Unravelling the Enigma of Citrin Deficiency: A Novel Holistic Multidisciplinary Approach

Citrin Foundation

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INTRODUCTION

Citrin deficiency (CD): A complex, heterogeneous condition with distinct differences to other urea cycle disorders (UCD).
Impact: Affects the malate-aspartate shuttle, impacting multiple metabolic pathways (Figure 1)[1].
Phenotypes: Multiple age-dependent and diverse clinical phenotypes despite identical mutations.
Symptoms differ across phenotypes: Strong food preference, prolonged jaundice, failure to thrive, hypoglycemia, fatty liver, fatigue, hyperammonemia.
Diet preference: Patients prefer high protein/fat, low carb diets[2].
Prevalence: High global prevalence based on carrier rates (1:31-65 in Asia), very likely underdiagnosed in the West[3,4].

Key questions & challenges

- Are there genotype-phenotype correlations?
- What triggers onset of adolescent & adult CD (AACD[3], previously termed CTLN2)? Why does ASS1 activity decline?
- Are there CD specific biomarkers?
- Why is fatty liver prevalent amongst patients?
- How to improve newborn screening for CD?
- Which biochemical aspects of CD are most amendable to treatment intervention?
- Lack of comprehensive global natural history studies.

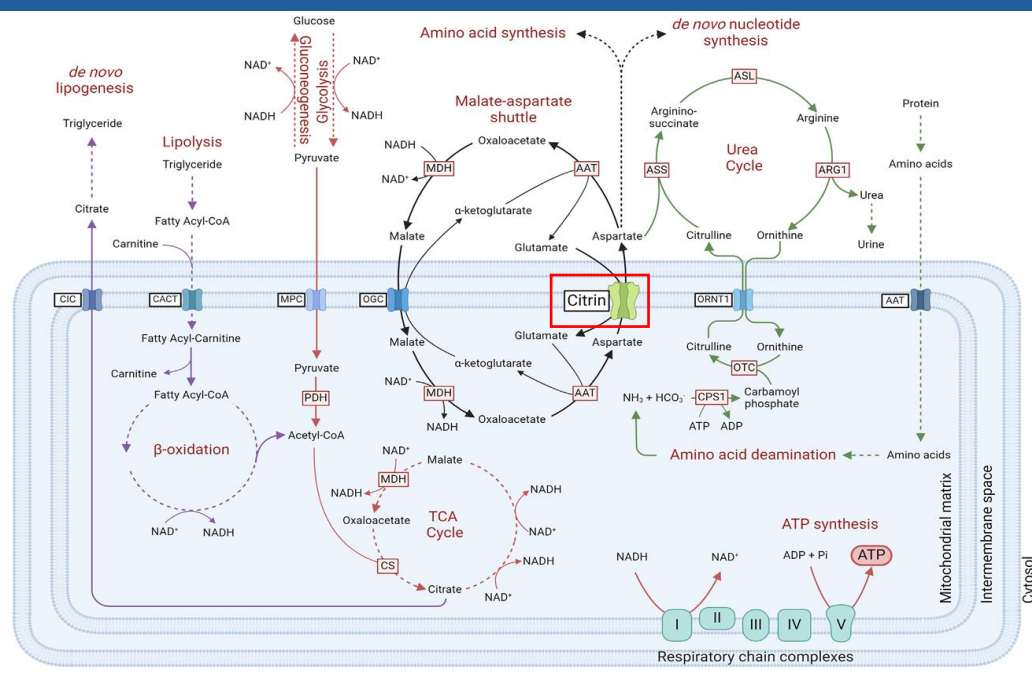


Figure 1. Representation of metabolic pathways affected in CD. Biochemical pathways involved in glycolysis/TCA cycle (red), malate-aspartate shuttle, amino acid & nucleotide synthesis (black), protein degradation & urea cycle (green), lipid metabolism (purple) are shown. Citrin is boxed in red (adapted from Vuković et al. 2024)[1].

METHODS

Comprehensive CD landscape review	**Global multi-disciplinary research & clinical consortium**	**Organize & support patient cohorts**	**Long-term commitment USD30m for first 10 years**	**Fund targeted research Avg. grant: USD300k to 500k**	**Establish strategic centers**

RESULTS

Key Areas Identified	Citrin Foundation Funded Projects
Understand phenotype & symptom heterogeneity	• Study the **impact of citrin pathogenic mutants**[5] on cellular expression, bioenergetics, & metabolism (PI: Edmund Kunji, Diana Stojanovski) • **Metabolic flux studies** in CD mouse models to investigate metabolic consequences (PI: Marc Hellerstein)
Biomarker discovery & functional assays	• **Global multi-center, multi-omics study** analyze >100 CD patient plasma samples (Global PI: Johannes Häberle, Kimitoshi Nakamura) • **Ureagenesis test** using $^{15}NH_4Cl$ to study ureagenesis functions in patients (PI: Johannes Häberle)
Understand cause of AACD onset	• ASS1 protein/mRNA assessment & quantification, proteomic **analysis of AACD patient liver samples** (PI: Masahide Yazaki, Johannes Häberle, Jorgina Satrústegui) • Transcriptomic analysis of AACD patient liver samples (PI: Ituro Inoue)
Develop new pre-clinical models	• **CD hepatocyte models**: HepaRG, patient-derived iPSC hepatocytes (PI: Johannes Häberle), HepG2 (PI: Edmund Kunji) • New **CD rodent models**: Aralar liver-conditional KO/citrin-KO mouse (PI: Laura Contreras, Araceli del Arco); Citrin-KO rat
Improve newborn screening	• Use of Arg, Cit, Ile+Leu, Tyr, C0/C5-DC to **improve sensitivity & specificity of NBS** for NICCD[6] (PI: Kimitoshi Nakamura, Jun Kido)
Establish strategic centers	• **CD Center of Excellence** (JP): Uncover patients, improve NBS, patient registry, natural history study (PI: Kimitoshi Nakamura) • **UCD Translational Research Center** (CH): Accelerate translational research and clinical development for UCD (PI: Johannes Häberle)

