) will impact feasibility of organoid molecular and physiological analyses. To what extent will the cell type composition and maturity in each organoid impact activity and drug response?



	<ul style="list-style-type: none"> • There is a lack of clarity on the uniqueness of PGBD cases, particularly given clinical characteristics beyond lithium responsiveness. Moreover, as all are of European ancestry, the study doesn't expand diversity of BD hiPSC collections. • The pitfalls identified are relatively simple (e.g. fibroblasts fail to reprogram, additional organoids required for sequencing) whereas potentially larger pitfalls (large batch effects between hiPSC cohort 1 and 2; Li+ impacts cellular signaling with minimal changes on gene expression; power to resolve inter-donor effects by MPRA) are not considered. • Batch effects are not addressed.
GWG Votes	Is the project feasible?
<p>Yes: 4</p> <p>No: 10</p>	<ul style="list-style-type: none"> • The project is feasible in terms of developing an understanding of the impact of cis regulatory elements in BD risk. • This qualified team of investigators includes staff and trainees to carry out all the proposed experiments. The application describes the investigators' team well, their role is identified, and there is no concern that the collaboration could be productive. • The team is qualified and appropriately staffed. • No, based on (i) concerns regarding the power of the study to see differences in responses; (ii) insufficient preliminary data; (iii) pitfalls could have been more seriously considered (e.g., batch effects). • There are no data to support feasibility regarding power to detect significant differences - this was a score driving concern for this reviewer. • Reviewers need more preliminary data to evaluate feasibility. • There were not a lot of preliminary data. • Pitfalls are not well thought out. • This is a massive body of work. It's unclear if it can be completed within the time frame and with the proposed staffing.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
<p>Yes: 4</p> <p>No: 10</p>	<ul style="list-style-type: none"> • The lines are all from individuals of European ancestry. The reasons for this are understandable, but separate aims could be incorporated that address ancestral heterogeneity and move the field forward with regards to studying a broader set of ancestral backgrounds. • As stated above there is a major shortfall in the DEI aspect of the project. The case for generating cell lines exclusively from people of European American ancestry was not well made. Could the investigators have focused on individuals from another ancestral group? • The proposed new iPSC lines will account for sex, and all the proposed lines will be from European-American ancestry. The notion of applicability could have been explored to a much greater extent. • The proposal does not include any attempt to incorporate diversity into their cell line selection. • A major concern. There are no attempts to use diverse cells lines; the DEI plan is insufficient. • There is a lack of diversity of cell lines used. • There is no diversity in the hiPSC cohort. The proposal has a limited description of outreach, partnership, and education. • The project is focused on European/American lines.



Application #	DISC4-16461
Title (as written by the applicant)	Discovery of Neuroimmune Mechanisms in Schizophrenia (SCZ) From Genes, to Proteins, to Circuits to in vivo Neuroimaging
Research Objective (as written by the applicant)	Converging evidence points to the synapse as the primary site of pathology in SCZ. We assembled a team of experts to discover abnormalities in synaptic physiology in SCZ using induced neuronal cells.
Impact (as written by the applicant)	If successful, the project will identify the defects in brain synapses in SCZ
Statement of Benefit to California (as written by the applicant)	By understanding the pathophysiology of schizophrenia at a molecular level, more precise and more effective medications might be developed some day. Future therapies might potentially mitigate the disease at its earliest stages, and thus prevent the manifestations of schizophrenia.
Funds Requested	\$12,307,472
GWG Recommendation	Tier 3: sufficiently flawed, cannot be resubmitted
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 3

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	3
Lowest	3
Count	15
Votes for Tier 1	0
Votes for Tier 2	0
Votes for Tier 3	15

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 4	<ul style="list-style-type: none"> • The project will take a deep dive into the possible biological mechanisms by which [named gene] reduces synaptic density in schizophrenia (SCZ).
No: 9	<ul style="list-style-type: none"> • At a fundamental level, the results of the project will likely provide important insight into the interaction of immune signaling and synaptic network function.



