"Treat the Symptoms. Cure the Disease"
CureGRIN's Research Roadmap
June 5, 2021

CureGRIN
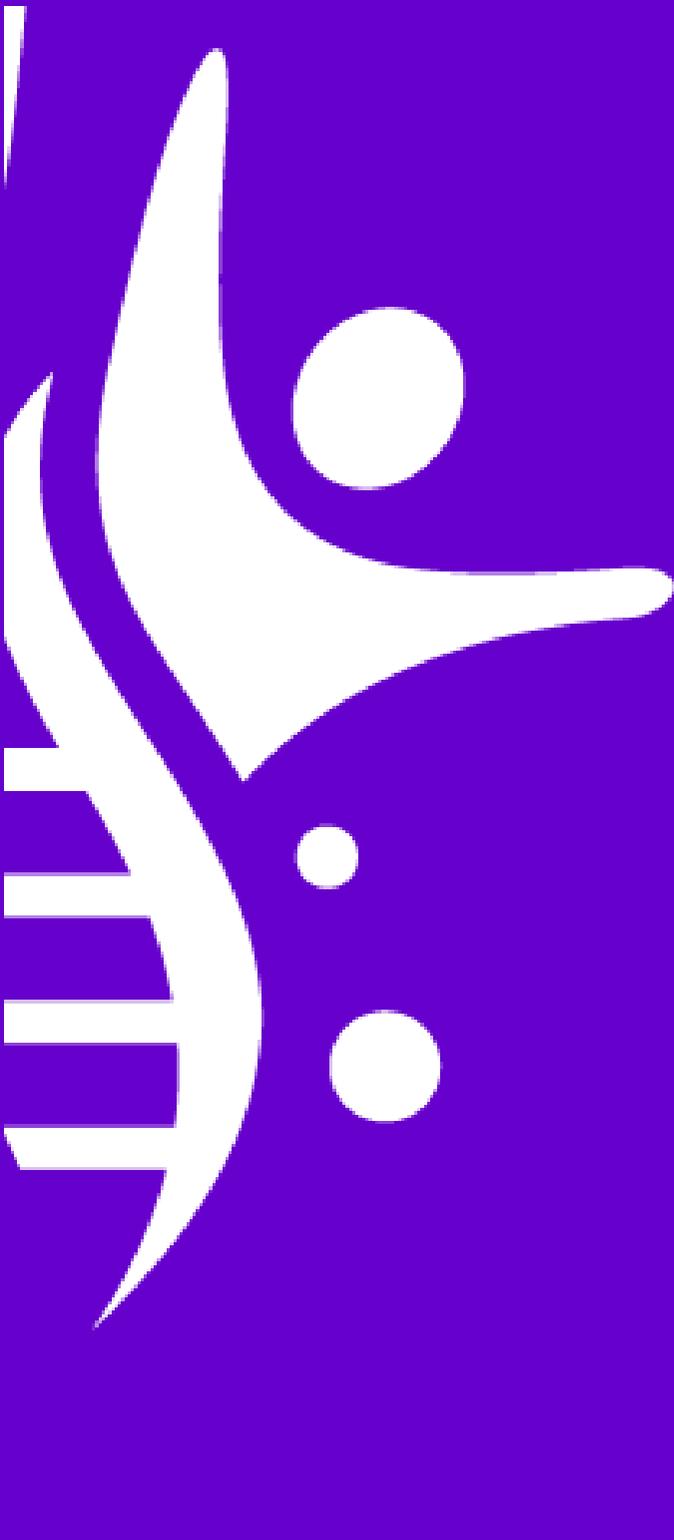### Funding Research for GRIN Disorder

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

info@curegrin.org

Treat the Symptoms.
Cure the Disease.

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EXECUTIVE SUMMARY

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.

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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:

• Intellectual Disability / Speech
• Epilepsy
• 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 functions 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 and molecules** 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

1. Conduct Detailed Research Audit → 2. Consult Families: Survey and Focus Groups → 3. Consult Researchers and Clinicians: Surveys, Roundtable Meetings

4. Create working group of stakeholders → 5. Identify Essential Questions → 6. Final Consultation with stakeholders

7. Presentation to GRIN Community (We Are Here) → 8. Identify best path to answer each question → 9. Follow chosen path to answer questions
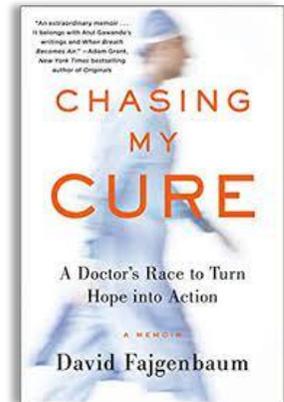
Research Roadmap Working Group Membership

- Keith McArthur, CureGRIN (Chair)
- Meagan Collins, CureGRIN
- Jillian Hastings-Ward, CureGRIN
- Liz Marfia-Ash, GRIN2B Foundation
- Carole Quennessen, GRIN2B Foundation
- Sandra Silva, GRIN2B Europe
- Amela Huskic, GRIN2B Europe
- Heather Cartwright, GRIA family representative
- Dr. Steve Traynelis, 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.
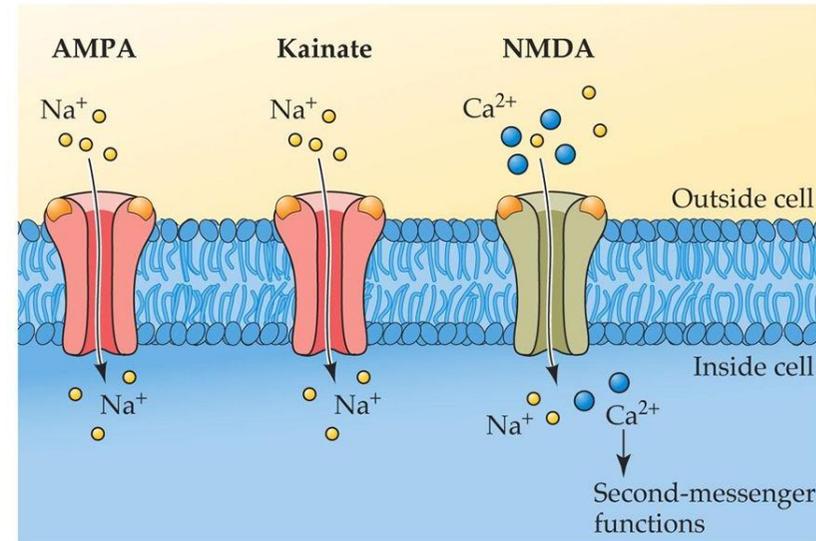
PSYCHOPHARMACOLOGY, Figure 7.4 © 2005 Sinauer Associates, Inc.

GRI–AMPA (GRIA1, GRIA2, etc.)

GRI–NMDA (GRIN1, GRIN2A, etc.)

GRI–Kainite (GRIK1, GRIK2 etc.)

NOTE: GRID genes play a role in the refinement of synapse formation and are part of the same family, but they may not function as ion channels in the same way (under investigation).

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%)

Treat Symptoms 29%

Find Cure 16%

Equal 55%

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%)

Narrow
21%

Broadest
29%

Equal
50%

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 (0.5%)	Breathing (2.5), Low Muscle Tone (1.4%), Vision	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.

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

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

- Families also also overlap between symptoms (eg. Mood / Behaviour and Neurostorms) allowing us to consolidate our categories.

