PURA syndrome Gap and Landscape Analysis

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Scope of Work: The goal of this document is to provide actionable recommendations to the PURA Syndrome Foundation (PSF) about how best to direct their research efforts for PURA syndrome. To this end, we have interviewed relevant key opinion leaders, reviewed published material and public databases, reviewed foundation provided materials, and integrated learnings from other related fields and disorders to inform our recommendations. This document has not been peer reviewed and is not intended to be a comprehensive review of PURA syndrome, the PURA gene or the PURA protein, and is not intended to inform treatment or practices. The activities we are suggesting should be considered alongside the organizational priorities, available funding, and bandwidth or resources needed to pursue these activities. PURA syndrome is a disease that affects a range of symptoms including neurodevelopmental delays, intellectual disability, muscle weakness, seizures, and eating difficulties. Priorities may shift over time as scientific understanding of the disease progresses and as therapeutic technologies mature. We recommend revisiting strategic plans and community priorities yearly to ensure PSF's goals are aligned with the investments being made. Due to the length of the document several sections repeat content since we assume the reader might skip around and we do not want key information to be missed. The intended audience for this document is the PURA Syndrome Foundation board members and their constituents. The writing style aims to be accessible to patients and families that are familiar with PURA syndrome. This document was written as a snapshot in time of what is known as of March 2025, this is not a living document.

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Priority Recommendations by category

1. Research & Therapeutic Opportunities
    A. Drug repurposing efforts and in-silico screening
    B. Focus on addressing fundamental questions about the molecular and cellular biology of PURA syndrome that have the potential to drive therapeutic development. Priorities include:
        i. PURA syndrome genetic mechanism of action- only haploinsufficiency, or is there dominant negative effect?
        ii. What are the cell types and organ systems of interest (brain regions, bones, muscles, etc.)
        iii. Molecular understanding of PURA dysfunction in bones and muscle, and neuromuscular junction
        iv. Impact of PURA overexpression as a therapeutic approach and whether this will cause toxicity
    C. Clinically relevant gaps include understanding how PURA impacts:
        i. Identification of biomarkers
        ii. Scientific tracking of treatments and outcomes of patients
        iii. Understanding pain perception and pain receptor function
        iv. Determine how PURA affects temperature regulation/sensitivity, metabolic and endocrine systems and bone health

2. Governance & Community Structure
    A. Patient Registry: in addition to the registry at Southampton, look into a registry that is:
        i. User friendly for patients and families as well as translatable
        ii. Easier for researchers to access the data
        iii. Has appropriate data security, privacy, and data sharing practices
        iv. Major emphasis should be placed on collection of data relevant to biological and clinical questions important for clinical trials and disease understanding.
    B. New SAB: An advisory board serves to advise the PSF on research they are interested in engaging in as well as provide updates on key research in the field. Regular meetings to discuss what the foundation is working on as well as address progress in the field are recommended.
    C. Biobanking efforts: Continue efforts to initiate German biobank and lean on learnings to create a compatible biobank in the United States.
    D. Collaborative engagement
        i. Collating a list of all resources, animal and cell models of PURA syndrome and how to access them
        ii. Facilitating communication between PURA syndrome organizations through regular meetings and timely updates on funding decisions (to avoid overlap) to facilitate the effective formation of a ‘federation’ of PURA groups

Short and long term goals

In the **short term** (1-2 years) we recommend prioritizing the following (those in italics would ideally be completed prior to the next PURA conference):

1. *Identify a registry partner, and begin launch process*
2. *Generate an RFP (request for proposals)- we recommend focusing on hypotonia/ the role of PURA at the NMJ*
3. *Establish a new SAB and goals for the SAB*
4. *Create an overview of PURA syndrome tools and resources for the website*
5. Initiate biobanking efforts in the US
6. Establish regular meetings with the other PURA foundations to facilitate alignment and open communication, especially around the following areas:
    A. Development and availability of PURA research, including PURA antibody and animal models
    B. ASO efforts
    C. Mechanism(s) of action (haploinsufficiency vs dominant negative)
7. Supporting further drug repurposing efforts, consider investing in new efforts
8. Landscape in-silico screening companies; determine the compound libraries used, if they provide validation, evaluate computational models, etc.
9. Consider establishing resources for the PURA community that explain the value of assistive devices that help with expressive language and how to obtain them

In the **long term** we recommend prioritizing:

1. Regular reporting from funded research, establish expectations around reporting, and frequency
2. Investments in clinical outcomes and PURA biomarkers, with careful and strategic considerations
3. Fund research into:
    A. Understanding PURA function at the neuromuscular junction
    B. PURA interactors and downstream pathways
    C. Pain perception and pain receptor function
    D. Temperature regulation and sensitivity
    E. Metabolic and endocrine systems
    F. Bone health
    G. Better understanding of communication deficits (aphasia, dysarthria, apraxia, or something else?)
4. Continue to promote patient registry, biobanking efforts

Long term goals will be added to/reprioritized as more short-term goals are addressed/ completed.

PURA Syndrome Overview

In 2014, two studies reported that a monogenetic neurodevelopmental disorder, later named PURA syndrome, was caused by sporadic mutations in the PURA gene (*1*, *2*). As of January 2025, it is estimated that around 700 patients worldwide are affected by PURA syndrome (https://purasyndrome.org/). This syndrome results in a range of symptoms including neurodevelopmental delays, intellectual disability, muscle weakness, seizures, and eating difficulties.

PURA Syndrome Diagnosis- Patient Experience

Since the initial description of PURA syndrome in 2014, the age at diagnosis has decreased significantly, as more patients are referred to genetic testing earlier. However, there may be older individuals who remain undiagnosed due to mild symptoms, limited access to genetic testing, or misdiagnosis, among other factors. As of 2025, some patients are diagnosed either before or shortly after discharge from the hospital after birth, due to the high incidence of NICU admissions for issues such as breathing difficulties, temperature instability, and other related complications (from community/family interviews). Often, older children are diagnosed after identification of endocrine issues leads to genetic testing (from community/family interviews).

The most common features of PURA syndrome include neurodevelopmental delays, speech delays, and intellectual disability, often accompanied by significant cognitive impairments (*3*–*5*). Many individuals with PURA syndrome also experience muscle weakness and hypotonia (low muscle tone), which can lead to difficulties with motor coordination and posture. Central apnea is common but many children outgrow it as they get older (*4*). Seizures or seizure-like symptoms are frequently observed, with anecdotal evidence suggesting that these events are often triggered by environmental changes, such as temperature fluctuations, bathing, or diaper changes, and typically begin around puberty. All children with PURA syndrome experience delayed motor development, and most do not achieve independent walking. Many children also struggle to develop meaningful speech, though some parents report good receptive language skills and success in using communication devices (from community/family interviews). Patients with PURA syndrome often exhibit slower growth, with families noting differences in puberty that are currently not well understood (from community/family interviews).

PURA patients present with unique immunological characteristics (from community/family interviews). Many seem to rarely get sick or have fevers, and unusually high antibody titers following vaccinations have been noted. Endocrine abnormalities have also been observed in a significant percentage of patients (*4*). In addition to these observations, some patients show altered pain perception, with reports of broken bones or other injuries occurring without complaint (from community/family interviews). Unfortunately, there is not much research on this symptom in PURA syndrome yet but it is noted in other neurodevelopmental disorders as well. Skeletal abnormalities such as scoliosis have been documented (*3*, *4*) and among those who undergo surgery to correct spinal curvature, there is anecdotal evidence suggesting that

this may improve seizure symptoms, although this does not hold true for all patients (from community/family interviews).

Common PURA syndrome symptoms reported in family/community interviews:

- Low muscle tone
- Breathing problems (apnea)
- Feeding difficulties
- Seizures and/or seizure-like movements
- Vision impairments
- Reduced bone density (Osteoporosis)
- Scoliosis
- Hiccups
- Fatigue
- Temperature instability (especially in newborns)
- GI issues
- Large birth weight

PURA Biology and Disease

Mechanism of action in disease

Heterozygous, de-novo mutations or deletions in the *PURA* gene cause PURA syndrome. In contrast to other monogenetic disorders, almost all reported mutations in this gene result in full disease penetrance (*6*). The majority of mutations in PURA syndrome patients result in early termination of the protein (nonsense, frameshift, etc.) (*3*, *5*). ***While many researchers believe that PURA Syndrome is caused by haploinsufficiency, the field is still conflicted with some researchers hypothesizing that there may be a subset of mutations that act as dominant negative mutations.***

Haploinsufficiency means the mutated protein is likely non-functional and rapidly targeted for degradation by the cell. Overall, haploinsufficiency results in a reduction in the amount of functional protein. Alternatively, a dominant negative mechanism of action causes the mutated gene to create a mutated protein that may still be functional but due to the mutation, the protein function may altered to take on either a new function or actively reduce the ability of the non-mutated PURA protein to function properly. This is a possibility for PURA syndrome because the PURA protein is known to dimerize (bind to itself or other proteins in groups of twos) (*6*). If the mutant protein is not identified by the cell as nonfunctional, it could bind to healthy PURA or other dimerization partners, thereby reducing the ability of the healthy copy of PURA to function.

