To date, NPKUA has provided nearly $6.5 million to fund over 85 grant projects. See some of the approved grant projects from the last few years:

2025 Grant Season

- **Novel Approaches to Achieving Permanent Therapeutic Gene Insertion in PAH-Deficient Mice**
  Cary Harding, M.D., Oregon Health & Science University

  Dr. Harding and his lab are working to develop a safe and permanent treatment for PKU using gene insertion. This specific project will examine two novel methods to insert a working copy of the phenylalanine hydroxylase (PAH) gene into the liver cells of a PKU mouse, which serves as a model for human PKU. Because this approach would be inserting a working copy of the gene, it is not specific to certain genetic variants, and it can be applied to any animal without a working copy of PAH. If effective, this project could provide the foundation for clinical studies in gene therapy for PKU.

- **AI4PKU study: Artificial Intelligence for PKU**
  Charlotte M.A. Lubout, M.D., University Medical Center Groningen

  Dr. Lubout and her team will utilize artificial intelligence (AI) to investigate if machine learning can aid in the management of newborns with PKU. This team will develop a machine learning model based on data from patient charts of newborn babies, inclusive of different disease severities, at two metabolic centers in the Netherlands. The goal of this project is to identify factors leading to variation in Phe levels in infants with PKU and to predict Phe values to provide subsequent diet advice to maintain levels in the recommended range. If this foundational project is successful, the proposed machine learning model can be validated and eventually used to guide diet advice in clinic for infants with PKU.

- **Defining Protein Requirements in Adults with PKU: Impact of Genotype and Medical Food Intake**
  Jessica Strosahl, Ph.D., Emory University

  Dr. Strosahl and her team propose to provide a more accurate, individualized assessment of protein requirements in adults with PKU by better understanding protein metabolism in PKU. To do this, they will use the indicator amino acid oxidation method, which is a minimally invasive test to determine how much of an amino acid someone actually needs in their diet. Specifically, the goals of this project are to determine the protein requirements in adults with PKU of varying disease severities and investigate how these requirements may change if someone has different amounts of

medical food and intact protein. If successful, the data gained from this project could help to update nutrition guidelines and treatment strategies to provide more personalized nutrition recommendations for PKU.

- **Proficiency in Verbal Discourse: A Proposed Outcome Measure for Clinical Trials in Phenylketonuria (PKU)**
  Susan Waisbren, Ph.D., Boston Children's Hospital

  Dr. Waisbren and her team plan to establish a tool that can detect changes in neurocognition in people with PKU after changes in treatment. Changes in blood phenylalanine (Phe) is the primary method of determining changes in symptoms after treatment updates, but this often fails to capture the patient-reported outcomes and differences in neurocognition. Therefore, this study team is working to create a tool that can measure these changes in symptoms associated with blood Phe levels. This project is a continuation of a previously NPKUA-funded research project by Dr. Waisbren and her team to use statistical and AI analyses to quantify language expression through the Proficiency in Verbal Discourse tool. The data from this project clearly differentiated adults with PKU from a control comparison group and was significantly corelated with changes in Phe. The goals of the present project are to confirm that this tool distinguishes between people with PKU from a comparison control group, show that within-person changes using this tool correlate with changes in blood Phe and demonstrate that this tool reflects patient-reported perceptions of functioning and quality of life. If successful, this project could be the foundational evidence needed to validate this tool to be used in clinical trials for PKU.

- **Engineered Native Gut Bacteria for Phenylketonuria Therapy**
  Amir Zarrinpar, M.D., Ph.D., University of California, San Diego

  Dr. Zarrinpar and his team are working to develop a novel therapeutic for PKU that will allow for long-term breakdown of dietary Phe in the gut. This team has examined previous strategies that have used engineered probiotics but were unable to engraft in the intestine long enough to be effective, ultimately making them unsuccessful. This highlights a gap in the understanding of how to achieve persistent colonization of live bacterial therapeutics in the gut. Therefore, the goal of this project is to develop a platform that uses a bacterial enzyme that stably engrafts in the gut and provides sustained enzyme activity to serve as a long-term therapeutic without requiring high-dose administration. To do this, Dr. Zarrinpar's group will engineer native *E. coli* strains to express phenylalanine ammonia-lyase (PAL), which is an enzyme that helps to break down Phe. If successful, the results of this project can provide the foundation for a gut-based microbial therapy for PKU and will establish a strategy to engineer long-term therapeutic microbial colonization, which can be applied to other metabolic and rare disease treatments.