	<ul style="list-style-type: none"> • However, it is less clear that the project will provide deep insights on how the processes these mechanisms contribute to the etiological or pathophysiological progression of the disease. • It's possible that the results will lead to the development of the interim biomarker for target engagement, but noting ReMIND-L's remit, it's more difficult to imagine how it will provide a cohesive framework for SCZ. • Yes, the project holds great significance to the SCZ field as <i>[named gene]</i> is one of the major risk factors for SCZ and the intraneuronal effects of <i>[named gene]</i> are currently unknown. • Yes – the feasibility of complement mechanisms as represented by cell lines and synaptic-focused PET. • The project might inspire more paired imaging-cell line experiments, and those might have beneficial effects. • The outputs are limited in sample size; they are (best case) more likely to be inspirational than immediately useful. • This proposal is unlikely to offer significant insights. • Significance and potential impact are challenging for reviewers to assess given the disorganized application. • The planned in vitro studies have problematic risk of confounding. • The project will product mass spec data and data from IHC microscopy, array tomography, miniflux microscopy, and electron microscopy. Data and code will be uploaded to institutional and lab repositories.
GWG Votes	Is the proposal innovative?
<p>Yes: 5 No: 8</p>	<ul style="list-style-type: none"> • The strength of this proposal is to use of imaging to examine phenotypes in human brains and in in vitro systems. However, the proposal could use further in-depth mechanistic studies into how <i>[named gene]</i> affects synaptic densities and better correlation between the clinical and in vitro work. • Looking for coherence between lines and neuroimages from the same individuals is fairly novel, even if the sample size is unlikely to support clear conclusions. • While the individual technologies are not novel, the conceptual and technical combination of approaches is solid. • The framework and hypothesis are only modestly innovative. It is primarily a continued deep dive based on the prior landmark paper from two applicant team members' labs. • The proposal has an excellent team of diverse expertise, particularly in neuroimmunology and proteomics. • There are aspects of innovation in the technical approaches. • The array tomography for postmortem SCZ samples appears to be new. • The applicants put their biosketches in the page designated for describing innovation. • There is no innovation section - the applicants included biosketches only. • The project will get into biochemical aspects but the connection to schizophrenia is lacking. Innovation is not high.
GWG Votes	Is the rationale sound?
<p>Yes: 4 No: 9</p>	<ul style="list-style-type: none"> • The rationale is sound as <i>[named gene]</i> is a major known risk factor for SCZ. • Yes, <i>[named gene]</i> has been strongly implicated in SCZ. However, it's not clear that the results of this will provide incisive knowledge on the pathophysiology of SCZ per se. • If you had to pick a single mechanism for SCZ, severe synaptic pruning is reasonable. • Based on other postmortem SCZ studies, as well as paired imaging-omics data the sample sizes here are optimistic and the power analysis is not sufficiently described, and there's no in-hand preliminary data on these populations that makes me believe the very optimistic sample sizes (with regard to every genomic, omic, and neuroimaging study published on related topics) are substantiated. • The evidence on synaptic density deficits in early-course SCZ seems solid. Based on their correlation figures, some intriguing results are also available, but these rely on small sample sizes. • The overall project has a reasonable scientific rationale.



	<ul style="list-style-type: none"> • Yes. However, the proposal misses major opportunities for considering what other mechanisms and proteins might be at work with this novel combination of imaged individuals. • There is concern that the sole use of the 2D iN's will not be robust enough to support good conclusions. • The presentation is disjointed. • GRN algorithms need more prelim data. • Mouse studies will be used to validate the functional effects of <i>[named gene]</i> manipulation.
GWG Votes	Is the project well planned and designed?
Yes: 1 No: 12	<ul style="list-style-type: none"> • Some portions of the project are well planned and designed, such as the application of AT and PET imaging. However, what is critically missing is how all of the components will synergize to increase the impact of the findings. Additionally, the iNs are a monoculture and the imaging is done on samples in a multicellular environment, thus interpretation of the results may fall short. • Additionally, there's no follow-up or validation of the detected GRNs nor any preliminary data on the ability to detect shared GRNs between all of the samples given that potential batch effects/variance which could lead to false positives. • The experiments themselves are all quite reasonable, but the overall flow and logic of the aims is a bit disjointed. A lot of the data will be collected, but it's not clear how it will all come together into a cohesive hypothesis on how <i>[named gene]</i> is involved in schizophrenia. • The components will provide additive evidence for each other, but it's not clear that the compilation of experiments will yield incisive or synergistic values. • The application lacks integration, clarity and coherence. That begin said, there is the nucleus of a good idea that's will require more effort and preliminary data to support. • The proposal does not include sufficient collaboration between the investigators on the project team. The proposal is not well organized. • There should be a lot of synergy, but the applicant team did not write an integrated and coherent proposal, so the synergy remains theoretical. • The writing and structure are scattered. • Strong preliminary data are lacking for some proposed approaches. • The aims are non-cohesive.
GWG Votes	Is the project feasible?
Yes: 1 No: 12	<ul style="list-style-type: none"> • The project is feasible as all of the investigators are experienced in the fields proposed. Also, the environment at the applicant institution is ideal for the successful execution of this project. • Overall, yes, but the reference to computers "with the latest Nvidia GPU" doesn't make sense (the GPU would have to auto-update). • This is a strong team well-suited to each other's areas of expertise. • A project management plan is proposed with frequent touchpoints and feedback opportunities. • The team has access to all the necessary resources. • The budget is reasonable. • No - the team has not been able to formulate a coherent plan, so it's unclear that they will form a strong team for the project. • If they can't develop an integrated plan there's not hope for carrying out a project. • There are concerns about the preliminary data and the network algorithms. • The organization of the proposal made it difficult to judge aspects of feasibility. • This is a disorganized proposal. • The team has no history of collaboration.
GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
Yes: 3	<ul style="list-style-type: none"> • While there is a standard DEI statement provided about DEI efforts at the institution, there is little explanation for DEI initiatives within the proposed project. There's a missed



