APRIL 2023

CITRIN FOUNDATION QUARTERLY NEWSLETTER

We are very pleased to be rolling out our new quarterly newsletter. This first issue will cover updates from last year, and future issues will then cover quarterly updates. This newsletter will cover research progress, publications, and other updates tailored to our professional network.

SCIENTIFIC SUPERVISORY BOARD (SSB) UPDATES

In May 2022, we welcomed the expansion of our Scientific Supervisory Board. Dr. Hannele Yki-Järvinen, MD, FRCP, who is a Professor of Medicine at the Department of Medicine, University of Helsinki, Finland, joined our Board last year. She focuses on internal medicine and endocrinology and is a global expert on non-alcoholic fatty liver disease (NAFLD).

Our SSB meets on a monthly basis under the leadership of Prof. Sir John Walker, the Chair of the Board, to steer the scientific direction of our Foundation, evaluate our ongoing research projects and assess new grant applications. Prof. Johannes Häberle and our co-founders, Barbara Yu and Yen How Tai are also members of the Board.

ADVISOR UPDATES

We welcomed two new advisors who joined our Foundation last year. Prof. Fumio Endo and Prof. Kimitoshi Nakamura from Kumamoto University, Japan, have joined us as our Special Advisor and Scientific Advisor respectively. They have both been instrumental in our efforts in Japan and Asia.

RESEARCH UPDATES AND UPCOMING RESEARCH PROJECTS

Over the past year, Citrin Foundation has continued to provide support to research projects towards better understanding citrin deficiency (CD) and to develop promising therapies. In the past year, the Foundation has doubled the number of research projects funded. We are also supporting the development of various cellular and animal models of CD to accelerate preclinical research and can provide these models to those who may be interested.

New projects commenced in 2022 in chronological order:

Prof. Edmund Kunji (University of Cambridge): Bioenergetic, metabolic, and morphological consequences of citrin pathogenic variants on liver cells and tissue.

Prof. Yukio Nagasaki (University of Tsukuba): Development of nanoparticle-encapsulated amino acids (alanine and aspartate) to enhance their bioavailability and to improve their efficacy as potential therapies for citrin deficiency.

Prof. Laura Contreras & Prof. Araceli del Arco (Autonomous University of Madrid): Development of a new citrin-KO mouse model with a liver-conditional aralar knockout.

Prof. Diana Stojanovski (University of Melbourne): Elucidating the biogenesis, trafficking, and mitochondrial import of citrin and the functional consequences of clinically reported citrin mutations.

Prof. Kimitoshi Nakamura (Kumamoto University): Establishment of a Citrin Deficiency Center of Excellence to develop a new method of NBS and use NGS to uncover more patients, setting up of a digital patient registry system, and to develop an official guideline for citrin deficiency.

Prof. Johannes Häberle and Prof. Gerald Schwank (University Children's Hospital Zürich & University of Zürich): Development of a new *in vitro* hepatic model of citrin deficiency and application of gene editing strategies to treat citrin deficiency.

Prof. Joseph Baur (University of Pennsylvania): Evaluation of small molecule compounds to alleviate NADH/NAD+ redox imbalance in citrin deficiency.

CLINICAL STUDIES

Global Omics Study

In order to find a good biomarker to track disease progress and evaluate potential treatments, Citrin Foundation has initiated a global omics study that aims to analyze blood samples from at least 100 citrin deficiency patients by applying a combination of transcriptomics, proteomics, metabolomics, and lipidomics. This landmark study involves multiple international study sites (JP, TW, SG, UK, USA) and is slated to begin in 2023.

Ureagenesis Function Test

The ureagenesis test, developed by Prof. Johannes Häberle and his team, accurately determines the ureagenesis functions in patients by measuring labelled amino acids in the urea cycle using stable isotope tracers. The test can determine whether urea cycle functions are compromised in patients and allow better prediction on prognosis. It has already been carried out on several CD patients with the Foundation's support and we are actively recruiting more participants.

GLOBAL CONFERENCE UPDATES

Our Foundation started to present at various conferences among relevant scientists and clinicians to raise awareness about citrin deficiency, share the work that we have been doing and expand our network.

International Conference on Ureagenesis Defects and Allied Conditions, Valencia, Spain. Oct 2022

Citrin Foundation sponsored the first-ever spotlight program for citrin deficiency. John Walker, Nobel Laureate and Chairman of our Scientific Supervisory Board, gave the keynote speech. Our co-founder, Barbara Yu introduced the Foundation and our unique approach to rare disease. This was followed by a series of lectures in which we discussed various aspects of the condition and highlighted our collaborative approach to solving citrin deficiency.