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**

Key Considerations for Gene Therapy

- Many types of gene therapy (including CRISPR and Antisense Therapy) require a personalized medicine for each variant.
- Many hundreds of variants have already been identified that cause GRI Disorder.
- GRI Disorder is usually heterogenomic, meaning the patient has one typical (wildtype) gene and one mutated gene.
- The two most common genetic varieties of GRI Disorder are:
  1. Both proteins get made. But the mutant ones cause harm in receptors.
  2. Only the "good" proteins get made, but there aren't enough of them for proper function of receptors.
- *The ideal gene therapy would require just one medicine per gene regardless of variant or genetic variety.*

Heterogenomic GRIN variant

Wildtype gene   **Variant gene**

Both proteins get made. But the mutant ones cause harm in receptors.

or

Only the good proteins get made. But there aren't enough.
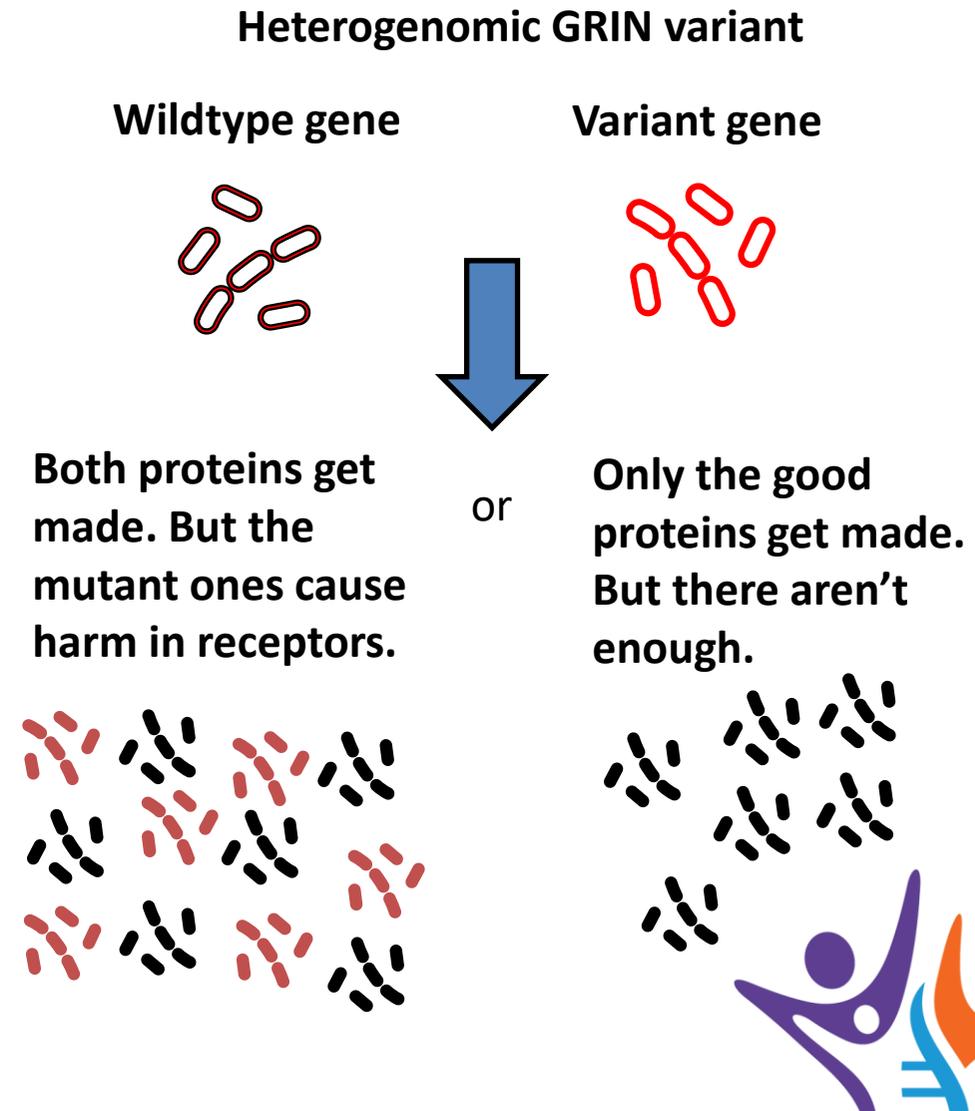
Knockdown and "Replace" Gene Therapy How It Works

KNOCKDOWN   **"REPLACE"**   **Rescued GRIN Expression**

Gene therapy is delivered through the central nervous system or directly into the brain.

The therapy contains two instructions.
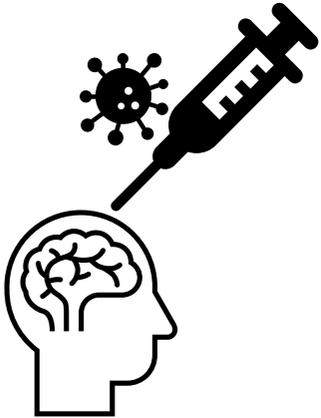
First, it tells the gene to stop making proteins from both copies of the patient's genes.

A small number may sneak through

Second, it introduces and boosts a synthetic copy of the gene.
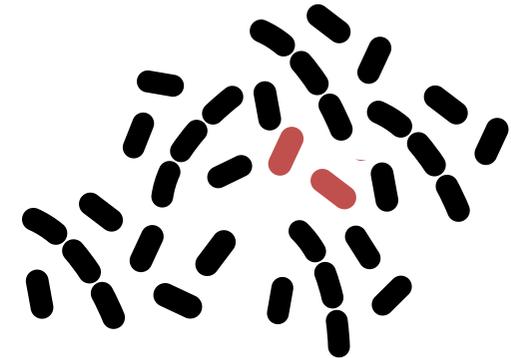
The result is that while some mutant proteins could still get made, they are vastly outnumbered by the wildtype proteins.

Knockdown and "Replace" Advantages and Questions

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

The 10 Essential Questions

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?
5. How can we deliver gene therapies for **larger genes**?
6. What are optimal **drugs and molecules** 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**?
10. What's are the **functional and phenotypic details** for each variant?

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. to indicate if treatments are working or to diagnose patients?

  – Biomarkers can be measured as an indicator of normal biological processes, pathogenic physiological processes, or indicate responses to a drug or other therapy.

  – Examples: seizure likelihood, 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 gray matter tissue in the brain).
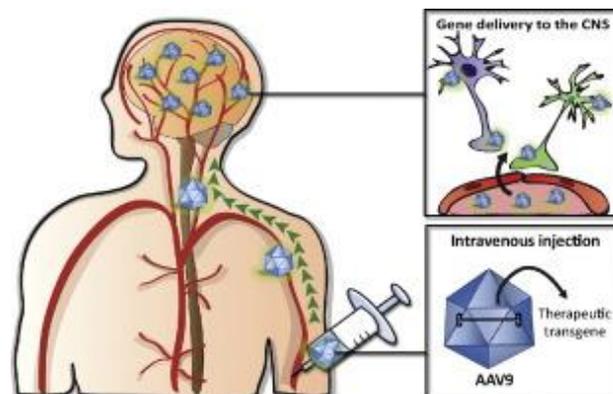
(Saraiva et al., 2016)

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's protein-coding nucleotides. (Largest splice variant)
GRIN1	GLuN1	9	2.8kb
GRIN2A	GLuN2A	16	4.3kb
GRIN2B	GLuN2B	12	4.4kb
GRIN2D	GLuN2D	19	4.0kb
GRIA1	GLuA1	5	2.7kb
GRIA2	GLuA2	4	2.7kb
GRIA3	GLuA3	X	2.7kb
GRIA4	GLuA4	11	2.7kb
GRIK2	GLuK2	6	2.7kb

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**
  - GRIN1 gain-of-function (c.1923G>A, p.Met641Ile)
    - Memantine reduced seizure burden (Xu et al., 2021).
  - 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**
  - GRIN2B gain-of-function mutations (Xenopus oocytes).
  - Negative allosteric modulator (NAM) of GluN2B-containing NMDA receptors (Mullier et al., 2017)
  - Infantile spasm syndrome:
    - Phase I completed
    - Phase II trial in patients with drug-resistant infantile spasms was not completed, due to low number of enrolled patients (Auvin et al., 2020).
    - One patient became spasm-free with treatment and two patients showed clinical improvement (Auvin et al., 2020).

