“Treat the Symptoms. Cure the Disease”

CureGRIN’s Research Roadmap

May 25, 2021 DRAFT

CureGRIN

Funding Research for GRIN Disorder

About CureGRIN Foundation

CureGRIN is a parent-run public charity dedicated to improving the lives of people living with GRIN Disorder around the world.

We work closely with Families, Scientists and the Medical Community to drive research that will lead to treatments and cures.

CureGRIN.org

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Treat the Symptoms.
Cure the Disease.

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EXECUTIVE SUMMARY

Executive Summary: Our Goal

In 2020, CureGRIN began engaging stakeholders (researchers, clinicians and families) to develop a Research Roadmap that will guide us for the next three years.

Our goal in developing a research roadmap:

To identify and prioritize **specific questions** that need to be answered in order to develop **cures and treatments** for GRI Disorder.

… in order to …

- Define how CureGRIN invests resources (time and money).
- Influence priorities for researchers, biotech and other GRIN advocacy organizations.

Executive Summary: What Families Want

We conducted surveys and focus groups to identify top priorities for GRIN patient families.

- GRIN families want us to balance our efforts between cures and treatments for GRIN Disorder, with a slight skew towards treatments (56% to 44%)
- To reflect family preference that we focus at least as much on symptoms, we've titled this roadmap: "Treat the Symptoms. Cure The Disease."
- GRIN families would like us to prioritize finding finding cures / treatments for three primary symptoms:
  1. Intellectual Disability / Speech
  2. Epilepsy
  3. Mood / Behavior / Neurostorms
- Other symptoms less often identified as priorities include Feeding / Digestion, Mobility, Sleep, Breathing, Hypotonia, Vision Impairment, Hearing Impairment.

Executive Summary: Our Approach

We identified three approaches to cures and treatments and decided to allocate effort & resources roughly as follows:

1. Gene Therapies (40%)
2. Drugs targeting NMDARs (30%)
3. Drugs targeting symptoms / downstream (30%)

- CureGRIN is particularly interested **in Knockdown-and-Replace Gene Therapy**. In theory, this technique could allow for a single medicine per gene which could help regardless of specific variant or type of mutation (nonsense, nonstop, deletion, etc.)

We determined that our approach should be broader than GRIN alone.

- GRIA Disorder and other GRI-diseases are in scope for the plan.

Executive Summary: 10 Essential Questions

We identified 10 critical questions that need to be answered in order to find cures and therapies for GRI Disorder.

1. What are the right **outcome measures?**
   - *How do we measure symptoms pre- and post-treatment?*
2. Can we find **biomarkers**?
   - *Are there ways that GRIN Disorder changes blood or another biological function that will be reversed with treatments / cures?*
3. Is a **cure possible** at any age?
   - *Only for young children or teens and adults too?*
4. What's best **delivery route** for gene therapy?
   - eg. Spinal Cord? Specific region of brain?
5. How can we deliver gene therapies for **larger genes**?
   - Larger genes can be more difficult for gene therapy

Executive Summary: 10 Essential Questions

6. What are optimal drugs targeting NMDARs and related ion receptors?
   - *Can drugs bring GRIN-related receptors into balance?*
7. Are there approved or late-stage drugs that could be repurposed for GRIN and related GRI Disorders?
   - *Could there be drugs out there already that will help?*
8. Which symptoms are due to receptors outside of the brain?
   - *GRIN genes are expressed in gut, lungs, nervous system, etc.*
9. Can we improve symptoms by targeting downstream?
   - *Eg. oxidative stress, neuroinflammation, nutrient sensing, etc.*
10. What's are the functional and phenotypic details for each variant?
    - *Functional Analysis and natural history by gene and variant*

Executive Summary: How We'll Answer These Questions

Now that we know the questions, it's time to find the answers. So what's next?

- CureGRIN will collaborate with stakeholders to identify the best path for each of the essential questions.
- Our goal is to answer each question as fully as possible by April 30, 2024
- Whether we make this date (or exceed it) will depend on a number of factors, including:
  - CureGRIN's ability to ramp up fundraising
  - Success in CureGRIN and research partners securing grants around the world
  - Commitment to collaboration across patient advocacy organizations, researchers, clinicians and industry.

Part 1

BUILDING A RESEARCH ROADMAP

Why a Research Roadmap?

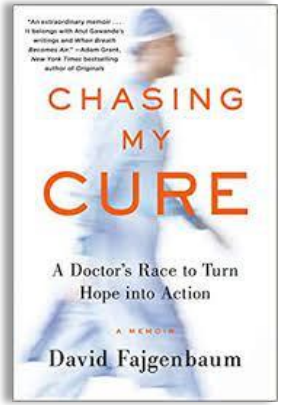
- CureGRIN was founded by a group of Parent Caregivers in 2019.
- In 2020, we embraced the “Fajgenbaum Model” (see next slide) and identified the need to prioritize our resources by identifying the essential questions we need to answer in order to find cures and treatments as quickly as possible.
- We engaged a broad range of stakeholders (families, researchers and clinicians) to identify these essential questions.

Fajgenbaum Model

Dr. David Fajgenbaum -- known as the "doctor who cured himself" – developed a unique approach to rare disease funding and research. CureGRIN is using this model for our three-year research roadmap.

Traditional Model

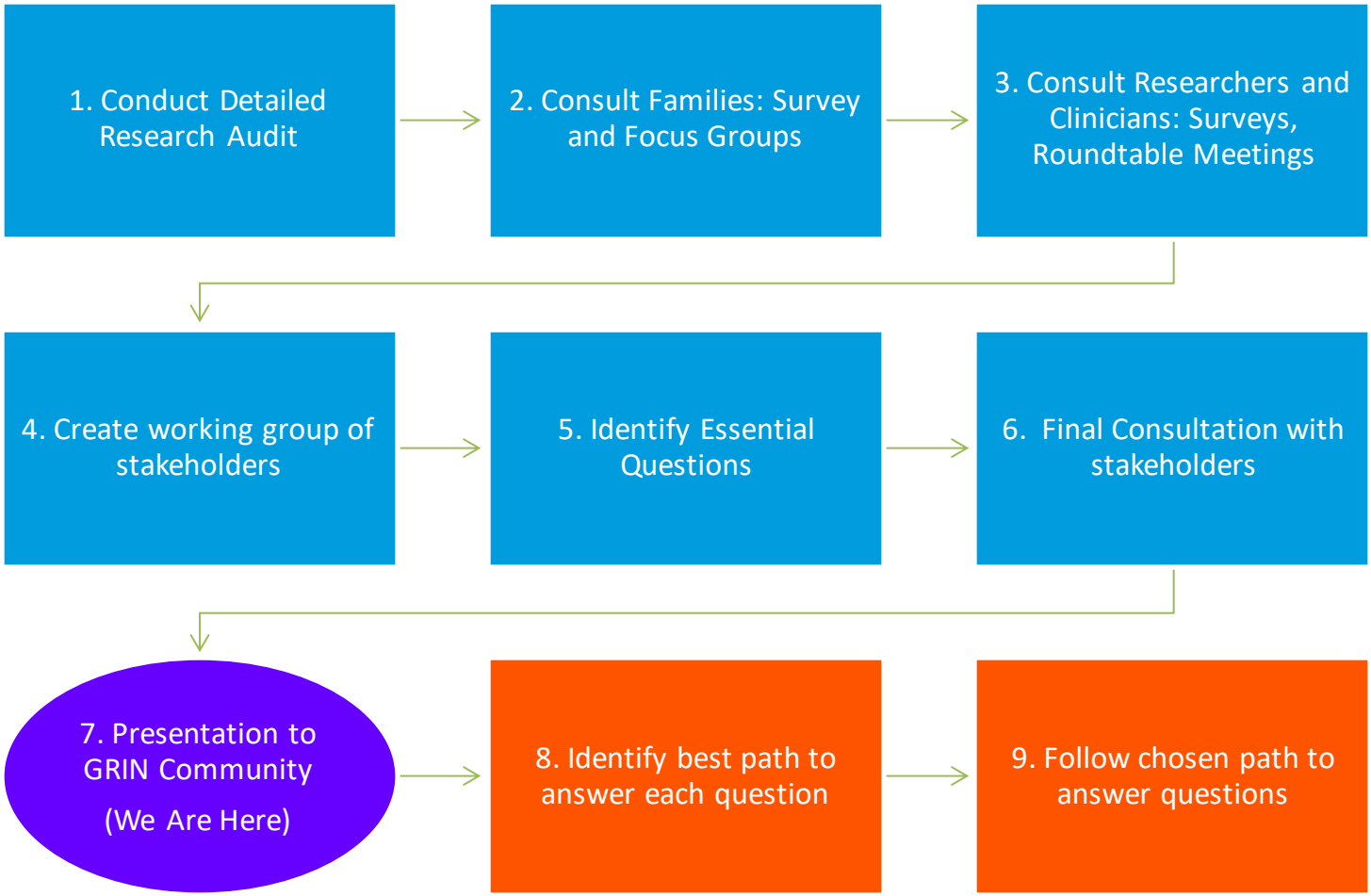
1. Fundraise.
2. Ask researchers to present ideas for how to spend funds.
3. Hand out to projects that sound most worthwhile.



Fajgenbaum Model

1. Consult with families, researchers and clinicians to identify research priorities.
2. Fundraise.
3. Recruit the best researchers to help answer top questions.

Our Research Roadmap Journey



Research Roadmap Working Group Membership

- Keith McArthur, CureGRIN (Chair)
- Meagan Collins, CureGRIN
- Jillian Hastings-Ward, CureGRIN
- Liz Marfiz-Ash, GRIN2B Foundation
- Carole Quennessen, GRIN2B Foundation
- Sandra Silva, GRIN2B Europe
- Amela Huskic, GRIN2B Europe
- Heather Cartwright, GRIA family representative
- Dr. Steve Treynelis, Emory University
- Dr. Amy Ramsey, University of Toronto
- Dr. Wayne Frankel, Columbia University
- Dr. Adi Barzel, Tel Aviv University

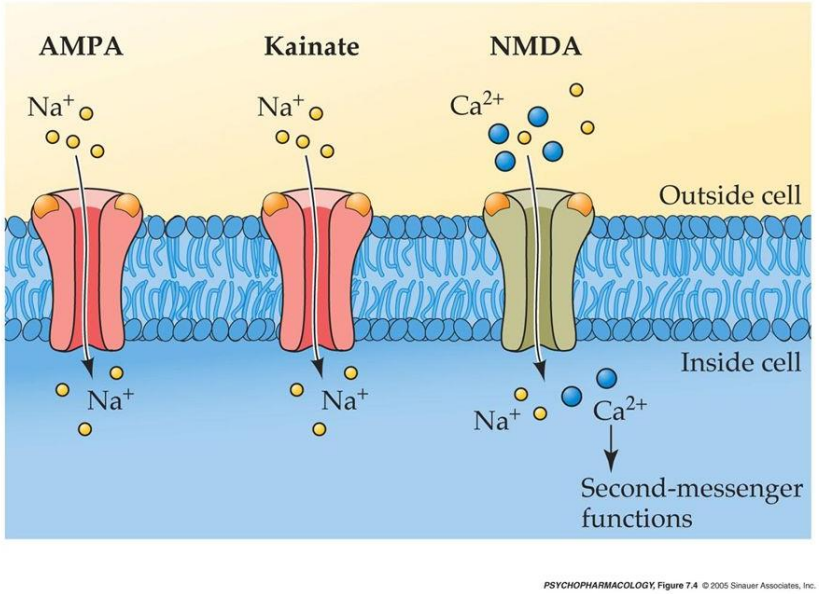
Timelines

- We have identified 10 essential questions to be answered in order to find therapies and cures.
- Working closely with GRIN stakeholders around the world, our aim is to have answers to these questions over the next 3 years.
- The precise timeline is contingent on a number of factors including how much money we can raise.
- Next steps involve identifying the right path to answer each question.