63rd Japanese Society for Inherited Metabolic Diseases (JSIMD), Kumamoto, Japan. Nov 2022

We presented to a large audience of clinicians and scientists with a strong interest in our work during the citrin deficiency symposium. We also co-hosted a citrin deficiency luncheon with Prof. Kimitoshi Nakamura, where clinicians with interest in CD were invited. Many of them showed interest in our global omics study which we will be rolling out later this year.

Society for Inherited Metabolic Disorders (SIMD) Annual Meeting, Salt Lake City, Utah. Mar 2023
We sponsored an exhibition booth at the conference. Our patient engagement team focused on networking and expanding our reach in the United States and had the pleasure of meeting some of our US-based members face-to-face.

The 6th Asian Congress on Inherited Metabolic Diseases (ACIMD), Bangkok, Thailand. Mar 2023
We gave a presentation at the conference on the Foundation's global approach towards solving citrin deficiency. We also held a successful meeting amongst leading CD clinicians in Asia. The first Citrin Deficiency Consortium for Asia was established during the meeting, with Prof. Kimitoshi Nakamura serving as Chair, Prof. Fumio Endo appointed as Special Advisor, and the Foundation as its Secretariat and Sponsor. The Consortium aims to meet regularly to work on initiatives such as uncovering more patients, creating a CD patient registry and exploring potential clinical studies on CD.

EVENTS

Evaluation of Existing Treatments for Citrin Deficiency
In March 2023, the Foundation held an online meeting to comprehensively review MCT and sodium pyruvate for CD as they are currently the most commonly used therapies to manage this condition. Prof. Kiyoshi Hayasaka and Prof. Masahide Yazaki were our guest speakers. The session was attended by the members of our Scientific Supervisory Board, and other leading experts in the field. The discussion covered the biochemical basis of these treatments, their clinical evidence and explored potential studies to further validate treatment efficacy and dosage optimization.

Expert talk series
Our Foundation regularly holds seminars by leading experts in their respective fields that are relevant to our aims. Upcoming talks:

- **Prof. Julian Sale,** the Lead of the Division of Protein and Nucleic Acid Chemistry at the Medical Research Council Laboratory of Molecular Biology, University of Cambridge. Topic: Gene Regulation. Date: May 16th, 2023
- **Prof. Ugur Sahin**, Founder and CEO of BioNtech. Topic: LNP and mRNA technology. Date: September 5th, 2023
- **Prof. Patrick Chinnery,** Professor of Neurology and Head of the Department of Clinical Neurosciences, University of Cambridge. Topic: Whole genome sequencing and alternative approach for rare diseases. Date: November 16th, 2023

GLOBAL CITRIN DEFICIENCY SYMPOSIUM

The Foundation is pleased to be hosting the second in-person Global Citrin Deficiency Symposium this autumn. This conference seeks to provide a comprehensive update of the latest scientific and clinical research findings on citrin deficiency, to foster in-depth discussions and spark further collaborations among those in our professional network.

This event will be held at **Magdalene College, University of Cambridge, UK from 18th Sep to 20th Sep**.
If you would like to know more or are interested to attend, please contact:
andreatan@citrinfoundation.org

PUBLICATIONS (FOUNDATION SPONSORED)

We are glad to announce three papers that were recently accepted for publication. These studies come from the groups led by Prof. Edmund Kunji (University of Cambridge), Prof. Paul Yen (Duke-NUS Graduate Medical School), and Prof. Jorgina Satrústegui (Autonomous University of Madrid) respectively, whose studies were funded by Citrin Foundation. Below, we provide a brief summary of the findings and significance of their publications:

Pathogenic variants of the mitochondrial aspartate/glutamate carrier causing citrin deficiency. Tavoulari et al. *Trends in Endocrinology & Metabolism* (2023)

The review provides an overview of CD from a biochemical perspective. It also describes the molecular movements of the citrin protein involved in the regulation and substrate (aspartate/glutamate) transport between the cytosolic and mitochondrial compartments. It discusses all known pathogenic mutations, providing useful predictions into the relationship between genetic mutations of the SLC25A13 gene and the associated structural defects and possible functional consequences.

Nicotinamide riboside rescues dysregulated glycolysis and fatty acid β-oxidation in a human hepatic cell model of citrin deficiency. Yau et al. *Human Molecular Genetics* (2023)

This study examines the therapeutic potential of nicotinamide riboside (NR) on a citrin-KO HepG2 cell line. The cells displayed metabolic impairments as seen in human CD such as increased cytosolic NADH/NAD+, reduced glycolysis, impaired fatty acid metabolism, and reduced mitochondrial respiration. Addition of NR improved NADH/NAD+ ratios, which subsequently improved glycolysis and fatty acid metabolism defects. These findings suggest that NR may have therapeutic potential in treating some of the metabolic defects seen in CD.