PURA Gene and Protein

The PURA gene encodes a ubiquitous protein known as purine-rich element binding protein A, which is involved in the regulation of various DNA and RNA-related processes including transcriptional and translational gene regulation as well as mRNA transport in neurons (*5*–*9*). This gene has also been implicated in postnatal brain development and neuroplasticity (*6*). PURA is primarily an intracellular protein, with its primary functions occurring in the nucleus. Studies also note postsynaptic and intracellular trafficking functions of PURA in the movement of RNAs. ***The intracellular nature of PURA (compared to proteins that are secreted) may be important for therapeutic considerations, depending on the modality and the targeted action of the therapeutic.***

The PURA protein is made of three conserved sequence regions (PUR repeats I, II, and III) and because PURA binds thousands of mRNAs, changes in PURA protein levels are known to alter mRNA abundance (*10*). PUR domains are thought to play a role in unwinding and winding of DNA and RNA and therefore are critical to expression. PURA has also been shown to function as a 'host factor' for RNA-based virus infection, meaning that normal PURA helps viruses hijack intracellular systems to drive their infection. This may be a reason why PURA syndrome patients experience less infections because normal PURA function is not as readily available to RNA-viruses (*11*, *12*). This may be of an area of interest for researchers to consider in more detail.

Recent evidence has also highlighted the importance of PURA for processing body (P-body) activity (*6*, *10*). P-bodies are organelles in the cytoplasm of cells that regulate gene expression by storing and degrading RNA. While the full range of specific RNAs regulated by PURA is still being studied, it is known that the PURA protein interacts with mRNAs that play crucial roles in neuronal development and function which highlights the importance of PURA in normal brain development and its role in maintaining the integrity of neurological processes. It also highlights the huge impact that loss of PURA has on proper cellular function. It also makes it very difficult to know if there a single mRNA or group of mRNAs that PURA regulates which are key to causing PURA syndrome symptoms. In addition to PURA syndrome, the PURA protein has also been implicated as a modulator of neurodegenerative disorders, such as fragile X-associated tremor/ataxia syndrome (FXTAS), and the amyotrophic lateral sclerosis (ALS) frontotemporal dementia spectrum disorder (*13*, *14*).

PURA cellular localization (primarily intracellular/nuclear):

- nucleus
- cytoplasm
- dendrites
- glutamatergic synapses
- neuronal cell body
- post-synaptic

Primary functions of the PURA protein include:

- DNA-binding transcription factor activity, binding, & repressor
- Double-stranded telomeric DNA binding
- RNA binding
- Single-stranded DNA binding
- Purine-rich negative regulatory element binding
- SMAD binding
- Dendritic transport of messenger ribonucleoprotein complex
- DNA replication initiation
- DNA unwinding
- Epithelial cell & lymphocyte proliferation
- mRNA regulatory element binding translation repressor activity

PURA functions specific to the central nervous system and brainstem:

- neuronal development
- neuroplasticity
- motor neuron development

PURA in the central nervous system (CNS)

Given the lack of information on PURA in different cell types, it is difficult to know if there are impacts of PURA mutations specific to cells of the CNS. The data derived from various databases does not show a consistent trend or preferential expression of PURA in glia compared to neurons but shows expression across all of these tissues. According to publicly available datasets derived from human tissue (see pink bar graph in Figure 1), PURA's expression in glial cell types may be important for function or disease pathology. For example, PURA transcript quantification from the Barres Lab RNAseq dataset (https://brainrnaseq.org/) shows PURA most highly expressed in oligodendrocytes, followed by neurons, and mature astrocytes. This is consistent with reports of decreased myelination, decreased pain perception, and hypointensities on MRI. Importantly, this profile of expression is different from mice. In mice (blue bar graph in Figure 1) PURA transcript expression is highest in neurons, followed by astrocytes, while myelinating oligodendrocytes is one of the lowest expressing cells. ***This profile may vary across mouse strains and should be considered when funding mouse studies where***

the brain cell type is important for understanding the biology or function of PURA being studied. Data from Protein atlas (proteinatlas.org) shows PURA transcript expressed across brain cell types, with cluster analysis highlighting a potential role of PURA in astrocyte function (see Figure 2). Species and cell type selection may play an important role when looking at PURA function. Overall, data show that PURA has low tissue and low cell type specificity, meaning it is not highly expressed in any one tissue or cell type and therefore, cell type selection may impact functional considerations. These data are also consistent with PURA playing an important role in basic cellular functions across all cell types.

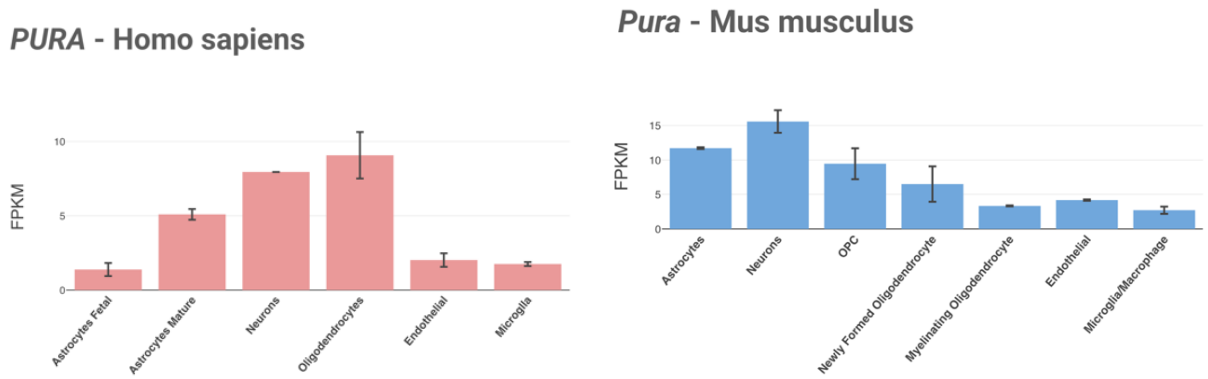
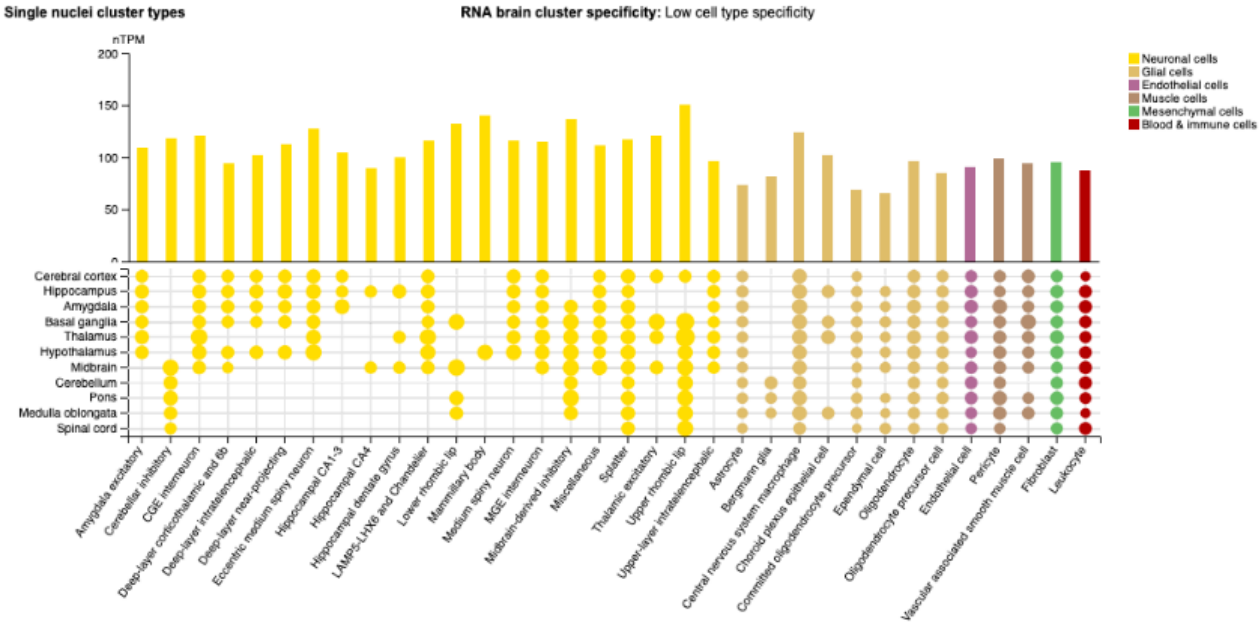


Figure 1. PURA transcript quantification across different brain cell types in human tissue (pink) and mouse tissue (blue). Data source: https://brainrnaseq.org/



PURA is part of cluster 19 **Astrocytes - Mixed function** with confidence[i] 1
451 genes in cluster

Go to interactive expression cluster page

15 nearest neighbours based on brain RNA expression

Neighbour[i]	Description[i]	Correlation[i]	Cluster[i]
FBXW4	F-box and WD repeat domain containing 4	0.9140	19
NUDT16	Nudix hydrolase 16	0.9123	19
NOP9	NOP9 nucleolar protein	0.9088	19
CASC3	CASC3 exon junction complex subunit	0.8807	13
FOXO4	Forkhead box O4	0.8614	13
TSR2	TSR2 ribosome maturation factor	0.8561	19
ZNF768	Zinc finger protein 768	0.8456	19
CDK11B	Cyclin dependent kinase 11B	0.8439	6
MAP4	Microtubule associated protein 4	0.8421	19
BCL2L2	BCL2 like 2	0.8281	19
MON1B	MON1 homolog B, secretory trafficking associated	0.8263	19
VEGFB	Vascular endothelial growth factor B	0.8263	19
CCDC97	Coiled-coil domain containing 97	0.8123	19
SAMD1	Sterile alpha motif domain containing 1	0.8105	19
PLEKHF1	Pleckstrin homology and FYVE domain containing 1	0.8088	19

Figure 2. PURA transcript quantification across different brain cell types and cluster analysis highlighting PURA's potential role in astrocytes. Genes shown in the table show the most similar genes in terms of expression. Datasource: proteinatlas.org

PURA at the NMJ

PURA syndrome has many similarities to other channelopathies which has led researchers to consider the role of the PURA protein at the neuromuscular junction (NMJ), similar to congenital myasthenic syndromes (CMS) (*15*). The NMJ is the interface between neurons and muscles that allows for communication between brain signals and muscle movement. At the

moment, there is limited information on the function of PURA at the NMJ but dysfunction at this site can cause muscle weakness, difficulty breathing, and difficulty swallowing among other things. This is characteristic of CMS which are a heterogeneous group of disorders characterized by impaired neuromuscular signal transmission and, according to some, include PURA syndrome (*16*). Further evidence that PURA syndrome has an NMJ component includes recent data from two PURA syndrome cases treated with pyridostigmine or salbutamol showing improvement in neuromuscular function. Pyridostigmine is an acetylcholinesterase inhibitor and salbutamol is short-acting β_2-adrenergic receptor agonist and are used to treat CMS patients. (*15*). Work is ongoing, although unpublished, in this area. Keeping as up to date as possible on the goals of the labs working in this area allows the PSF to fund relevant research while avoiding duplicating efforts unnecessarily.