All GRI Genes are in Scope

- **Including GRI genes will accelerate our path to treatments / cures for all GRI Disorder patients.**
- **Here's Why**
  - AMPA and NMDA receptors are part of a single mechanism
  - Significant overlap in researchers
  - Similar symptoms
  - Similar paths to a cure
  - GRIN, GRIA, GRIK (and possibly GRID) can be thought of as a larger family of GRI Disorders.
  - Genes from all four groups have been identified as connected to autism.
  - GRIA Disorder families have raised more than $50,000 for research through CureGRIN

Note: Funds raised by GRIN families will continue to be dedicated for GRIN-specific Research.



GRI–AMPA (GRIA1, GRIA2, etc.)
GRI–NMDA (GRIN1, GRIN2A, etc.)
GRI–Kainite (GRIK1, GRIK2 etc.)

NOTE: It's not clear if GRID genes are part of the same family.

What are GRI Disorder?

Glutimate

Ionotropic

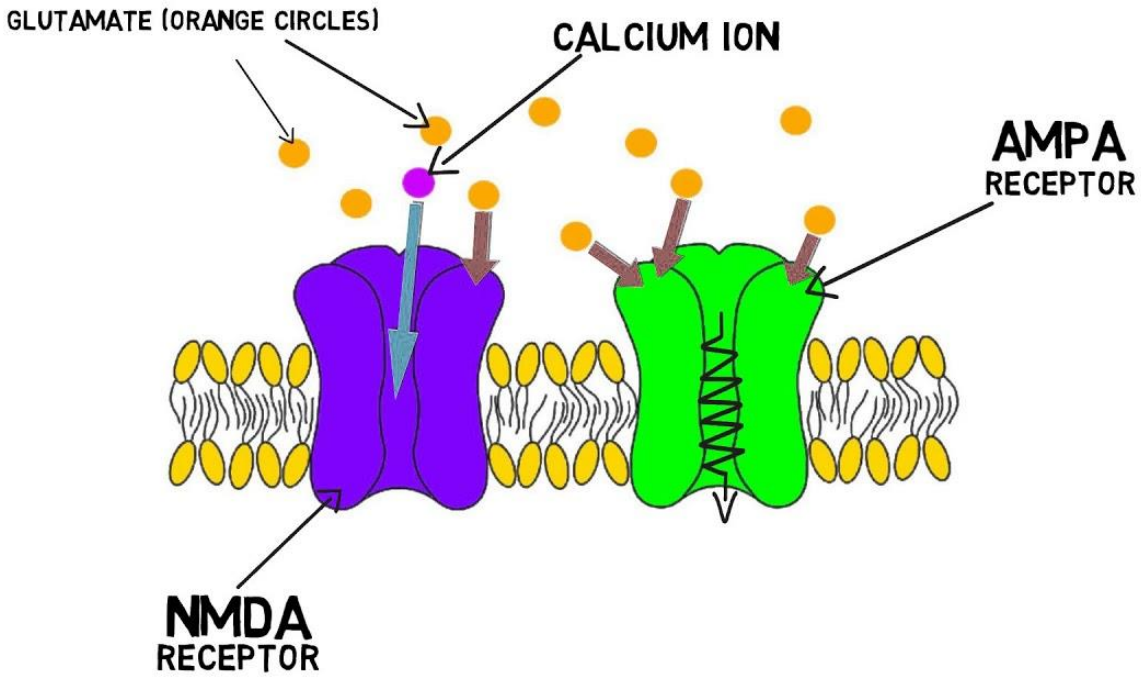
Receptors

GRI – AMPA (GRIA)

GRI – NMDA (GRIN)

GRI – Kainite (GRIK)

GRI – Delta (GRID)



Part 2

FAMILY PRIORITIES

Family Consultation

- CureGRIN conducted a family survey in 2020. We received responses from 197 GRIN families and 15 GRIA families.
- We also conducted 8 family focus groups based on the following themes:
  - Intellectual Disability & Speech
  - Epilepsy
  - Mood, Behavior & Neurostorms
  - GRIN1
  - GRIN2A
  - GRIN2B
  - GRIN2D
  - GRIA

What symptoms does your child have? Please include all symptoms even if their connection to GRIN Disorder is unknown.

All GRIN (n=197)

1. **Intellectual Disability (91%)**
2. **Speech (80%)**
3. **Low Muscle Tone (72%)**
4. **Sleep Challenges (66%)**
5. **Mood / Behavior (53%)**
6. **Epilepsy / Seizures (49%)**
7. **Mobility Impairment (49%)**
8. **Constipation (50%)**
9. **Visual Impairment (45%)**
10. **Digestive (39%)**
11. **Neurostorms (33%)**
12. **Feeding Tube (22%)**
13. **Breathing (12%)**
14. **Hearing Impairment (5%)**

GRIN1 (n=71)

1. ID (94%)
2. Speech (84%)
3. Sleep (74%)
4. Mobility (73%)
5. Low Muscle Tone (71%)
6. Epilepsy (59%)
7. Constipation (59%)
8. Visual Impairment (54%)

GRIN2B (n=80)

1. ID (96%)
2. Speech (84%)
3. Low Muscle Tone (74%)
4. Mood / Behavior (65%)
5. Sleep (63%)

GRIN2A (n=36)

1. ID (74%)
2. Epilepsy (69%)
3. Speech (69%)
4. Low Muscle Tone (63%)
5. Sleep (63%)
6. Mood/behavior (51%)

GRIN2D (n=10)

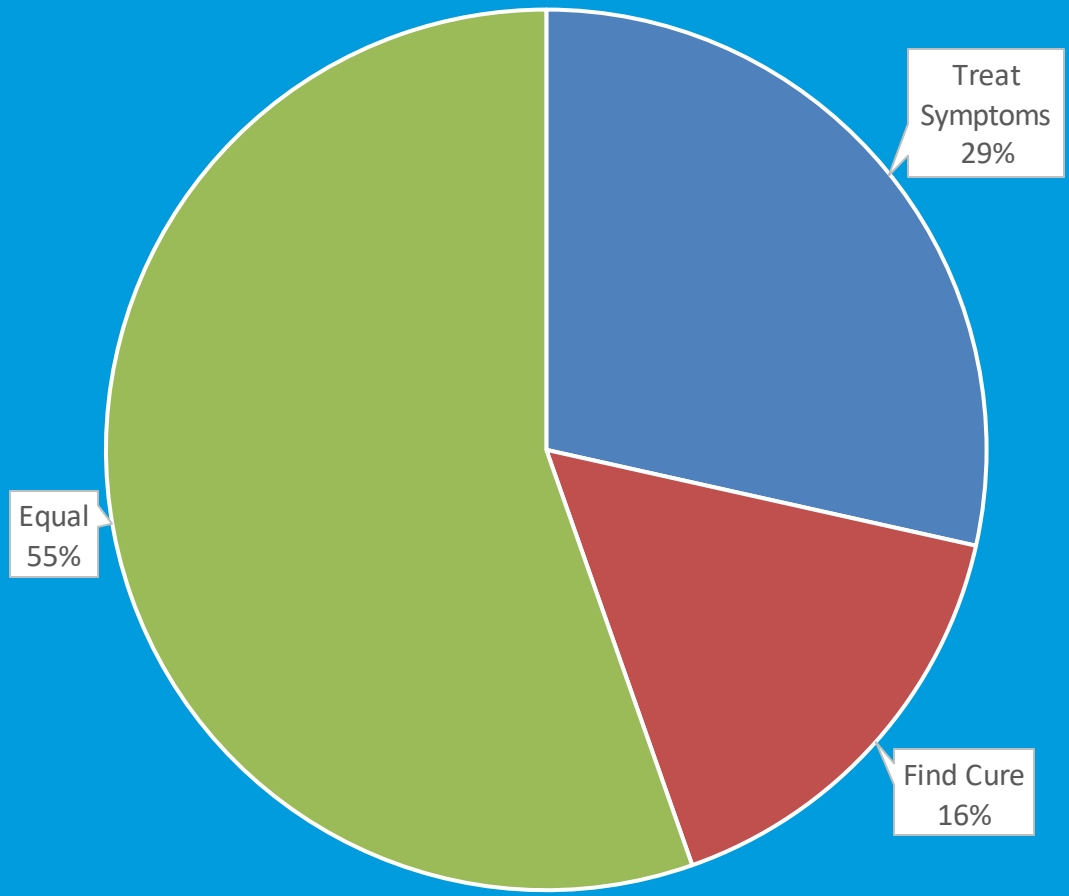
1. Low Muscle Tone (80%)
2. ID (80%)
3. Epilepsy (80%)
4. Mobility (80%)
5. Visual Impairment (70%)
6. Speech (07%)

* Responses >50% included here

Balance Cure and Treatments

GRIN families want us to prioritize both finding long-term cures and shorter term treatments for symptoms.

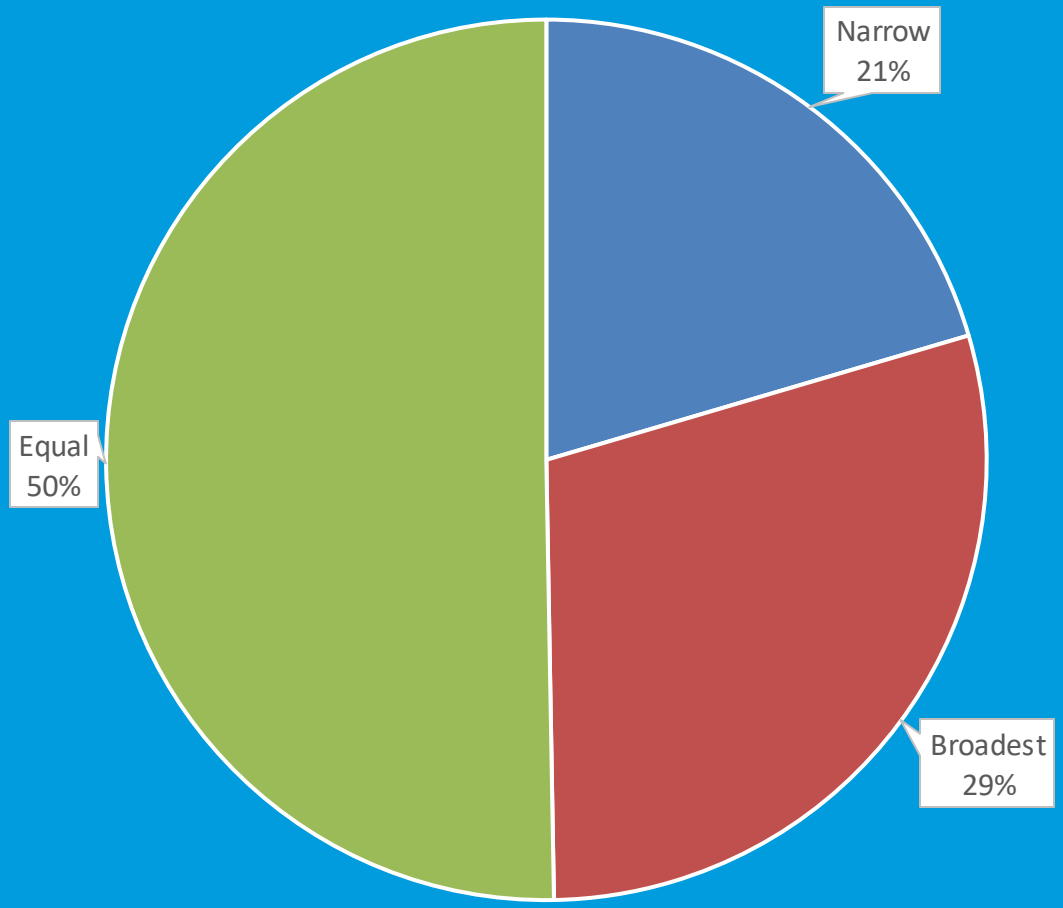
If we have to choose, a slight edge to treatments (56%) over cures (44%)



Balance Cure-all with Cure-some

GRIN families want us to balance taking our time on long-term solutions that help the most kids vs. finding faster treatments or cures that help a smaller number

If we have to choose, a slight edge to a broad focus (54%) over faster for smaller group (46%)



Where GRIN Families Want Us to Focus

Tier	SINGLE MOST IMPORTANT SYMPTOM TO TREAT	All GRIN (n=197)	GRIN1 (n=72)	GRIN2A (n=36)	GRIN2B (n=80)	GRIN2D (n=10)	ALL GRIA (n=15)
1	Intellectual Disability / Communication	44%	43%	23%	56%	30%	14%
1	Epilepsy	22%	20%	43%	10%	60%	40%
1	Mood / Behavior / Neurostorms	17%	16%	20%	20%	0%	40%
2	Feeding & Digestion	5%	7.2%	0	1.2%	0	0%
2	Mobility	5%	5.7%	5.7%	2.5%	10%	0%
2	Sleep	4%	4.3%	2.9%	3.7%	0%	0%
3	Other	Breathing (1.5%), Low Muscle Tone (1.5%), Vision (0.5%), Hearing	Breathing (2.5), Low Muscle Tone (1.4%),	Breathing (2.8%), Low Muscle Tone (2.8%)	Low Muscle Tone (1.3%), Hearing (1.3%)	NA	Low Muscle Tone (7%)

Focus Group Takeaways

During focus groups, families provided additional detail on symptoms and discussed their priorities. Three key themes came up in multiple sessions:

- Families noted that quality of life was an important consideration in prioritizing symptoms. Some noted they thought their children could have good quality of life with physical disabilities or visual impairment, but not with severe seizures or neurostorms.