<p>No: 10</p>	<p>opportunity to expand on diversity and inclusion opportunities, particularly in patient recruitment.</p> <ul style="list-style-type: none">● There is no professional or scientific interest expressed in supporting diverse populations anywhere in the application. Including the university-level statement is not even close to sufficient.● The proposal does not include a plan for including or increasing diversity.● This is lacking; it's a generic statement.● Only a broad DEI statement is attached.● The proposal does not support diversity in sample collection.● The project does not take demographic factors into careful consideration.● The DEI section did not include any explicit DEI outreach and educational activities in the track records of the project team.
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Application #	DISC4-16273
Title (as written by the applicant)	Multidimensional investigation of neuropsychiatric disability: Aggressive behavior challenges (The 'MIND-ABC' Study)
Research Objective (as written by the applicant)	Unravel the mechanisms underlying aggressive/injurious behavior, a common neuropsychiatric symptom in neuropsychiatric, genetic, and developmental disorders, using MPS III as a model.
Impact (as written by the applicant)	Unraveling mechanisms offers insights applicable to broader neuropsychiatric disorders where aggression is symptomatic. Dysregulation of dopamine signaling emerges as a promising therapeutic target.
Statement of Benefit to California (as written by the applicant)	This project fills a gap for the scientific community studying neuropsychiatric, neurological, and developmental disorders where aggressive/injurious behavior manifests as a symptom. By using MPS III as a model, this study may deepen understanding of similar behaviors in other brain disorders, guiding targeted treatments. The project directly benefits MPS III patients and alleviates burden on caregivers managing this frightening and dangerous symptom.
Funds Requested	\$11,610,067
GWG Recommendation	<i>Tier 3: sufficiently flawed, cannot be resubmitted for 6 months</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 3

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	3
Lowest	3
Count	14
Votes for Tier 1	0
Votes for Tier 2	0
Votes for Tier 3	14

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.