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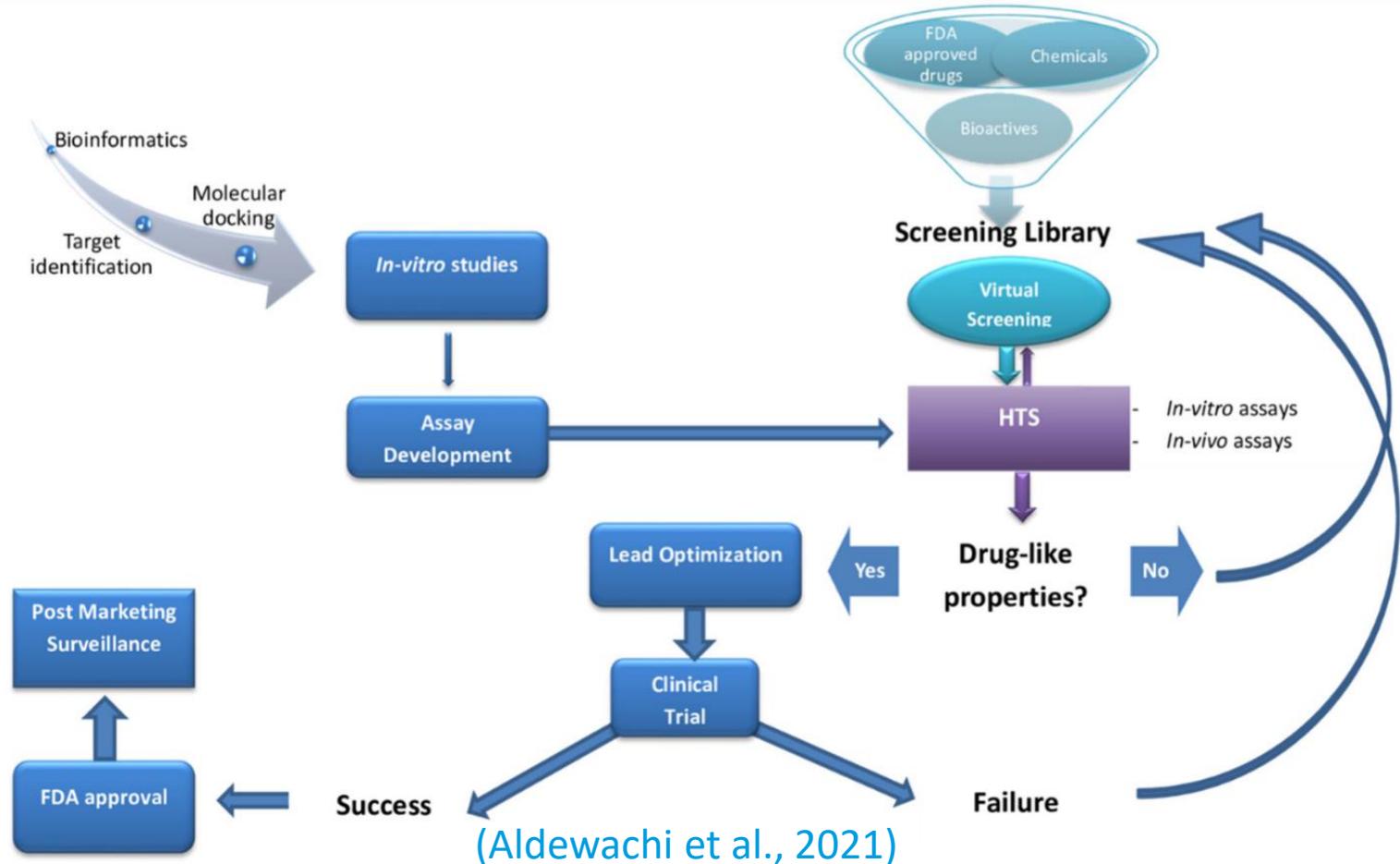
8. WHICH SYMPTOMS ARE DUE TO RECEPTORS OUTSIDE THE BRAIN?

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, yet expression in other tissues is lower.

NMDARs Beyond the Brain

- For example, is hypotonia caused by NMDARs in the brain or the somatic nervous system? Are respiratory issues caused by hypotonia originating in brain neurons or localized in respiratory system?

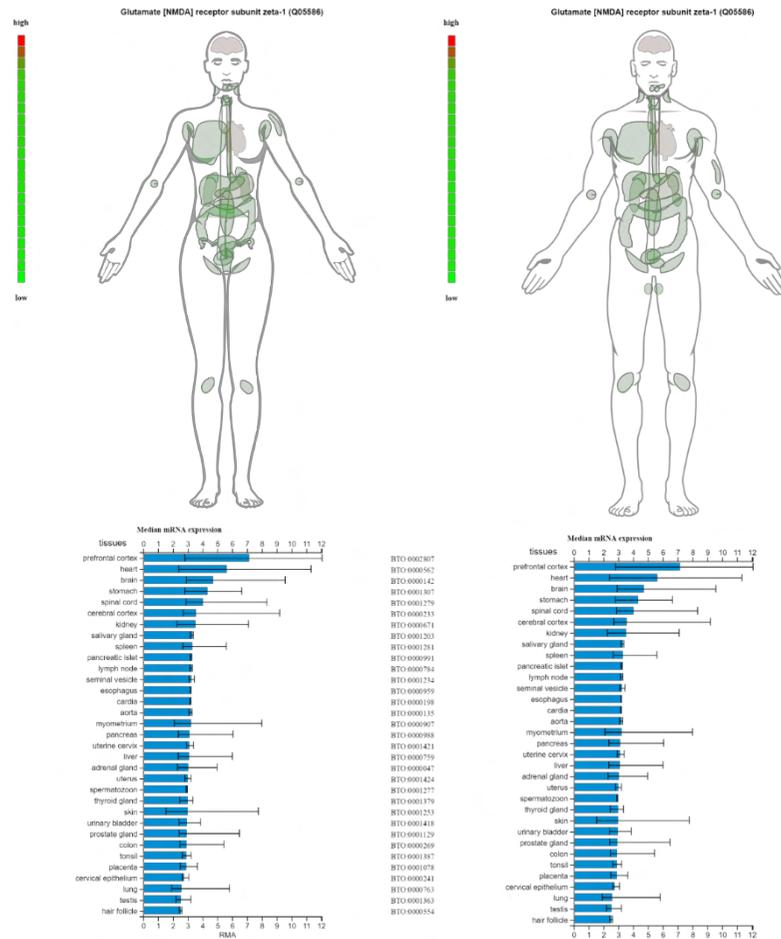- NMDARs are highly expressed throughout the central nervous system (CNS), but less information is known about NMDAR function outside of the CNS.

  - 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

- 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 lack of balance of free radicals (oxygen-containing molecules with electrons) and antioxidants in the body, which can lead to cell and tissue damage.
  - **Neuroinflammation** is an **immune response** of cells in the brain. Some inflammation protects neurons from damage, but too much **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 key cell growth regulator that is important for **cellular metabolism and growth**.
  - **Epigenetic markers** tell your genes when to **turn on or off**.
    - Two types of epigenetic markers: **chemical** (e.g., methylation) and **protein** (e.g., histones).
    - By way of epigenetic markers, **environmental factors** (e.g., diet, stress, prenatal nutrition, etc.) can imprint on genes that can be passed onto the next generation.