- Families emphasized that symptoms are interrelated. For example, vision impairment impacts mobility and severe gastro pain gets in the way of learning.

- Families indicated that while there are already several possible epilepsy treatments, research that could answer which are best for GRIN Disorder would be welcome.

Part 3

THREE PATHS TO TREATMENTS AND CURES

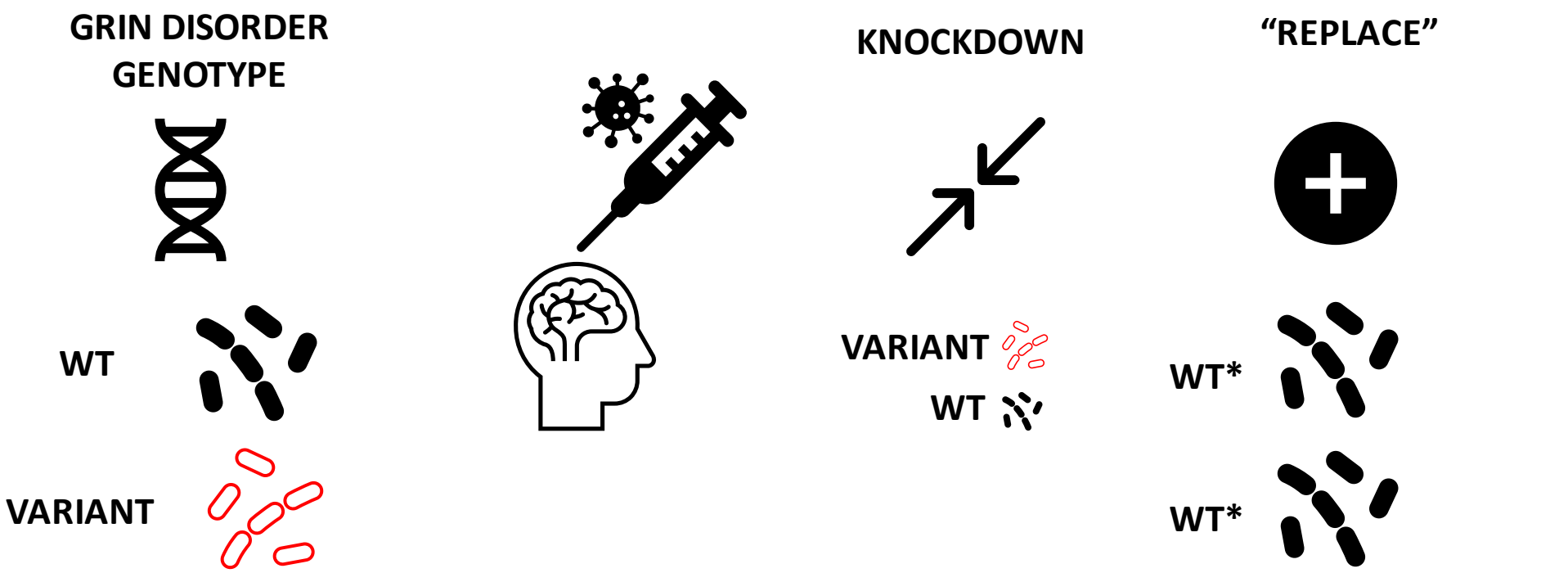
Defining Treatments and Cures

- A **cure** is a medical intervention that would reduce a range of symptoms by rebalancing function of the NMDA receptor through gene therapies or pharmacological treatments.
- A **treatment** is a medical intervention that can help with one or more related symptoms by targeting NMDARs or specific symptoms.

A Three-Tiered Approach

Gene Therapy (40% Effort)	Drugs Targeting NMDARs (30%)	Drugs Targeting Symptoms (30%)
• Prime candidate: Knockdown and Replace • Other candidates: ASO, boost / reduce,mRNA CRISPR, etc.	• Agonists, Antagonists, PAMs, NAMs, etc.	• Epilepsy drugs, psychiatric drugs, drugs to treat downstream symptoms, etc.
CURE	**CURE / TREATMENT**	**TREATMENT**

Lead Candidate
Knockdown and "Replace" Gene Therapy



Knockdown & Replace Gene Therapy

Advantages

- Potential of single treatment for each gene, regardless of variant type (missense, nonsense, etc.) function (LOF or GOF) and location (protein change).
- Potential to fully reverse phenotype in target cells.

Key Questions

- Potentially very expensive. Who pays for treatments?
- Unknown which symptoms could be reversed in children / teens / adults.
- Can we deliver to enough NMDAR cells?
- How to deliver larger genes?
- How long to get regulatory approval?

Part 4

THE 10 ESSENTIAL QUESTIONS

Executive Summary
Top 10 Questions

CureGRIN has identified 10 critical questions that need to be answered in order to find cures and therapies:

1. What are the right outcome measures?
2. Can we find biomarkers?
3. Is a cure possible at any age?
4. What's best delivery route for gene therapy?
   - eg. Spinal Cord? Specific region of brain?
5. How can we deliver gene therapies for larger genes?
6. What are optimal drugs targeting NMDARs and related ion receptors?
7. Are there approved or late stage drugs that could be repurposed for GRIN and related GRI Disorders?
8. Which symptoms are due to receptors outside of the brain?
9. Can we improve symptoms by targeting downstream?
   - Eg. oxidative stress, neuroinflammation, nutrient sensing, etc.
10. What's are the functional and phenotypic details for each variant?
    - Eg. Gain of Function vs. Loss of Function, clinical presentation, etc.

1. WHAT ARE THE RIGHT OUTCOME MEASURES?

Outcome Measures

- We want to make sure that researchers are measuring the things that are most important to us. By agreeing a set of "outcome measures", research from different studies can all be compared together.
- What are suitable **outcome measures** to determine benefit from treatment? How should these be measured for future pre-clinical studies / clinical trials?
  - Examples of outcome measures:
    - Reduction of seizure frequency/severity
    - Assessment scores:
      - Vineland test
        - » Measures behavior of intellectual and developmental disabilities, autism, and developmental delays
      - Wechsler Adult Intelligence Scale (WAIS)
        - » Measures intelligence /cognitive ability in adults and older adolescents
      - Social Communication Questionnaire
        - » Measures autism spectrum disorder symptomatology (rates lifetime and current characteristics)
      - Gross Motor Function Measure-66
        - » Measures changes in gross motor function over time

Outcome Measures

- Example of clinical trial outcome measures (clinicaltrials.gov)
  - L-Serine clinical trial
    - Dose tolerability
    - Change in mental age with Vineland Adaptive Behavior Scales
    - Change in Bayley Scales of Infant and Toddler Development
    - Efficiency of the treatment measured by change in the cognitive assessment (Wechsler Intelligence Scale)
    - Change in the Achenbach System of Empirically Based Assessment (ASEBA) System of Empirically Based Assessment (ASEBA)+
    - Change in Gross Motor Function Measure-88
    - Change in Social Communication Questionnaire (SCQ)
    - Change in the Sleep Disturbance Scale for Children (SDSC)
    - Microbiota composition

2. CAN WE FIND BIOMARKERS?

Biomarkers

- Biomarkers are biological, chemical, or physical parameters that can be measured in the body.
- Can we find **biomarkers** in blood, urine, skin cells, cerebral spinal fluid, brain waves, etc.?
  - Biomarkers can be measured as an indicator of normal biological processes, pathogenic physiological processes, or indicate responses to a drug or other therapy.
  - Examples: oxidative stress, psychological stress, metabolics, nutrition, etc.

3. IS A CURE POSSIBLE AT ANY AGE?

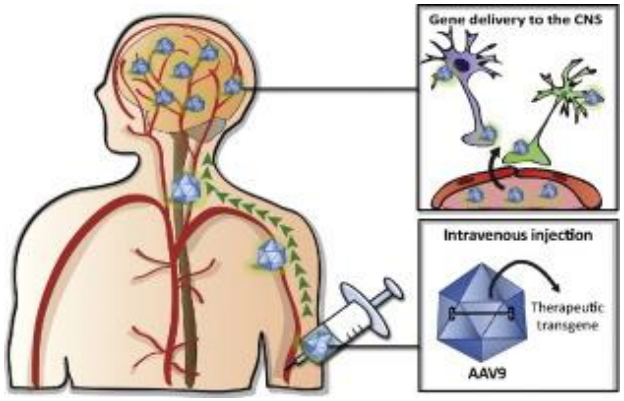
Research in mice suggests that adults can overcome developmental deficits caused by GRIN Disorder

- Adult LoF Grin1 mouse model phenotype was rescued using gene editing (with Cre recombinase) (Mielnik et al, 2020)
  - First, the study **suggests that it is possible to reverse the developmental consequences of changes in NMDAR expression levels**, leading to changes in brain wiring.
  - Second, the study suggests that the reversal of developmental consequences **can happen in adulthood**.
- We do not know exactly how or when interventions must be administered for GRIN/GRIA patients to have big improvements. Have the deficits altered circuitry in a permanent fashion? Has the human brain wired around deficits in such a way that reversal has unanticipated outcomes?

4. WHAT'S THE BEST DELIVERY ROUTE FOR GENE THERAPY?

Genetic Treatments

- What is the best method to **administer gene therapies**?
  - Because the nervous system is very complex, there are obstacles to delivering the ideal gene therapy.
  - Some obstacles include the blood brain barrier (BBB) and how invasive the particular delivery method is
  - Some types of administration to be studied:
    - **Intravenous** (inject medicine into the vein)
    - **Intracerebroventricular** (inject medicine into cerebrospinal fluid in the brain)
    - **Intraparenchymal** (inject medicine into cells in the brain).



(Saraiva et al., 2016)

5. HOW CAN WE DELIVER GENE THERAPIES FOR LARGER GENES?

GRI Genes and AAV limits

Adeno-Associated Virus Vectors (AAVs) have a carrying capacity of about 4.7kb of nucleotides. The “instructions” for Knockdown-and ”Replace” gene therapy contain approximately 1.1kb of code, resulting in a maximum viable gene size of approximately 3.6kb.

