



“Jordan’s Syndrome” PPP2R5D Prospectus

Executive Summary

Mutations in the gene *PPP2R5D* have recently been described as a cause of neurodevelopmental disorders including autism, intellectual disabilities, behavioral challenges, and seizures. All of the mutations described to date have been the result of new mutations that arose in the child and were not inherited from a parent. To date there have been 36 patients with thirteen different mutations and these mutations are co-located in a very specific place in the PPP2R5D protein. PPP2R5D plays an important role in regulating key neuronal and developmental brain processes. Now that we have identified the causal gene responsible for the neurobehavioral challenges in these children, we have an opportunity to potentially use this gene as a target for future therapies and/or an opportunity to correct the gene through gene editing. However, before we can focus on therapeutic interventions, there are many foundational studies that must be performed to understand the mechanism by which mutations in *PPP2R5D* lead to their effects on the brain and whether or not these effects can be reversed. Because related genes are thought to be involved in cancer, some work has already been done to understand how to target these genes for the treatment of cancer. We may be able to apply some of the knowledge gained from studying cancer to the study of the brains in children with *PPP2R5D* mutations.

We have assembled a team of investigators who are experienced in a range of relevant activities to study *PPP2R5D* biology including human geneticists, neurologists, psychologists, biochemists, protein modelers, model organism researchers, and cancer biologists who study this or related genes who can work together to better understand the mechanism of *PPP2R5D* disease and to develop possible treatments for the future.

Initial Research Elements

To enable the research team to be maximally effective and efficient, we need to develop several reagents and answer a few fundamental questions about the effects of *PPP2R5D* mutations to empower this research and provide direction for future studies.

1. To catalyze the research, we need to make four mouse models that have the corresponding human genetic mutations so that we have a mouse model of the human disease that will allow us to directly examine the brain, perform invasive studies that are not possible in patients, and eventually provide an animal in which we can test new treatments.
2. In addition, we need to collect cells from human patients and make induced pluripotential stem cells and differentiate these cells into neurons (brain cells) that can be studied in the laboratory. This will enable us to study the biology of *PPP2R5D* mutations and will be necessary to perform high throughput drug screens and test several different biochemical models of how mutations in *PPP2R5D* cause this condition.
3. It will be necessary to have the three-dimensional structure of the protein to better understand how the four different mutations all cause the same effect, and we will need the three-dimensional protein structure for chemists to develop compounds to target the mutated protein and make a drug.

4. Finally, we need to identify more patients with the condition and collect clinical and genetic data to better understand the clinical problems that are not yet fully enumerated for this rare disorder.

It is often difficult to predict exactly how long experiments will take and when breakthroughs will occur, but we envision that it will require 6-10 years (research and clinical trials) to develop a treatment for this condition given that we do not yet understand how mutations in *PPP2R5D* lead to the brain disorder.

Research Byproducts

Because we know that the mutations of *PPP2R5D* are connected with or linked to pathways involved in other medical conditions, it is possible that our research could provide significant findings regarding how these mutations are involved with or causal to those conditions.

Those conditions include:

- 1. Intellectual Disability & Autism Spectrum Disorder:**

Intellectual disability (ID) and autism spectrum disorder (ASD) often occur together in a wide number of neurodevelopmental disorders (NDD) sharing similar genetic causes. Our findings from understanding the biology and impact of *PPP2R5D* mutations will provide the research community with valuable tools to study how altered neuronal and developmental brain processes lead to the deficits in cognition and behavior associated with NDD. Similarly, identifying pharmacological interventions in our study will open up the door for applicability to NDD broadly, particularly to those caused by genes that have shared therapeutic targets with *PPP2R5D*.

- 2. Alzheimer Disease:**

Several reports have highlighted that protein phosphatase 2A (PP2A), of which *PPP2R5D* is a common regulatory subunit (see below), may play a crucial role in the pathogenesis of Alzheimer disease (AD). AD is characterized histopathologically by the presence of amyloid plaques and neurofibrillary tangles (NFTs). The major protein constituent of NFTs is abnormally hyper phosphorylated tau, a microtubule-associated protein. Among the various phosphatases which regulate the phosphorylation of tau, PP2A is by far the most important enzyme. In postmortem AD brain samples, PP2A enzyme activity is decreased. In addition, the expression of several PP2A subunits is abnormally altered in an AD model cell line. The inhibition of abnormal tau hyperphosphorylation is considered a promising therapeutic target for the development of disease modifying drugs for AD. The findings in our study will further the understanding of PP2A's role in AD pathogenesis, and may shed light on additional therapeutic targets.

