

JOURNEY TO A CURE: RESEARCH UPDATE 2020



In partnership with:



SUMMARY AND BACKGROUND

This document presents an update on research efforts undertaken and achievements during the first 18 months of an international research project led by Jordan's Guardian Angels into the PPP2R5D gene mutation also known as Jordan's Syndrome. Research was expanded to include studying two neighboring genes, PPP2R1A and PPP2R5C. It is believed that this family of genes are closely related and studying them as a group is essential to unlocking the mystery of the disease. The progress in this short time period has been unprecedented as the team moves with a great sense of urgency working to improve the lives of the children and families affected and follow linkages to other diseases. Working collaboratively, the research team has accomplished in this short time, what would normally take 4-6 years.

Key milestone achievements:

- The team has developed state of the art tools needed to create both a treatment for Jordan's Syndrome and a cure.
- Those tools developed include:
 - Antibodies and nanoparticles
 - Mice models with several variants of Jordan Syndrome mutations
 - Pluripotent stem cell lines for use in testing treatments, etc.
 - Structural models of the PPP2R5D gene and mutation locations on the gene
- The research team has successfully edited the mutation out of the gene in cells developed from children samples, proving Jordan's Syndrome is reversible.
- PPP2R1A mice models have been created and the number of patients described in literature has been extended for each of PPP2R1A and PPP2R5C genes.

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RESEARCH HIGHLIGHTS – THE FIRST 18 MONTHS

- Each of the 10 institutions partnering with Jordan’s Guardian Angels have built a strong team of researchers dedicated to the project. As a result, **no less than 100 researchers are involved in the project.**
- Remarkable progress has been made related to **deciphering the molecular mechanisms** associated with Jordan’s Syndrome.
- Individuals with Jordan’s Syndrome continue to be enrolled in the **natural history studies.**
- The team has developed **key tools, cell lines, mouse models, and structural insights** to better understand the cellular functions and the pathobiology of the PPP2R5D variants.
- **Three paths are being explored for treating the genetic disorder.** These include reversing the mutation through gene editing, restoring the signaling pathways through existing or new drugs, and identifying drugs that specifically target the variant PPP2R5D and restore normal function and regulation to the holoenzyme.
- **Alpacas have been used to develop antibodies** and nanobodies to increase our understanding of the physiology and pathophysiology of PPP2R5D enzymes.
- DNA base editing system (CRISPR-BE4) has been used to create a human cell line that precisely mimics the PPP2R5D variation. This allows **head-to-head comparisons to be made in the normal cells and the variant cells** to determine how the variant cells differ.
- The research team has successfully edited the mutation out of these cells, proving that **Jordan’s Syndrome is reversible.**
- Mice models have been developed and the results suggest that they are faithful models of Jordan’s Syndrome in that they **recapitulate the most common symptoms** of the disorder.
- Induced pluripotent stems cells have been developed from skin cells and blood samples collected from the children. These cells are now being differentiated and ultimately **developed into neurons and even into mini-brains called organoids.**



Nanoparticles

Ultrafine unit with dimensions measured in nanometres and comparable in size to subcellular structures

Induced Pluripotent stem cells

Adult cells that have been genetically reprogrammed to an embryonic stem cell. These iPSCs add useful diagnostic tools that help our researches study derived neurons through the reprogramming of the cells.

Holoenzyme

An active form of enzyme consisting of its protein component and its coenzyme. Enzymes are proteins that speed up (or catalyze) cellular chemical reactions.

CRISPR

Technology that can be used to edit genes. It allows researchers to alter DNA sequences