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.
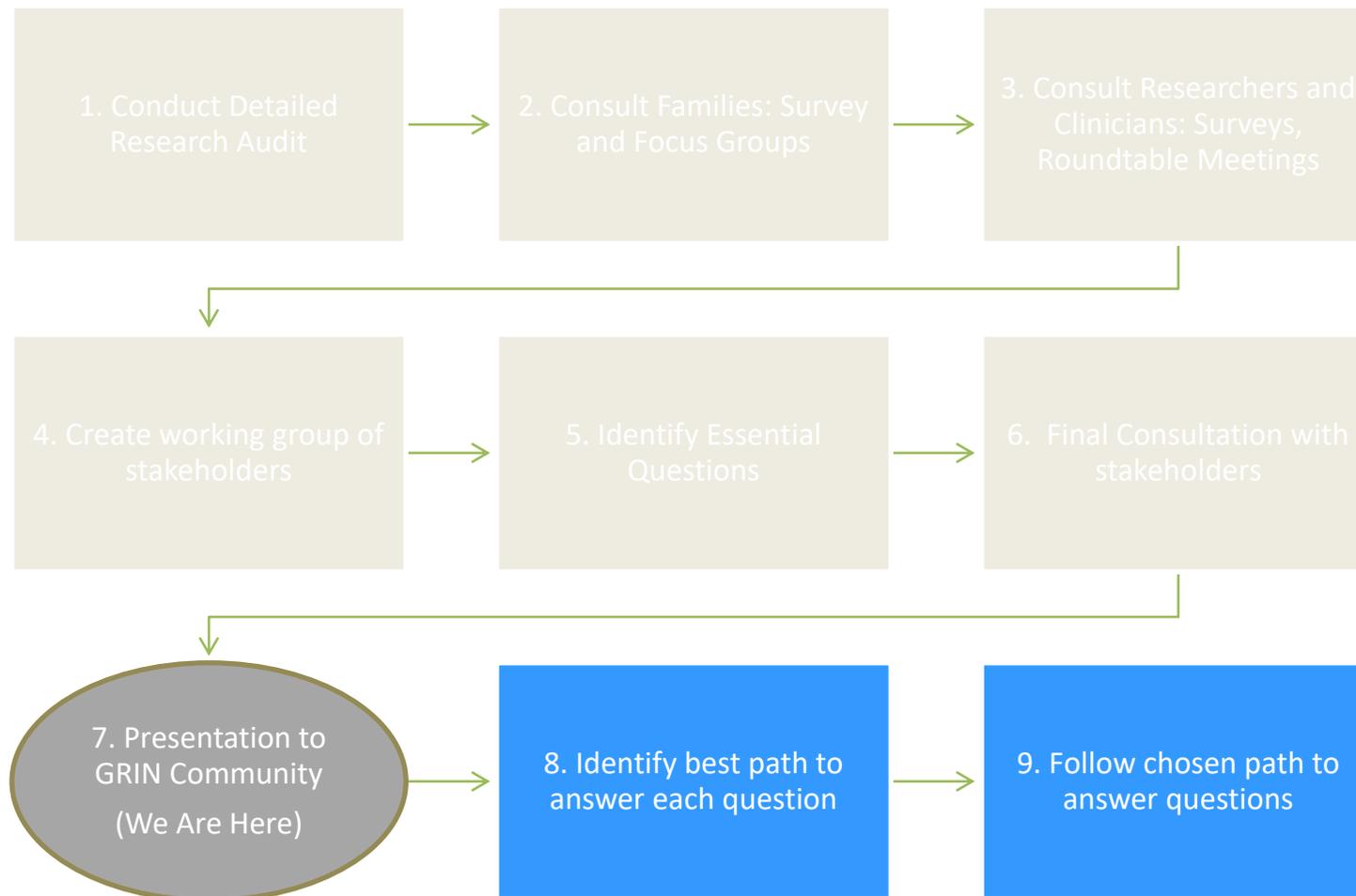
Part 5

WHAT'S NEXT?

Our Research Roadmap Journey

```
┌─────────────────────┐      ┌─────────────────────┐      ┌─────────────────────┐
│ 1. Conduct Detailed │ ───► │ 2. Consult Families:│ ───► │ 3. Consult          │
│    Research Audit    │      │    Survey and Focus │      │    Researchers and  │
│                     │      │    Groups           │      │    Clinicians:      │
│                     │      │                     │      │    Surveys,         │
│                     │      │                     │      │    Roundtable       │
│                     │      │                     │      │    Meetings         │
└─────────────────────┘      └─────────────────────┘      └─────────────────────┘

┌─────────────────────┐      ┌─────────────────────┐      ┌─────────────────────┐
│ 4. Create working   │ ───► │ 5. Identify         │ ───► │ 6. Final            │
│    group of         │      │    Essential        │      │    Consultation     │
│    stakeholders     │      │    Questions        │      │    with             │
│                     │      │                     │      │    stakeholders     │
└─────────────────────┘      └─────────────────────┘      └─────────────────────┘

   ⬭                         ┌─────────────────────┐      ┌─────────────────────┐
 7. Presentation to    ───►  │ 8. Identify best    │ ───► │ 9. Follow chosen    │
 GRIN Community              │    path to answer   │      │    path to answer   │
                             │    each question    │      │    questions        │
 (We Are Here)               └─────────────────────┘      └─────────────────────┘
```

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 9

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:

Willingness of GRIN community to raise funds	Success in CureGRIN and research partners securing grants	Commitment to collaboration across patient advocacy organizations, researchers, clinicians and industry.

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HOW YOU CAN HELP US ACHIEVE OUR PLAN

SCIENTISTS AND PHYSICIANS

1 **BE ESSENTIAL**
Consider how to nudge your research towards the 10 Essential Questions.

2 **KEEP US INFORMED**
Let us know how your research is going.

3 **WORK WITH US**
Let us know if your planning to apply for GRIN-related research grants. We can help!

4 **COLLABORATE**
Participate in GRI Research Roundtable Discussions.

5 **STAY CONNECTED**
Join our mailing list at curegrin.org/newsletter and follow us on social media

HOW YOU CAN HELP US ACHIEVE OUR PLAN

GRIN FAMILIES

1 SUBMIT YOUR CHILD'S DATA
REGISTRY
Complete the registry for your region:
curegrin.org/participate-in-research

2 CREATE YOUR
FUNDRAISER
Get Moving for GRIN - July
Count Me GRIN - November
Facebook Fundraiser
Get Moving for GRIN 360
Giving Tuesday
curegrin.org/fundraising

3 SAVE THE DATE
GRIN CONFERENCE
APRIL 21-24, 2022 IN BOSTON
More details coming soon
curegrin.org/2022-grin-conference

4 VOLUNTEER
Do you have special skills and talents that could
help CureGRIN advance it's mission of finding
treatments and cures for GRIN Disorder?
Contact info@curegrin.org

5 STAY CONNECTED
Join our mailing list at curegrin.org/newsletter and follow us on social media

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. Ian Coombs, Dr. Mark Farrant, Dr. Stephen Traynelis, Dr. Angie Serrano, Dr. Allan Bayat and Dr. Berardo Rinaldi.

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

Research Audit: Table of Contents

- **Introduction**
- **NMDA Receptors**
  - Overview of history of NMDAR research
  - GRIN genes
  - Identification of variants
  - Descriptive studies (classified in research system/tissue where this is assessed):
    - NMDAR studies
      » General biology, Processing, Assembly, Mapping Studies
    - General Interactome Studies
  - Promoters/Enhancers
  - Spatiotemporal expression
  - GRIN expression in other tissues (beyond the nervous system)
  - NMDAR expression in other cells/tissues
  - Other diseases connected to functioning of NMDARs
  - Animal models generated and phenotype description/characterization
  - Pharmacological agonists/antagonists
- **GRIN Disorder**
  - Overview
  - Patient registries
  - Phenotype characterization of patients
  - Identification of genotype-phenotype correlations
  - Animal models generated with patient variants
  - Functional/mechanistic studies:
    - LoF Studies
    - GoF Studies
    - Test of small molecules ameliorating molecular/biological phenotype
  - Pharmaceutical and Biotechnology Companies of Interest

Research Audit: Table of Contents

- **AMPA Receptors**
  - Key historical AMPAR discoveries
  - GRIA genes
  - Identification of variants
  - Descriptive studies
  - AMPAR studies
  - AMPAR protein partners
  - Promoters/Enhancers
  - Expression patterns
    - GRIA expression in the brain
    - GRIA expression in other tissues
  - Pharmacological agonists/antagonists

- **GRIA Disorder**
  - Overview
  - Patient registries
  - Animal models generated with patient variants
  - Functional/mechanistic studies:
    - LoF Studies
    - GoF Studies
  - Test of small molecules ameliorating molecular/biological phenotype

- **GRIK Genes**

- **GRID Genes**

- **Conclusions**

- **References**

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.
- Groups in Paris (Asher) and the USA showed that the **magnesium block of NMDAR**s is highly voltage-dependent and that NMDARs have a **high permeability to calcium**.
- Ray Diongledine showed **glycine is a required co-agonist**, making NMDARs the first receptor to require two activating agonists.
- 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 (Mishina, Nakanishi), Germany (Seeburg), and the USA (Heinemann).
- 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
- Resurgence of drug discovery centered on subunit-selective allosteric modulators (driving pharmaceutical companies to restart NMDAR programs)

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).