Genotype-Phenotype Correlations

Predictive Models

Both missense (one amino acid change) and nonsense (or mutations that lead to early stop codons and therefore shortened proteins) have been identified in PURA syndrome patients. Missense mutations are classified into discrete categories based on their predicted effect on the protein function (pathogenic, likely pathogenic, variants of unknown significance (VUS), or benign mainly) via machine learning tools. Currently, for PURA syndrome and other diseases, clinicians and genetic counselors are using industry standard machine learning tools such as Alpha fold 3 (Google Deep Mind) (*17*).

There is not a strong relationship between genotype and phenotype among patients with PURA syndrome, which is fairly common for neuro-developmental disorders. This often indicates that there are additional factors (besides just the singular mutation) that determine the severity of the disease including other genetic interactions and environmental factors (*18*) or not enough data (or the right type of data) to identify patterns or subgroups within a disease.

PURA mutations

There were differing opinions among the researchers interviewed about why so few low-severity patients have been identified. One hypothesis is that patients with more mild PURA syndrome phenotypes have yet to be diagnosed, not that they don't exist. Because the diagnosis of PURA syndrome is defined by genetic testing of the PURA gene, it is very possible that patients with more mild symptoms are not referred for genetic testing. Increases in genetic testing may begin to address this hypothesis and hopefully identify additional patients that could benefit from a conclusive diagnosis. Country-wide phenotyping/genotyping sequencing efforts are underway in Iceland, Finland, UK, Estonia, and USA which may provide a way to look at all the variation in the PURA gene across a population and determine if there are more mild

phenotypes associated with a specific subset of mutations, those not captured by current clinical efforts to sequence patients.

Another hypothesis is that the PURA gene is not amenable to even minor changes in sequence and that any mutation in PURA causes moderate to severe disease. Given the disease severity of the majority of PURA syndrome patients, this is a very possible reality. Another piece of data that supports this hypothesis is information from the gnomAD database (https://gnomad.broadinstitute.org/). The Genome Aggregation Database (gnomAD) is a collection of exome and whole genome sequencing data from a variety of large-scale sequencing projects. It aims to collect data on genetic variation found in relatively healthy groups of people. This is a great place to learn how frequently mutations in specific genes occur in these populations. It is worth noting that because these are taken from various studies, it is possible that some disease-causing variants are present if that disease was not selected against in the recruitment process. As of February 2025, there are zero loss-of-function mutations of PURA in the gnomAD database. This strongly indicates that heterozygous loss-of-function variants are not tolerated in these control populations. In line with this hypothesis, there are over 150 missense variants identified which is significantly fewer than what would be expected by random chance for PURA (396 missense mutations predicted by random chance). This indicates an intolerance to variation for missense mutations of PURA in these control populations.

Compared to other proteins, PURA is a very small protein and it is reasonable that any mutation could affect the entire protein or critical function because the PURA protein is not a complex, multi-functional, multi-domain protein. This is illustrated in the 3D protein structure of PURA (see Figure 3) where pathogenic mutations (in red) are mapped to the protein. Blue indicates the areas of the protein that do not have pathogenic mutations. This is also illustrated in the heat map below the 3D protein structure which shows a linear map of the PURA protein from amino acid 1 to 322 and where the pathogenic mutations occur (in red), at the beginning and end of the disordered areas of the protein while the functional core of the protein which contains the PUR domains appears to be intolerant of mutations. Further work to understand the domains of PURA and predict the impact of specific mutations is ongoing in the lab of Dr. Dierk Niessing.

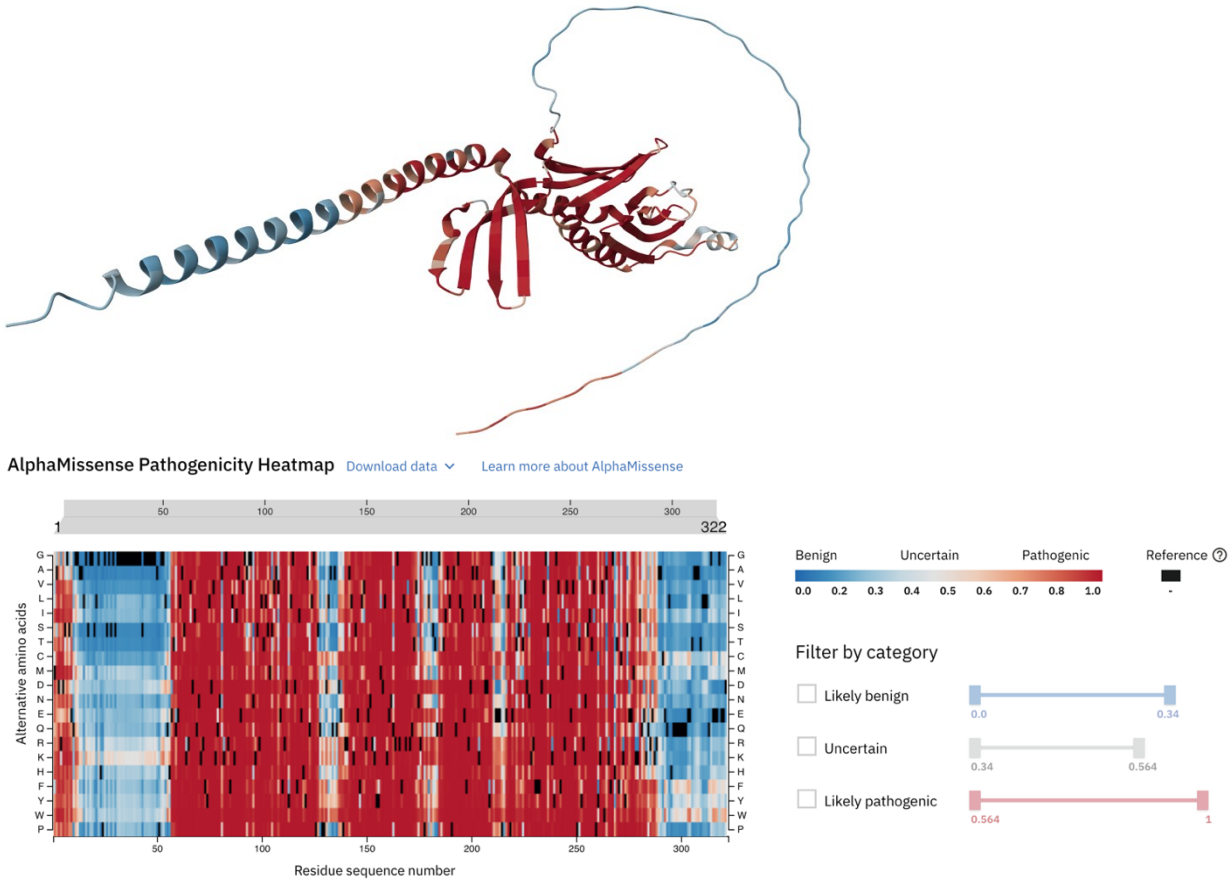


Figure 3. 3-Dimensional structure of PURA protein (top) and a heatmap representing the tolerance of each amino acid position of PURA to mutation. Data source: https://alphafold.ebi.ac.uk/

Therapeutic Considerations for PURA Syndrome

Developing a genetic therapeutic to treat PURA syndrome is difficult since PURA is a ubiquitous protein, that regulates the expression of a large number of other RNAs. Finding a treatment may require fine tuning of the PURA gene itself, targeting a specific function of PURA, targeting downstream targets of PURA, or addressing specific symptoms without the goal of impacting PURA function itself. Developing a diverse portfolio of therapeutic approaches for PURA syndrome can increase the likelihood of finding an effective treatment. Below is a general list of different therapeutic modalities or approaches to drug development. Not all of these modalities are applicable to PURA treatment, we advise making prioritization decisions based on feasibility and biological considerations.