Organoid

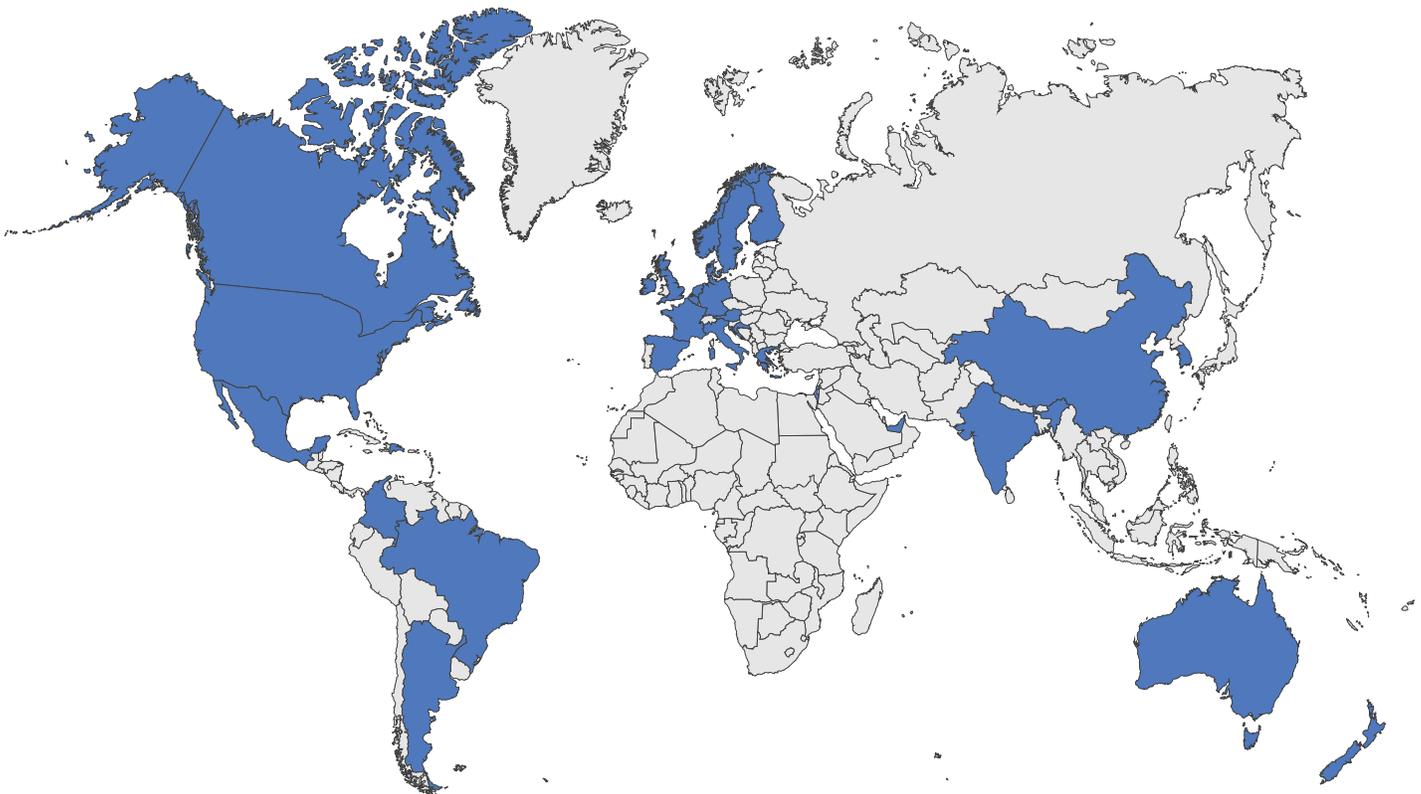
3D cell structures that are a miniaturized and simplified version of an organ produced in vitro

Refer to Appendix B for a full summary of the findings to date.

FAMILIES UPDATE (AS OF MARCH 2020)

- The PPP2R5D community has grown to **more than 140 families from 24 different countries**.
- More than half the population affected by the mutation are **children under 12 years of age**. This skewed age distribution creates challenges in fully understanding the long-term effects of the mutation.
- The online Facebook community continues to grow at a fast rate. The numbers of **families connected has tripled in the first 18 months**, with many references to the Facebook page coming from medical practitioners from throughout the world.
- **Families face a wide range of challenges** including learning difficulties, communication challenges, sleep disorders, epilepsy, anxiety disorders, gastrointestinal challenges, and more.
- **The second family conference was held in March, 2019 in California**. 50 of our families travelled long distances to attend. JGA's entire research team was present as well. The video recap from the event can be found at <https://vimeo.com/332816369>.