2024 Grant Season

- **Cell Therapy for Phenylketonuria**
  Markus Grompe, M.D., Oregon Health & Science University

  Dr. Grompe and his team are working on a new treatment for PKU. Their research started in 2021 and focuses on using the hepatocyte liver cells for cell therapy. These cells are designed to avoid being attacked by the immune system so that they can be transplanted from one person to another. To optimize the growth of the transplanted cells, this team has altered these liver cells so that they can survive and grow when exposed to acetaminophen, that can kill liver cells in high doses. In tests with mice that have PKU, this method has successfully lowered phenylalanine (Phe) levels in the blood for extended periods of time.

  Next, the researchers will work on creating human liver cells that can hide from the immune system and won't be rejected after transplantation. If this works, they will test these cells in mice with PKU and later move on to testing in larger animals with more advanced immune systems.

- **Novel Approaches to Achieving Permanent Gene Correction in PAH-Deficient Mice**
  Cary Harding, M.D., Oregon Health & Science University

  Dr. Harding and his team have been working on this project since 2018 to develop a safe and lasting cure for PKU using gene editing focused on the liver. In this project, they are testing a method that uses CRISPR/Cas9 technology to insert a new gene into the livers of mice with PKU. This gene produces phenylalanine hydroxylase (PAH), the defective enzyme in people with PKU. If the gene insertion works, the team will use a special technique involving acetaminophen to allow the edited liver cells to grow preferentially over the unedited cells. The goal is to increase concentrations of these cells in the liver to allow for enough phenylalanine hydroxylase enzyme to metabolize Phe and lower blood Phe levels in the PKU mice.

- **The Burden of PKU on both the disease and its treatments: Asking the right questions toward the first PKU-PROEM**
  Francjan van Spronsen, M.D., Ph.D., University of Groningen

  Dr. van Spronsen and his team are creating and validating a new questionnaire to understand the full impact of living with PKU. This tool will be used worldwide and will include questions about both how PKU affects daily life and how treatments impact patients. To make the questionnaire, they will gather the right words and phrases to describe these experiences. This will then be used to develop a validated Patient Reported Outcome and Experience Measure (PROEM) questionnaire that will assess the full burden of PKU on daily life. Dr. van Spronsen has already brought together healthcare

providers, researchers, and patient advocacy groups from around the world to help create this tool.

2023 Grant Season

- **Development of Novel Psychological Assessment Tools & Anxiety Intervention for PKU**
  Shawn E. Christ, Ph.D., University of Missouri

  The focus of this research is to explore the efficacy and feasibility of a short-term skills-based intervention (Show ME FIRST) on anxiety and depression in adolescents with PKU. Additionally, this team plans to assess the validity of a novel assessment tool created by their research team to capture real time neurocognitive and psychological function.

- **Cell Therapy for Phenylketonuria**
  Markus Grompe, M.D., Oregon Health & Sciences University

  This research is a continuation of Dr. Grompe's grant project starting in 2021. This ongoing project explores the methods of overcoming two current challenges with hepatocyte transplantation as a cure for PKU: insufficient cell replacement levels to achieve correction of systemic phenylalanine levels, and issues related to immunosuppression. To combat insufficient cell replacement levels, their team has developed a system of cell expansion via gene-edited liver cells, hepatocytes, that are resistant to the drug acetaminophen. These gene-edited hepatocytes will preferentially expand over the non-gene edited cells that are not resistant to acetaminophen toxicity. This approach has proven to provide long-lasting therapeutic correction of blood phenylalanine levels in a PKU mouse model. Secondly, their team plans to generate immune stealthy hepatocytes to prevent immune rejection in allogenic hepatocyte transplantation.

- **Structural Insights into the Regulation of Phenylalanine Hydroxylase in Phenylketonuria**
  Kushol Gupta, Ph.D., University of Pennsylvania

  This research aims to gain a detailed mechanistic understanding of the regulation of the PAH enzyme by creating a synthetic version of two *PAH* gene mutations that can rescue the mutated PAH enzyme increasing its enzymatic activity. They hope this will allow for a determination of the molecular basis of allosteric regulation of the PAH enzyme and a better understanding of how clinical mutations affect PAH enzyme regulation. This approach can potentially lead to development of future treatments for PKU.

- **Novel Approaches to Achieving Permanent Gene Correction in PAH-Deficient Mice**
  Cary O. Harding, M.D., Oregon Health & Sciences University

  This research is part of an ongoing project from Dr. Harding since 2018. His work aims to investigate the therapeutic effectiveness of two possible approaches in a PAH-deficient mouse model of human PKU: CRISPR-Cas9 facilitated gene integration of a phenylalanine *hydroxylase (PAH)* gene into the hepatocyte genome of PAH-deficient mice, or a novel gene editing strategy called Programmable Addition via Site-specific Targeting Elements (PASTE) that could improve the frequency of gene editing.