Research Audit: AMPAR History

1980s	1990s	2000s	2010s

1980s

- **Single-channel recordings** of glutamate receptors including receptors activated by kainate(Cull-Candy and Usowicz, 1987; Jahr and Stevens, 1987; Nowak et al., 1984).
- Drugs CNQX and DNQX developed to distinguish AMPA/KAR signalling from NMDARs (Honore et al., 1988).

1990s

- Between 1989 and 1992, Hollmann and Heineman performed research on the cloning of cDNAs encoding glutamate receptor subunits (Hollmann and Heinemann, 1994). **GluA1-4 identified (GRIA1-4).**
- GluA2 identified as functionally critical due to RNA editing which affects the channel's ion selectivity filter. GluA2-containing receptors are Ca^{2+} impermeable (CI-AMPARs) while those which lack GluA2 are Ca^{2+}-permeable (CP-AMPARs) (Burnashev et al., 1992).
- Alternative splicing region discovered (the flip/flop cassette) present in all four genes. Flop forms display faster decay kinetics. (Sommer et al., 1990).
- AMPAR non-competitive inhibitor GYKI 52466 discovered (Donevan & Rogawski, 1993).
- The AMPAR positive allosteric modulator cyclothiazide identified (Bertolino et al., 1993).
- Block of CP-AMPARs by intracellular polyamines discovered (Bowie & Mayer 1995; Kamboj et al., 1995).
- AMPAR properties can be modulated by phosphorylation (Derkach et al., 1999; Banke et al., 2000).

2000s

- AMPAR ligand binding domain structures resolved in the presence of agonists and antagonists (Armstrong and Gouaux, 2000)
- **Stargazin**, the first transmembrane AMPAR regulatory subunit (TARP) shown to be critical for expression of synaptic AMPARs. (Chen et al., 2000)
- TARPs are a family of six proteins (Tomita et al., 2003; Soto et al., 2009).
- The cornichons, another family of AMPAR auxiliary proteins identified (Schwenk et al., 2009).
- Full-length AMPAR structure produced (Sobolevsky et al., 2009).

2010s

- The AMPAR proteome reveals over 40 associated proteins involved in receptor biosynthesis, trafficking and modulation (Schwenk et al., 2012).
- **Perampanel** licensed for treating certain types of epilepsy (2012-2016).
- **Structures of native AMPA receptors** elucidated by cryo-EM (Zhao et al., 2019).

Research Audit: AMPAR expression

- **GRIA expression in the brain**
  - AMPARs are found throughout the brain, however there is variation in the relative expression of AMPAR subunits by brain region and within different cell types within each region.
  - In terms of total GRIA mRNA detected, GRIA2 is the most strongly expressed gene brain-wide,
  - GRIA4 is expressed at high levels in a number of brain regions including the **cerebellum, thalamus, hypothalamus** and **myencephalon**.
  - GRIA1 and 3 are the lesser expressed subunits throughout the brain but are **enriched in the hippocampus**.

- **GRIA expression in other tissues (beyond the nervous system)**
  - The GRIA genes are, in general, expressed at extremely low levels outside of the central nervous system. However, this does not preclude an important role in certain non-neuronal cell types.
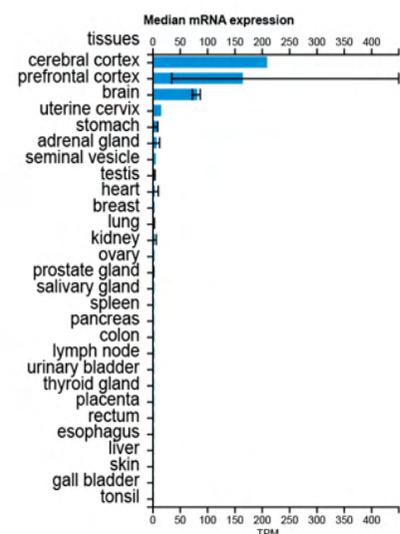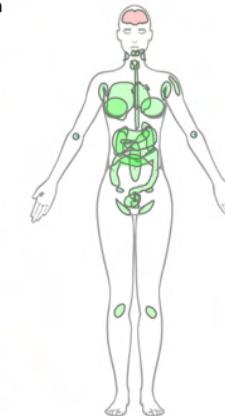

Glutamate receptor 1 (P42261)
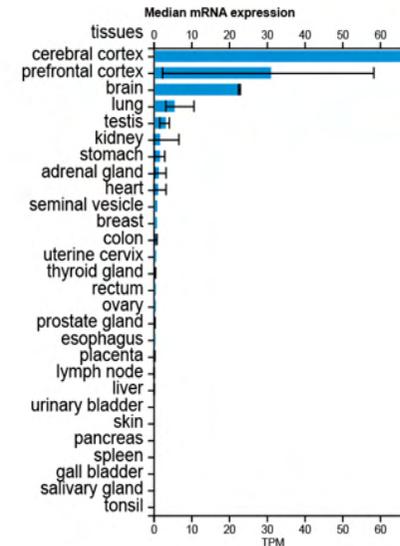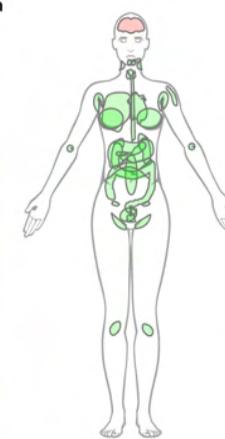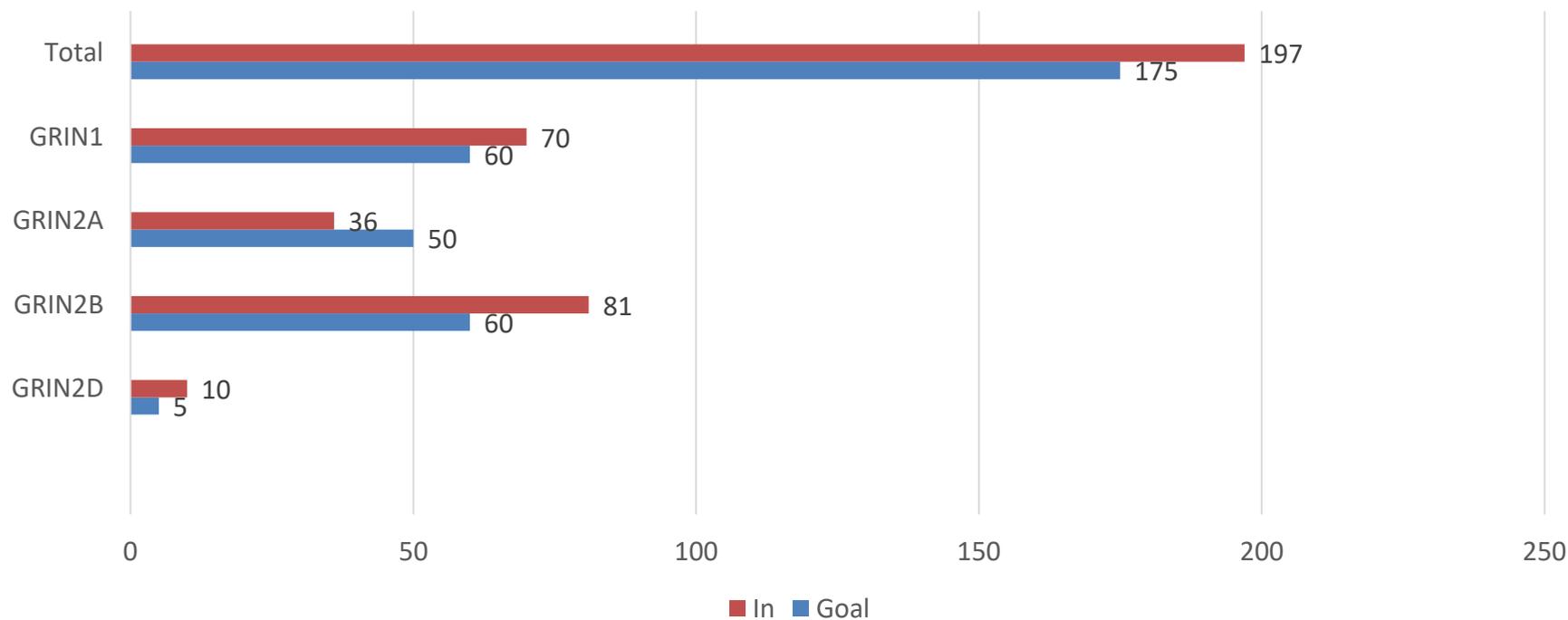