DISTRIBUTION OF CASES WORLDWIDE



More than **140 cases** in nearly **30 countries***

Likely approximately **250,000 undiagnosed cases** worldwide

*USA, England, Canada, Ireland, Croatia, Colombia, Denmark, Sweden, Scotland, Australia, Spain, Dominican Republic, Israel, Finland, Norway, France, The Netherlands, New Zealand, Greece, Brazil, Austria, United Arab Emirates, Germany, Argentina, Italy, Isle of Man, India, China, Korea, Belgium

UPDATE ON OUTREACH AND AWARENESS EFFORTS

- Our website traffic is increasing at a substantial rate and our videos have been **shared and seen by tens of thousands of people all over the world**.
- **We have given dozens of presentations to thousands of people** in various groups that have local, regional and international reach.
- Multiple domestic and international media members have covered the group and the research efforts. **Major media outlets are telling our story:**
 - CBS Sacramento: *Local Girl May Hold Key to Unlocking Disorders and Diseases* (<https://www.youtube.com/watch?v=wmfS6374zpA>)
 - Sacramento Bee: *How UCD Research Could Unlock Clues to Alzheimer's* (<https://www.sacbee.com/news/local/health-and-medicine/article217165060.html>)
 - FOX Denver: *Denver-area Girl Has Rare Genetic Condition* (<https://vimeo.com/280295552>)
 - BBC: *Girl, 10, one of few in world with PPP2R5D condition* (<https://www.youtube.com/watch?v=r6Qwefr3Kk4>)
- JGA has **championed additional publications** on the gene such as the Gene Reviews article at www.ncbi.nlm.nih.gov/books/NBK536360/
- The foundation has **established multiple partnerships to support our efforts and continue with spreading awareness** such as with Simons Search Light Foundation, New York Stem Cell Foundation, Rare Science, and Next Generation of Science Standards.
- **Strong JGA representation for rare diseases events across multiple states** such as California, Washington D.C., and Colorado.



JGA families visit Washington, D.C., in August 2017

JGA PROJECT UPDATE SUMMARY

18 MONTH PROGRESS REPORT - JULY 1, 2018 – DECEMBER 31, 2019

Compiled from the individual Team Progress Reports By Jan Nolte, 02/11/2020

BACKGROUND

In 2015, it was recognized for the first time that a single point mutation in a subset of genes, encoding a family of proteins called Protein Phosphatase 2A (PP2A), could be the cause of an inborn (neuro)developmental disorder, characterized by a spectrum of symptoms, ranging from developmental delay (delayed motoric development and speech), moderate to severe intellectual disability, low muscle tone, to behavioral problems, and sometimes, epilepsy. PP2A proteins are expressed in all human tissues, and act as modifiers/regulators of other proteins (called their 'substrates'), of which they can remove a small chemical group (called 'a phosphate'). The presence or the absence of this phosphate group determines the biological activity of these substrates – in other words: it renders these proteins active or inactive. PP2A phosphatases can have positive or negative effects on many different cell and tissue functions. The complexity of 'the PP2A system' mainly derives from their structure, as a functional PP2A in fact consists of three proteins: a catalytic C subunit (which actually removes the phosphate), a regulatory B subunit (which will determine which substrates can be modified), and a scaffolding A subunit (which forms the bridge between the C and the B subunit).

In the PP2A-related neurodevelopmental disorders, either the gene encoding the C subunit (PPP2CA), or the gene encoding the A subunit (PPP2R1A), or two (of all together 15) genes encoding a specific B subunit (PPP2R5D and PPP2R5C) can be affected. So far, the general idea about the effects of the mutations in these PP2A genes, is that the mutation creates a loss-of-function, leading to a dysfunctional subunit that can no longer perform all its functions, including the removal of the phosphate from its substrate(s). However, there is an overall lack of knowledge about which substrate or, more likely, which substrates, a specific PP2A complex modulates and which cell or tissue function or more likely, functions, this modulation affects. In brain, the PPP2R5D- or PPP2R5C-containing PP2A complexes may affect several substrates, and thus several neuronal or brain functions.

Thus, if we aim to therapeutically intervene in order to reverse the effects of the PP2A gene mutations, we need to understand which substrates and pathways are regulated by the affected PP2A complexes. A great amount of progress has been made in this area since the project began.