Gene	Protein	Chromosome	Size of Gene
GRIN1	GLuN1	9	
GRIN2A	GLuN2A	16	
GRIN2B	GLuN2B	12	
GRIN2D	GLuN2D	19	
GRIA1	GLuA1	5	
GRIA2	GLuA2	4	
GRIA3	GLuA3	X	
GRIA4	GLuA4	11	
GRIK2	GLuK2	6	

To be Confirmed

AAV Gene Therapy

- GRIN1 and GRIA genes likely fit into AAV. What is the **best vehicle to deliver** GRIN2 genes?
  - Some genes are too big to fit into the AAV (the optimum genome-packaging capacity is ~4.7Kb).
  - There are only 2 FDA approved AAV gene therapies.
    - Luxturna was approved in 2017 for a rare inherited retinal dystrophy.
    - Zolgensma was approved in 2019 for spinal muscular atrophy.
  - Research with biotech companies will need to be done to assess what is the right strategy for delivery of gene therapy for GRIN2A, GRIN2B, and GRIN2D

6. WHAT ARE OPTIMAL DRUGS TARGETING NMDARS AND RELATED ION RECEPTORS?

NMDAR Agonists/Antagonists

- **Agonist**: a substance that binds to and **activates a receptor** (protein that receives signals), producing a biological response. For example, glutamate is an agonist of the NMDAR. There are 2 types of agonists:
  - Endogenous agonists are naturally produced in the body (such as hormones and neurotransmitters).
  - Exogenous agonists are external factors (such as drugs).
- **Antagonist**: a substance which interferes with or inhibits the physiological action of the receptor. An example of an NMDAR antagonist is memantine.

NMDAR PAMs/NAMs

- **PAMs** (positive allosteric modulators): act to enhance the function of a receptor. PAMs work in the presence of agonist (a substance, like glutamate, that activates a receptor) but are not able to directly activate the receptor in the absence of the agonist.
  - GRIN FACT: Some of the classes of PAMs investigated in relation to GRIN disorder are: Spermine, Pregnenolone Sulphate, 24 (S)-HC (this one is now in clinical trials at Sage Therapeutics – called SAGE 718). The hurdle has been to find PAMs that target specific subtypes (NR1, NR2A, NR2B, NR2D).
- **NAMs** (negative allosteric modulators): A molecule that blocks the binding of the agonist (activator) to a receptor. A NAM binds to a site on the receptor that is different than the spot where the agonist binds.

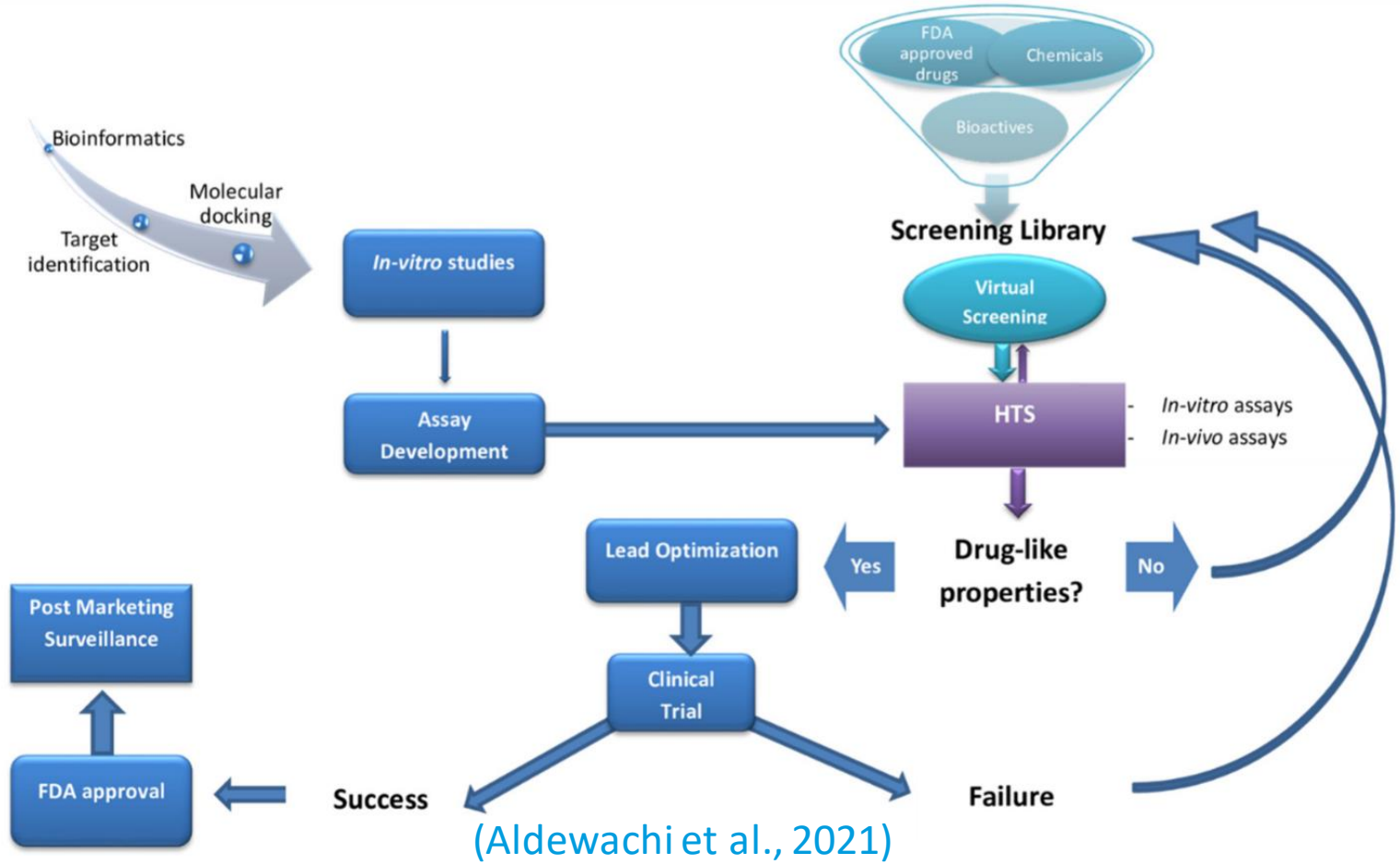
Examples of Studies on NMDAR Agonists/Antagonists/PAMs/NAMs

- **Memantine**
  - GRIN2A gain-of-function (c.2434C>A; p.L812M)
    - A memantine dosage of ~0.5 mg/kg per day was administered, and the treatment resulted in **decreased seizure frequency** (Pierson et al., 2014).
  - GRIN2A gain-of-function (c.1930A>G (S644G)) (Amador et al., 2020)
    - Treated with off-label memantine and dextromethorphan
    - Memantine started at 2 years old and following treatment, the daily **seizure burden was reduced by ha**lf (Amador et al., 2020)
  - GRIN2B gain-of-function
    - Memantine treatment offered to patients after functional confirmation of a gain-of-function variant retaining memantine sensitivity in vitro (Platzer et al., 2017)
    - Oral memantine treatment: doses of 0.5–0.6 mg/kg body weight/day (Platzer et al., 2017)
- **L-Serine**
  - GRIN2B loss-of-function (P553T)(Soto et al., 2019)
    - For 4 weeks, patient was given a dose of 250 mg/kg per day. Then, the dose was increased to 500 mg/kg per day. The dose was administered by dietary supplements (3) and was mixed with food or drink.
    - **Improvements in motor impairments, cognition, and communication** was noted after 11 and 17 months of L-Serine dietary supplementation.
  - L-Serine Clinical Trial in GRIN LOF: Tolerability and Efficacy of L-Serine in Patients With GRIN-related Encephalopathy (https://clinicaltrials.gov/ct2/show/NCT04646447)
  - Led by Barcelona GRIN Team (Spain)
- **Radiprodil** (Xenopus oocytes)
  - GRIN2B gain-of-function mutations
  - Negative allosteric modulator of GluN2B-containing NMDA receptors (Mullier et al., 2017)
  - Phase I completed. Phase II trial in children with Infantile spasm-trail was not completed, low number of enrolled patients.

7. ARE THERE APPROVED OR LATE STAGE DRUGS THAT COULD BE REPURPOSED FOR GRIN AND RELATED GRI DISORDERS?

High-throughput Screening

- What approved drugs and late-stage development candidates can be **repurposed** for GRIN / GRIA? (Would we need high-throughput screening for each separate variant?)

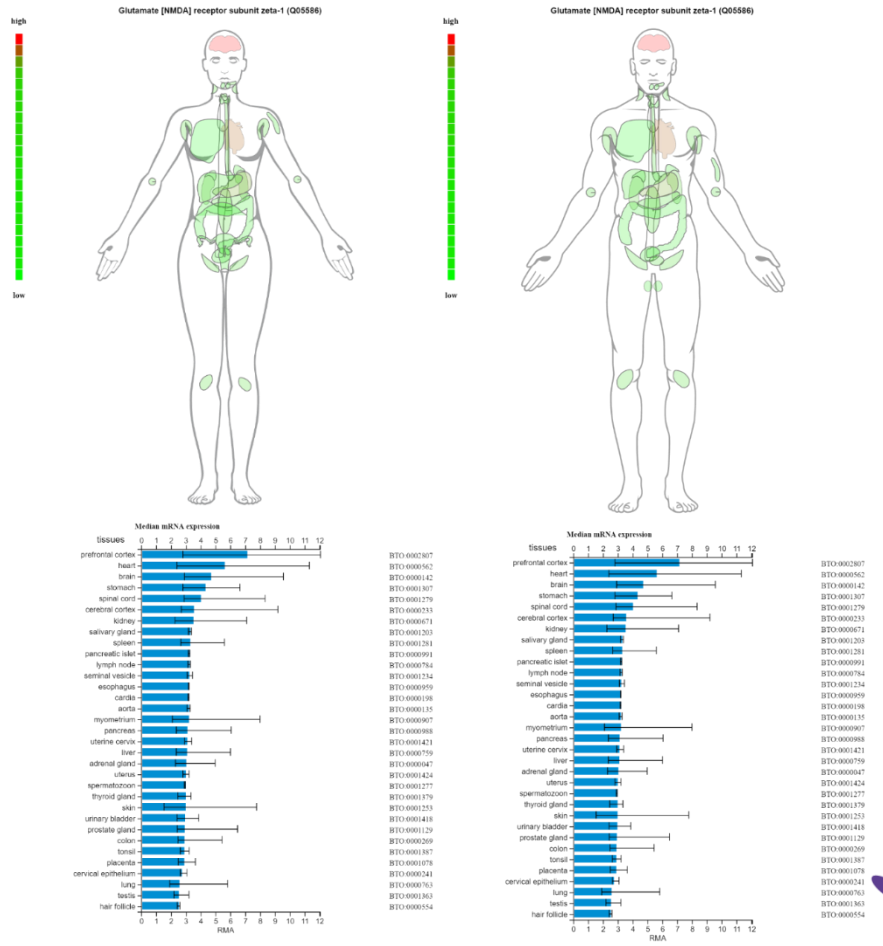


(Aldewachi et al., 2021)

8. WHICH SYMPTOMS ARE DUE TO RECEPTORS OUTSIDE THE BRAIN?

NMDARs Beyond the Brain

- What harm is caused by variant NMDARs **outside of the brain**?
  - mRNA expression
    - Highest expression in the brain
    - GRIN genes are expressed outside of the brain as well



NMDARs Beyond the Brain

- Is hypotonia caused by NMDARs the brain or in somatic nervous system? Are respiratory issues caused by hypotonia originating in brain neurons or localized in respiratory system?
  - NMDARs are highly expressed throughout the central nervous system (CNS), but less information is known about NMDAR function outside of the CNS.
  - Dong et al. (2021) studied the expression of NMDARs in the human pulmonary artery.
    - The **pulmonary artery** works to carry **deoxygenated blood from the right side of the heart to the lungs**.
    - Previous research has indicated that the **NMDAR subunits GluN1 and GluN2A-D** are expressed throughout the lungs and trachea.
    - One cell type which expresses NMDARs in the lungs are pulmonary airway **smooth muscle cells**.
    - When **NMDARs are activated** in these pulmonary airway smooth muscle cells, **calcium (Ca2+) is released and the airway contracts**. Yet, if there is too much activation of the NMDARs in the lungs, **acute nitric oxide-dependent injury** can occur.
  - CureGRIN has been notified of a very small number of unexpected events related to respiratory issues in our patient community. Therefore, this study and other future studies will be very important for our understanding of respiratory-related events in patients with GRIN disorder.