Therapeutic Modalities Overview:

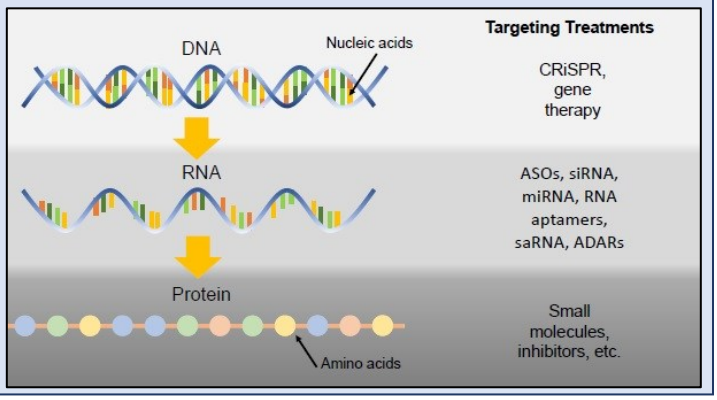
1. Small molecule screening: when screening compounds already approved by the FDA this is called drug repurposing. Examples include Tylenol and Zyrtec. While cost effective and sometimes the most efficient path to find a drug, this approach is what we call a “fishing

expedition." Usually large libraries of chemical compounds are tested to see if they have an effect on a disease-relevant outcome in cells lines. For example, a screen might test a library of a thousand or tens of thousands of different compounds to see if the compounds affect a DNA repair mechanism when exposed to a DNA damaging toxin. Those compounds that protect the cell against damage would be studied further. While this can be a cost-effective approach when an effective compound is identified, there isn't always an effective compound identified or the outcome is not truly disease specific. Also, if there is a potential drug identified, it isn't always clear what the chemical or molecular mechanism of action is. It is also critically important to have a phenotype (whether in a cell or animal model) to test against.

2. Biologics (antibodies, soluble proteins, hormones): These are large protein-based molecules that are usually injected into the patient and target circulating proteins. Because of their size they do not usually penetrate cells easily or cross certain barriers in the body and therefore they must exert their effects outside of the cell. This limits their utility to certain tissues or localized use.
3. Cell therapies (bone marrow transplants, or iPSC delivery): The injection into or replacement of cells in the body is called cell therapy. Sometimes a functional cell is put into the body when the previous cells die off or are dysfunctional, and sometimes cells are taken from the body and treated with a gene therapy and then put back into the body.
4. Gene therapies and genome editing (AAV, lentivirus, CRiSPR): These approaches deliver genetic material (such as a functional gene) or directly edit the mutation in a genome.
5. RNA therapeutics (siRNAs, ASOs, aptamers, etc): Targeting RNA is sometimes easier than delivering DNA, especially when the aim is to decrease the expression or function of a protein. RNA targeting therapeutics utilize a diverse and growing number of approaches that can exert different effects on the RNA itself including upregulation, downregulation, and allele-specific targeting to name a few.

Inside of a cell, DNA is transcribed into RNA before being translated into protein (Figure 4). Therefore, a mutation in DNA can result in the mutation being carried into the RNA transcript. This mutation in turn may result in a change in amino acid in the protein which could affect function of the protein. Because only one copy of the RNA transcript for the mutated version of the protein is different from the wildtype (non-mutated) RNA transcript, it is possible to target this RNA specifically. This opens the door for RNA therapeutics. Additional methodologies can target DNA or protein.

Figure 4. Dogma of DNA



Therapeutic development currently in progress for PURA Syndrome

Currently there are several ongoing efforts to develop and/or identify treatments for PURA syndrome. Some of them are being spearheaded by other PURA foundations (see section on Ongoing work in other Foundations). Briefly, some of those efforts include:

- PURA Gene replacement therapy (AAV)
- Initial ASO discussions
- Drug repurposing including Ravicti, pyridostigmine, and salbutamol
- In silico screening efforts

Additional efforts are underway to lay the groundwork for future therapeutic development. Fly and worm screens can identify a phenotype in those animals to use for drug screens and research into mouse models could also identify testable phenotypes. Work to identify the impact of PURA overexpression will be very important for determining if gene therapy is a viable option for treatment.

From our discussions with researchers, clinicians, and families, there continue to be several areas where additional therapeutic investigations are suggested, including in silico and in vitro screening and drug repurposing efforts. But there are obstacles that we suggest need focus in order to overcome before undertaking some of these efforts, or, that better understanding may facilitate therapeutic target identification. For example, given the currently unknown downstream impacts of PURA mutations on gene, mRNA, and protein expression it is difficult to determine what is the key molecule or pathway to target. Until there is further clarification on these biological processes, it is likely that small molecule and drug repurposing studies will be the most likely to identify therapies to help reduce symptoms of PURA syndrome. This is discussed in further detail in Section Recommendations for Research.

AAV Gene Replacement Therapy

PURA is primarily intracellular, but with noted postsynaptic functions. This is important for therapeutic considerations. It is not expected that PURA is secreted and therefore cells receiving PURA gene replacement will not cross-correct cells that are not transduced by AAV, but there may be some treatment that affects other cells through postsynaptic and downstream network effects. Currently there is work ongoing, funded by Jack's Tomorrow, to develop a gene therapy which would aim to deliver a functional copy of the *PURA* gene. However, there are concerns among PURA researchers about a gene replacement therapy for PURA syndrome due to the known, tight regulation of *PURA* expression. Dysregulation of *PURA* expression, namely *PURA* overexpression, has been found in several types of cancer including breast cancer and esophageal squamous cell carcinoma (*7*). Studies, including gene therapy, that aim to increase the expression of *PURA* should consider possible oncogenic effects of

treatment. We recommend that PSF get regular updates from Jack's Tomorrow on this program, including data on efficacy testing in PURA syndrome animal models and identified side effects of PURA overexpression. There are concerns about the impact of overexpression on cells as there are no published cases of *PURA* duplication in humans. However, since PURA is under such tight regulation, he has optimism that overexpression will be counterbalanced in the cell (from an interview with Dr. Jim Dowling). Additionally, AAV delivery is not effective at delivering the gene to every cell in a targeted area so the concerns of overexpression are unlikely to affect the entire brain, but rather on a cell-by-cell basis. This would be something to consider closely in a toxicology study looking at the histopathology of cells that were transduced by the gene therapy compared to non-transduced neighboring cells.

Of note with regards to PURA and cancer, there are also reports that low levels of PURA have been found in some types of cancer including AML, Myeloid neoplasia (MN), hormone-refractory prostate cancer (HRPC), and breast cancer (*7*). This indicates that proper regulation of PURA expression is crucial for proper cell function. Keep in mind that there have not been reports that PURA is an oncogenic gene, so dysregulation of its expression is likely something downstream from a cancer-causing event and not the causative event itself. This link to cancer may open the possibility of researching PURA in the context of cancer which may open up additional lines of funding.

Challenges of AAV-PURA

- Targeting the right brain area
- Delivering AAV-PURA to enough cells in the brain to provide sufficient treatment and symptom relief
- Targeting the right cells is also a concern. PURA is likely to play a role in neurons, oligodendrocytes, and other glial cell types. Some AAV capsids are better at transducing one cell type over another. Studies of PURA syndrome have noted a role in gliogenesis, expression in oligodendrocytes, and delayed myelination (by MRI) (*19*) as well as pain sensing defects, temperature sensing defects, and motor neuron defects which point to a function of PURA in brainstem as well as myelination that may be important for symptom presentation and treatment.

Recommendations for Research

A goal of this document is to provide concrete recommendations to the PSF for how to best direct their research efforts and investments. To this end, we have reviewed published literature, interviewed stakeholders (including researchers, clinicians, and families), and integrated information from other related fields and disorders to inform our recommendations. A significant part of the review process involves identifying key gaps in biological understanding. From interviews with key PURA researchers, we have identified and prioritized

gaps in the biological and clinical aspects of PURA that could be key for drug development efforts.

Summary of Key Basic Biology Research Questions

- **Molecular and cellular biology**:
  - Is haploinsufficiency the only mechanism of PURA syndrome or is there evidence that there could be a dominant negative effect?
  - In what cell types is PURA expressed (for example brain regions, neurons vs glia, bones, muscles, etc.)?
  - What is the impact of PURA haploinsufficiency on embryological development?
  - What is the mechanism that causes bones and muscles/NMJ to be impacted by PURA haploinsufficiency?
  - What are the repercussions of PURA knock down on protein expression (may be time-dependent, developmental-stage dependent as well)?
  - Are neurons differentiating inappropriately? Brain could be wired inappropriately, would be difficult for gene therapy to help this mechanism but could impact other symptoms that are not 'hard wired'
- **Clinically relevant**
  - What is the impact of PURA knock down on temperature regulation and sensitivity?
  - How does PURA haploinsufficiency impact the functioning of pain receptors and pain perception?
  - What impact does PURA have on the endocrine system? What treatments help overcome this?
  - How does PURA impact bone health? Is this a side effect of the seizure medication or is lack of PURA bad for bones?
  - What is the function of PURA at the NMJ and is this a function at pre- or post- synaptic sites or within the synaptic cleft?

Key Biological and Clinical Focus

After reviewing the published research and interviewing families and researchers, we have assembled a prioritized list of key focus areas for PURA syndrome. This prioritization takes into account what is currently ongoing in the labs of researchers interviewed as well as an understanding of the work initiated by other PURA syndrome foundations (for more information on the activities of other Foundations, see Ongoing work in other Foundations). Since those activities are already ongoing, we recommend prioritizing research into other areas of PURA syndrome. PURA syndrome is a relatively newly defined syndrome and there is much about the clinical presentation and biological mechanism that remains unknown. There are

areas of research that it might make sense for the PSF to overlap in funding or support with other PURA foundations, but we recommend the PSF efforts largely focus on the areas of biological understanding that are not currently being addressed or prioritized by other groups and are critical to therapeutic development. We have broken down priorities into clinical and biological research areas. Since the PSF represents the PURA syndrome community at large, we recommend prioritizing activities that have the potential of impacting the most debilitating symptoms and the greatest number of community members. The following are prioritized based on communications with the PSF and community members. Primary clinical concerns identified through interviews we conducted with families and researchers in order of importance are: (1) seizures, (2) communication, (3) hypotonia, and (4) patient registry.