UPDATES

Holoenzyme interactions and cryo-EM structures

The Xing lab at University of Wisconsin-Madison is investigating how ID (intellectual disability) mutations affect processes that in turn affect the level of the PPP2R5D (B'S) holoenzymes. They showed that the PPP2R5D holoenzyme is not prone to be recycled, unlike other holoenzymes. The mutant holoenzymes, however, interact stronger with PP2A-specific methylesterase (PME-1), making them more vulnerable to the recycling process. The ultimate goal here is to learn whether specific targeting of the molecular processes controlling holoenzyme biogenesis and recycling could be helpful to restore PPP2R5D function.

The team predicted that ID mutations might alter substrate specificity of the PPP2R5D holoenzyme. They identified and characterized specific substrates of the PPP2R5D holoenzyme. CREB is crucial for memory formation and regulation of metabolism. They showed that all mutant holoenzymes have reduced phosphatase activity toward CREB. Surprisingly, all mutants lost specific binding to CREB, except E198K, a variant that has the most severe symptoms.

They hypothesize that E198K might cause Jordan's Syndrome by altered holoenzyme behavior. These results will allow the team to design assays to screen compounds specifically targeting E198K, E200K, and E420K.

High-resolution structures of the PPP2R5D holoenzyme ID mutants would help understand disease mechanisms and correct the mutations with atomic precision. Dr. Derek Taylor from Case Western Reserve University, who was approached by Richard Honkanen 2-3 years ago to determine the structure of the PPP2R5D holoenzyme, acquired the cryo-EM electron density map for the wild type. The resolution is low for the unique residues of PPP2R5D. He expects to model ~30 out of 180 unique residues based on differences of cryo-EM maps of holoenzyme with different truncations. The Xing lab is in the process of determining the cryo-EM structures of the wild type holoenzyme and three forms of the E198K mutant holoenzyme.

Antibody generation

The goals of the Wadzinski lab at Vanderbilt University have been to develop, characterize, and utilize PPP2R5D-directed antibodies and nanobodies to increase our understanding of the physiology and pathophysiology of PPP2R5D enzymes. Alpacas have been used as the host animal for the production of antibodies and nanobodies because these animals express both conventional antibodies and heavy-chain-only antibodies from which the nanobodies can be derived. To date the Wadzinski team has developed a plethora of PPP2R5D antibodies/nanobodies. The PPP2R5D-directed polyclonal antibodies and nanobodies were isolated from the plasma and peripheral blood mononuclear cells, respectively, of alpacas immunized with specific PPP2R5D peptides or proteins. The antibodies/nanobodies are currently being utilized in a number of different applications to elucidate the structure, function, and regulation of PPP2R5D enzymes.

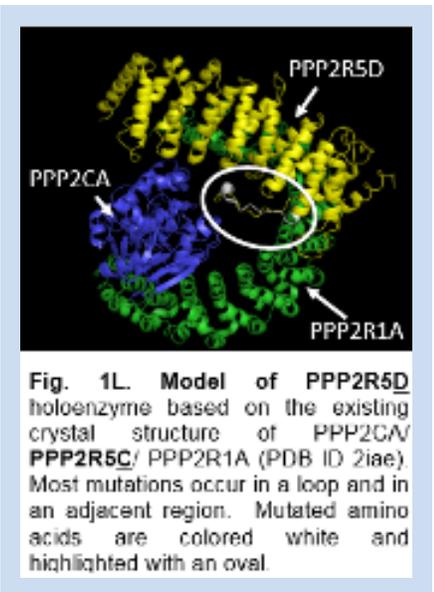
The lab will also be testing the ability of select nanobodies to modulate PPP2R5D activity, with the goal of identifying nanobodies that specifically modulate (activate or inhibit) PPP2R5D holoenzymes without influencing other PP2A holoenzymes. They anticipate that these biomolecules could be leveraged to develop potential therapeutic strategies for JS.

The team made phosphor-specific antibodies to test whether there are any differences in the phosphorylation/dephosphorylation of wild type versus variant forms of PPP2R5D. Furthermore, they are performing pilot studies to test whether these assays can be developed into a high throughput screen (HTS) to identify biomolecules and/or small molecules that specifically target PPP2R5D. The ultimate goal would be to identify a small molecule that restores normal function to the variant forms of PPP2R5D in cells, which could then be tested in mouse models of mutated PPP2R5D and, hopefully one day, in human patients harboring such mutations. Since PPP2R5D has been linked to Alzheimer's Disease, various cancers, and autism, the collective efforts of the JGA team will also provide new insights into these conditions as well.