Glutamate receptor 2 (P42262)

Appendix B

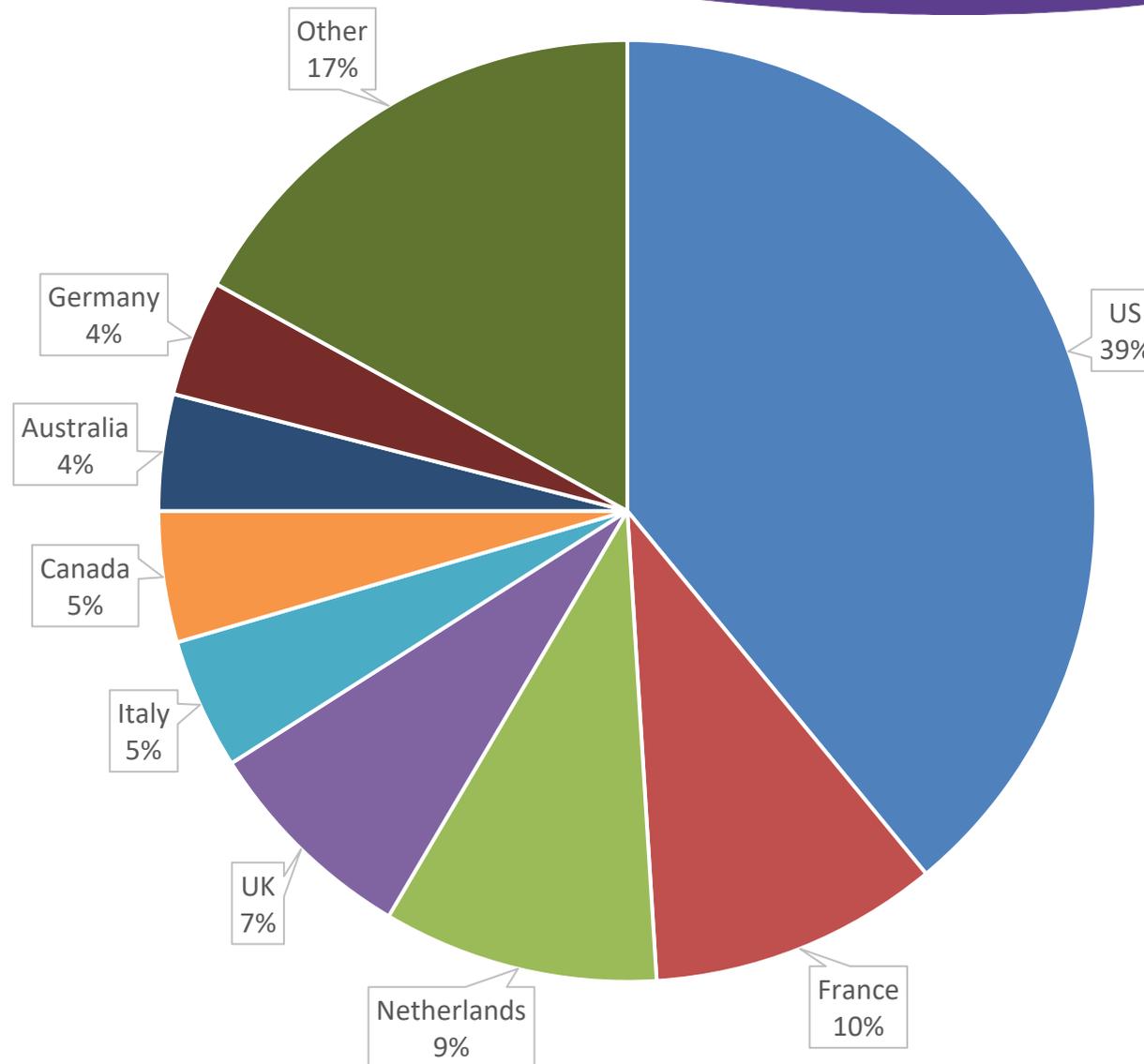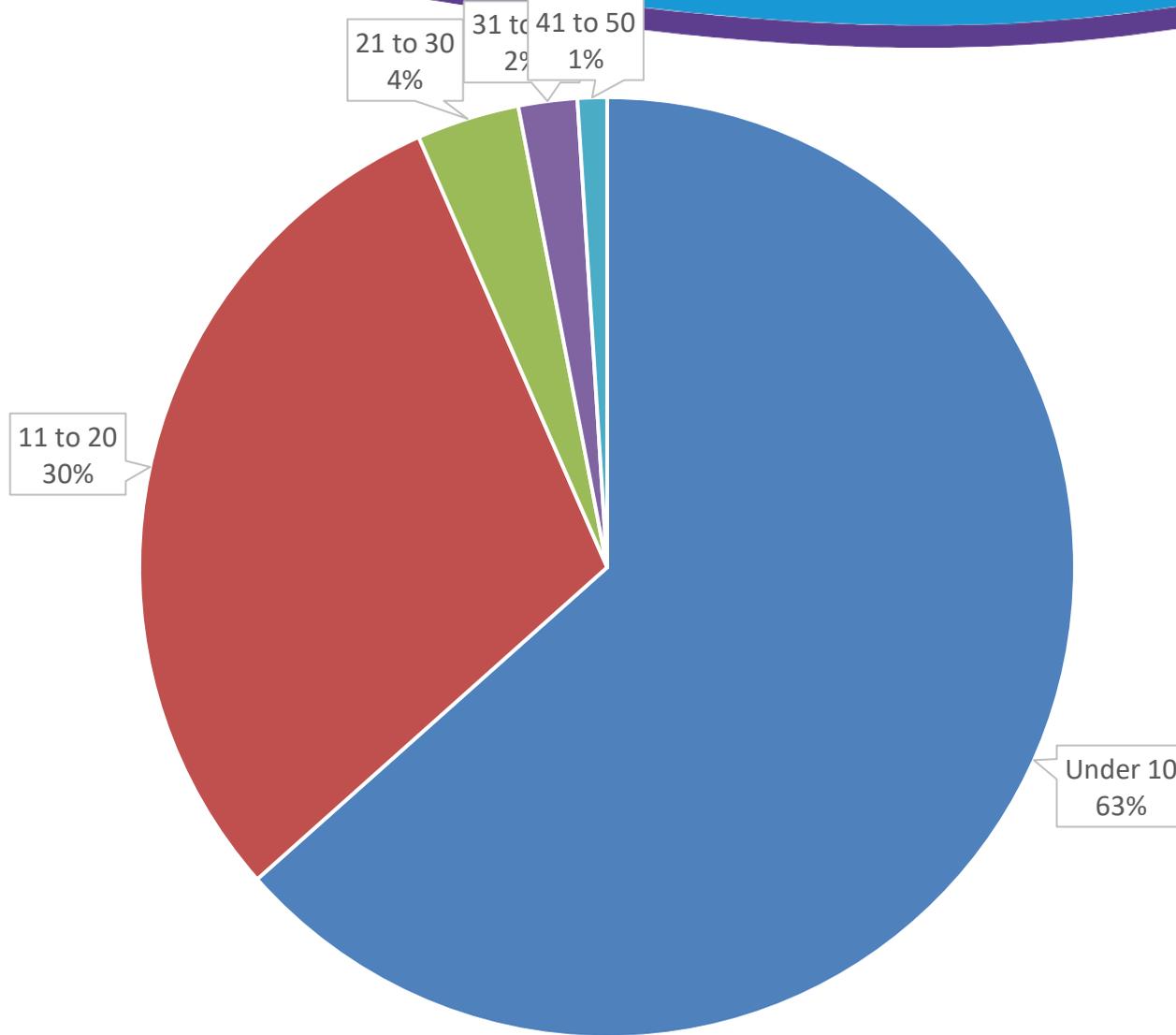# DETAILED FAMILY SURVEY RESULTS