Exogenous aralar/slc25a12 can replace citrin/slc25a13 as malate aspartate shuttle component in liver. González-Moreno et al. *Molecular Genetics & Metabolism Reports* (2023)

This study explores the therapeutic potential of substituting citrin expression with its protein isoform, aralar in a mouse model of CD. Exogenous aralar expression in hepatocytes from citrin-KO mice was beneficial in reducing cytosolic NADH/NAD+ ratios, as high cytosolic NADH/NAD+ is thought to contribute to the pathology of CD. Hepatic mitochondria isolated from citrin-KO mice with a liver-specific transgene for aralar expression had increased malate aspartate shuttle (MAS) activity when compared to citrin-KO mice. Collectively, these results support the hypothesis of replacing citrin with aralar in the liver to improve redox balance and could serve as a potential therapy for CD.

If you are interested in reading more publications supported by Citrin Foundation, please kindly visit our publications page at https://citrinfoundation.org/publications/.

Other Citrin Deficiency-related Publications

The following list details some of the recent publications on citrin deficiency from various independent groups over the past year:

1. The prognosis of citrin deficiency differs between early-identified newborn and later-onset symptomatic infants. Chen et al. *Nature Pediatric Research* (Apr 2023)
2. The mutation spectrum of SLC25A13 gene in citrin deficiency: identification of novel mutations in Vietnamese pediatric cohort with neonatal intrahepatic cholestasis. Nguyen et al. *Journal of Human Genetics* (Jan 2023)
3. Dynamic changes of metabolic characteristics in neonatal intrahepatic cholestasis caused by citrin deficiency. Zhang et al. *Frontiers in Molecular Biosciences* (Aug 2022)
4. Improved diagnosis of citrin deficiency by newborn screening using a molecular second-tier test. Chen et al. *Molecular Genetics and Metabolism* (Aug 2022)
5. Usefulness of serum BUN or BUN/creatinine ratio as markers for citrin deficiency in positive cases of newborn screening. Suzuki et al. *Molecular Genetics and Metabolism Reports* (Mar 2022)
6. Clinical manifestation and long-term outcome of citrin deficiency: Report from a nationwide study in Japan. Kido et al. *Journal of Inherited Metabolic Disease* (Feb 2022)

MEMBERSHIP COHORT UPDATES

In the past year, our global membership has grown significantly, with patients and their family members from Australia, Austria, Canada, China, Hong Kong, Indonesia, Japan, Malaysia, Philippines, the US, and the UK joining our global patient community which now has more than 160 members.

Our Foundation also established a global physician registry for clinicians with CD patients which currently consists of more than 60 clinicians internationally, from countries and regions such as Europe, Japan, Korea, Vietnam, the Middle East, the US, and the UK.

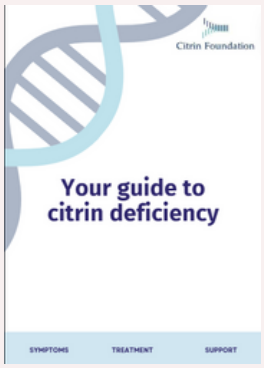
PATIENT ENGAGEMENT INITIATIVES AND RESOURCES

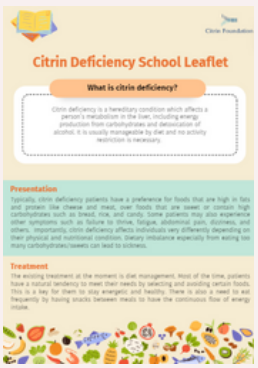
Patient Leaflets

We have developed the following leaflets for patients:

- A CD booklet explaining the condition, current treatment and management, available support, and ways in which patients could get involved with the Foundation.
- School leaflet with tips on how teachers can support students living with citrin deficiency.
- A resource for school children to give to their classmates.
- Snack suggestion sheet with easy food and drink ideas.

All leaflets are available to download from our patient website.





Global Digital Community

Last October, we launched our digital community on social media. We share once a week and this includes diet tips, patient stories, and research updates and showcases our involvement with conferences. You can find us on Instagram, Twitter, and Facebook.

Peer Support Group

We also launched our global peer support community in January 2023. This takes the form of an informal chat group of other parents and adult patients where patients and families can exchange ideas and build a support network.

Events

- English-speaking patient and family webinar December 2022.
- Japanese-speaking patient and family webinar March 2023.

Recipes

In January, we launched a new initiative focused on gathering and creating recipes suitable for CD patients. You can view our recipe bank at https://patient.citrinfoundation.org/en/our-recipes/.

Thank you for being a part of the Foundation's journey to finding a cure for citrin deficiency. We welcome suggestions on how we can improve our resources as well as ideas for new projects to explore.

If you would like to get in touch, please email us at info@citrinfoundation.org for general inquiries or grants@citrinfoundation.org for grant inquiries.

You can look forward to the next edition of our newsletter in July.