Protein interactions- targets for PPP2R5D

The Janssens Lab at KU Leuven stably expressed six different PPP2R5D variants (P53S, E198K, E200K, Q211P, D251H and E420K) and the non-mutated PPP2R5D in normal human embryonic epithelial cells and used them for different overall comparative screenings (interactomics, phosphoproteomics, lipidomics), yielding, so far, at least three interesting research lines for future follow-up:

- I. A protein called liprin- α 1 came out as an interesting 'hit', as both its interaction with PPP2R5D, as well as its phosphorylation was affected by most of the PPP2R5D mutations. Liprins are important players in the generation of synapses – these are the contact sites between two neurons, or between a neuron and a muscle cell, and are thus functionally important to transmit signals between neurons, or between neurons and muscle. Although not well-studied, liprin- α 1 phosphorylation may be an important way to regulate liprin function in this process of synapse generation, and a dysregulation of liprin phosphorylation may thus contribute to impaired neuronal transmission, or potentially, the low muscle tone that is observed in the affected patients.
- II. Determined that PPP2R5D itself is also subject to phosphorylation on different sites, and that these phosphorylations



are significantly changed in the PPP2R5D variants. As PPP2R5D phosphorylation may determine the associated phosphatase activity and/or the stability of PPP2R5D, these observations certainly warrant further follow-up.

- III. Different PPP2R5D variants lost C subunit binding to different extents, and more importantly, that there was not always a concordance between the remaining C subunit binding and the phosphatase activity that could be measured on specific (artificial) peptide substrates. This would imply that diminished C binding would not necessarily result in a loss-of-function, but might actually also promote phosphatase activity of the mutated PPP2R5D towards specific substrates – thereby in part contradicting the current idea that the mutations would mainly cause a loss-of-function of PPP2R5D. Moreover, the discordance between the degree of C subunit binding deficiency to a specific variant, and the overall clinical severity of patients expressing that variant, further seems to confirm this view. Thus it remains of utmost importance to identify not only the PPP2R5D substrates in cell and mouse models which might be less dephosphorylated by the variants, but also those which might be more dephosphorylated by the variants.

To determine how the PPP2R5D variants alter normal function at a cellular level, the Honkanen lab has modified a recently developed genomic DNA base editing system (CRISPR-BE4) to create a human cell line that precisely mimics the E420K variant (one of the mutations identified in the children). The cell line is genetically identical to parent cell with the exception of the single base change in PPP2R5D. This allows head-to-head comparisons to be made in the normal cells and the variant cells to determine how the variant cells differ.

From cell - based studies, the team has learned that the PPP2R5D E420K gene is translated. The variant protein is assembled into PPP2A-phosphatases in the cells. Both the normal and variant PPP2R5D proteins form a complex with PPP2CA, which is known to act by removing phosphate from signaling proteins to control a cell response to growth factors and hormones.

The Honkanen lab at University of South Alabama used a non-biased method to compare the changes in protein phosphorylation that occur, conducting a head-to-head comparison of global protein phosphorylation in the normal and PPP2R5D E420K variant cells. The data set generated is complex, containing >25,000 phosphopeptides, and the analysis of the data is ongoing. The preliminary data analysis indicates that the E420K variant cells have altered metabolic properties. For example, the variant cells have a signaling pathway that is normally associated with starvation “turned on” when the cells have been fed a large amount of sugar. The team is exploring ways to turn off the signals that are aberrantly turned on.

ANIMAL MODELS

Murine Embryonic Stem Cell lines

The first step in the general research effort is to build ‘models’ of the PP2A-related neurodevelopmental disorders – this means to genetically manipulate either cells (this can be stem cells, neurons, fibroblasts,...), or mice, to introduce the very same mutation into the very same gene that is affected in the patients. This, by itself, is already a big effort, as at least four PP2A genes are affected, and for each gene, many different variants do occur in the patients. Once established, the team members can study these models in larger depth to better understand the disease mechanisms, at the phenotypical as well as at the molecular level - meaning, we first describe how the disease models differ from the models without introduction of the mutation in terms of their behavior and characteristics, and then we try to understand at the molecular level what the underlying cause(s) of this different behavior is (are).