Clinical prioritizations for research:

1. The number one concern among families interviewed is seizures. Many families expressed interest in being involved in a future clinical trial aimed at reducing seizures (as of February 2025 from caregiver interviews). This is good to know because there is no point in designing a clinical trial if there is no interest from the community in being involved. While the PURA Foundation Australia has a seizure focus, we recommend PSF also prioritizing seizure research given the emphasis from your community members. This is an area where we do suggest strategic overlap in funding with another foundation and coordinated discussion between foundations on groups funded, type of research, and outcomes.
   We also recommend continuing to learn from other neurodevelopmental seizure disorders, as the PSF is already doing. There is much that can be learned from that ongoing work including drug repurposing. However, the specific mechanism that causes the seizures in PURA patients (for example an ion channel expression, malformed connections in the brain, dysregulated neurotransmitters, etc.) remains unknown. A better understanding of this mechanism would open the potential to identify better therapeutic targets. The seizures that people with PURA syndrome have are different from many other seizure disorders. Focusing on the differences and those aspects of disease and seizures that are specific to PURA can help identify treatments as well as mechanisms. Some specific characteristics of PURA seizures include:
   - The reflexive nature of the seizures (meaning that seizures often occur in response to specific stimuli such as change in environment, pain, temperature or light changes, etc.). We recommend looking into treatments used by other disorders with reflexive seizures. A better understanding of seizure triggers provides insight on the pathways that are not functioning properly. Identifying if any animal model has these types of seizures could be very useful for testing treatments.

- The seizure-like activity that many patients experience that often precedes an epilepsy diagnosis. This may highlight certain brain pathways or biomarkers that could be used to determine treatment or monitor treatment efficacy.
- The impact of hormones on the initiation of epilepsy. Interviews with families highlighted changes in the pre-pubescent or pubescent time in PURA patients that triggers the initiation of seizure-like or seizure activity. Further exploration of this link could identify a mechanism or pathways to target for treatment. This should be explored through registry efforts, clinical research, and basic biology research in more detail.

2. For most caregivers interviewed, the second most important aspect of PURA syndrome that they wished could be addressed was communication. Currently, PURA Syndrome Australia is working on a study about communication in PURA patients. Additionally, there are devices or device-based apps that can help patients communicate. ***Patients with PURA syndrome often have higher receptive language than expressive language indicating that they understand more than they can communicate. The levels of hypotonia in many patients may prohibit easy communication due to impacts on word formation and vocalization. Detailed classifications of the cause of the communication deficits (for example aphasia vs apraxia vs dysarthria) have not been extensively reported.*** We suggest further conversations with Speech Language Pathologists (SLPs), and possible fMRI analysis for identifying speech deficit manifestations. ***Interviews with the community suggest that using assisted devices with eye-tracking may enable more expressive communication.*** Many of these devices can even account for differences in vision including strabismus and gaze direction. Advocating for the use of such devices or highlighting which devices have been useful for patients would be a good resource for the community. Assistive communication devices may be available through public school IEP (individualized education program) plans or insurance following recommendations from a speech therapist. The ability of PURA patients to communicate could give insight into the patient experience and potentially identify additional needs and treatments. ***Patients have been documented to express pain and even oncoming seizures through these devices (from interviews with families/community).***
3. Addressing hypotonia is also a key focus for many families. We suggest working with clinicians to generate a standard of care to increase muscle development in patients. This may also likely aid bone density development. We also suggest further investigation into the role PURA is playing at the NMJ to determine if there are targetable pathways to decrease hypotonia in people with PURA syndrome.
4. ***Finally, among researchers and scientists interviewed, there was general consensus that more data needs to be collected on the effect of different medications PURA patients have used.*** This may be a long-term goal but should be a key consideration as the PSF designs their new patient registry as that can be a basis for beginning to understand all the medications people with PURA are or have been on, how effective

they have been, what side effects were observed, and if they needed to switch medication at some point. A caregiver-reported patient registry can assist a community with tracking new medications and outcomes across the population

Biological prioritizations for research:

1. What is the function of PURA peripherally? This includes at the NMJ as well as at pain and temperature receptors. Understanding the role PURA plays in the proper formation of these nerves and nerve terminals could also elucidate the role PURA plays at nerve terminals in the central nervous system which could have implications for other symptoms of PURA syndrome, including seizures.
2. What is the role of PURA in embryological development? The Xenopus model is a great model to begin answering this question (see section on Xenopus). Understanding how PURA haploinsufficiency affects development can give insight into disease mechanisms as well as therapeutic targets. Even if PURA syndrome begins early in embryogenesis, some pathological effects continue to progress past this period. This type of research can identify targetable pathways before symptoms emerge, biomarkers for early diagnosis, or compensatory pathways that could be targeted for treatment later.
3. Which proteins does PURA interact with at the NMJ? Identifying specific interactors and function at the NMJ will help prioritize therapeutics for hypotonia and maybe even reflex seizure symptoms. Understanding how these effects intersect with acetylcholine (Ach) function at the NMJ may provide insights into repurposing drugs used in other similar disorders.
4. Which proteins does PURA interact with in the central nervous system? Identifying specific interactors and function in the CNS will help prioritize therapeutics. In the CNS it will also be important to consider cell types. PURA's function in neurons may differ from its function in glial cells etc. Additionally, understanding PURA's pre- and post-synaptic role and role at the synaptic cleft may help in targeting dysfunction.

Are there ways we could increase the expression of the non-mutated PURA protein? Traditional gene therapy approaches aim to increase expression of the wild-type (or non-mutated) version of a gene of interest. This is often done by exogenously expressing the gene using a viral vector in a region of the body or a subset of cells. However, there are other ways of increasing the body's own expression of the healthy copy of the gene in conditions such as PURA syndrome that are caused by a mutation in only one copy of a person's PURA genes. For example, there has been work in Friedreich's ataxia to identify an ASO that could increase the stability of the healthy mRNA. This would then increase the production of healthy protein, thereby increasing expression of functional PURA (*19*). This is a type of ASO that upregulates gene activity, usually through increasing mRNA stability or increasing the activation of transcription. If no one is actively working on it, we recommend considering development of an ASO to increase the stability of the healthy copy of PURA. There are other ways to target increasing expression of the functional copy of PURA in the body, see table in Potential Future Therapeutic Targets.

Primary Obstacles and Considerations for Therapeutic Development

Given the goal of the PSF to generate therapeutics for PURA syndrome, we would like to highlight the obstacles that we have identified that may make this more difficult. Having the correct research tools can accelerate therapeutic development. If, for example, an antibody cannot be developed, there are work arounds that can be discussed. Many of the tools highlighted below would impact multiple therapeutic modalities (small molecules, genetic therapies, etc.).

Summary of Primary Obstacles

1. Lack of a consistent, specific antibody for the PURA protein
2. No completed Natural History Study or patient registry
3. Lack identified biomarkers of disease
4. Ubiquitous expression and function of PURA may complicate delivery or targeting

Biological Understanding/Drug Development

Recent advances by multiple labs to create mouse models of PURA syndrome have generated models with seizure phenotypes (from key opinion leader (KOL) interviews). This is exciting news as it may be an opportunity to test any new or new-to-PURA therapeutics for efficacy in reducing seizure activity. ***Since so many other groups and labs are working on this model, we do not think it should be a priority for the PSF to develop an animal model. However, it is important to keep an open line of communication with these partners to continue to understand the models and how the models can be shared with other researchers in the future.***

As of February 2025, current research into specific localization or interacting partners of the PURA protein are hindered by the lack of a robust commercially available antibody that is consistent and specific to PURA. However, there may be work ongoing through Jack's Tomorrow to generate a working antibody. Keeping informed on the progress of this antibody will be important both for sharing resources and for prioritization of research. At this time, there are key biological questions about the pathogenesis and clinical manifestations of PURA syndrome that the PSF could consider focusing on. Since there is currently no commercially available antibody that works well for PURA, we recommend deprioritizing efforts that rely on this resource. If one does become available, there may be a shift in priorities.

Clinical Development

Key gaps in the understanding of PURA syndrome itself exist that could present difficulties in the design and execution of any clinical trial for PURA. A true Natural History Study (NHS) is the

gold standard for identifying the clinical manifestation of PURA syndrome over time. However, NHSs are long and costly and are usually limited in the number of patients they enroll. It is possible to find clear clinical outcomes in other ways, including from a patient registry. We recommend continuing to support the work of Dr. Hunt and the current NHS while pivoting the majority of efforts to the generation of a direct to caregiver patient registry. Although patient registries may not have the depth of clinical data that a NHS has, they are an easier way to collect relevant information from a larger spectrum of the population (See section on Patient Registry for more details).

Another gap is a PURA syndrome or symptom-related biomarker (from an interview with Dr. Dierk Niessing). Biomarkers can be used for diagnosis or determining disease state. They can be very helpful in the design and execution of a clinical trial when they are a clear indicator of therapeutic effectiveness. We recommend continuing to monitor the field, including other seizure disorders, and learning about advances in phenotyping and diagnostics that could be advantageous to the PURA syndrome community.