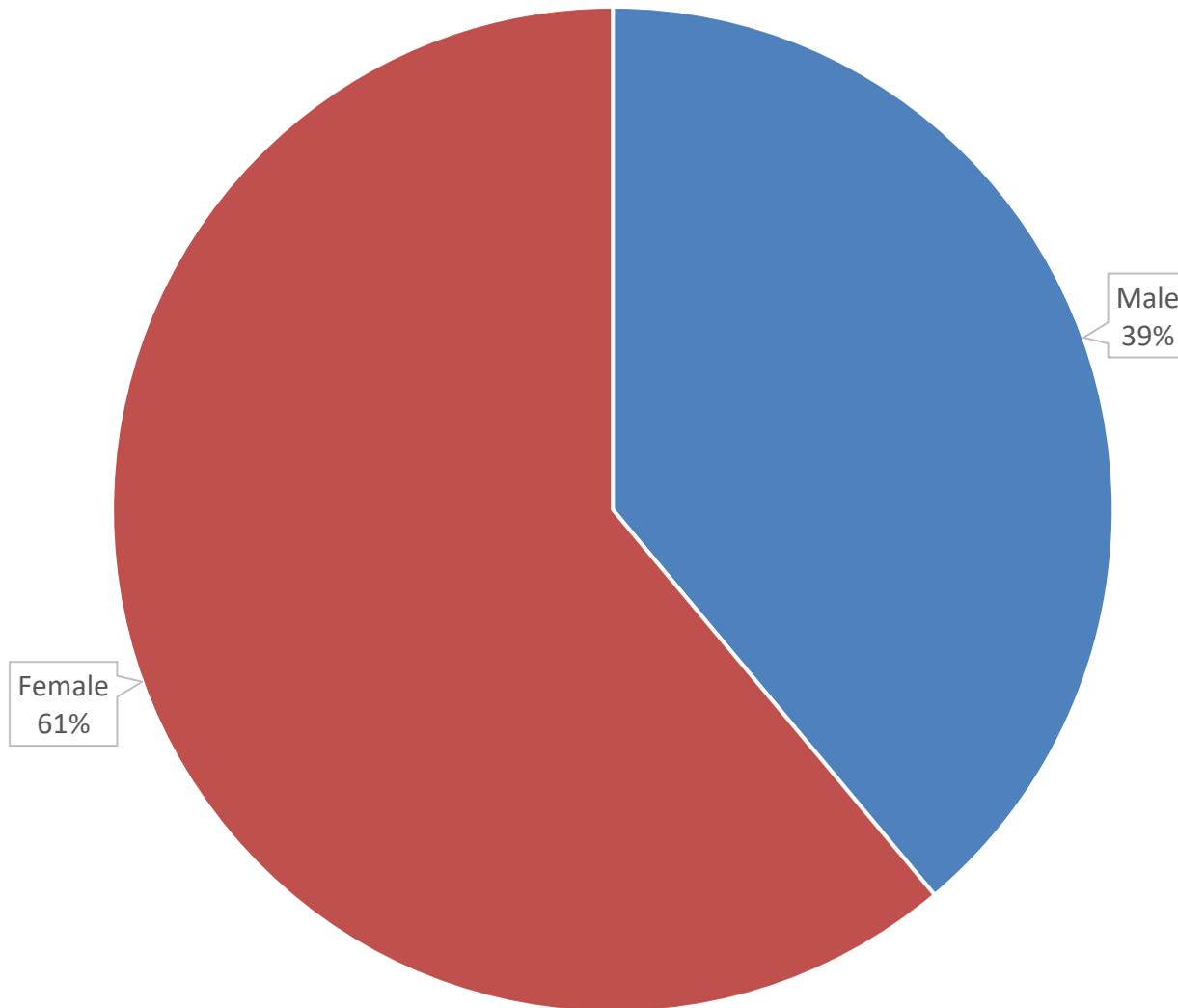# GRIN Survey Submissions by gene

Responses from Five Continents

Age of Patients

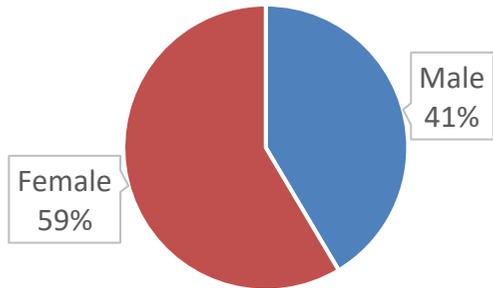# Sex of Patients

Sex by Gene

GRIN1
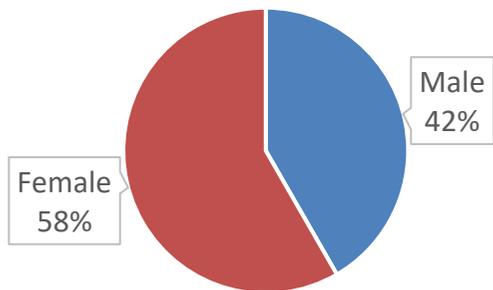
Male
41%

Female
59%

GRIN2B

Male
36%

Female
64%

GRIN2A

Male
42%

Female
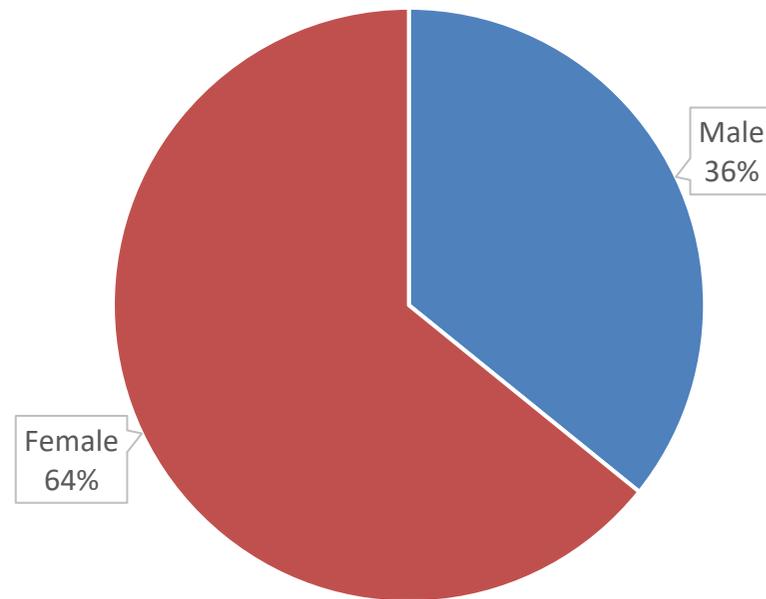58%

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%)

Treat Symptoms 29%

Find Cure 16%

Equal 55%

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%)