At KU Leuven, the Janssen lab has now developed 3 such models, in which they performed several studies and obtained interesting results: (1) they made a mouse embryonic stem cell model with introduction of the E198K variant into the PPP2R5D gene. They used these stem cells for differentiation into neurons. They noticed that the mutant cells rather tended to take an indirect path toward neuronal differentiation, which may make them slower in neuron formation by the time of birth, than the unaffected stem cells. This observation would fit well with the neurodevelopmental delay observed in PPP2R5D-affected children harboring this E198K mutation.

Mouse models

The Janssens lab at KU Leuven generated two mouse strains, with a targeted insertion of either the PPP2R1A M180T, or the PPP2R1A R182W variant. Upon further crossing of these mice with mice expressing Cre recombinase, the offspring will actually express the variants, and thus will be proper disease models for PPP2R1A-affected patients that can be further analyzed during the remainder of the project.

During the reporting period, the Strack Lab has made all three of the initially proposed constitutively-mutant, gene-editing (CRISPR knock-in) mouse alleles and successfully bred them for several generations. Two lines of these gene edited mice, E198K and E200K have already been shared with consortium member Hugh Xia at the University of Rochester, who will record their brain activity. The third line, E420K, will be shipped to him soon. These mouse models carry a single point mutation in one of the two PPP2R5D alleles, which mimics the condition of affected individuals.

The Strack lab at Iowa University has also made one of the inducible mouse models of JS. Without further genetic intervention, these mice have two normal ("wild-type") copies of the PPP2R5D gene. However, when bred to mice that carry a gene manipulating enzyme (Cre recombinase) or when this enzyme is introduced via a virus, one wild-type copy is converted to the E198K mutation, again mimicking the human condition. Because expression of Cre recombinase can be controlled in a tissue- and development-specific fashion, these inducible E198K mice will allow identification of where and when expression of the mutated PP2A regulatory subunit causes abnormal cognitive development and other symptoms of JS. This information will be very important for the development of therapies.

The Strack Lab has further begun to characterize the three "constitutive" mouse models (E198K, E200K, and E420K). The results suggest that they are faithful models of JS in that they recapitulate the most common symptoms of the disorder, including cognitive impairment, increased seizure risk, small stature, and increased head size. Moreover, the severity of the symptoms in the three mouse models mirrors what is seen in patients, with the E198K and E420K mutations causing more debilitating impairments than the E200K mutation.

The Xia Lab at University of Rochester used the mouse models created by the Strack lab to study which step of the neuronal communication is altered by variants in PPP2R5D and what substrates are affected in neurons and in which brain region. Currently they have the two mouse models mimicking human PPP2R5D variant E198K and E200K, respectively. They make fresh brain slices from these mice and load them, after recovery from slicing, onto an electrophysiological workstation ("Rigs") to record neuronal communications.

Preliminary data indicates that the ability of neuron I to release glutamate is reduced in E200K mouse, but this effect does not occur with E198K mouse. On the other hand, there does not seem to be major changes in neuron II. Interestingly, we found that the capacity of neuronal communication to change (strengthen or weaken, aka synaptic plasticity) does not change appreciably in E200K mouse, but this needs to be determined more rigorously and studies are ongoing.

The Xia team also performed biochemical studies to determine which proteins/substrates are affected by PPP2R5D variants. The prominent results are a decrease/increase of phosphate levels (exact term is phosphorylation level) on GSK3beta protein in E198K and E200K mouse model, respectively (GSK3beta is an important protein in synaptic transmission, plasticity and neuron survival). These results indicate that the two variants behave in an opposite manner in neurons and their effect on GSK3beta is likely not a direct effect, meaning PPP2R5D variants could act on other proteins to affect GSK3beta. Assessments are ongoing. This is an interesting lead since GSK3beta modulating drugs are in trials for other indications.