9. CAN WE IMPROVE SYMPTOMS BY TARGETING DOWNSTREAM?

Downstream Effects

- Certain aspects of GRIN/GRIA symptoms could possibly be attributed to other things involved in the pathway.
- Which symptoms can be ameliorated by combinations of substances that together target **downstream effects** including 1) Oxidative stress 2) Neuroinflammation 3) Mitochondrial / nutrient signaling dysfunction (IGF, mTOR, etc.) 4) Excitatory / Inhibitory Imbalance 5) Methylation & other epigenetic markers?
  - **Oxidative stress** is an imbalance of free radicals (oxygen-containing molecules with electrons) and antioxidants in the body, which can lead to cell and tissue damage.
  - **Neuroinflammation** is a **basic immune response** of cells in the brain. Physiological concentration of inflammation protects neurons from damage, but **excessive neuroinflammation intensifies neuronal damage**.
  - **Mitochondrial dysfunction** occurs when the **mitochondria don't work as well** as they should and can be caused by exposure to certain environmental factors or genetic abnormalities.
  - Protein kinase mechanistic target of rapamycin (**mTOR**) is a central cell growth regulator important for **cellular metabolism and growth**, with a wide range of environmental inputs as part of the mTOR complexes.
  - **Epigenetic markers** tell your genes to **switch on or off**.
    - There are two types of markers: **chemical** (e.g., methylation) or **protein** (e.g., histones).
    - Through epigenetic markers, environmental factors like diet, stress, and prenatal nutrition, can make an imprint on genes passed from one generation to the next.

10. WHAT'S ARE THE FUNCTIONAL AND PHENOTYPIC DETAILS FOR EACH VARIANT?

Functional Effects of GRIN/GRIA Variants

- What are the **functional & phenotypic** in vivo (live organism)/ in situ (inside an organism) a consequences of each GRIN / GRIA variant?
  - Continue to do **functional testing** on each identified variant (CFERV, Barcelona GRIN team, etc.)
  - For improved diagnosis and effective treatment, we need better understanding of the function of GRIN/GRIA variants.

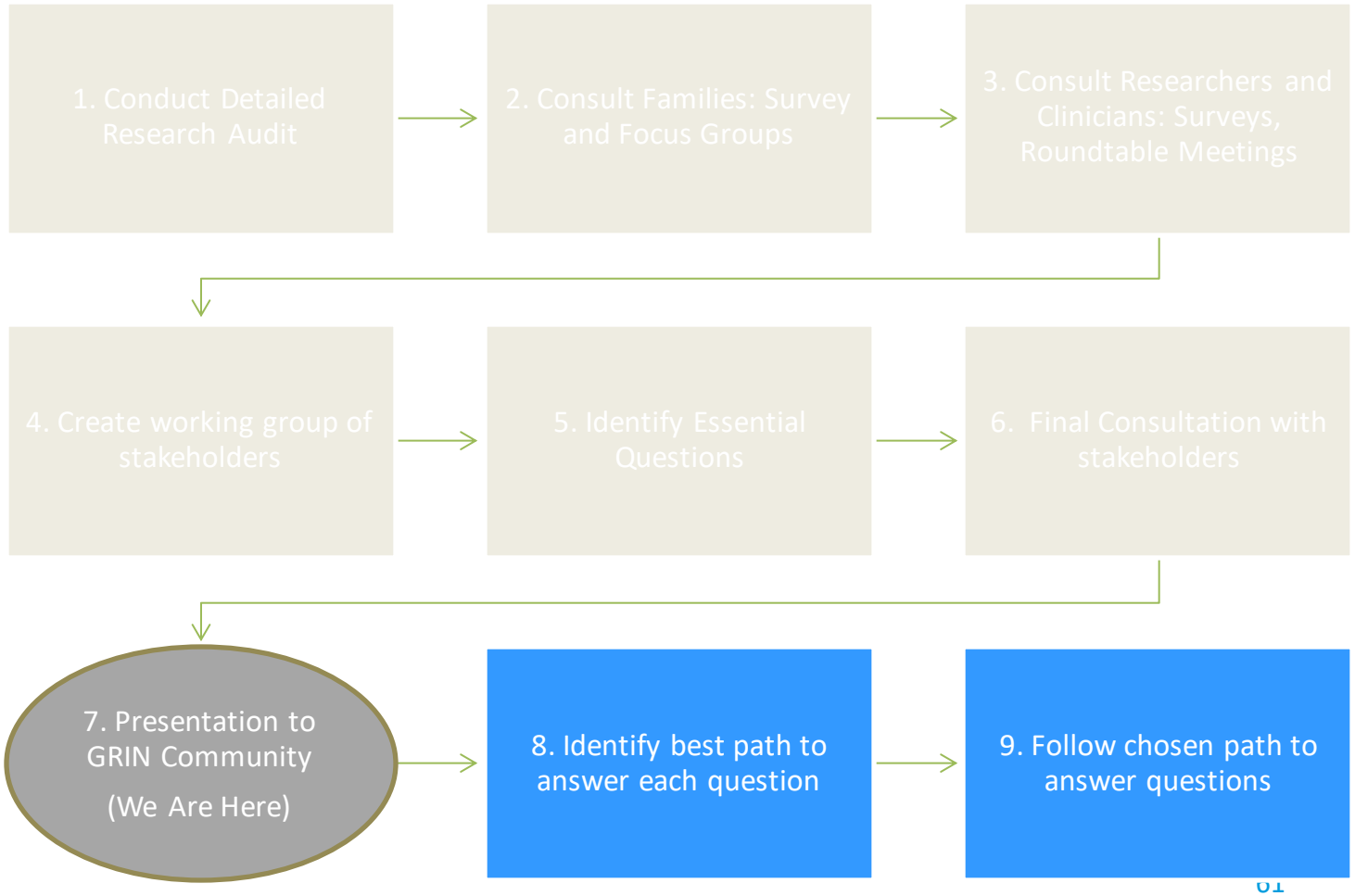
Phenotypic Effects of GRIN/GRIA Variants

- How does a particular variant impact brain development and neuronal / synaptic function / receptor function (e.g. Are there fewer or more receptors / neurons / synapses)?
  - We do not know exactly how the brain's architecture is changed by GRIN/GRIA variants.
  - We can do further analysis on existing animal models, perform natural history studies, and set up a brain donation program for our GRIN Angels to better understand changes in the human brain.

Part 5

WHAT'S NEXT?

Our Research Roadmap Journey



Step 8: Identify Best Path to Answer Each Question

- CureGRIN will collaborate with stakeholders to identify the best path for each of the essential questions.
- This could involve:
  - Setting up a question-specific working group
  - Partnering with other rare disease orgs and / or pharma / biotech
  - Identifying grant opportunities and working with researchers to pursue these
  - Awarding at least $800,000 in grants by April 30, 2022, and millions more over the next three to five years

Step 8: Follow chosen path to answer questions

- Our goal is to answer each question as fully as possible by April 30, 2024
- Whether we make this date (or exceed it) will depend on a number of factors, including:
  - CureGRIN's ability to ramp up fundraising
  - Success in CureGRIN and research partners securing grants around the world
  - Commitment to collaboration across patient advocacy organizations, researchers, clinicians and industry.

APPENDIX A: RESEARCH AUDIT SUMMARY

GRIN Research Audit

CureGRIN Research coordinator Meagan Collins conducted a research audit in 2020. Thanks to the support of multiple researchers and family members who provided support including Dr. Graham Collingridge, Dr. Stephen Traynelis and Dr. Angie Serrano.

What follows is a summary of key findings. The full audit is available here:

[LINK TO COME]

Research Audit: Table of Contents

Research Audit: NMDAR History

1960s

- Glutamate and similar amino acids found to **excite brain cells**.
- Synthesis of N-methyl-D-aspartate (NMDA) (Watkins, 1962).
- John Olney showed that glutamate could also be neurotoxic (**excitotoxicity**).

1970s

- Jeff Watkins and Hugh McLennan synthesized selective **NMDAR antagonists**, such as D-AP5, and used these to prove that **NMDARs contribute to synaptic excitation** in the central nervous system.
- Jeff Watkins and Richard Evans discovered that magnesium ions are potent NMDAR **antagonists**.

1980s

- David Lodge discovered that ketamine and phencyclidine are NMDAR antagonists.
- Graham Collingridge showed that NMDARs trigger changes in synapse strength (i.e., **long-term potentiation**).
- Richard Morris showed that NMDARs are important for **learning & memory**.
- Brain Meldrum discovered that NMDAR could protect against seizures and stroke-induced cell death.
- Single-channel recordings of glutamate receptors (Cull-Candy and Usowicz, 1987; Jahr and Stevens, 1987; Nowak et al., 1984).

1990s

- In 1993, Bliss & Collingridge determined that long-term potentiation was generated by activation of **NMDARs in the hippocampus** (Bliss & Collingridge, 1993).
- The **primary structure** and **genetics** of the NMDAR subunits were identified by cloning, by groups in Japan and Germany.
- Ifenprodil found to be a highly selective antagonist for NMDARs containing the GluN2B subunit, heralding the search for **subtype selective NMDAR modulators**.
- Endogenous steroids, such as pregnenolone, and endogenous cholesterols found to act as negative and/or positive allosteric modulators (**NAMs and PAMs**) of the NMDA receptor.
- Structural analysis of NMDARs (Armstrong et al., 1998).

2000s

- **Memantine** (Namenda) licensed for the treatment of Alzheimer's disease. Scientists at Merz (Germany) showed how this low potency NMDA receptor antagonist could slow the decline in cognition in some patients.
- Discovery of **NMDA receptor encephalitis**. This autoimmune condition is usually due to antibodies raised against the patient's GluN1 and results in psychosis, memory impairments, seizures and dyskinesias.

2010s

- **Structure of the NMDAR determined.**
- (S)-Ketamine licensed for the treatment of depression by Johnson & Johnson

Research Audit: Other diseases connected to functioning of NMDARs

- **Schizophrenia**
  - Lower expression of the GluN1 and GluN2C subunits has been reported in the prefrontal cortex tissue of schizophrenia patients (Weickert et al., 2013; Catts et al., 2016; Bygrave et al., 2019).
  - In addition, the GRIN2A and GRIN2B genes have been identified schizophrenia risk genes (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Allen, 2008; Bygrave et al., 2019).
- **Anti-NMDAR encephalitis**
  - Complex syndrome characterized by neuropsychiatric symptoms and cerebrospinal fluid antibodies against the GluN1 subunit (Dalmau et al., 2019).
- **Major depressive disorder**
  - Excessive activity of NMDARs induced by stressors could result in clinical depression (Marsden, 2011) .
- **Alzheimer's Disease (AD)**
  - NMDAR transmission is affected in AD (Mota et al., 2014).
  - Aβ accumulation may activate NMDARs during the early stages of AD progression (Parameshwaran et al., 2008).
  - GluN2B-containing NMDARs are activated by Aβ, resulting in an increase in calcium (Ferreira et al., 2012).
  - **Memantine** is an FDA approved treatment for moderate to late-stage AD (de Oliveira et al., 2014).
- **Amyotrophic lateral sclerosis (ALS)**
  - Calcium influx by NMDARs can trigger cell death resulting in ALS-related motor neuron death (Peng et al., 1998; Nguyen et al., 2011).
- **Huntington's Disease (HD)**
  - Higher extrasynaptic NMDAR activity and dysregulated intracellular calcium signaling persists in early HD (Cowan et al., 2008; Okamoto et al., 2009).
- **Parkinson's Disease (PD)**
  - The abundance of GluN1 and GluN2B subunits of NMDARs in PD is decreased in striatal membranes (Johnson et al., 2009).