Potential Future Therapeutic Targets

There are several areas of research that we recommend focusing to facilitate therapeutic development. Drug repurposing efforts that are ongoing or in development are a great way to identify an already approved drug that could be used by PURA patients. However, there are also some open questions on the function of PURA that could be targeted with therapeutics. Areas Odylia highlights for increased research are:

Open area of research	How this can aid therapeutic development
What are the metabolic impacts of *PURA* mutations? Can they be distinguished from neurodevelopmental impacts? (from discussion with Dr. Dierk Niessing)	Identifying the metabolic impacts could lead to the identification of treatments such as dietary modifications, enzyme supplements, or metabolic cofactors that could supplement treatments
Is it possible to increase the expression of the non-mutant PURA gene in patients?	This would allow for the increase of PURA expression without using gene therapy to artificially express PURA using a viral vector. There are several technologies that exist, such as: ASOs, CRISPR activation (CRISPRa), overexpression of transcription factors (TFs) that naturally regulate the target gene or small activating RNAs
What is the PURA interactome (what proteins does PURA interact with)?	A better understanding of this could indicate in which cells PURA is acting (this is especially true if the proteins are cell-type specific). It could also identify potential druggable targets.

Are there network effects of PURA haploinsufficiency?	Identifying the overall networks that a dysfunctional due to a reduction in PURA expression could identify druggable pathways.
How does PURA expression change pain signaling?	Pain is an important indicator for health and wellbeing. If pain is not properly signaled, then there is a potential for exacerbation of an injury. Therefore, proper pain sensation is important for overall health. Additionally, if pain is a trigger for seizures in PURA syndrome patients, then understanding how this system works could identify a druggable molecule, cell type or pathway.
What is PURA's function at the NMJ?	Given what is currently known about PURA function at the NMJ and similarities between other neurological and NMJ disorders (such as ALS and Fragile X), consideration of the following NMJ therapeutic approaches may yield viable therapeutic targets: (1) Neurotrophins, (2) acetylcholinesterase inhibitors (to prolong action of acetylcholine), (3) CCLC-1 chloride channel inhibitors, (4) Sympathetic stimulation. Experiments that focus on understanding where the dysfunction occurs (pre-synaptic, post-synaptic, synaptic cleft) may yield specific targets for PURA.

Resources and Tools

Cell lines

As of February 2025, there are cell lines in several PURA research labs including the labs of Dr. Jim Dowling and Dr. Dierk Niessing. As PSF continues to build their presence as a resource for the community, we recommend keeping an updated list of which labs work with PURA cell lines and the lab's process for sharing. In addition to animal models, cell lines can be a useful tool both for understanding biology as well as for testing therapeutics. According to an interview with Dr. Jim Dowling, he is working with PURA patient-derived fibroblasts with the aim of identifying a phenotype in these cells. In the future he is interested in further investigating cellular organelles for structural phenotypes in these lines and is interested in potentially differentiating these cells into neurons or muscle cells. If a clear phenotype is identified in these patient cell lines, then this endpoint might be useful for future drug screening efforts.

Animal Models

Animal models provide a means to study the effect of genetic mutations on behavior and biology, as well as test therapeutics for efficacy, safety, and toxicity. The existence of an effective translational animal model enables disease communities to test therapeutics on relevant models prior to moving into human testing. *While this lowers risks that arise if a treatment is moved directly into human trials without animal testing, models can also have the added benefit of accelerating discovery of new therapeutics, decreasing wasted funding, and increasing pharma interest in a specific disease.* Animal models are validated based on certain criteria and can have predictive, face, and/or construct validity.

- Predictive validity: a measure of how well a model can be used to predict currently unknown aspects of human disease (e.g. correlation between the animal and human as it relates to therapeutic outcomes)
- Face validity: a measure of how well a model replicates the disease phenotype in humans (e.g. does the animal model exhibit all the symptoms and behaviors found in the human condition?)
- Construct validity: how well the mechanism of disease in the humans is matched in the animal model (e.g. does the underlying biology match between the human and the animal)

While it is ideal to have an animal model with predictive, face, and construct validity, this is rarely possible. Important findings can still be made using an animal model which does not fully recapitulate the human condition.

Animal Models for PURA Syndrome

Overview of current animal models for PURA syndrome:

1. There are a number of PURA mouse models developed or currently being phenotyped for PURA syndrome. These models are primarily in use to for gene therapy and seizure medication development.
2. So far, the mice heterozygous for PURA KO do not show strong, reproducible phenotypes (as of January 2025) while animals homozygous for PURA KO do have a phenotype. It is important to keep in mind that this does not directly mimic the human condition and indicates that the mouse is more tolerant to perturbations in the PURA gene than humans are. This does not negate their utility as a model of disease but is important to keep in mind when interpreting data.
3. Since there is already a concerted effort to generate and characterize these mouse models it creates an opportunity for the PSF to focus on different animal models to tackle different obstacles for PURA.

We recommend that the PSF keep a list of all animal models and research tools available to the PURA research community on their website, or easily accessible by the community. As new models are developed and characterized, we recommend updating this list. The PSF can also list which of the resources and tools are available to the community and who to contact or where to go to get access. Below is a place to start for this asset list. We recommend keeping an open communication with researchers so the PSF can continue to build up this list for years to come. This can be a very useful resource for researchers to best know what is available for sharing.

Resource	Description	Location	Sharing Protocol Established?
Cell lines	Patient-derived cell lines	Dr. James Dowling, University of Toronto	
Biobank		Germany	
		Australia	
Mouse Models	PURA Knockout	Temple University	Unknown
	PURA Knockout	University of Helsinki	
	PURA model(s)	Munich	
	PURA Knock in	Florey Institute, Australia	
	PURA Knockout	Jackson Labs	Available (https://www.jax.org/strain/038424)
	PURA Conditional KO	Jackson Labs	Available (https://www.jax.org/strain/038423)
Xenopus Model	PURA Knockout	Dr. Matt Guille, University of Portsmouth	
Zebrafish Model	PURA Knockout	Bettina Schmid	
Patient Data	Natural History Study	University of Southampton	Yes
	Patient Registry	TBD	
Antibody	Unknown	UVM and Johns Hopkins	In progress

Non-mouse Animal models

The fruit fly, or *Drosophila melanogaster*, is a model that has been used by geneticists for decades. Because of this, many research techniques have been developed which make it a useful model for studying a genetic disorder such as PURA syndrome. Flies reproduce rapidly and develop to adulthood quickly, so scale-up of fly colonies is quick and cost-effective

(compared to mammalian disease models). The family of purine-rich element-binding (PUR) protein is highly conserved from plants to humans (*9*). Flies do have the PURA gene, but they are missing PURB and PURG. This means that they may be missing some key regulation and interactions of PURA so they are unlikely to be an ideal model for understanding PURA biology as it relates to PURA syndrome in humans. Additionally there are existing Xenopus (frog/tadpole) models of PURA syndrome in the lab of Dr. Matthew Guille that could be used to investigate a number of key biological questions, as well as therapeutic testing.

Organizational Strategy

As rare disease patient advocacy organizations are largely run by volunteers, it is ideal to take advantage of the expertise that already exists within the PURA syndrome community. Odylia recommends reaching out to the already existing PURA community to determine if there are additional people who are interested in volunteering time. This can be done through parents and caregivers, as well as, extended family and friends to tap into relevant skill sets that are otherwise expensive to hire and helpful to an organization such as graphic designers, lawyers, coders, marketers, etc. Identifying critical needs and 'making the ask' is an easy way to engage the community without directly needing to ask for monetary donations.

In addition to the PURA community already established, there is a lot that can be gained by combining forces with other rare disease patient advocacy groups. In the Related Patient Groups section below we compiled several related diseases and their affiliated patient advocacy group. Additionally, we hope to increase the researchers interested in PURA syndrome and PURA biology. In the Academic Groups section we aimed to identify researchers previously unknown to the PSF who are doing work highly relevant to aspects of PURA syndrome and PURA syndrome drug development. As new research is funded by the Foundation it may be worthwhile to have discussions with these and other researchers to broaden the field of PURA research.