Narrow
21%

Broadest
29%

Equal
50%
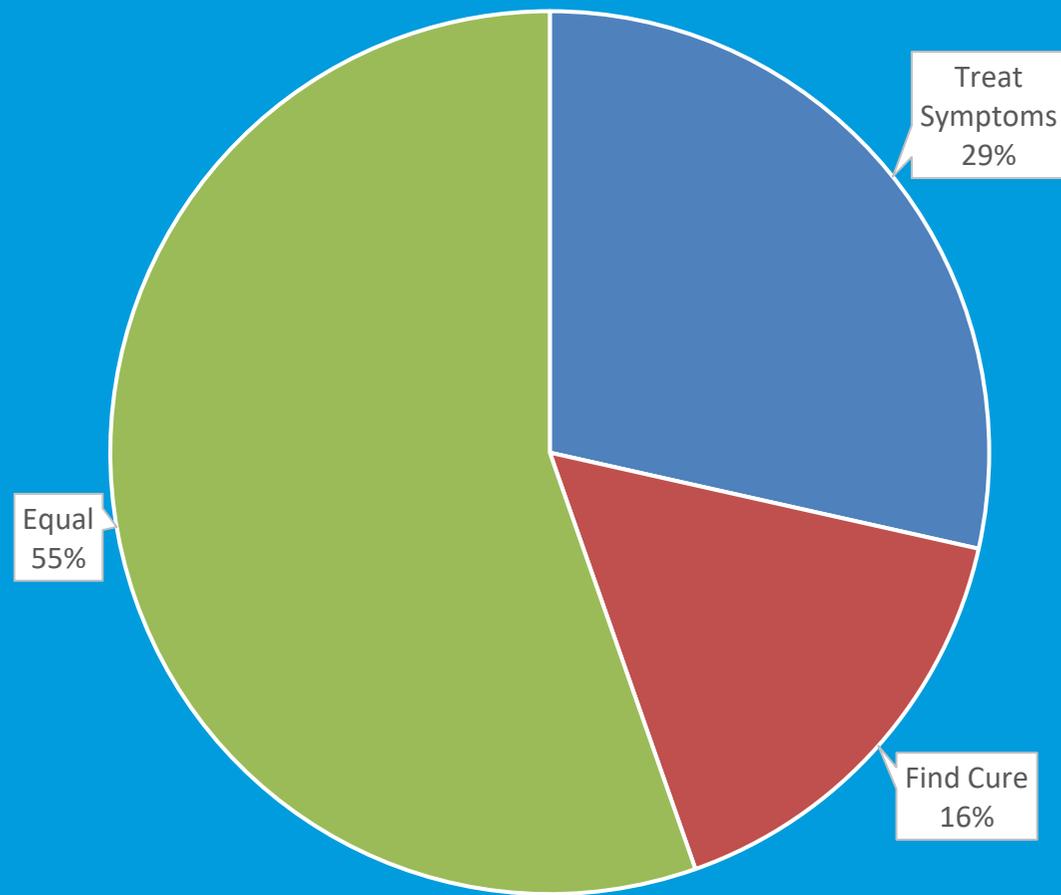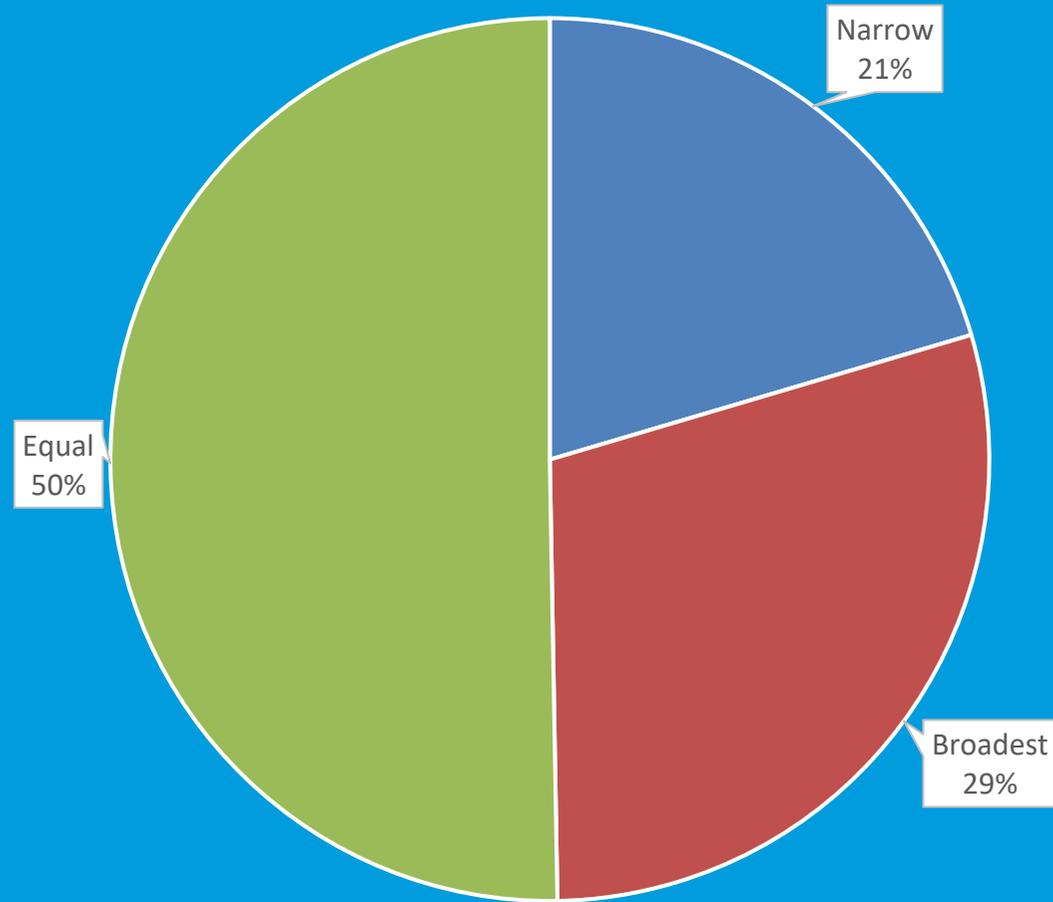
GRIN1 Summary N = 72	Does your child have this symptom? (Red = Top 3)	What's the most important symptom to help? (Red = Top 3)
Intellectual Disability	**94%**	**34%**
Speech	**84%**	9%
Low Muscle Tone	71%	1%
Sleep Challenges	**74%**	4%
Mood / Behavior	41%	4%
Constipation	59%	1%
Epilepsy / Seizures	59%	**20%**
Mobility Impairment	73%	6%
Visual Impairment	54%	1%
Digestive	50%	3%
Neurostorms	47%	**11%**
Feeding Tube	32%	1%
Breathing	11%	3%
Hearing Impairment	9%	0%

GRIN2A Summary N = 36	Does your child have this symptom? (Red = Top 3)	What's the most important symptom to help? (Red = Top 3)
Intellectual Disability	**74%**	**17%**
Speech	**69%**	6%
Low Muscle Tone	63%	3%
Sleep Challenges	63%	3%
Mood / Behavior	51%	**11%**
Constipation	46%	0%
Epilepsy / Seizures	**69%**	**42%**
Mobility Impairment	28%	6%
Visual Impairment	42%	0%
Digestive	31%	0%
Neurostorms	34%	9%
Feeding Tube	5%	0%
Breathing	20%	3%
Hearing Impairment	9%	0%

GRIN2B Summary N = 80	Does your child have this symptom? (Red = Top 3)	What's the most important symptom to help? (Red = Top 3)
Intellectual Disability	**96%**	**40%**
Speech	**84%**	**15%**
Low Muscle Tone	**74%**	1%
Sleep Challenges	63%	4%
Mood / Behavior	65%	**15%**
Constipation	50%	1%
Epilepsy / Seizures	30%	10%
Mobility Impairment	36%	2%
Visual Impairment	35%	0%
Digestive	32%	0%
Neurostorms	21%	5%
Feeding Tube	13%	5%
Breathing	5%	0%
Hearing Impairment	1%	1%

GRIN2D Summary N = 10	Does your child have this symptom? (Red = Top 3)	What's the most important symptom to help? (Red = Top 3)
Intellectual Disability	**80%**	**30%**
Speech	60%	0%
Low Muscle Tone	**80%**	0%
Sleep Challenges	50%	0%
Mood / Behavior	30%	0%
Constipation	30%	0%
Epilepsy / Seizures	**80%**	**60%**
Mobility Impairment	**80%**	**10%**
Visual Impairment	70%	0%
Digestive	40%	0%
Neurostorms	30%	0%
Feeding Tube	40%	0%
Breathing	40%	0%
Hearing Impairment	0%	0%

GRIA Summary N = 15	Does your child have this symptom? (Red = Top 3)	What's the most important symptom to help? (Red = Top 3)
Intellectual Disability	**80%**	**14%**
Speech	**93%**	0%
Low Muscle Tone	53%	7%
Sleep Challenges	60%	0%
Mood / Behavior	**73%**	**40%**
Constipation	27%	0%
Epilepsy / Seizures	46%	**40%**
Mobility Impairment	40%	0%
Visual Impairment	13%	0%
Digestive	20%	0%
Neurostorms	20%	0%
Feeding Tube	7%	0%
Breathing	7%	0%
Hearing Impairment	0%	0%

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 (0.5%),	Breathing (2.5), Low Muscle Tone (1.4%), Vision	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 important at all) and 3 (very important)

- In the following slides the highest ranked questions are marked in red, the middle tier in black and the lowest tier in grey.

- Following this exercise, the working group discussed all questions to distil the 10 most essential.

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)

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