- 3. Cancer:**

PP2A regulates major cell signaling pathways, and reversible protein phosphorylation plays an essential role in regulating these pathways. Deregulation of the delicate balance between protein kinases and protein phosphatases is a well-recognized mechanism by which cells escape self-limiting signals, eventually resulting in malignant transformation. Changes in the PP2A holoenzyme assembly, activity and, substrate specificity have a direct role in disease and are an important contributor to the maintenance of cancer. PP2A holoenzyme (with specific subunits) is believed to have a tumor suppressive role. Various studies revealed that restoration of PP2A activity is beneficial to some cancer patients. Findings from our study may provide additional evidence to support this and may potentially identify new targets for drug interventions.

Scientific Proposal Detail

Protein phosphatase 2 (PP2A), one of the major Ser/Thr phosphatases is a known regulator of growth and differentiation and a suspected tumor suppressor. A trimeric enzyme of catalytic (C), scaffolding (A), and variable regulatory subunits (B, B', B'', B'''), PP2A can exist in >90 subunit combinations in mammalian cells, presumably with distinct localization, substrates, and regulatory mechanisms. A series of de novo mutations in PP2A first identified in 2015 define two new classes of autosomal-dominant intellectual disability. The most common class is caused by recurrent missense mutations in one of the 12 PP2A regulatory subunit genes, *PPP2R5D*, the product of which, B' δ predominates in human testes and brain. The mutations we have observed in *PPP2R5D* are not randomly distributed but are present in four very specific amino acids. All four of these amino acids are highly conserved among the PP2A subunit family, and all change a negatively charged acidic glutamic acid (E) to a positively charged basic lysine (K), and are predicted to disrupt the PP2A subunits binding and impair the dephosphorylation capacity. The same de novo *PPP2R5D* mutations cause human overgrowth, a syndrome commonly associated with intellectual disability and autism.

We need to identify molecular mechanisms by which recurrent de novo mutations in *PPP2R5D* (B' δ) cause neurodevelopmental disorders. **Because some neurodevelopmental disorders are reversible, our ultimate goal is to develop new pharmacological interventions.** We hypothesize that de novo mutations in *PPP2R5D* cause neurodevelopmental disorders by a novel change-of-function mechanism. Specifically, the basic amino acids introduced into an acidic substrate-binding surface may alter PP2A substrate specificity to impair some and favor other dephosphorylation events. This in turn may enhance growth/proliferative signaling pathways over those that mediate cell cycle exit, differentiation, and morphogenesis. We will delineate consensus sequences for dephosphorylation by wild-type and mutant PP2A enzymes, identify their cellular substrates by quantitative phosphoproteomics, and identify relevant phenotypes in cell models of neuronal development.

We will define the impact of de novo mutations in B' δ on PP2A substrate specificity. To identify cellular substrates, we will introduce the most common B' δ mutation (E198K) into human cell lines by genome editing, and will then characterize their phosphor-proteome. We will characterize aberrant growth factor signaling and neuronal differentiation in cell models of *PPP2R5D* mutations. We will characterize the cellular phenotypes of de novo B' δ mutations in vitro and in vivo.

Small molecule PP2A inhibitors that target the mutant substrate binding site may effectively suppress aberrant phosphatase activity without affecting the normal enzyme.

We envision that it will take approximately one year to develop the necessary reagents and establish the infrastructure for the investigative team. We need to make four mouse models that have the corresponding human genetic mutations so that we have mouse models of the human disease that will allow us to directly examine the brain, perform invasive studies that are not possible in patients, and eventually provide an animal in which we can test new treatments. After the mouse models are made we will carefully characterize the brain development and behavioral phenotypes in the mice. This will be performed with a combination of observational behavioral tests as well as microscopic examination of all regions of the brain.

Once we are able to determine what portions of the brain are affected, we will biochemically determine the altered phosphor-proteins associated with the *PPP2R5D* mutation and determine whether they change over time. We will also create a mouse model of the most common E198K mutation with a tamoxifen inducible Cre which will allow us to turn off the mutation by addition of tamoxifen in the drinking water. This will allow us to determine whether or not we are able to reverse the phenotype and to determine if there are limits to the time points at which it is possible to reverse the brain dysfunction. These experiments will be critical to understand reversibility and whether or not drug or other treatments will ultimately be effective.

In addition to the mouse model, we will develop a fly model for the four *PPP2R5D* mutations. We will characterize the neurobehavioral phenotypes in the flies. Flies have the advantage of being able to reproduce more quickly and can be used for basic biochemical experiments for proteins that are highly conserved across species. These initial pilot studies will help us to determine whether or not the fly will be a reasonable model to be used in future scientific strategies.

We will collect cells from human patients and make induced pluripotential stem cells and differentiate these cells into neurons (brain cells) that can be studied in the laboratory. Once we have the differentiated neurons, they will be characterized to determine exactly what types of neurons they are. We will then determine the difference in phosphor-proteins and study the downstream protein pathways with a combination of tandem mass spectrometry and RNA sequencing. Understanding the targets of these proteins will help us to determine whether or not it is possible that one of these downstream proteins would be the best therapeutic target.

