# Pyruvate dehydrogenase complex deficiency (PDCD) essentials including current trials/research and prospects for newborn screening

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**MitoAction Expert Series**

November 1, 2024

# Conflicts of Interest

Ultragenyx

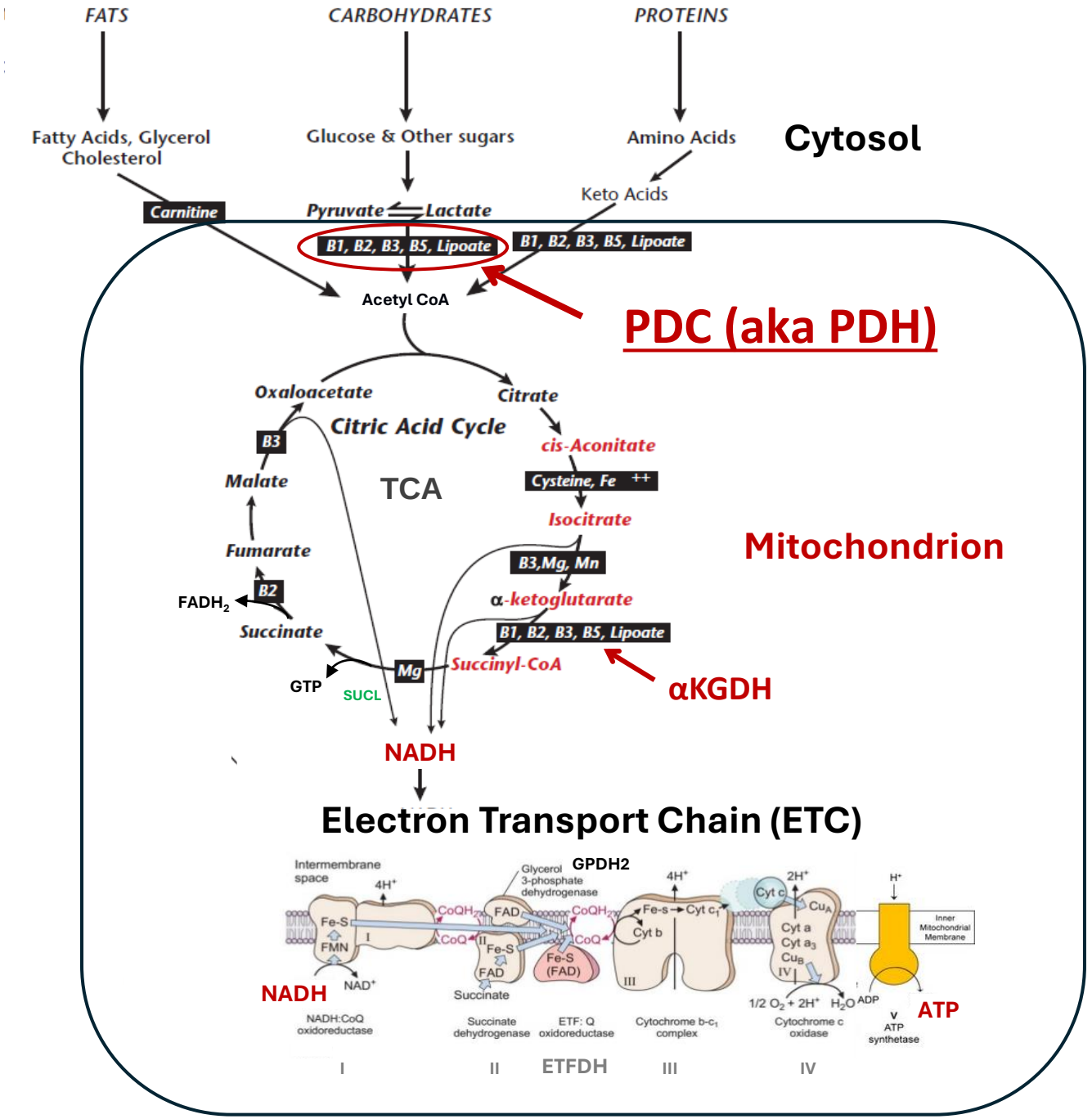
# Outline

- **PDCD essentials**
- **Active trials and investigations at UPMC**
  - PDCD Natural History Study
  - fNIRS for PDCD
- **Newborn Screening (NBS)**
  - Overview
  - Limitations
  - Adding a new disorder
  - RUSP
  - PDCD NBS prospects
- **Protein-specific target-based small molecules to restore PDC function**