The Xia team also showed an increase of a nuclear plasticity protein called cAMP/Calcium regulatory element binding protein (CREB) in the E200K mouse model. CREB is a protein promoting neuronal communication, neuron survival, learning and memory. As E200K variants have a milder effect on human intellectual development than E198K variant, they will test the possibility that this upregulation of CREB does not occur in E198K mouse. CREB could also be a druggable target.

INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS

Cell Line Generation

The Stem Cell Core at UC Davis specializes in the creation of induced pluripotent stem cells that are created from skin cells of those affected with neurological conditions. These cells have the ability to be differentiated into any type of cell, including neurons and even into mini-brains called organoids. The Stem Cell Core has created five patient specific lines harboring four of the common Jordan Syndrome Mutations. All of these cell lines have passed our rigorous quality control for measures of potency in addition to karyotypic stability. We are currently in the process of “correcting” the mutations to create a type of cell referred to as an isogenic control. These control cells are critical for understanding the impact of PPP2R5D mutations in neurons, the most affected cell type.

Disease Modeling

The Fink Lab at the UC Davis Institute for Regenerative Cures is developing novel therapeutic interventions targeted at the underlying genetic cause of the syndrome. They specialize in the creation and validation of targeted interventions using DNA-binding domains such as CRISPR/Cas9. The manner in which the lab uses these tools is to not directly cut the DNA, but rather to use the system to specifically target specific sequences of the DNA or RNA and to recruit specific proteins to that site that would result in a therapeutic benefit.

For Jordan’s Syndrome specifically, the lab has focused on an approach targeted the gene directly at the DNA level to modify expression of the variant or healthy allele, the RNA using an interference strategy to reduce expression of PPP2R5D selectively, or to “correct” the RNA transcript using a novel CRISPR system. They have completed preliminary studies of each approach in patient-derived skin cells and determined that correction at the RNA-level holds the most promise. This approach will next be optimized and tested in the patient iPSC, and patient iPSC-derived neurons.

Patients and phenotype

The Janssens lab at KU Leuven extended and described the number of patients, affected in PPP2R1A, or PPP2R5C, of which so far, few patients had been described in the scientific literature. This is an important step in the general understanding of rare diseases such as these: one indeed needs, first of all, to ‘increase the numbers’ in order to better understand the clinical characteristics of the disease, the specific nature of the different variations/mutations that occur in these genes, how these are linked with each other, etc. This is vital information, not only to better understand the disease mechanisms, but also to improve the clinical management of the patients and to increase the number of diagnoses: the more patients can be accurately described, the better recognizable the disease will become.

Based on the above findings, the Janssens lab is currently preparing two papers, one on 33 new PPP2R1A patients, with 17 new variants, and one on 6 new PPP2R5C patients, with 4 new variants, which we all characterized in terms of PP2A subunit binding and phosphatase activity, and for which we described the clinical characteristics for each individual. For the PPP2R1A patients, the major conclusion was the occurrence of at least two subgroups in terms of disease severity, with one group clearly being more severely affected than the other (both clinically and biochemically, nicely correlating).

This finding also formed the basis for the development of two PPP2R1A mouse models, each model expressing a variant representative for one of both subgroups (with M180T representing the least affected group, and R182W representing the more severely affected group). For the PPP2R5C patients, the major conclusion was their striking similarity with PPP2R5D patients, both clinically and biochemically. However, it still remains to be clarified why PPP2R5C mutations overall seem to be even more rare within the population than PPP2R5D-affected individuals.

The Mirzaa lab at Seattle Children’s Hospital studies brain overgrowth or Megalencephaly (MEG), a developmental disorder often associated with multiple comorbidities including epilepsy, intellectual disability (ID) and Autism Spectrum Disorders (ASD). Recently, mutations of the large PP2A phosphatase family of genes have been identified in children with a wide range of neurodevelopmental disorders including MEG, ID and ASD, with mutations of PPP2R5D (aka. Jordan syndrome) found in >70 families worldwide, to date.

The team's preliminary studies have shown that PPP2R5D regulates important cell growth pathways including the critical PI3K-AKT-MTOR pathway. Activating mutations of key components of this pathway (PIK3CA, AKT3, MTOR, PTEN) are known to cause similar features (MEG, ID and ASD) in children, a critical observation as many inhibitors of this pathway are known that have been used to treat body overgrowth and dysplasia phenotypes as well as cancer. This raises the exciting possibility of using these molecularly targeted therapies for PPP2R5D related disorders.