Depending on the results of the above studies and as the field of gene editing in humans progresses, we will position ourselves to perform gene editing experiments first in the mouse model and then potentially in humans. It is difficult to predict if and when the field will be ready to pursue gene editing for neurological conditions.

As the above mechanistic scientific work is progressing, we will continue to collect data on patients as they are identified with *PPP2R5D* mutations. We anticipate that the number of patients will increase substantially as clinical exome sequencing is performed more frequently. We will have a study website and a family Facebook page to aggregate patients with *PPP2R5D* mutations.

As patients are identified, we will review their genetic test reports to confirm that they in fact have bona fide mutations. If they fulfill the study entry criteria, we will collect clinical data for medical records as well as standardized interviews and clinical evaluations. This clinical information that we aggregate will be immediately helpful to families and will be shared with them through family webinars and family meetings on at least an annual basis. These families will be invited to provide us with blood samples and/or skin biopsies to perform the studies outlined above.

Scientific Team

Dr. Wendy Chung (Columbia University)-overall study principal investigator

Dr. Chung is currently the Director of the Clinical Genetics Program and the Clinical Cancer Genetics Program for Columbia University Medical School. She also holds the Kennedy Family Professorship of Pediatrics.

Megan Cho (GeneDx)-genetic counselor

Megan Cho received her Master of Science in 2009 from John Hopkin's University and is currently the Research Program Manager of Whole Exome Sequencing at GeneDx.

Clinical collaborators

Dr. Ghayda Mirzaa (Seattle Children's Hospital)

Dr. Mirzaa is board certified in Pediatrics, Clinical Genetics (MD), and Clinical Molecular Genetics. She is also the recipient of the Peter Duncan Fellow Award in 2012 and the 2013 John M. Opitz young investigator award.

Dr. Veerle Janssens (KU Leuven)

Dr. Janssens received her PhD in Medical Sciences, and a Master in Biochemistry. She is also the author of "Promoter Analysis and Characterization of Novel Splice Variants of the human Phosphotyrosol Phosphatase Activator Gene".

Basic science collaborators

Stefan Strack (University of Iowa)

Dr. Strack is the Associate Chair of Research, a Professor of Pharmacology, and a Professor of Pathology at the University of Iowa. He received a Master of Sciences, Computer Science, and a PhD Biology from the State University of New York at Albany.
Protein phosphatase 2A in neuronal signal transduction

Richard Honkanen (U South Alabama)

Ser/Thr phosphatase inhibitors and has been pursuing high throughput screens recently.

Dr. Honkanen holds a PhD from the University of Georgia and was awarded the 2011 NIH Directors Transformative RO1 Award

Yongna Xing (U Madison)

PP2A structural biologist

Dr. Xing holds a Master in Genetics from the university of Fudan, China, a PhD from Rutgers University as well as being an Associate Professor of Oncology at the University of Madison Wisconsin.

Brian Wadzinski (Vanderbilt)

PP2A cell biology in Drosophila

Dr. Wadzinski holds Bachelor of Science degrees in Chemistry and Biochemistry, and a PhD in Pharmacology. He is also an Associate Professor of Pharmacology at Vanderbilt University and has published over eighty papers.

Houhui (Hugh) Xia (U Rochester)

PP1 in the nervous system. He's an expert in mouse models with altered PPase activity in the brain and does electrophysiology and behavior

Dr. Xia is an Associate Professor of Cell Biology and Anatomy Neuroscience at the University of Rochester. He received his Master of Science from the University of Minnesota, a PhD from the University of California San Francisco and did his Post doctorate work at Stanford University. Dr. Xia was awarded the 2004-2006 NARSAD Young Investigator Award and also for 2006-2008.

Budget/Timeline

Our initial estimates are that it will take approximately 6-10 years to complete the studies outlined. It will take approximately \$10 million and three to five years to identify a compound that could be tested in human clinical trials. Once a lead compound is identified, we would partner with a pharmaceutical company to begin formal clinical trials and it generally takes at least five years between the time a compound is used first in humans until it is FDA approved. Below is an estimated budget for this project.

Function	Budget
Clinical registry and biorepository	1,000,000
Phosphoprotein characterization	\$400,000
Biochemical studies	\$400,000
Structural protein characterization	\$250,000
iPSC generation and differentiation and characterization	\$300,000
High throughput drug screening in vitro	\$2,000,000
Mouse model generation and characterization	\$1,250,000
Mouse clinical trials	\$3,000,000

Figure 1. Outline of three foundational experiments to enable studies in PPP2R5D. 1) Creation of induced pluripotential stem cells and neurons to facilitate high throughput drug screens in vitro. 2) Creation of mouse models to study the brain and behavior in vivo. 3) Determination of the protein structure for PPP2R5D and location of mutations to predict mechanism of action.