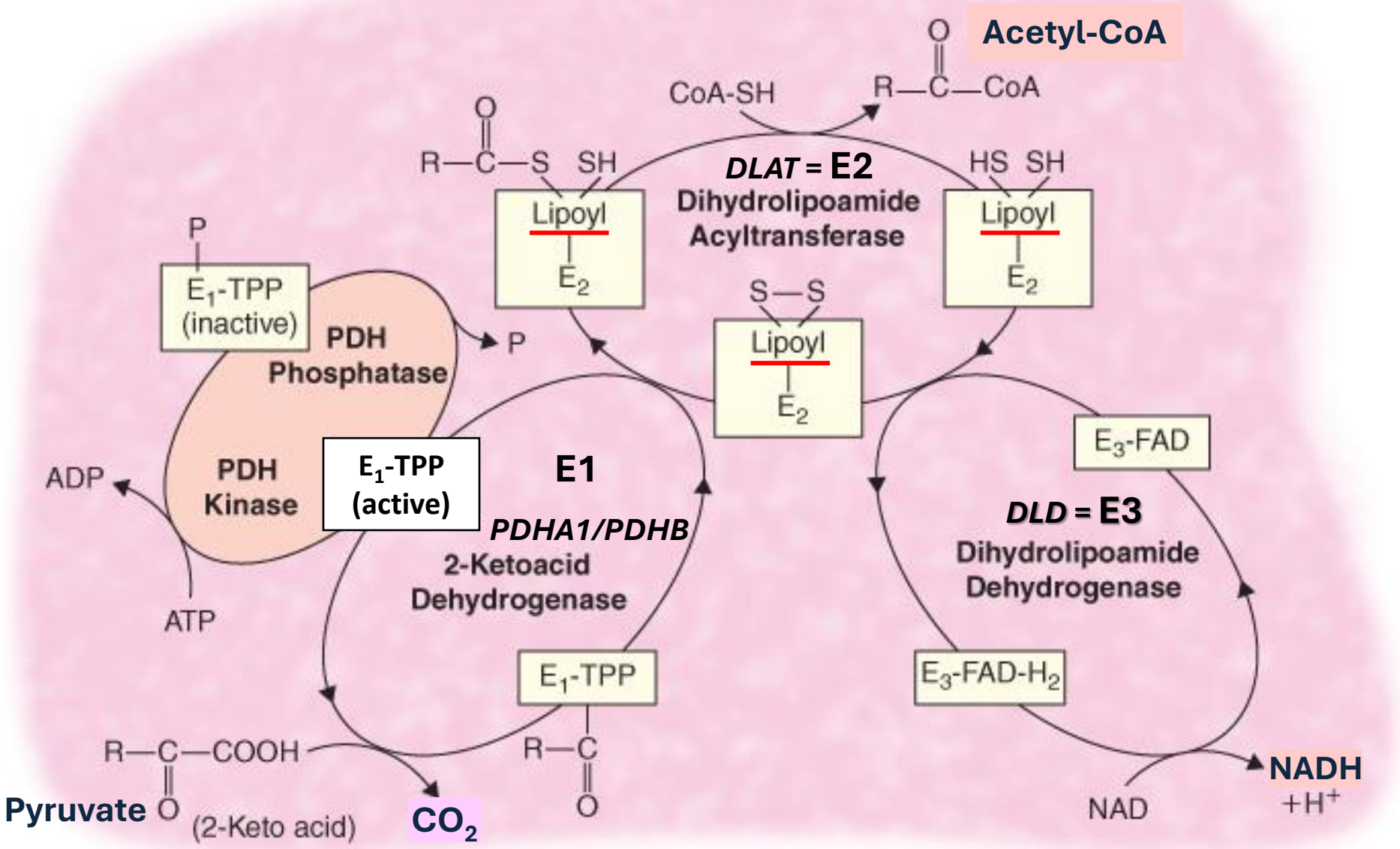
# PDCD Takeaways

- A **mitochondrial** neurometabolic disorder of carbohydrate (glucose) oxidation that mostly affects the brain and leads to decreased ATP production. **An energy deficit disorder.**
  - PDC is a large **multienzyme mitochondrial matrix complex**.
  - **>38 genes associated with functional (enzymatic) PDCD.**
  - **About 85% of cases** are due to mutations in **X-linked *PDHA1*** gene.
  - **At least 3 subclasses.**
- Major cause of **primary congenital lactic acidosis**[1]
- **Second most common genetically-resolved mitochondrial disorder in the NAMDC Registry**[2]
  - Second only to *POLG* and *POLG-related* mitochondrial disorders.
- **INCIDENCE: At least 1 in 40,000 live births annually** affected with PDCD in North America (Ohio, Pennsylvania)[3,4]
  - Similar to VLCAD (1:40,000 OH), GA-I (1:60,000 OH), or MMA 1:50,000-80,000.
- **LIMITED therapeutics. Ketogenic diet currently main (*untargeted*) option.**