Dr. Chung and team at Columbia University have continued to enroll individuals with PPP2R5D mutations from around the world in our natural history study. They confirmed eligibility by reviewing genetic test reports, and collected parent reported information about their child. Parents provide baseline information during a medical history interview and provide an assessment of their child's adaptive development through the standardized Vineland Adaptive Behavioral Scale conducted by telephone with a genetic counselor or research assistant. To date 78 families are registered, 68 of whom have provided copies of their genetic test reports, and 57 of whom have completed their baseline telephone interviews. In addition, blood samples have been collected on 33 individuals, lymphoblastoid cell lines made on 16, and iPSCs generated from 5.

Dr. Chung's team has identified individuals with 14 different genetic variants in PPP2R5D. The majority of the variants are clustered at amino acids 197, 198, 199, and 251 in the acidic substrate specificity loop. They hypothesize that these mutations alter the substrate specificity of the phosphatase.

Clinically, the most common features across the 57 PPP2R5D individuals include developmental delay/intellectual disability, autism, ADHD, behavioral challenges, hypotonia, macrocephaly, difficulty with coordination, seizures, visual impairment including strabismus, scoliosis, short stature and/or difficulty gaining weight, gastroesophageal reflux, constipation, and diarrhea.

Although the numbers are still limited, it appears that individuals with the E200K mutation have milder intellectual disabilities when they are children. Recently it was discovered that 3 individuals with this mutation have an atypical form of Parkinson's disease with onset in the 20s and 30s that was ultimately fatal in one patient. Neuropathology at the time of autopsy for the one deceased patient demonstrated severe neuronal loss and gliosis in the substantia nigra pars compacta, as well as marked vascular injury of the subcortical white matter and bilateral striatum. In collaboration with Dr. Cornelis Blauwendraat at the NIH, Dr. Chung's team found that mutations in PPP2R5D are not a common cause of Parkinson's disease and were not detected in over 5000 patients with Parkinson's Disease.

SUMMARY

Since July 2018, the JGA team has made remarkable progress related to deciphering the molecular mechanisms associated with Jordan's syndrome. The team has developed key tools, cell lines, mouse models, and structural insights to better understand the cellular functions and specific substrates for the PPP2R5D holoenzymes, as well as the pathobiology of the PPP2R5D variants. From biochemical studies, we now know that PPP2R5D encodes a protein that acts as a regulator to control the activity and /or substrate specificity of a PPP2A-type serine/threonine phosphatase. PPP2A-phosphatases are a family of ~80 enzymes that collectively control many critical functions within a cell, including the regulation of signaling networks controlling how a cell responds to hormones and growth factors (e.g. insulin). Therefore, PPP2A phosphatases help control metabolism, cell growth, differentiation, senescence, and programmed cell death.

The ultimate goal of the JGA team is to develop therapeutics to treat patients with JS. We envision three different routes for treating genetic disorders such as JS. One path is to "fix" the variant DNA by changing the mutated DNA base to a normal base. A second approach would be to identify the signal transduction pathways altered in cells expressing the variant PPP2R5D and then try to restore those signaling pathways to near normal using existing or new drugs. A third approach would be to identify drugs that specifically target the variant PPP2R5D holoenzyme (AB'8C) and restore normal function and regulation to the holoenzyme. All three approaches are simultaneously being developed by the JGA team, with excellent progress demonstrated in the 18 month progress report.

ABOUT US

Jordan's Guardian Angels is a Sacramento based non-profit foundation working to unlock some of our greatest medical mysteries. We are leading groundbreaking international research into a recently discovered mutation on the gene PPP2R5D, known as Jordan's Syndrome. It causes global developmental delays and is linked to autism, Alzheimer's, cancer and other conditions.

We've united families around the world. In partnership with major research institutions, we're on a mission to make a better future for our children, and potentially millions more, through research that world-renowned medical experts believe will change the world.

Our mission: To conduct research seeking answers to rare genetic mutations affecting children and adults, and assist and improve the quality of life for children and families.



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