Related Patient Groups

Disease	Gene(s) Responsible	Similarity to PURA	Symptoms	Foundation
Fragile X Syndrome	FMR1	RNA processing and P-body dysfunction	intellectual disability, attention deficit and hyperactivity, anxiety, autistic behaviors, seizures, speech delay	FRAXA Research Foundation: https://www.fraxa.org/

DDX3X Syndrome	DDX3X	RNA processing and P-body dysfunction	intellectual disabilities, seizures, autism, low muscle tone, brain abnormalities, and slower physical developments, range of verbal and motor dysfunction	DDX3X Foundation: https://ddx3x.org/
Congenital Myasthenic Syndrome (with seizures)	CHRNE	NMJ defects and seizure	extreme fatigue and profound muscle weakness, Can impact ability to see, swallow, smile, walk, breathe, or engage in normal, everyday activity	Myasthenia gravis foundation of America: https://myasthenia.org/
SATB2-associated syndrome	SATB2	speech development expressive vs receptive, as it relates to muscle function, issues with bone dysfunction	intellectual disability, attention deficit and hyperactivity, anxiety, autistic behaviors, seizures, speech delay, bone defects	SATB2 Gene Foundation: https://satb2gene.org/
Smith-Magenis Syndrome (SMS)	RAI1	experience with Coriell Biobanking effort, sleep disturbances	Intellectual disability, sleep disturbances, behavioral issues (aggression, hyperactivity), self-harming behaviors	PRISMS: https://www.prisms.org/about-sms/what-is-sms/
Mowat-Wilson Syndrome	ZEB2	hypotonia, epilepsy/seizures, feeding difficulties, sleep disturbances, delayed or absent walking	Intellectual disability, delayed development, heart defects, genital and urinary tract abnormalities, seizures, intestinal disorder called Hirschsprung disease	Mowat-Wilson Syndrome Foundation: https://mowat-wilson.org/
Rett Syndrome	MECP2	reflex seizures, protein function, hypotonia, communication difficulties, feeding and	Loss of purposeful hand movements, loss of speech, motor impairments (ataxia), seizures, intellectual disability, repetitive hand movements (wringing)	International Rett Syndrome Foundation: https://www.rettsyndrome.org/ ; Rett syndrome Research Trust: https://reverserett.org/

		growth difficulties		

Ongoing work in other Foundations

Like many other neurodevelopmental disorders, there is more than one Foundation that supports research and drug development for PURA syndrome. Understanding priorities of the other Foundations will enable PSF to most effectively allocate and focus resources. Just because these organizations are focused on this work does not mean the PSF cannot also engage in research in these areas. However, we would recommend not attempting to do the exact same research but instead working in collaboration or in support of these programs where it is of strategic value to the community. Facilitating communication between organizations through regular monthly or quarterly meetings and timely updates on funding decisions (to avoid overlap) can facilitate the effective formation of a 'federation' of PURA groups. Highlighting this coordination across groups is important for increasing patient community and research engagement. We suggest communicating on the PSF's website about these points of intersection or integration across the various foundations.

As mentioned in previous sections, Odylia also recommends that the PSF keep track of the resources generated by these and other organizations such as antibodies, animal models, cell lines, etc. so PSF is a resource for all PURA researchers to find tools that will help make their research easier. This could be done with minimal effort and aligns with the PSF's goal of sharing information, and being a hub of information for the PURA community at-large. This will create a space where researchers can see who else is working in the space, with the goal of increased collaboration and transparency.

There is a biobank in Australia (operated by PURA Syndrome Australia) and the PSF is actively involved in the generation of a biobank in Germany. Ideally, these biobanks would be in communication to attempt to collect the same types of samples and in similar ways. ***We recommend requesting the biobanking protocol and consent forms from the Australian and European groups (if not already shared) to review the information to see if the protocol and consents are similar to those used in the selected US biobank. Clarification on if and how samples could be shared among them could benefit all biobanks and researchers. Convening the various groups to review the biobanking efforts in the different countries can increase collaboration and attempt support biobanking efforts that are as consistent as possible across the international efforts for PURA.***

Academic Groups

Investigating new therapeutics and advancing knowledge of the pathways disrupted in PURA patients requires dedicated clinicians, researchers, and patient families working together. While there are already many dedicated researchers studying PURA, it is always good practice to

reach out to additional researchers who work in related fields or whose research interests intersect with the PURA community. We always recommend expanding your research network whenever possible to encourage fresh ideas and critical feedback on current approaches. These investigators have expertise that could be helpful in continuing progress towards solving some of the predominant concerns of PURA patients and their families.

Sarah Slavoff, Ph.D.
Biomolecular Design and Discovery, Yale University

- P-body biology and targeting
- RNA processing
- "chemical biology to elucidate spatial and structural regulation of mRNA degradation inside living cells as a mechanism of gene expression regulation, to identify sites and cellular roles of non-canonical translation initiation, and to understand the scope and regulation of small open reading frame (smORF) translation across evolution."

https://pubs.acs.org/doi/epdf/10.1021/acs.biochem.7b01162?ref=article_openPDF
https://slavofflab.yale.edu/research

Sarah Elsea, Ph.D.
Molecular and Human Genetics, Baylor College of Medicine

- Neurodevelopmental disorders with sleep and metabolic dysfunction or food-related behaviors

https://www.bcm.edu/people-search/sarah-elsea-21035

Stephen Kolb, M.D., Ph.D.
Professor of Neurology and Biological Chemistry and Pharmacology, The Ohio State University Medical Center

- Devoted to the understanding of molecular pathways that, when altered, result in diseases of the motor neuron.
- Particularly interest in alterations in RNA metabolism

https://osuchildrensmusclegroup.org/faculty-research/stephen-kolb/

PURA patient registry

Current efforts to recruit patients to the patient registry run by Southampton has successfully increased the amount of collected patient data. However, there seem to be barriers to accessing this dataset and it has been reported as an onerous undertaking by many families despite efforts to make it easier through hiring someone to walk through the forms with families. Unfortunately, this has resulted in low participation both for initial enrollment and for ongoing engagement or follow-up. This is a missed opportunity resulting in important data going uncollected, and inaccessible. Therefore, after discussions with the PSF board and other PURA syndrome caregivers, there may be an opportunity to partner with another registry.

Ideally this registry would be user friendly, translatable to other languages (in addition to English), have the capability to collect longitudinal data, be accessible to interested researchers, and has appropriate data security and privacy. The registry landscape has changed significantly over the last 5 or 10 years providing options that were not previously available. Major emphasis should be placed on collection of data relevant to biological and clinical questions important for clinical trials and disease understanding.

Scientific Advisory Board and Clinical Advisory Board

Given the recent changes to the global research network (GRN), the PSF has a great opportunity to engage old and new researchers to its Scientific Advisory Board (SAB). An advisory board serves to advise the PSF on research priorities, as well as provide updates on key research in the field. It is not necessary to include all PURA researchers in an advisory board, some may not have the time to commit to this role in which case we advise to ask if they would be willing to advise in a less formal way if the PSF would like to keep a line of communication open. For the SAB, we recommend regular meetings to discuss what the foundation is working on as well as address progress in the field. The PSF should have clear goals for these meetings. It is helpful to send out slides or materials you hope to discuss in advance of the meeting so advisors can come prepared. Because it can be hard to get multiple researchers on a call at the same time it is important to utilize all the time available in the most constructive, informative way possible.

Additionally, there may also be an opportunity to have a separate group of clinicians or more clinically minded researchers who make up a Clinical Advisory Board (CAB). This group can engage on discussions around patient treatment, standard of care, gaps in understanding or effectiveness, as well as patient data collection efforts, and clinical trial design. This group could be a subset of the SAB or a stand-alone group, whatever makes the most sense given interest, availability, and priorities. Caregiver interviews highlighted an interest in PSF supporting a formal clinicians' network with a true clinic, ideally multiple doctors, to build infrastructure around PURA treatment. Since the PURA syndrome population is so small, an ideal way to do this may be to team up with other similar diseases.

Website Informational Materials

There is always an opportunity to build up informational material on a website. Given PSF's position with the community and the other organizations that exist to support PURA research, we recommend positioning the PSF website as a hub of information. To that end, the PSF website should be a place where not only patients and families but also researcher and clinicians go to learn about updates in PURA. Collating a list of all animal and cell models of PURA syndrome including genotype, phenotype, and other research tools, and ways to access them would be a wonderful resource for the community. Finally, since there is more than one foundation supporting PURA syndrome research, it is important to be very clear on your

website what the PSF does. Being transparent about your collaborations and your specific mission will hopefully clarify within the community who the PSF is and what they stand for. This is especially true for the patient registry and the Biobanks that PSF supports. These efforts are critical for supporting fundraising over time as a clear and up-to-date website communicates to constituents the PSF is a good steward of their donations and support.

References

1. D. Hunt, R. J. Leventer, C. Simons, R. Taft, K. J. Swoboda, M. Gawne-Cain, the DDD study, A. C. Magee, P. D. Turnpenny, D. Baralle, Whole exome sequencing in family trios reveals *de novo* mutations in *PURA* as a cause of severe neurodevelopmental delay and learning disability. *J. Med. Genet.* **51**, 806–813 (2014).

2. S. R. Lalani, J. Zhang, C. P. Schaaf, C. W. Brown, P. Magoulas, A. C.-H. Tsai, A. El-Gharbawy, K. J. Wierenga, D. Bartholomew, C.-T. Fong, T. Barbaro-Dieber, M. K. Kukolich, L. C. Burrage, E. Austin, K. Keller, M. Pastore, F. Fernandez, T. Lotze, A. Wilfong, G. Purcarin, W. Zhu, W. J. Craigen, M. McGuire, M. Jain, E. Cooney, M. Azamian, M. N. Bainbridge, D. M. Muzny, E. Boerwinkle, R. E. Person, Z. Niu, C. M. Eng, J. R. Lupski, R. A. Gibbs, A. L. Beaudet, Y. Yang, M. C. Wang, F. Xia, Mutations in PURA Cause Profound Neonatal Hypotonia, Seizures, and Encephalopathy in 5q31.3 Microdeletion Syndrome. *Am. J. Hum. Genet.* **95**, 579–583 (2014).

3. W. Dai, Y. Sun, Y. Fan, Y. Gao, Y. Zhan, L. Wang, B. Xiao, W. Qiu, X. Gu, K. Sun, Y. Yu, N. Xu, A 25 Mainland Chinese cohort of patients with PURA-related neurodevelopmental disorders: clinical delineation and genotype–phenotype correlations. *Eur. J. Hum. Genet.* **31**, 112–121 (2023).