[1]Kerr and Bedoyan (2017) PDC/TCA chapter 2nd Edition *Pediatric Endocrinology and IEMs*, 2017, [2]Barca *et al.* (2020) Neurol Genet **6**:402, [3]Bedoyan *et al.* (2020) *JIMD Rep* **56**:70, [4]Verma et al. (2023) *MGGM*, PMID: 37688338


FATS
CARBOHYDRATES
PROTEINS
Fatty Acids, Glycerol
Cholesterol
Glucose & Other sugars
Amino Acids
Keto Acids
Cytosol
Carnitine
Pyruvate
Lactate
B1, B2, B3, B5, Lipoate
B1, B2, B3, B5, Lipoate
Acetyl CoA
PDC (aka PDH)
Oxaloacetate
Citrate
Citric Acid Cycle
B3
cis-Aconitate
Malate
TCA
Cysteine, Fe ++
Isocitrate
Fumarate
B3,Mg, Mn
Mitochondrion
FADH2
B2
α-ketoglutarate
Succinate
B1, B2, B3, B5, Lipoate
GTP
SUCL
Mg
Succinyl-CoA
αKGDH
NADH
Electron Transport Chain (ETC)
GPDH2
Intermembrane space
4H+
Glycerol 3-phosphate dehydrogenase
Fe-S
FMN
NAD+
CoQH2
CoQ
FAD
Fe-S
Fe-S (FAD)
Succinate
Fe-s
Cyt c1
Cyt b
Cyt c
CuA
Cyt a
Cyt a3
CuB
2H+
H+
Inner Mitochondrial Membrane
1/2 O2 + 2H+
H2O
ADP
ATP
NADH
NADH:CoQ oxidoreductase
Succinate dehydrogenase
ETF: Q oxidoreductase
Cytochrome b-c1 complex
Cytochrome c oxidase
ATP synthetase
I
II
ETFDH
III
IV
V