- **Can Care of Adult PKU Be Improved with Additional Dietary Large Neutral Amino Acids: A Protocol for an Nof-1 Study**
  Shoji Yano, M.D., Ph.D., University of Southern California

  This research will use Phenylalanine (Phe), Tyrosine (Tyr), and the Phe/Tyr ratio as biomarkers, as well as other clinical assessments to determine the impact of large neutral amino acids (LNAAs) on the outcomes of PKU adults. LNAAs have historically been used as a treatment to reduce the amount of Phe that can be taken up by the brain through competition with other neutral amino acids across the LATI transporter. Their team hopes to identify the appropriate use of LNAAs to improve neurocognitive symptoms and possibly allow increased dietary protein intake. This study was previously awarded grant funding from the NPKUA but has requested additional funds to increase enrollment in hopes of providing more definitive study results.

2022 Grant Season

- **Cell Therapy of Phenylketonuria**
  Markus Grompe, M.D., Oregon Health & Sciences University

  The research to be conducted will explore methods of overcoming two current challenges with hepatocyte transplantation as a cure for PKU: insufficient cell replacement levels to achieve correction of systemic phenylalanine levels, and issues related to immunosuppression. To combat insufficient cell replacement levels, their team has developed a system of cell expansion via gene-edited hepatocytes that are resistant to the hepatotoxic effects of the drug acetaminophen. These gene-edited hepatocytes will preferentially expand over the non-gene edited cells that are not resistant to acetaminophen toxicity. This approach has proven to provide long-lasting therapeutic correction of blood phenylalanine levels in a PKU mouse model. Secondly, their team plans to generate immune stealthy hepatocytes to prevent immune rejection in allogenic hepatocyte transplantation.

- **Novel Approaches to Achieving Permanent Gene Correction in PAH-Deficient Mice**
  Cary Harding, M.D., Oregon Health & Sciences University

  This project aims to investigate the therapeutic effectiveness of two possible approaches in a PAH-deficient mouse model of human PKU: CRISPR-Cas9 facilitated gene integration of a phenylalanine *hydroxylase (PAH)* gene into the hepatocyte genome of PAH-deficient mice, or a novel gene editing strategy called Programmable Addition via Site-specific Targeting Elements (PASTE) that could improve the frequency of gene editing.

- **Identification of linguistic markers revealing severity of symptom in phenylketonuria: A novel measurement tool for clinical trials**
  Susan Waisbren, Ph.D., Boston Children's Hospital

  This research explored the effectiveness of the Cookie Theft Picture Task, which is a standardized instrument used to quantify parameters of spontaneous speech. Dr. Waisbren and her team are interested to understand if this tool could be used to measure the subtle deficits associated with neurocognitive functioning in individuals with PKU. If proven effective, the Cookie Theft Picture Task could be used to capture the benefits of developing treatments.

2021 Grant Season

- **Intervention Targeting PKU Cerebral Energy Deficit and Oxidative Stress**
  Steven F. Dobrowolski, Ph.D., University of Pittsburgh School of Medicine

- **Cell Therapy for Phenylketonuria**
  Markus Grompe, M.D., Oregon Health & Sciences University

- **Novel Approaches to Achieving Permanent Gene Correction in PAH-Deficient Mice**
  Cary Harding, M.D., Oregon Health & Sciences University

- **Understanding the Molecular Basis for Hyperphenylalanemia Toxicity and Developing Therapeutic Options for PKU Patients**
  Erik Koppes, Ph.D., Children's Hospital of Pittsburgh

- **Can Care of Adult PKU Be Improved with Additional Dietary Large Neutral Amino Acids-an N-of-1 Study**
  Shoji Yano, M.D., Ph.D., University of Southern California, Keck School of Medicine

2020 Grant Season

- **Generation of the First Placenta Stem Cell Bank for Phenylketonuria Treatment**
  Roberto Gramignoli, Ph.D., Karolinska Institutet

- **Novel Approaches to Achieving Permanent Gene Correction in PAH-Deficient Mice**
  Cary Harding, M.D., Oregon Health & Sciences University

- **Understanding the Molecular Basis for Hyperphenylalanemia Toxicity and Developing Therapeutic Options for PKU Patients**
  Erik Koppes, Ph.D., Children's Hospital of Pittsburgh

- **Validation of the NIH Toolbox for Use in Phenylketonuria Clinical Trials**
  Desiree White, Ph.D., The Washington University

- **Curing Hyperphenylalaninemia by Engineering Tractable Native Bacteria**
  Amir Zarrinpar, M.D., Ph.D., The University of California, San Diego