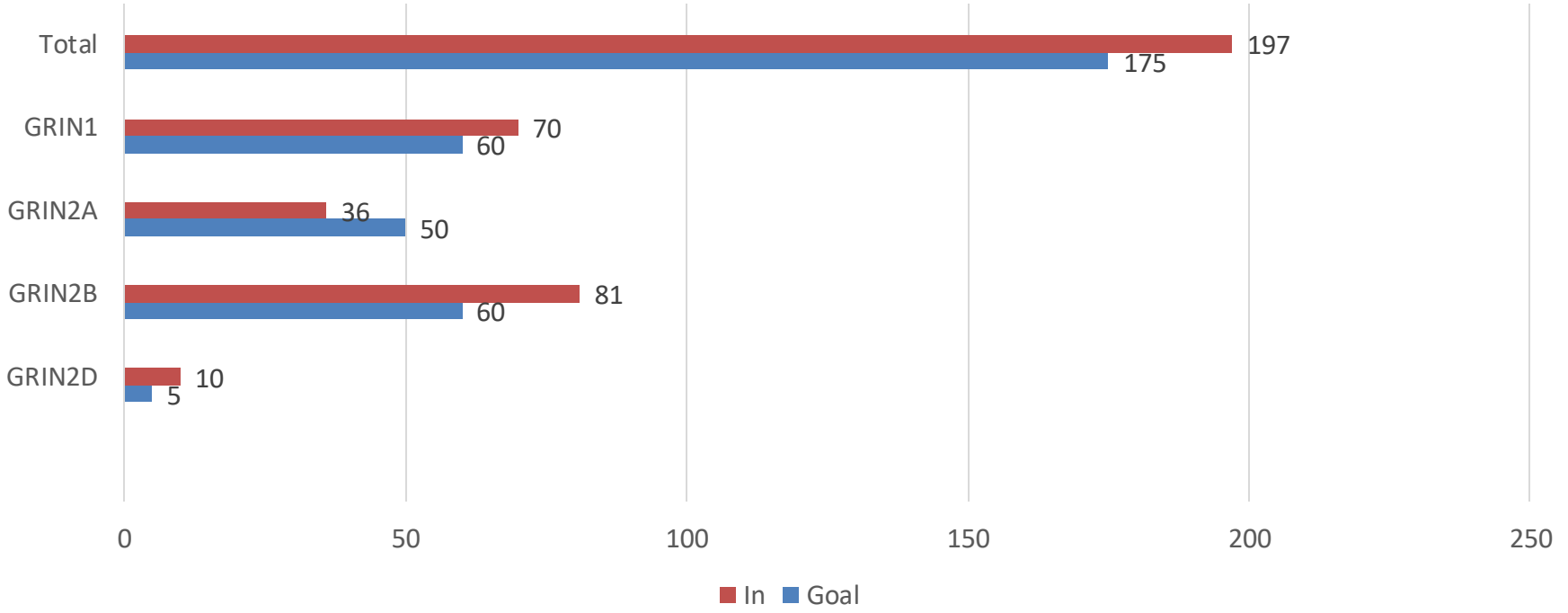
Research Audit: NMDAR expression in other cells/tissues

- NMDARs are expressed in **neuronal cells** and non-neuronal cells which include **glial cells, endothelium, bone, kidney, pancreas**, etc. (Hogan-Cann & Anderson, 2016).
- Functional NMDARs are expressed by astrocytes which are adept to responding to glutamatergic input and **neuroinflammatory** processes (Dzamba et al., 2013; Sofroniew, 2009; Ting et al., 2009).
- Endothelial NMDARs are found to contribute to the **functioning of the blood–brain barrier (BBB)**. When glutamate levels are unregulated in brain, this can be toxic to neurons, can **damage the functioning** of the endothelium and the BBB (András et al., 2007; Basuroy et al., 2013).
- NMDARs expressed by osteoblasts have been shown to lead to higher **bone mineralization** and **deposition of bone matrix** (Hinoi et al., 2003; Li et al., 2011).
- Renal NMDAR activity has been shown to **stimulate vasodilation** in the glomerulus, which influences blood flow, filtration, and reabsorption in the proximal tubule in the renal system (urinary system) (Deng and Thomson, 2009; Anderson et al., 2011; Sproul et al., 2011).
- NMDARs expressed by **insulin-producing islet β cells** in the pancreas and contribute to the function of β cells (Inagaki et al., 1995; Molnár et al., 1995; Marquard et al., 2015).
- **Smooth muscle cells express NMDARs in the lung** and may contribute to inflammatory bronchiole hyper-reactivity (Antošová and Strapková, 2013; Anaparti et al., 2015).