# Pyruvate Dehydrogenase Complex (PDC)



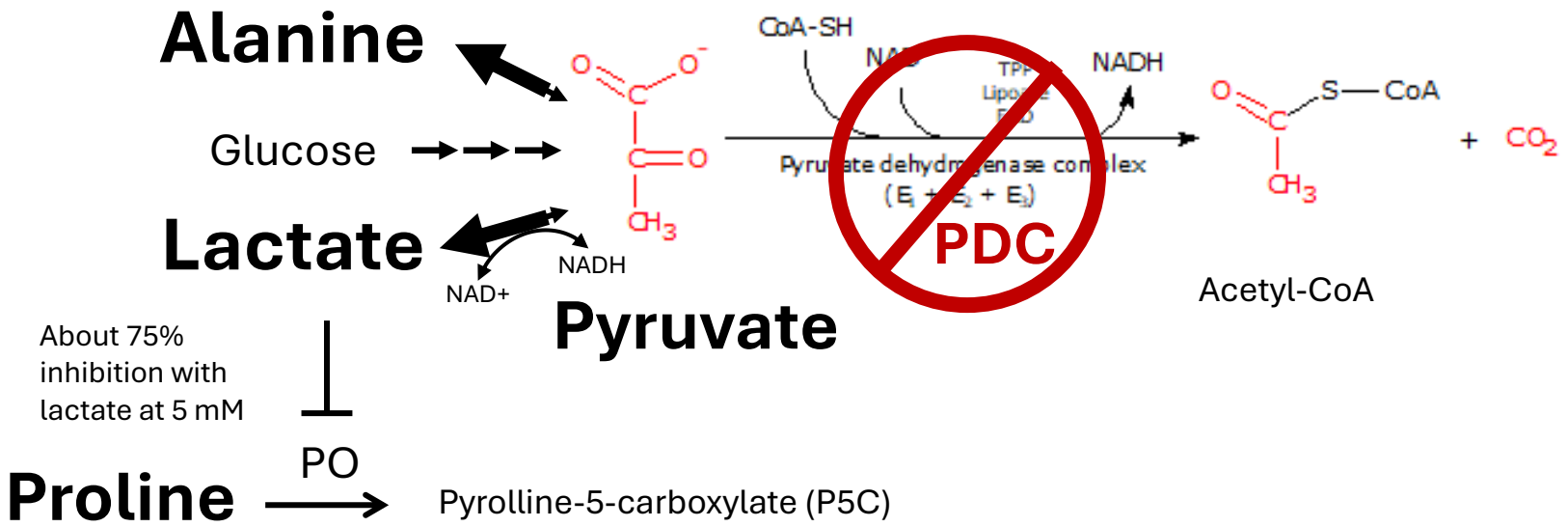

Kerr and Bedoyan (2017) PDC/TCA chapter 2nd Edition *Pediatric Endocrinology and IEMs*, 2017

# Human NAD-dependent dehydrogenases with lipoyl moieties or shared E3 subunit

| # of carbons in **α-ketoacid** substrate | Enzymatic reaction | Subunits/regulation (gene MIM number) | Affected metabolic pathway | |
|---|---|---|---|---|
| **3C** | Pyruvate dehydrogenase complex (PDHC) | E1 alpha (*PDHA1* MIM300502, *PDHA2*)<br>E1 beta (*PDHB* MIM179060)<br>→ E2 (*DLAT* MIM608770)<br>E3 (*DLD* MIM238331)<br>→ E3BP (*PDHX* MIM608769)<br>PDHC kinases (*PDK1*, *PDK2*, *PDK3* MIM300906, *PDK4*)<br>PDHC phosphatases (*PDP1* MIM605993, *PDP2*, *PDPR*) | Connects glycolysis with the Krebs cycle | |
| **5C** | α-Ketoglutarate dehydrogenase (α-KGDH) | E1 (OGDH)<br>→ E2 (DLST)<br>E3 (DLD MIM238331) | Krebs-cycle enzyme | **TCA αKGDH** |
| **6C** | 2-Oxoadipate dehydrogenase (2-OADH) | E1 (DHTKD1 MIM614984)<br>→ E2 (DLST)<br>E3 (DLD MIM238331) | Lysine degradation | |
| **6C/5C** | Branched-chain ketoacid dehydrogenase (BCKDH) | E1 alpha (*BCKDHA* MIM608348)<br>E1 beta (*BCKDHB* MIM248611)<br>→ E2 (*DBT* MIM248610)<br>E3 (*DLD* MIM238331)<br>BCKDH kinase (*BCKDK* MIM614901)<br>BCKDH phosphatase (*PPM1K* MIM611065) | Leucine, isoleucine, valine degradation | **MSUD** |

