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Dear ICOC Members,

We were delighted have our application recommended for funding. We are very excited about this project and looking forward to making important discoveries into ID Syndromes and provide hope to families suffering from these devastating diseases. The reviewers made thoughtful comments that we would like to incorporate to improve our approaches.

"Is there a particularly vulnerable neuroprogenitor population? If it also/instead affects neurons, why are there not continued neurodegeneration phenotypes for these patients? There is a period of regression for Rett patients, but not for the other syndromes like BOS or KAT6A/B. Do the neurons have premature senescence but then stop senescing so there's not further degeneration?"

At this point we have only observed physiological deficits in neurons for Rett, BOS and KAT6A, not at the progenitor stage of differentiation. This is consistent with the clinical data as the patients have normal head circumference at birth and microcephaly develops later. It should be noted that these are not typically described as neurodegenerative disorders, instead the microcephaly is thought to be due to retraction of neuronal arbors such as dendrites as described in the preliminary data for this proposal. Regardless, our plan is to look for DNA damage and repair defects at hiPSC and NPC stages as well.

"If the mechanisms presented such as telomere dysfunction cause neurologic dysfunction then why do patients with clear primary telomere dysfunction monogenic syndromes (such as mutations in TERC) have no neurologic dysfunction, even into adulthood?"

In mice lacking TERC activity, phenotypes do not emerge for three generations, but when they do the major defect is dendritic complexity, as we show for these ID Syndromes. Furthermore, this phenotype can be reversed by deletion of P53, which is also consistent with our data using Pifithrin to inhibit P53. On the other hand, we have not yet observed defects in telomeres in Rett Syndrome, but we are not ruling it out for the other ID Syndromes until we can test to see if the senescence we see in BOS and KAT6A neurons is due to DNA damage, telomere defects, or other stressor as described in the application.

"It would have been very compelling to include a non-epigenetic disorder with a known mechanism of disease (there are many, including many with microcephaly), as opposed to Down syndrome, as a comparator."

This is a great idea, and Valerie Arboleda, our collaborator, is well suited to find such patients and derive lines to test this hypothesis. In the meantime, we will collaborate with Ranmal Samarasinghe at UCLA who has developed hiPSC lines and neural organoids from patients with mutations in SCN8A (see attached letter). We previously collaborated with Dr. Samarasinghe to define electrophysiological dysfunction in Rett organoids, and reversal of these defects by P53 Inhibition, as described in the proposal. These patients have Intellectual Disability and SCN8A is ion channel, thus providing another example of an ID Syndrome not related to epigenetic regulation to compare to Rett, BOS and KAT6A.

"There is a lack of statistics and power calculations."

We did not include a formal section to describe our statistical methods, but for each experiment described, we perform three separate studies with multiple biological replicates from cell lines of each disease from multiple genetic backgrounds. In addition, in many cases we are comparing isogenic lines, namely wildtype and mutant from the same genetic background, which obviates the need for power analysis typically used when comparing groups of unrelated normal versus affected individuals.

"The timeline for the project - i.e., what will happen when - is not clearly presented. A Gantt chart, or a timeline clearly showing a plan for year 1, 2, and 3 would be highly beneficial."

Our plans are as follows:
Activities 1 and 2 will be complete in Year 1
Activities 3, 4, 5 and 6 will be initiated in Year 1
Activities 3 and 5 will be complete in Year 2
Activities 4 and 6 will be complete in Year 3

We would like to reiterate our appreciation of the thoughtful comments provided by the reviewers, they have helped shape the plan for success. We are excited to accelerate this work with CIRM support and make important inroads into the etiology of these devastating syndromes.

Sincerely,

William Lowry

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Department of Neurology
David Geffen School of Medicine at UCLA
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March 22, 2023

Dear Bill,

I am happy to provide human induced pluripotent stem cell lines harboring mutations in SCN8A as well as control lines to bolster your study of DNA damage and Intellectual Disability Syndromes. We have been using these lines to create organoids and study the physiology that results from loss of the sodium channel SCN8A. This is a model of an epilepsy syndrome that is also accompanied by Intellectual Disability, so it can serve as an alternative ID Syndrome model for your studies. In fact, I am very curious to see if neurons from these lines also harbor DNA damage. Good luck on your application, I look forward to working with you!

Regards,

Ranmal Samarasinghe, MD/PhD
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