<p>GWG Votes</p> <p>Yes: 2</p> <p>No: 10</p>	<p>Does the project hold the necessary significance and potential for impact?</p> <ul style="list-style-type: none"> • The project is designed to produce several outcomes that may significantly benefit the broader research community by enabling the formulation and testing of novel hypotheses in the field of neuropsychiatric disorders, particularly those related to aggressive/injurious (A/I) behaviors. • Creation of open-access databases containing comprehensive clinical, genetic, proteomic, and neuroimaging data will be a major asset. The integration of neuroimaging with proteomic data provides a unique dataset that can be used to link molecular changes with structural and functional changes in the brain. • Successful completion of the project may provide insights into the mechanisms underlying aggressive/injurious (A/I) behaviour in Mucopolysaccharidosis (MPS) III. • In this project, the applicants propose to explore mechanisms underlying aggressive/injurious (A/I) behavior, a common symptom in many neuropsychiatric disorders. This is a significant problem - for affected individuals, their carers/families, healthcare professionals and entire society - but underlying mechanisms are still not known. • Successful completion of this project may extend to a wider array of neuropsychiatric disorders. However, the applicants propose using a neurodegenerative disorder as an working example - and aggression in neurodegenerative disorders may not be the same as in neuropsychiatric ones (e.g., reactive vs proactive). • The project aims to unravel the complex neurobiological and genetic underpinnings of A/I behaviors observed in neuropsychiatric disorders. By using MPS III as a model, the research could lead to a better understanding of how genetic mutations affect neural circuits and lead to behavioral outcomes. This could significantly enhance our understanding of similar mechanisms in more prevalent neuropsychiatric conditions. However, my concern here is usage of a neurodegenerative disorder. For example, aggression associated with Parkinson's or Alzheimer's is not the same (neither behaviorally nor mechanistically) as aggression in Autism Spectrum Disorder (which was shown also to have rewarding properties for some individuals). • Statements on how the data will be applied to other disorders are overstated. • Aggressive/injurious behaviour is a serious symptom of several developmental and neuropsychiatric disorders and a better understanding of its causes may help identify individuals at risk and develop treatments. If successful, this study would most likely provide insights into MPS III and potentially some other developmental disorders. I am not convinced that findings would generalise to other neuropsychiatric conditions.
<p>GWG Votes</p> <p>Yes: 2</p> <p>No: 10</p>	<p>Is the proposal innovative?</p> <ul style="list-style-type: none"> • The proposal encompasses a number of different technologies. However, I am concerned that the rationale is not always clear, and the choice of approaches is not sufficiently matched to the questions and data at hand. • It is very hard to judge given the claims and broadness of the application. • No. This proposal is not, contrary to applicant's claims, using any novel methodologies - nor combining the "old" ones in a novel way. • The proposal combines clinical evaluations with genetic analyses, leveraging the expertise of pediatric endocrinologists and geneticists. This approach ensures that insights into A/I behaviors are grounded in both observable clinical outcomes and underlying genetic factors. The involvement of stem cell biologists to develop and analyze iPSC-derived organoids introduces a layer of cellular and molecular understanding. • By linking clinical phenotypes directly with genetic, proteomic, and neuroimaging data, the project hopes to establish direct correlations between molecular changes and their clinical manifestations. • The applicants propose to explore A/I behavior in MPS III patients - but in a rather "classical" way and by using well established methodologies. • The proposal will use largely established protocols to explore the molecular phenotypes associated with A/I phenotypes in MPSIII patients.
<p>GWG Votes</p>	<p>Is the rationale sound?</p>



<p>Yes: 1 No: 11</p>	<ul style="list-style-type: none"> • The overall project and its subprojects are designed with a robust scientific rationale, focusing on understanding the mechanisms of aggressive/injurious (A/I) behaviors in neuropsychiatric disorders, particularly MPS III. However, a caveat is the MPS is neurodegenerative disorder. Also, it seems as if some sub aims/projects are there just "to be there" - some appear not to be necessary. • The dependence on behavioral assessments, which are often subjective, might introduce variability that could weaken the association between clinical phenotypes and neuroimaging findings. While the use of dMRI is justified, it primarily focuses on structural connectivity. Incorporating functional MRI could enhance understanding of dynamic brain functions related to behaviors, providing a more comprehensive picture. • The success heavily relies on the ability to effectively integrate highly heterogeneous data, which requires sophisticated bioinformatics tools and expertise that are not sufficiently detailed in the proposal. • There seems to be a lack of detailed methodology on how data integration will be handled to address the complexity and scale of the data. This could be a significant hurdle in realizing the full potential of the integrated analysis. • Furthermore, this project relies on 20 individuals with a rare MPS III disorder. As explained, some individuals with MPS III show aggression and some do not - which may be influenced by other factors not related to genetics and that is not explored in this project. Also, literature on animal models of neurodevelopmental disorders show involvement of different circuits/mechanisms dependent on genetic mutation - so generalizability of findings here is questionable. • Rationale for doing MRS is unclear. The applicants are not clear where exactly (and why) the voxels will be positioned nor they provide any explanation for the metabolites they propose to measure. • There is no rationale as to why particular metabolites will be examined. • The basic rationale for some of the analyses was not entirely clear in many cases. • I have some concerns about the scientific rationale. This program is based on a small number of individuals with a rare disease. The cause of their developmental disorder is monogenic (and known). However, that does not imply that the outcome of interest, A/I behaviour, has a similar (genetic) architecture. More likely, it is a complex multifactorial outcome. At that point 20 individuals with the outcome and 20 without are too small a sample size to gain significant insights using omics etc.
<p>GWG Votes</p>	<p>Is the project well planned and designed?</p>
<p>Yes: 0 No: 12</p>	<ul style="list-style-type: none"> • The project is structured around a series of interconnected aims designed to elucidate the mechanisms underlying aggressive/injurious (A/I) behavior in neuropsychiatric disorders, using MPS III as a model. • The integration of diverse biological assessments is a strength, ensuring a multi-dimensional understanding of A/I behavior. However, the reliance on neuroimaging and the complexity of the omics technologies might pose challenges in terms of data integration and interpretation. The success of this aim heavily depends on the robustness of the statistical models and the ability to handle vast datasets, which might require more explicit detailing of data management strategies. • Comparing organoids from different diseases introduces variables that must be carefully controlled and interpreted. • While the aim to integrate clinical, organoid, and mouse model study results is crucial for holistic understanding, the success of such integration relies heavily on advanced data analytics capabilities and the compatibility of data sets across different experimental designs. The proposal could be strengthened by specifying the methodologies for data integration and analysis, ensuring that they are capable of handling the expected heterogeneity and complexity of the data. • The project proposal outlines some potential pitfalls and challenges associated with investigating aggressive/injurious (A/I) behaviors in neuropsychiatric disorders using MPS III as a model, but it doesn't fully detail specific contingency plans or alternative approaches for each identified issue. • The basic rationale for some of the analyses was not entirely clear in many cases - for example, Subaim 2 of Aim 1 notes that certain enzymes are known to play roles in