# PDCD: Biochemical Consequences

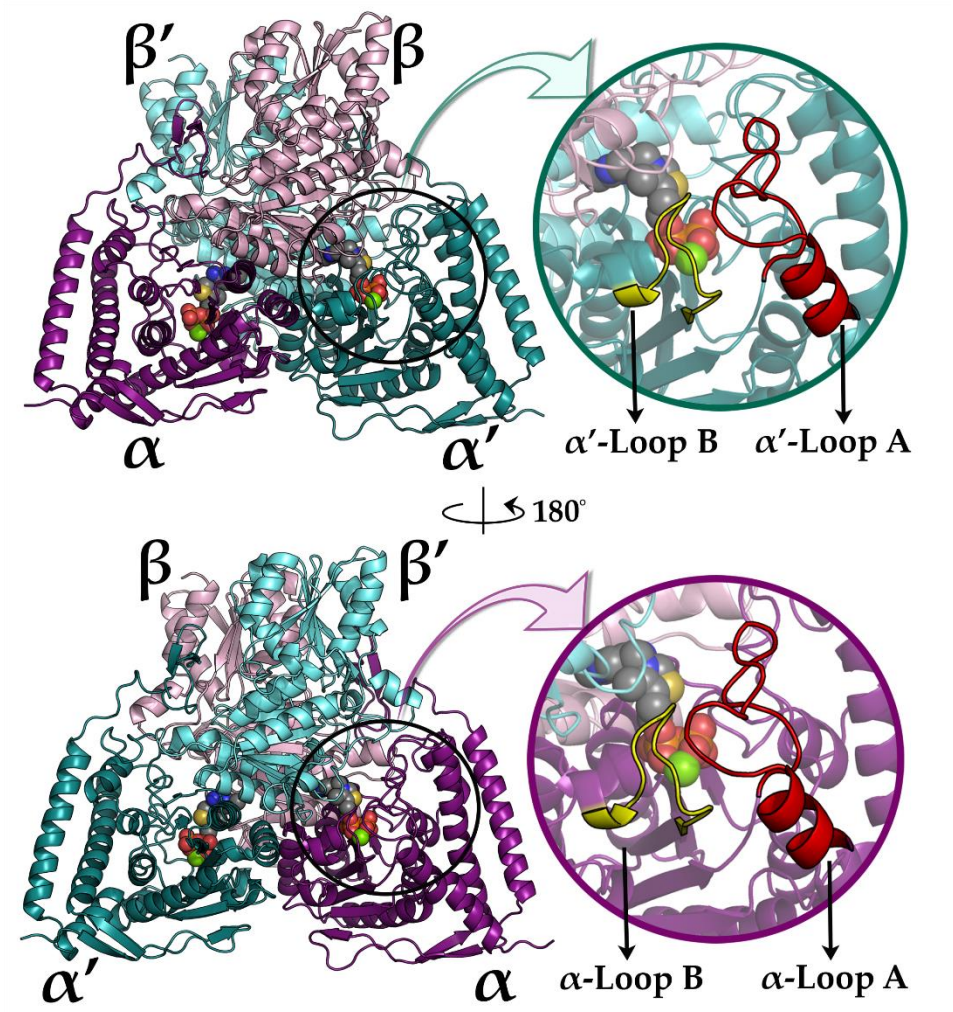
**Functional (enzymatic) deficiency of PDC**