4. M. R. F. Reijnders, R. Janowski, M. Alvi, J. E. Self, T. J. Van Essen, M. Vreeburg, R. P. W. Rouhl, S. J. C. Stevens, A. P. A. Stegmann, J. Schieving, R. Pfundt, K. Van Dijk, E. Smeets, C. T. R. M. Stumpel, L. A. Bok, J. M. Cobben, M. Engelen, S. Mansour, M. Whiteford, K. E. Chandler, S. Douzgou, N. S. Cooper, E.-C. Tan, R. Foo, A. H. M. Lai, J. Rankin, A. Green, T. Lönnqvist, P. Isohanni, S. Williams, I. Ruhoy, K. S. Carvalho, J. J. Dowling, D. L. Lev, K. Sterbova, P. Lassuthova, J. Neupauerová, J. L. Waugh, S. Keros, J. Clayton-Smith, S. F. Smithson, H. G. Brunner, C. Van Hoeckel, M. Anderson, V. E. Clowes, V. M. Siu, T. Ddd Study, P. Selber, R. J. Leventer, C. Nellaker, D. Niessing, D. Hunt, D. Baralle, PURA syndrome: clinical delineation and genotype-phenotype study in 32 individuals with review of published literature. *J. Med. Genet.* **55**, 104–113 (2018).

5. K. M. Johannesen, E. Gardella, C. E. Gjerulfsen, A. Bayat, R. P. W. Rouhl, M. Reijnders, S. Whalen, B. Keren, J. Buratti, T. Courtin, K. J. Wierenga, B. Isidor, A. Piton, L. Faivre, A. Garde, S. Moutton, F. Tran-Mau-Them, A.-S. Denommé-Pichon, C. Coubes, A. Larson, M. J. Esser, J. P. Appendino, W. Al-Hertani, B. Gamboni, A. Mampel, L. Mayorga, A. Orsini, A. Bonuccelli, A. Suppiej, J. Van-Gils, J. Vogt, S. Damioli, L. Giordano, S. Moortgat, E. Wirrell, S. Hicks, U. Kini, N. Noble, H. Stewart, S. Asakar, J. S. Cohen, S. R. Naidu, A. Collier, E. H. Brilstra, M. H. Li, C. Brew, S. Bigoni, D. Ognibene, E. Ballardini, C. Ruivenkamp, R. Faggioli, A. Afenjar, D. Rodriguez, D. Bick, D. Segal, D. Coman, B. Gunning, O. Devinsky, L. A. Demmer, T. Grebe, D. Pruna, I. Cursio, L. Greenhalgh, C. Graziano, R. R. Singh, G. Cantalupo, M. Willems, S. Yoganathan, F. Góes, R. J. Leventer, D. Colavito, S. Olivotto, B. Scelsa, A. V. Andrade, K. Ratke, F. Tokarz, A. S. Khan, C. Ormieres, W. Benko, K. Keough, S. Keros, S. Hussain, A. Franques, F. Varsalone, S. Grønborg, C. Mignot, D. Heron, C. Nava, A. Isapof, F. Borlot, R. Whitney, A. Ronan, N. Foulds, M. Somorai, J. Brandsema, K. L. Helbig, I. Helbig, X. R. Ortiz-González, H. Dubbs, A. Vitobello, M. Anderson, D. Spadafore, D. Hunt, R. S. Møller, G. Rubboli, the PURA study group, *PURA*- Related Developmental and Epileptic Encephalopathy: Phenotypic and Genotypic Spectrum. *Neurol. Genet.* **7**, e613 (2021).

6. M. Proske, R. Janowski, S. Bacher, H.-S. Kang, T. Monecke, T. Koehler, S. Hutten, J. Tretter, A. Crois, L. Molitor, A. Varela-Rial, R. Fino, E. Donati, G. De Fabritiis, D. Dormann, M. Sattler, D. Niessing, PURA syndrome-causing mutations impair PUR-domain integrity and affect P-body association. *eLife* **13**, RP93561 (2024).

7. S. Yu, C. Jiang, Y. Yang, F. Cheng, F. Liu, C. Liu, X. Gong, Purine-rich element binding protein alpha: a DNA/RNA binding protein with multiple roles in cancers. *Mol. Med.* **31**, 20 (2025).

8. K. Khalili, L. D. Valle, V. Muralidharan, W. J. Gault, N. Darbinian, J. Otte, E. Meier, E. M. Johnson, D. C. Daniel, Y. Kinoshita, S. Amini, J. Gordon, Pur␣ Is Essential for Postnatal Brain Development and Developmentally Coupled Cellular Proliferation As Revealed by Genetic Inactivation in the Mouse. *MOL CELL BIOL* **23** (2003).

9. J. J. López-Rivera, L. Rodríguez-Salazar, A. Soto-Ospina, C. Estrada-Serrato, D. Serrano, H. M. Chaparro-Solano, O. Londoño, P. A. Rueda, G. Ardila, A. Villegas-Lanau, M. Godoy-Corredor, M. Cuartas, J. I. Vélez, O. M. Vidal, M. A. Isaza-Ruget, M. Arcos-Burgos, Structural Protein Effects Underpinning Cognitive Developmental Delay of the PURA p.Phe233del Mutation Modelled by Artificial Intelligence and the Hybrid Quantum Mechanics–Molecular Mechanics Framework. *Brain Sci.* **12**, 871 (2022).

10. L. Molitor, M. Klostermann, S. Bacher, J. Merl-Pham, N. Spranger, S. Burczyk, C. Ketteler, E. Rusha, D. Tews, A. Pertek, M. Proske, A. Busch, S. Reschke, R. Feederle, S. M. Hauck, H. Blum, M. Drukker, P. Fischer-Posovszky, J. König, K. Zarnack, D. Niessing, Depletion of the RNA-binding protein PURA triggers changes in posttranscriptional gene regulation and loss of P-bodies. *Nucleic Acids Res.* **51**, 1297–1316 (2023).

11. L. G. Chepenik, A. P. Tretiakova, C. P. Krachmarov, E. M. Johnson, K. Khalili, The single-stranded DNA binding protein, Pur-alpha, binds HIV-1 TAR RNA and activates HIV-1 transcription. *Gene* **210**, 37–44 (1998).

12. D. C. Daniel, M. J. Wortman, R. J. Schiller, H. Liu, L. Gan, J. S. Mellen, C.-F. Chang, G. L. Gallia, J. Rappaport, K. Khalili, E. M. Johnson, Coordinate effects of human immunodeficiency virus type 1 protein Tat and cellular protein Puralpha on DNA replication initiated at the JC virus origin. *J. Gen. Virol.* **82**, 1543–1553 (2001).

13. B. Swinnen, W. Robberecht, L. Van Den Bosch, RNA toxicity in non-coding repeat expansion disorders. *EMBO J.* **39**, e101112 (2020).

14. J. G. Daigle, K. Krishnamurthy, N. Ramesh, I. Casci, J. Monaghan, K. McAvoy, E. W. Godfrey, D. C. Daniel, E. M. Johnson, Z. Monahan, F. Shewmaker, P. Pasinelli, U. B. Pandey, Pur-alpha regulates cytoplasmic stress granule dynamics and ameliorates FUS toxicity. *Acta Neuropathol. (Berl.)* **131**, 605–620 (2016).

15. M. Mroczek, S. Iyadurai, Neuromuscular and Neuromuscular Junction Manifestations of the PURA-NDD: A Systematic Review of the Reported Symptoms and Potential Treatment Options. *Int. J. Mol. Sci.* **24**, 2260 (2023).

16. K. Ohno, B. Ohkawara, X.-M. Shen, D. Selcen, A. G. Engel, Clinical and Pathologic Features of Congenital Myasthenic Syndromes Caused by 35 Genes—A Comprehensive Review. *Int. J. Mol. Sci.* **24**, 3730 (2023).

17. J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, O. Ronneberger, L. Willmore, A. J. Ballard, J. Bambrick, S. W. Bodenstein, D. A. Evans, C.-C. Hung, M. O'Neill, D. Reiman, K. Tunyasuvunakool, Z. Wu, A. Žemgulytė, E. Arvaniti, C. Beattie, O. Bertolli, A. Bridgland, A.

Cherepanov, M. Congreve, A. I. Cowen-Rivers, A. Cowie, M. Figurnov, F. B. Fuchs, H. Gladman, R. Jain, Y. A. Khan, C. M. R. Low, K. Perlin, A. Potapenko, P. Savy, S. Singh, A. Stecula, A. Thillaisundaram, C. Tong, S. Yakneen, E. D. Zhong, M. Zielinski, A. Žídek, V. Bapst, P. Kohli, M. Jaderberg, D. Hassabis, J. M. Jumper, Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).

18. A. McNeill, Good genotype-phenotype relationships in rare disease are hard to find. *Eur. J. Hum. Genet.* **30**, 251–251 (2022).

19. Y. Li, J. Li, J. Wang, D. R. Lynch, X. Shen, D. R. Corey, D. Parekh, B. Bhat, C. Woo, J. J. Cherry, J. S. Napierala, M. Napierala, Targeting 3' and 5' untranslated regions with antisense oligonucleotides to stabilize frataxin mRNA and increase protein expression. *Nucleic Acids Res.* **49**, 11560–11574 (2021).

Interview References

Odylia would like to that all the researchers, clinicians, PURA Syndrome Foundation Board Members, and caregivers who took the time to speak with us during this process. Below is a list of all the researchers/clinicians spoken to and the date of the interview. The identities of the caregivers and families will remain confidential.

Dr. Dierk Niessing, Helmholtz-Munich University; November 6th 2024

Dr. Matt Guille, University of Portsmouth; November 7th 2024

Dr. Jim Dowling, UT; November 19th 2024

Dr. David Hunt, University of Southampton; December 20th 2024