	<p>breaking down aggression-associated neurotransmitters, and then proteomics (on PBMCs), transcriptomics (on buccal swabs), ATAC-seq, Hi-C, EM-seq and whole genome sequencing will be done – what specifically will be learned from, for example, Hi-C, on a very small number of samples of patients and controls?</p> <ul style="list-style-type: none"> • It was surprising that the known genetics of MPS III was not clearly described up front and that some hypotheses about how mutations in the genes known to be mutated in different subtypes might be causing variable A/I symptoms. • One gets the sense that assays are being proposed because they can be done (in core facilities for example), but there is little consideration of the limitations associated with different types of data – a lot of space is dedicated to outlining certain details. For example, a paragraph on mouse and human genome assemblies is included, with no obvious connection to the proposed WGS of the recruited patients. • The data integration plan is not adequate. There is a field dedicated to developing machine learning methods for data integration to improve our ability to detect biological information – this may not be needed here, but it is important to acknowledge what can be done within the existing team. • There is a large number of novel technologies that may not all be justified. Some pitfalls, e.g., sample size, are not properly addressed. • Collaborative work across these different domains could be innovative and the disease may provide a good case study. • The application is too broad. • Some claims are overstated.
<p>GWG Votes</p>	<p>Is the project feasible?</p>
<p>Yes: 2 No: 10</p>	<ul style="list-style-type: none"> • The proposed team for this project is highly qualified and appropriately staffed to achieve the proposed outcomes. The team composition includes experts from diverse fields such as stem cell biology, pediatric endocrinology, neuroscience, biostatistics, and epidemiology, which are essential to the multidisciplinary nature of the research. • The applicant's stated plan to pinpoint treatment and intervention opportunities for all disorders with A/I behavior is overstated. • Many of the experiments rely on core facilities; resources seem to be in place. • The team includes some renowned scientists and significant breadth to address the questions. • Yes, the infrastructure and resources are appropriate. • Being at the top end of the funding limit for the ReMIND-L program, the proposal should promise more than exploratory work. At that cost, a realistic chance to offer more definitive insights about mechanisms, ideally with relevance to treatment, can be expected. • There is a clear plan for the planned collaborations.
<p>GWG Votes</p>	<p>Does the project uphold the principles of diversity, equity and inclusion (DEI)?</p>
<p>Yes: 7 No: 5</p>	<ul style="list-style-type: none"> • The project plan and design demonstrate an awareness and intent to account for diversity across race, ethnicity, sex, gender, and age. However, MPS is a rare disorder, so this is not easy to achieve. • While the direct applicability of the project's outcomes to diverse ancestries and underserved populations might be limited due to the specific nature of MPS III, the fundamental insights gained could indirectly benefit these groups by advancing the overall understanding of aggressive behaviors in neuropsychiatric disorders. • That is not the focus of this project. Given the small number of participants it is not clear whether the findings will generalize beyond this specific disease, so generalizability to diverse groups is a question mark. • The proposal is focused on a rare disease, so it is difficult to consider broader issues of race, sex, gender, etc. • There are no concerns around DEI. However, this project does not have a clear focus on this.