**Normal: Lactate/Pyruvate ratio (10-20)**

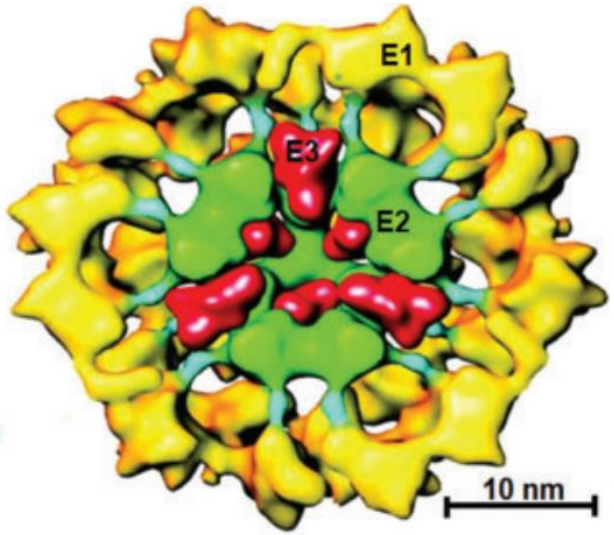
# E1 structure: Dimer of αβ heterodimer

E1α (361 aa) 40.2 kDa
E1β (329 aa) 35.9 kDa
**E1** (αβ/α'β') **152.3 kDa**




https://www.rcsb.org/structure/3exe
DOI: 10.2210/pdb3EXE/pdb
Resolution: 1.98 Å

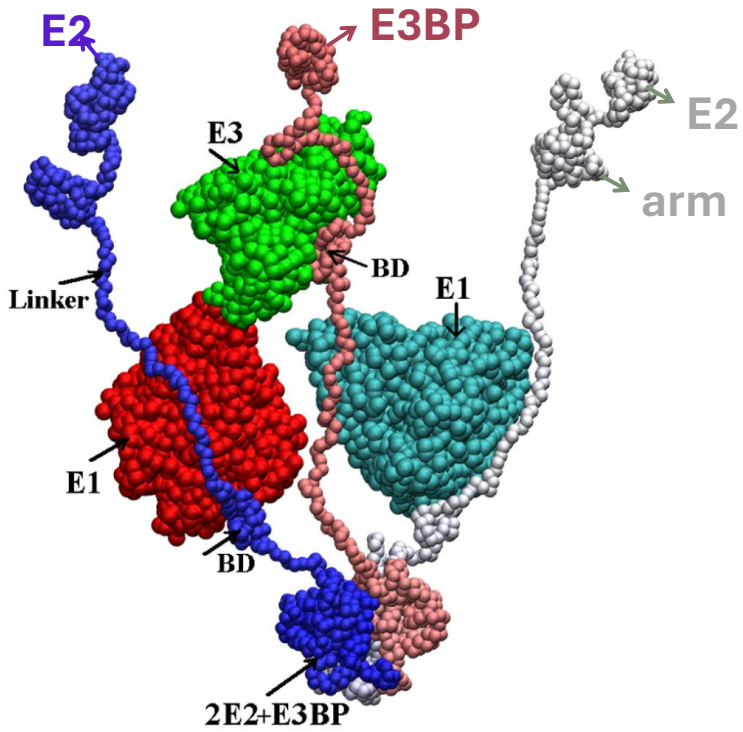
# PDC superstructure

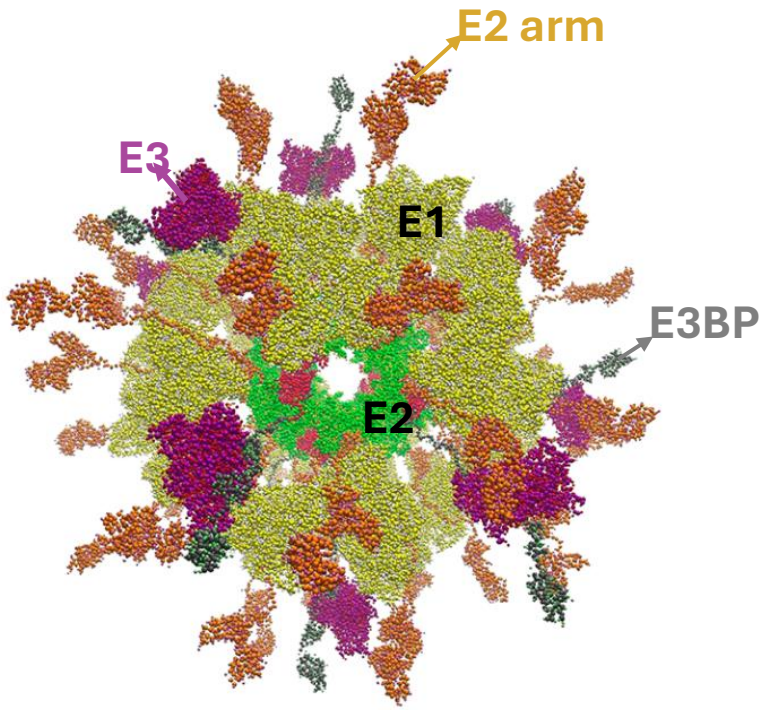



9.5 MDalton eukaryotic complex contains multiple copies of 3 enzymatic components:

E1: 30 copies
E2: 60 copies (core)
E3: 12 copies (incl. BP)

Stacpoole (2017) *J Natl Cancer Inst* 109




Hezaveh et al. (2018) *JCIM* 58:362-9.

# PDCD: Clinical Features

- **Broad** clinical presentation[1]
  - Neonatal onset (usually severe)
  - Infantile-early childhood form (usually moderate)
  - Late onset in children and adults (usually mild)
- **Clinical features**[1] (**heterogeneous in clinical presentation and clinical course**)
  - Hypotonia 53-**89**%
  - Developmental delay 69-**83**%
  - Ventriculomegaly 35-**85**%
  - Corpus callosum abnormalities 15-**55**%
  - Seizures 16-**57**%
  - Leigh syndrome 12-**35**%
  - Ataxia 17-**22**%
  - Visual impairments (cortical, optic nerve hypoplasia/atrophy, nystagmus, strabismus)
  - Hearing impairments
  - Other features include alternating hemiplegia, Guillain-Barre-like symptoms, episodic paroxysmal exercise-induced dystonia or ataxia, or other neuropathy/myopathy
  - **Neurocognitive impairments and neurobehavioral abnormalities are common and variable**
- **About 4% of patients with PDCD (due to *PDHA1*) have normal brain imaging (CT/MRI) findings**[2]
- **Females have better survival,** but surviving females are more severely affected than males.[1,2]
  - Survival (2012): About 40% die <3 mo, 60% die <1 yo, **90% die <4 yo**.[2]
- **Median** and mean ages of diagnosis about **12 months** and 31 months, respectively[1,3,4]

[1]DeBrosse *et al.* (2012) *MGM* **107**:394-402, [2]Patel *et al.* (2012) *MGM*, **106**:385-94,
[3]Sofou *et al.* (2017) *JIMD*, **40**:237-45. [4]Shin *et al.* (2017) *MGM*, **122**:61-66.