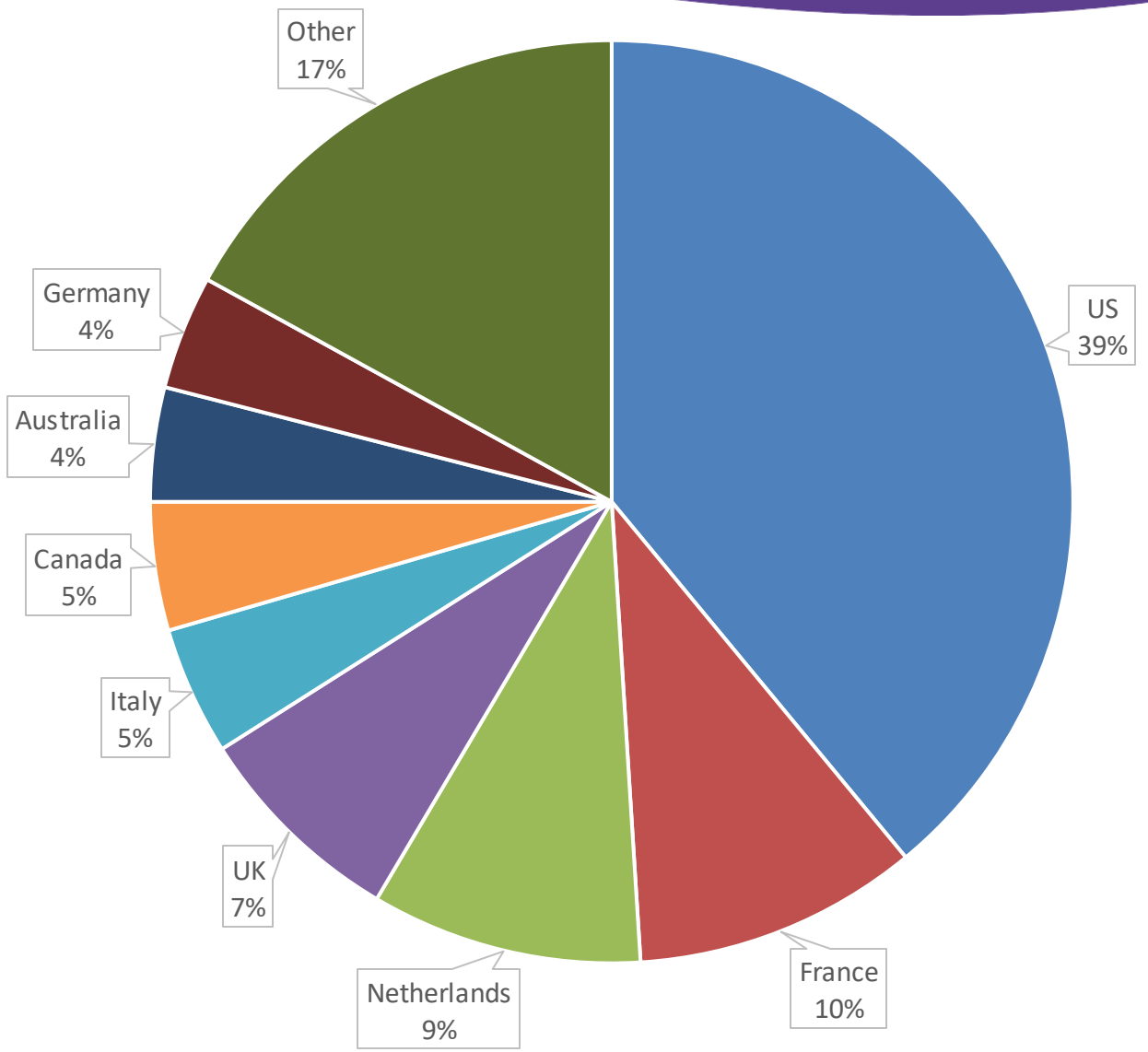
Appendix B

DETAILED FAMILY SURVEY RESULTS

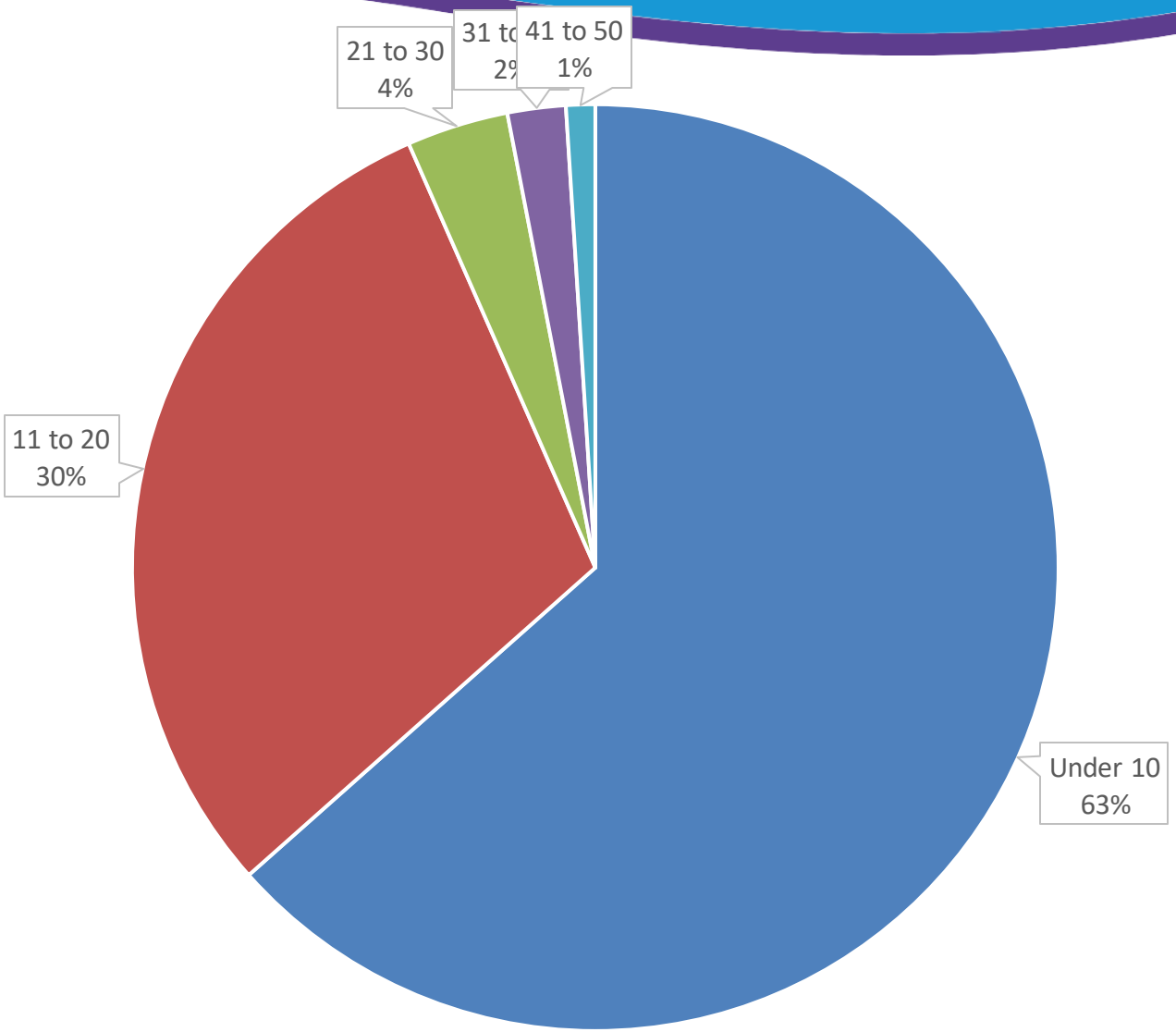
GRIN Survey Submissions by gene



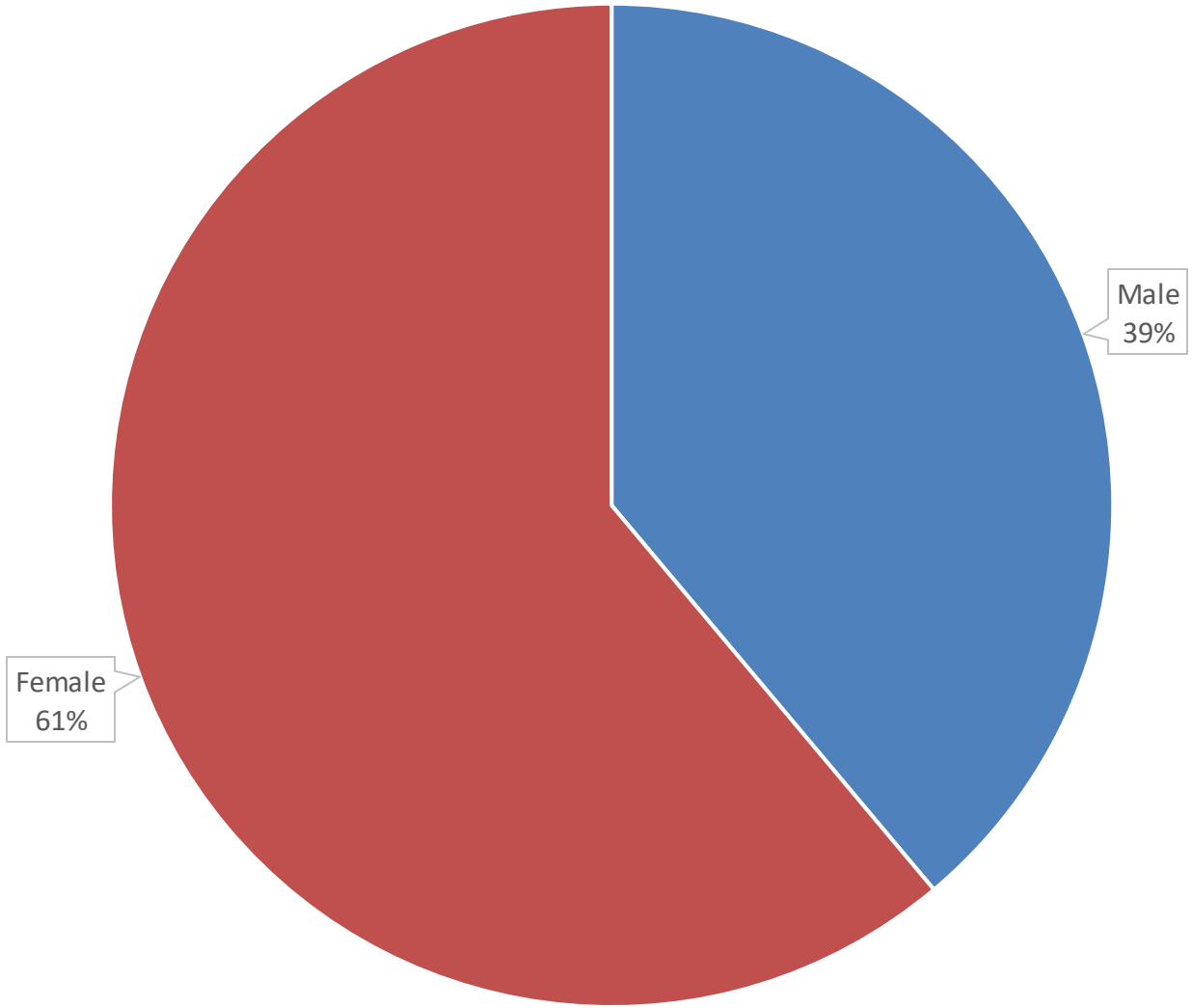
Responses from Five Continents



Age of Patients

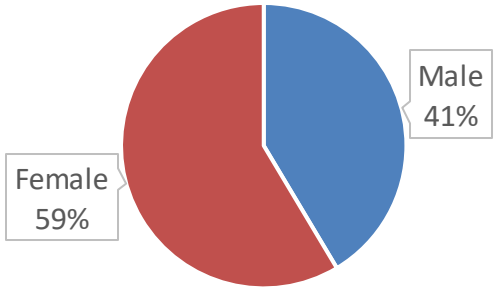


Sex of Patients

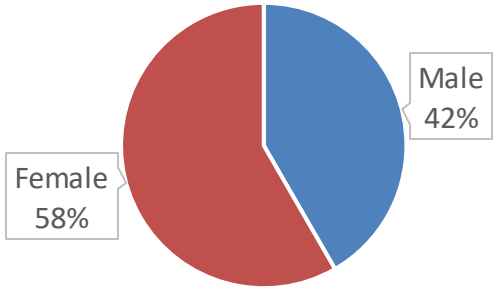


Sex by Gene

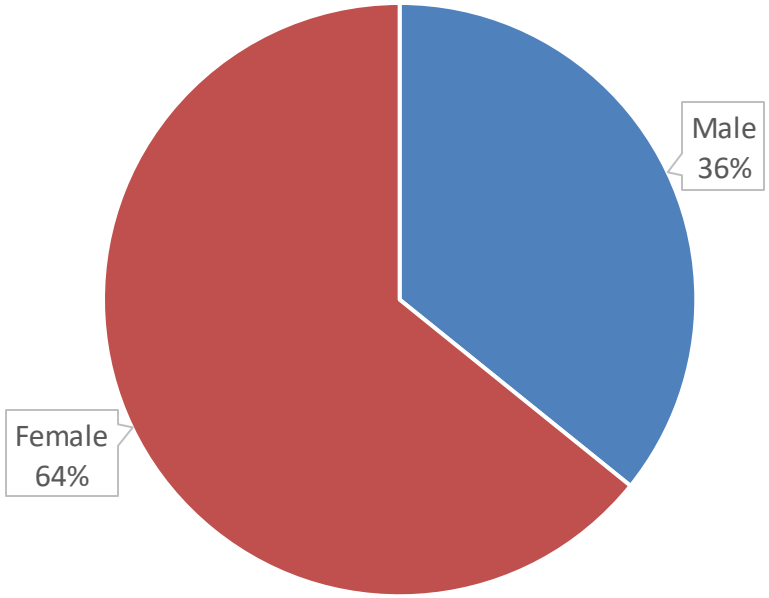
GRIN1



GRIN2A



GRIN2B



What symptoms does your child have? Please include all symptoms even if their connection to GRIN Disorder is unknown.

All GRIN (n=197)

1. **Intellectual Disability (91%)**
2. **Speech (80%)**
3. **Low Muscle Tone (72%)**
4. **Sleep Challenges (66%)**
5. **Mood / Behavior (53%)**
6. **Epilepsy / Seizures (49%)**
7. **Mobility Impairment (49%)**
8. **Constipation (50%)**
9. **Visual Impairment (45%)**
10. **Digestive (39%)**
11. **Neurostorms (33%)**
12. **Feeding Tube (22%)**
13. **Breathing (12%)**
14. **Hearing Impairment (5%)**

GRIN1 (n=71)

1. ID (94%)
2. Speech (84%)
3. Sleep (74%)
4. Mobility (73%)
5. Low Muscle Tone (71%)
6. Epilepsy (59%)
7. Constipation (59%)
8. Visual Impairment (54%)

GRIN2B (n=80)

1. ID (96%)
2. Speech (84%)
3. Low Muscle Tone (74%)
4. Mood / Behavior (65%)
5. Sleep (63%)

GRIN2A (n=36)

1. ID (74%)
2. Epilepsy (69%)
3. Speech (69%)
4. Low Muscle Tone (63%)
5. Sleep (63%)
6. Mood/behavior (51%)

GRIN2D (n=10)

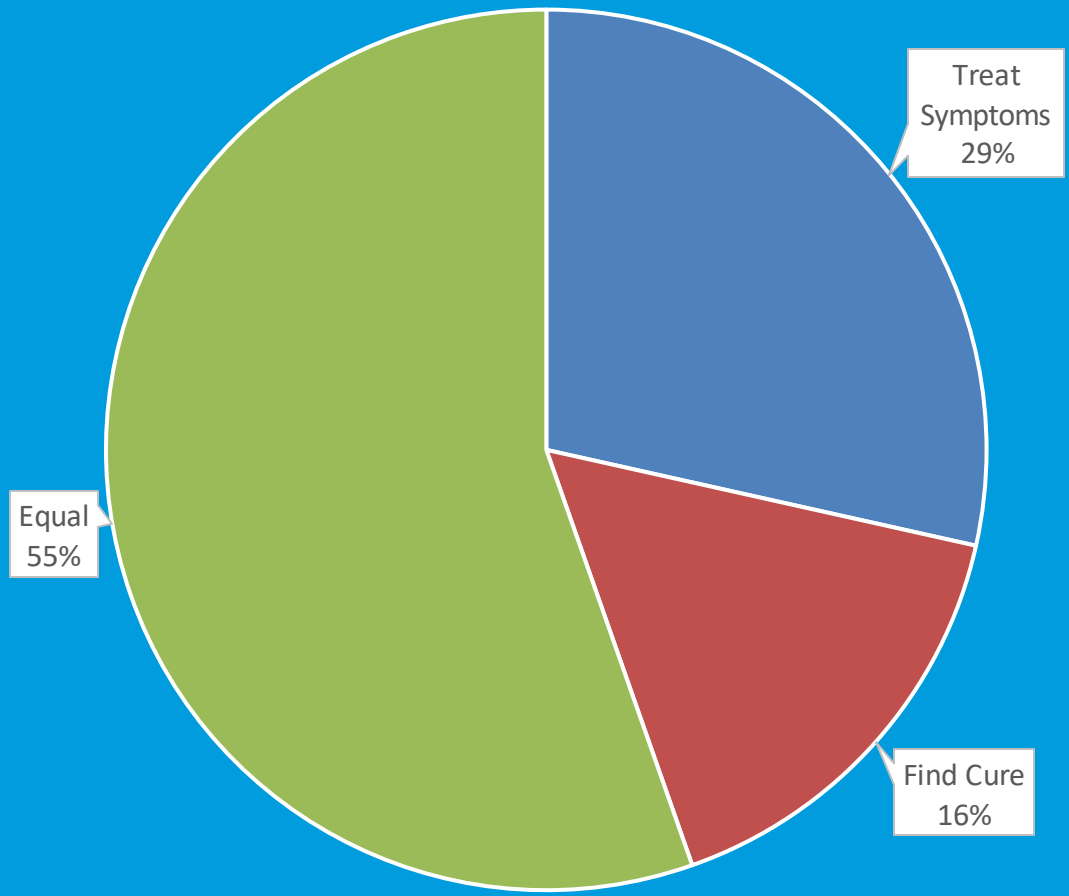
1. Low Muscle Tone (80%)
2. ID (80%)
3. Epilepsy (80%)
4. Mobility (80%)
5. Visual Impairment (70%)
6. Speech (70%)

* Responses >50% included here

Balance Cure and Treatments

GRIN families want us to prioritize both finding long-term cures and shorter term treatments for symptoms.