Application #	DISC4-16468
Title (as written by the applicant)	Epigenetic & infectious pathways affecting autism spectrum disorder & developmental disability in children exposed to maternal COVID-19 in pregnancy.
Research Objective (as written by the applicant)	This proposal aims to characterize the mechanisms responsible for a higher rate of severe developmental delay and autism spectrum disorder (ASD) in children born to mothers with COVID-19 in pregnancy.
Impact (as written by the applicant)	Our studies will help develop biomarkers and identify therapeutic targets to prevent, treat and potentially cure children with neurodevelopmental delays and/or ASD
Statement of Benefit to California (as written by the applicant)	We believe that our proposal will be transferrable to the population of California, in aiding early detection/predisposition for Autism Spectrum Disorders (ASD). COVID-19 exposure in pregnancy will continue to be an ongoing scenario and through our proposal, we hope to identify potential biomarkers, their mechanisms, and pathways, which can be translated into possible therapeutic treatments for ASD and developmental delays.
Funds Requested	\$10,853,604
GWG Recommendation	<i>Tier 3: sufficiently flawed, cannot be resubmitted</i>
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 3

Up to 15 scientific members of the GWG score each application. The final score for an application is the median of the individual member scores. Additional parameters related to the score are shown below.

Highest	2
Lowest	3
Count	14
Votes for Tier 1	0
Votes for Tier 2	1
Votes for Tier 3	13

- 1- The application has exceptional merit and warrants funding.
- 2- The application needs improvement and does not warrant funding, but may be resubmitted to address areas for improvement if the ARS does not approve the application for funding following the GWG’s review; or
- 3- The application is sufficiently flawed and does not warrant funding or the possibility of resubmission.

KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRP for clarity.



GWG Votes	Does the project hold the necessary significance and potential for impact?
<p>Yes: 8 No: 4</p>	<ul style="list-style-type: none"> ● It has been well established that infection during early pregnancy leads to increased risk for developmental or neuropsychiatric disorders. The impact of COVID-19 pandemic on the brain and mental development of children born from infected mothers during pregnancy remains not well understood. Given the continuous pandemic, it is critical to gain more knowledge of the impact of COVID-19 on maternal-child health. ● The project aims to investigate the potential link between maternal COVID-19 infection and increased risk of neurodevelopmental disorders (NDD) in children, such as autism spectrum disorder (ASD) and developmental disabilities (DD). ● By characterizing clinical outcomes, viral presence, and proteomic profiles, this study could elucidate the pathogenesis behind the potential link between maternal COVID-19 and increased DD/ASD risk, leading to potential therapeutic targets. ● The proposed work will generate clinical data from both mother and children on the inflammation profile; and the generation of large numbers of patient iPSC lines and CRISPR edited lines. In addition, transcriptome, genome and epigenome datasets from iPSC based cellular models and mouse models will be generated. ● The successful completion of the project may potentially lead to the identification of early biomarkers or the identification of target for treatment of SARS-CoV2 related DD and ASD. The proposed study is to gain insight on associated molecular and cellular changes, not causal effect. The study is mainly focused on general inflammation signatures and molecular pathways, and targeting these pathways may not be specific. ● The project will use a fairly unique cohort of mother infant pairs to study the possible link between SARS-CoV-2 and a several-fold increase in DD/ASD in the kids. The first aim will cover i) further characterization of outcomes (language, motor and cognition) and frequency of ASD in exposed versus control children, ii) sensitive assessment of signs for the virus (direct versus indirect), and iii) proteomics analysis. ● The cohort is very interesting and valuable, so extending the characterization will be valuable. The cohort and Aim 1 are strong, while the rest is less convincing. ● Aim 2 will generate iPSC lines from ~30 individual each followed by 2D and 3D differentiation. Assessment via imaging and genomics and perturbation will follow. As for the past decade, the challenge here is using in vitro models to study complex neurological disorders. There is no evidence that maternal infection triggers epigenetic alterations in the fetus that are preserved through reprogramming and differentiation. ● Aim 3 will use mouse models to study mechanism, assess behavior and genomic alterations as well as various inhibitors to rescue the neurodevelopment phenotypes. Further genetic engineering of human cell lines to probe specific pathways and candidates. While improving experimental feasibility, the jump to mouse models is not as impactful for the human condition. ● Aim 4 will use existing reference data and computational analysis with less clear goals. One objective is to design biomarkers and prioritize iPSC lines. The overall goal is data integration, but the potential for impact is less convincing. ● The collection of mother/infant pairs and the resources around this are compelling. ● The cohort is valuable, but everything else proposed has limitations.
GWG Votes	Is the proposal innovative?
<p>Yes: 7 No: 5</p>	<ul style="list-style-type: none"> ● The innovative aspect of the proposal is the unique mother-child pairs from both California and Brazil that have been longitudinally followed over time. The methodologies for data analysis are mostly standard, and sometimes lack sufficient detail. ● The possibility of a viral infection during pregnancy affecting the unborn child is real and Aim 1 will provide strong data to judge the risk. All other aims are going back to more basic ideas that are less innovative. ● Although the main goal of the proposed research is to understand how maternal infection during pregnancy leads to DD and ASD in children, the hypothesis to be tested is not entirely clear. ● The project integrates patient-derived and animal models. ● The proposal largely uses standard tools and pipelines. The cohort is the most unique and valuable aspect.