KEY TARGETED THERAPEUTIC AREAS

Restore redox balance
Evaluate efficacy of novel compounds (KL1333, citrate, ethyl pyruvate) to restore NADH/NAD+ levels (PI: Joseph Baur, USA)
Targeting G3P pathway to alleviate CD pathology (PI: Marc Prentki, Canada)

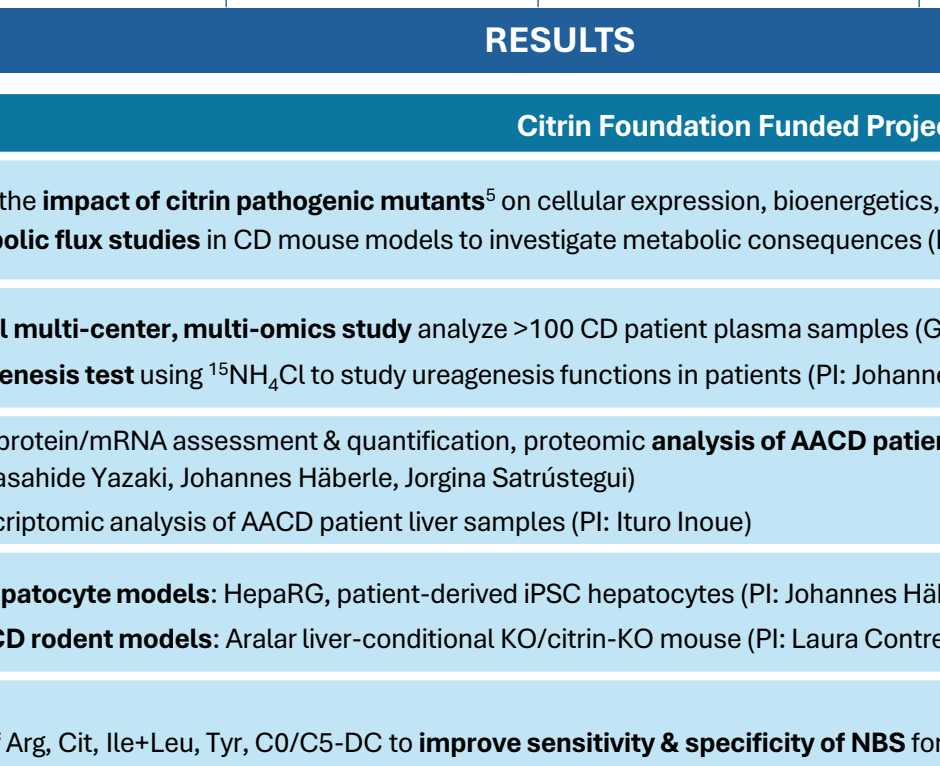
Evaluate existing treatments[1]
(PI: Johannes Häberle, Switzerland; Jun Kido, Japan)

Substitute citrin with aralar[8]
(PI: Laura Contreras, Araceli del Arco, Spain)

Enhance cytosolic aspartate supply[9]
Develop nanoparticle-encapsulated amino acids with enhanced bioavailability
(PI: Yukio Nagasaki, Japan)

mRNA therapy[7]
Evaluate LNP-mRNA constructs with improved delivery & target expression
(PI: Edmund Kunji, UK; Diana Stojanovski, Australia)

Gene editing
Develop prime & base editing therapies for common CD mutations (Figure 3)
(PI: Gerald Schwank, Johannes Häberle, Switzerland)



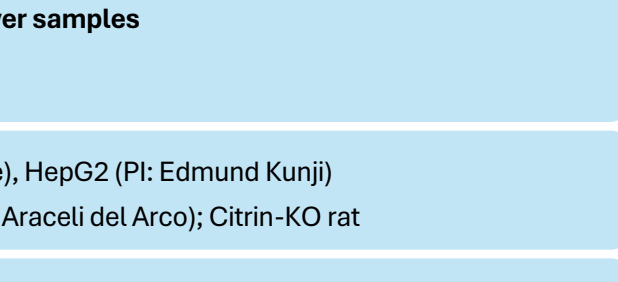


Figure 3. Distribution of common CD mutations according to major regions of Southeast Asia. (adapted from Tavoulari et al. 2023)[5].

Figure 2. Overview of the current & prospective treatment options for CD. Red indicates pathways affected in CD. Current & prospective treatment options & their effects are marked in green & yellow respectively. Specific disease aspects where therapies are being developed for by Citrin Foundation are highlighted (figure adapted from Vuković et al. 2024)[1].

CONCLUSIONS

- CD is a complex and intriguing condition that is relevant to multiple research disciplines.
- We welcome those who are interested to apply new technologies to solve this condition.

References:
1. Vuković et al. 2024 *JIMD*
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COI statement: The authors declare no conflicts of interest