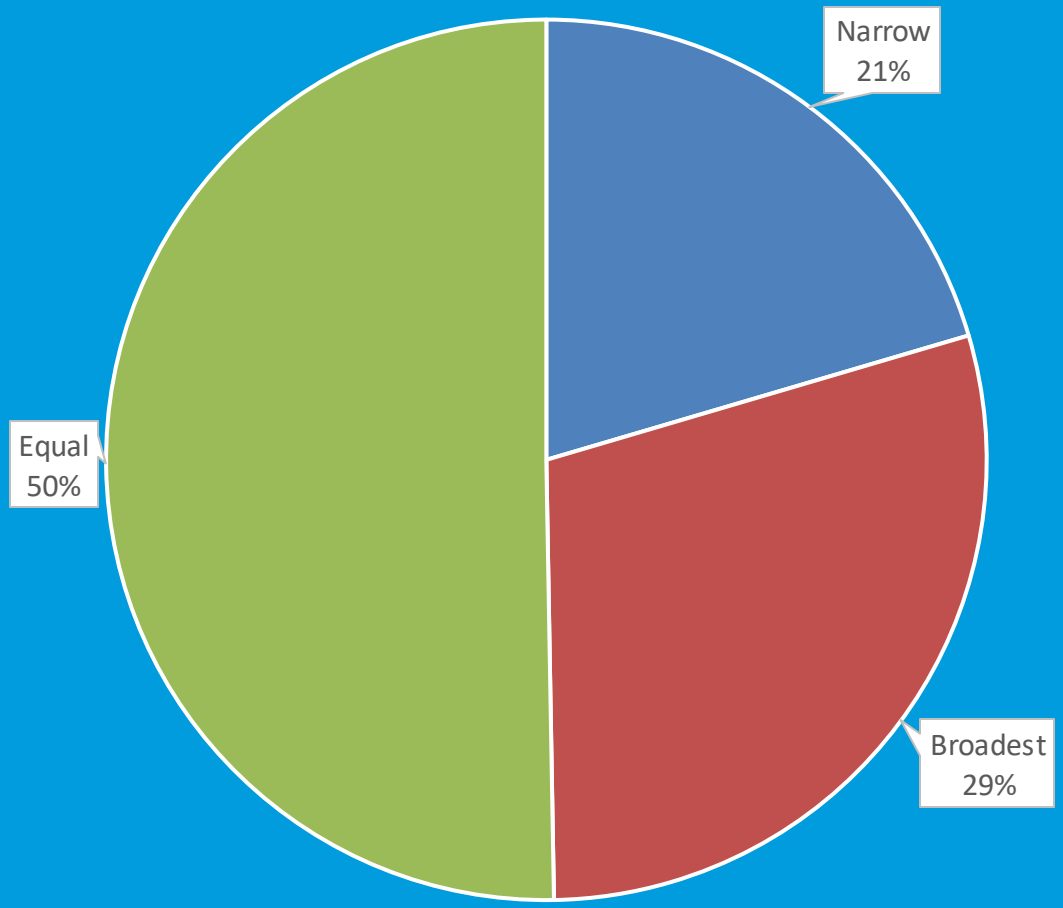
If we have to choose, a slight edge to treatments (56%) over cures (44%)



Balance Cure-all with Cure-some

GRIN families want us to balance taking our time on long-term solutions that help the most kids vs. finding faster treatments or cures that help a smaller number

If we have to choose, a slight edge to a broad focus (54%) over faster for smaller group (46%)



Where GRIN Families Want Us to Focus

Tier	SINGLE MOST IMPORTANT SYMPTOM TO TREAT	All GRIN (n=197)	GRIN1 (n=72)	GRIN2A (n=36)	GRIN2B (n=80)	GRIN2D (n=10)	ALL GRIA (n=15)
1	Intellectual Disability / Communication	44%	43%	23%	56%	30%	14%
1	Epilepsy	22%	20%	43%	10%	60%	40%
1	Mood / Behavior / Neurostorms	17%	16%	20%	20%	0%	40%
2	Feeding & Digestion	5%	7.2%	0	1.2%	0	0%
2	Mobility	5%	5.7%	5.7%	2.5%	10%	0%
2	Sleep	4%	4.3%	2.9%	3.7%	0%	0%
3	Other	Breathing (1.5%), Low Muscle Tone (1.5%), Vision (0.5%), Hearing	Breathing (2.5), Low Muscle Tone (1.4%),	Breathing (2.8%), Low Muscle Tone (2.8%)	Low Muscle Tone (1.3%), Hearing (1.3%)	NA	Low Muscle Tone (7%)

Appendix C

SUMMARY OF ALL RESEARCH QUESTIONS CONSIDERED

Summary

- Members of the Working Group and Scientific Advisory Board were asked to score each question between 0 (not very important) and 3 (not important at all)
- In the following slides the highest ranked questions are marked in red, the middle tier in black and the lowest tier in grey.

NMDA Structure, Assembly and Function

- Are there situations where individual proteins get made, but tetramers get degraded? What is result? Fewer receptors? More wildtype receptors? More receptors made with alternate 2/3 proteins? (2.60)
- What is structure and function of C-terminal domain (CTD) and what is its role in disease? How sensitive is the CTD to feedback control (particularly when intracellular Ca2+ is high)? (2.60)
- How do GRIN subunits interact? How does a mutation impact the function of the other subunits? (2.56)
- What interactions are taking place at the transmembrane (TMD)? (2.53**)**
- Which subunit proteins are freely available, and which are created at slower rates, limiting the assembly of the tetramer? (2.47)

Mechanisms & Pathways

- Are certain neuron types more affected than others? (e.g. inhibitory vs. excitatory) (2.69)
- Are some brain regions more affected than others by disease-causing variants? (2.63)
- Could boosting 2A / 2C / 2D/ 3A / 3B expression help for 2B patients and vice versa? (2.63)
- Why is phenotype so similar for GOF & LOF? Is it because brain has wired around the disease or another reason? (2.59)
- Which symptoms can be ameliorated by combinations of substances that together target 1) Oxidative stress 2) Neuroinflammation 3) Mitochondrial dysfunction or Nutrient signaling (IGF, mTOR, etc.) 4) Excitatory / Inhibitory Imbalance 5) Methylation & other epigenetic markers

Understanding GRIN Beyond the Brain

- How is GRIN manifesting itself in other parts of the body? (2.63)
- GRIN genes are highly expressed in gut. Is this playing role on gastro symptoms? Effect on microbiota? (2.60)
- Is hypotonia caused by NMDA receptors the brain or in muscle cells? (And are respiratory issues caused by brain-related hypotonia or localized in respiratory system?) (2.56)
- Can we find biomarkers in blood, skin cells or cerebral spinal fluid? (2.38)
- What is the relationship of CNS to non-CNS (or even non-neuronal) NMDARs in the pathobiology? (2.27)
- GRIN genes are highly expressed in heart cells. What are effects? (2.27)
- Effects of NMDARs in heart / vascular system? (2.27)

Symptoms

- How can we rescue / improve Intellectual Disability and Speech? (2.81)
- How can we rescue / improve epilepsy? (2.81)
- How can we rescue / improve mood, behavior and neurostorms? (*)
- What are the underlying cellular, molecular and network mechanisms for the various symptoms? (2.75)
- What is the phenotype (in a mouse model) of a given variant and what is the underlying molecular basis? Which can we map to human symptoms / outcomes? (2.69)
- What symptoms can we realistic address or target? (2.67)
- How can we rescue / improve feeding & digestion? (2.63)
- How can we rescue / improve mobility? (2.69)
- How can we rescue / improve sleep? (2.56)

Ability to reverse deficits / Timing of Treatment

- Can we reverse deficits (safely) with genetic or pharmacological treatments or have the GRIN deficits altered circuitry in a permanent fashion? (2.81)
- Can adult mice with 2A, 2B, 2D, GRIA be phenotypically rescued like they can for GRIN1? What about GRIN1 GOF? (2.71)
- Are there cellular and molecular mechanisms that are in common that can drive early therapeutic interventions?(2.50)
- What is the developmental origin of GRIN Disorders? (1.86)
- When is the right time to start therapies - prenatal? Can there be a positive effect regardless of when therapies begin? (2.44)
- How to delay or prevent symptoms? Are the symptoms reversible, or is it essential to provide a treatment before they appear? (2.38)
- How Can we Promote Early Diagnosis? (2.38)

Understanding Potential treatments

- What are suitable outcome measures to determine benefit from treatment? How should these be measured for future pre-clinical studies / clinical trials? (2.94)
- Will NMDAR agonists / antagonists / PAMs / NAMs prove useful for symptom management? (2.79)
- Precision Medicine: What drug/therapy is needed for specific variants in the different GRIN genes? (2.73)
- What existing and approved drugs can be repurposed for GRIN? (Would we need high-throughput screening for each separate variant?) (2.69)
- What substances can be used to modulate calcium inflow through the NMDAr? (2.33)
- Can we target promoters / enhancers of GRIN genes? (2.20)

Understanding DNA / RNA Treatments

- Where is best place to deliver gene therapies to CNS or brain region? (2.64)
- GRIN1 can easily fit into AAV. How could other genes be delivered? (2.60)
- Can allele-specific knockdown of GRIN RNA be used therapeutically? (2.33)
- Can CRISPR-based gene therapy restore function in adult mice with pathological human NMDAR variants? If so, how can this be optimized? (2.07)

Understanding Variants

- What are functional & phenotypic in vivo / in situ consequences of each GRIN variant? Can they be categorized into defined groups? (2.73)
- How does a particular variant impact brain development and neuronal / synaptic function / receptor function (e.g. Are there fewer or more receptors / neurons / synapses)? (2.69)
- Are there robust biomarkers that can stratify patients into groups that predict effective treatment strategies (ie loss of function, gain of function, complex gain/loss phenotype)? (2.67)
- If variant is disease-causing but functional analysis shows no change in electrophysiology, what is mechanism of disease? (2.40)
- Can we profile the full biological effects (phenotype) of the deleterious NMDAR variants we find in patients? (2.40)

Understanding Variants (cont.)

- Are there etiological underpinnings in common among gain-of-function GRIN mutations? Are there etiological underpinnings in common among loss-of-function GRIN mutations? (2.36)
- What is the neuronal network net effect of GRIN-variants? (2.27)
- Are there verified GRIN2C, GRIN3A or GRIN3B variants? (1.93)

1. Defining Success

- What are suitable **outcome measures** to determine benefit from treatment? How should these be measured for future pre-clinical studies / clinical trials?
- Can we find **biomarkers** in blood, urine, skin cells, cerebral spinal fluid, brain waves, etc?

2. Is a Cure Possible?

- Can we **safely reverse deficits** with genetic or pharmacological treatments?
  - Have the deficits altered circuitry in a permanent fashion?
  - Has the human brain wired around deficits in such a way that reversal has unanticipated outcomes?

3. Gene Therapies

- Where is **best route of administration for delivery of** gene therapies? Through CNS? Intrathecal? To specific brain regions?
- GRIN1 and GRIA genes like fit into AAV. What is the **best vehicle to deliver** GRIN2 genes?

4. Pharmacological therapies

- Will NMDAR / AMPAR **agonists / antagonists / PAMs / NAMs** prove useful for symptom management?
- What approved drugs and late-stage development candidates can be **repurposed** for GRIN / GRIA? (Would we need high-throughput screening for each separate variant?)

5. Symptoms

- What harm is caused by variant NMDARs / AMPARs **outside of the brain**?
  - Is hypotonia caused by NMDA receptors the brain or in somatic nervous system? Are respiratory issues caused by hypotonia originating in brain neurons or localized in respiratory system?
  - GRIN genes are highly expressed in gut. Is this playing role on gastro symptoms? Effect on microbiota?
  - GRIN appears to be highly expressed in the heart. What are consequences?
- Which symptoms can be ameliorated by combinations of substances that together target **downstream effects** including 1) Oxidative stress 2) Neuroinflammation 3) Mitochondrial / nutrient signaling dysfunction (IGF, mTOR, etc.) 4) Excitatory / Inhibitory Imbalance 5) Methylation & other epigenetic markers

6. Understanding Variants

- What are **functional & phenotypic** in vivo / in situ consequences of each GRIN / GRIA variant?
  - Can they be categorized into defined groups?
  - How does a particular variant impact brain development and neuronal / synaptic function / receptor function (e.g. Are there fewer or more receptors / neurons / synapses)?
  - How?
    - Functional Analysis
    - Natural History Studies