	<ul style="list-style-type: none"> • Aim 2-4 are standard ways that have been used in the past to study ASD and other pathologies. Whether or not the cohort of exposed and unexposed participants' lines will be game changing is unclear. • The application utilizes typical technologies. • The project uses multiple established technologies. • The study is an observational cohort, which limits the ability to establish causality between maternal COVID-19 exposure and neurodevelopmental outcomes. Confounding factors may influence the observed associations.
GWG Votes	Is the rationale sound?
Yes: 3 No: 9	<ul style="list-style-type: none"> • The proposal is based on the preliminary finding that there is a drastic increase in DD and ASD in children born from mothers infected with SARS -CoV2 during pregnancy. Better understanding of how children develop DD or ASD is well justified. While the clinical data are compelling, the data related to iPSC or animal models are not really directly related to SARS-CoV2. Most of the data were shown to support potential feasibility. • Aim 1 has some supporting data although numbers are still small and effect not huge. All others lack convincing data. • The rationale for Aim 1 is very convincing. Aim 2 seems high risk and unlikely to be very meaningful. Same for Aim 4. Aim 3 has some mechanistic potential. • For Aim 2, the hypothesis to be tested is that "gestational exposure to SARS-CoV-2, directly or indirectly, produces an inflammatory environment in the developing fetal brain, where the innate immune cells respond by secreting inflammatory cytokines that affect neural development and functions by producing long lasting genetic and epigenetic changes leading to neuropsychiatric complications in infants". There is no supporting evidence suggesting that infection leads to genetic changes. • Aim 2 does not have the necessary rationale. • For Aim 3, the rationale for focusing on two particular pathways is not clearly articulated. • The application does not sufficiently describe the rationale for doing the experiments. • The project has significant limitations, such as limited sample size for some subgroups; potential selection bias in cohort recruitment; challenges in long-term follow-up and retention; confounding factors not accounted for (e.g., genetics, environmental exposures, socioeconomic status, maternal comorbidities); biomarker analysis is exploratory and may not yield clinically actionable markers. • While the plan aims to assess viral presence and transfer, it does not directly investigate the mechanisms by which SARS-CoV-2 might contribute to neurodevelopmental deficits; the findings may not generalize to other populations/settings; the lack of pre-pandemic controls could make it challenging to disentangle the potential effects of the pandemic environment from the specific effects of SARS-CoV-2 exposure. Variability in patient-derived samples may complicate data interpretation. • The plan does not explicitly mention strategies to mitigate potential batch effects that may arise from generating and analyzing iPSC lines from multiple subjects over an extended period. There are potential technical limitations in reprogramming efficiency and differentiation fidelity, as well as issues with maintaining the stability and functionality of 3D organoids over time. • Infecting pregnant mice with SARS-CoV-2 will help in evaluating developmental & neurobehavioral outcomes. Various mouse models allow for investigating the roles of specific genetic factors and pathways in SARS-CoV-2-associated neurodevelopmental disorders. The plan includes direct interventions that could establish causal links between viral infection, inflammation, & neurodevelopment.
GWG Votes	Is the project well planned and designed?
Yes: 3 No: 9	<ul style="list-style-type: none"> • The study leverages a cohort of mother-infant pairs recruited during the COVID-19 pandemic. The infants have been followed for neurodevelopmental assessments, and a biorepository of maternal-infant specimens has been created. Their preliminary analyses uncovered altered inflammatory profiles, higher rates of respiratory distress in infants, and associations between maternal COVID-19 and DD/ASD.



	<ul style="list-style-type: none"> • Characterizing clinical outcomes, viral presence, and proteomic profiles could elucidate the pathogenesis behind the potential link between maternal COVID-19 and increased DD/ASD risk, leading to potential therapeutic targets. • Aim 1 is well structured and will collect valuable information on the cohort and answer a few critical aspects (direct versus indirect effects). Aim 2 and 4 outline a plan but without a convincing goal. Aim 3 is adequate. • Excellent foresight was employed in obtaining this cohort. Aim 1 is well designed and of value. • Aim 2 is not well designed. • Experiments proposed for the first three aims are to expanding clinic studies (Aim 1), iPSC based 2d and 3D organoid models for cellular and epigenetic profiling (Aim 2) and mouse models for inflammation and behavioral changes (Aim 3), respectively. Aim 4 will be data analysis for the first 3 aims. The aims are not well integrated. Many of the aims are just a collection of experiments. • It is not clear how data generated from clinic and model systems can be cross compared and validated, as experiments are at different developmental stages and in different model systems. While in many places in the proposal the COVID-19-related DD and ASD are referred to as idiopathic, experiments in Aim 3 will test the synergistic effects of genetic and environmental interactions as a cause using mouse models, without a strong justification. • Potential pitfalls and alternative approaches are discussed for each of the aims, yet not in depth. As written, each of the subprojects is more like an independent project testing a different hypothesis. The synergy does not appear to be very strong. • The plan relies on data from multiple sources, which may have varying quality and consistency. Harmonizing these datasets and ensuring their quality could be a significant challenge. Integrating multiple data types can lead to complex results that may be difficult to interpret.
<p>GWG Votes</p>	<p>Is the project feasible?</p>
<p>Yes: 3 No: 9</p>	<ul style="list-style-type: none"> • The necessary resources, including cores, are available for the proposed study. • Overall, everything seems feasible. No major innovation is needed; these are all standard and established approaches. • The project team consists of several investigators, additional key personnel, and consultants. One Key Data Personnel is leaving the institution at the end of 2024 but will continue as a consultant with relevant expertise and experience in their field. • The lead investigator for each of the aims is qualified and has the relevant expertise to carry out the proposed experiments. Most of the team members for Aim 4 are TBD. • The administrative core will create infrastructure for all projects, establish and maintain contractual arrangements, and ensure optimal fiscal management. It will also provide leadership, communication, dissemination, coordination, and support for the proposed investigation across multiple disciplines and institutions. The proposal integrates four main projects across two institutions. • For Aim 2 technical feasibility is discussed, but little time spent on exploring whether anything may be visible after reprogramming. These are not germ-line/genomic alterations, but transient exposures. • Aim 2 does not have evidence of feasibility. • The infrastructure is extensive. A description of the computing environment for data analyses should have been included. • The budget is appropriate.
<p>GWG Votes</p>	<p>Does the project uphold the principles of diversity, equity and inclusion (DEI)?</p>
<p>Yes: 12 No: 0</p>	<ul style="list-style-type: none"> • This study is relevant because COVID-19 has disproportionately affected underserved communities and worsened maternal and infant health disparities. • Yes, this would clearly benefit underrepresented minorities. • The research project compares the effects of maternal SARS-CoV-2 infection in pregnancy on infant neurodevelopment in two cohorts: one from California, and one from Brazil. Both locations are characterized by high diversity and health inequities.



	<ul style="list-style-type: none">• The mother-child pairs recruited to this study are from California and Brazil, with diverse representation. The main description of DEI is on the clinical part, which is excellent. However, there is no description on investigators' efforts for DEI.• The group has provided maternal-infant health care for 30 years to pregnant women and infants living with HIV, chronic health conditions, mental health illnesses, and patients experiencing homelessness and substance use disorder. They have worked closely with obstetrical and neonatal colleagues to address the needs of their participants, especially persons of color, during the pandemic. The group also completed cultural competence and DEI training.
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