# Therapeutic Approaches for PDCD

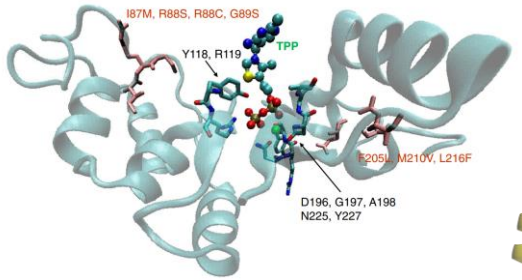
- **Provide the missing product – Acetyl-CoA**
  - *Untargeted*; e.g., ketogenic diet (**KD**) (mainstay tx for PDCD), etc…
- **Restore the defective component of complex**
  - protein (E1α)-specific, *target-based* approach (**our plan; research**)
- **Replace the defective component of complex**
  - Enzyme replacement therapy (ERT; gene-specific)
    - BBB-permeable and targeting mitochondrial matrix by ERT are issues (not tried)
  - Gene therapy (gene-specific) (research)
- **Utilize regulators of PDC (e.g., DCA – under FDA review)**
  - Molecules that inhibit PDH kinase – maintain/maximize PDC activity
    - DCA used in primary-specific PDCD and PDH phosphatase (PDP1) deficiency
  - Restore/replace PDH phosphatases – restore regulation PDC activity (not tried)
- **Provide cofactor of PDC (e.g., thiamine)**
  - For thiamine-responsive cases

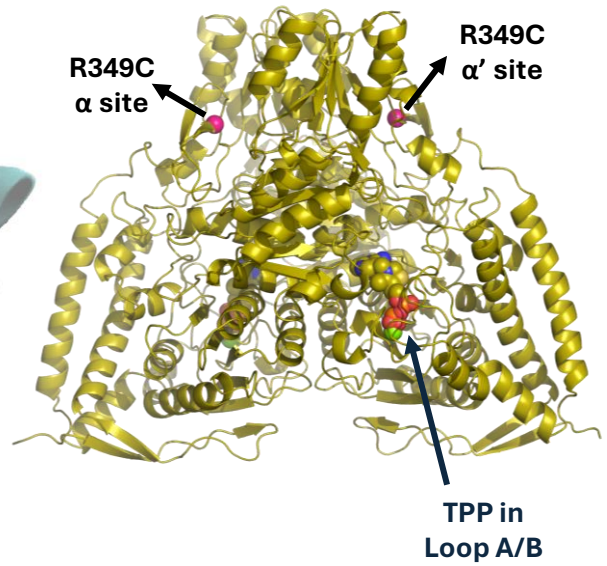
# Thiamine-responsive substitutions in E1α (*PDHA1*)[1-6]

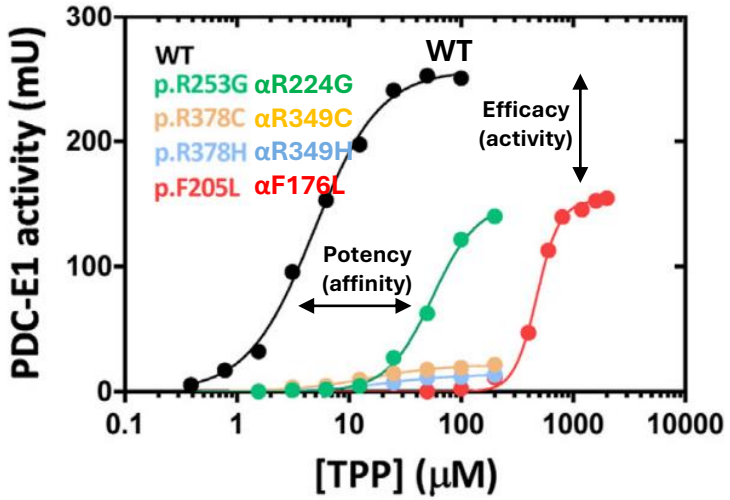
- p.H44R (mature His15)[5]
- p.V71A (mature V42)[3,5]
- p.I87M (mature Ile58)[2,5]
- p.R88S, p.R88C (mature Arg59)[2,5]
- p.G89S (mature Gly60)[2,5]
- p.C101F (mature Cys72)[3,5]
- p.Y161C (mature Tyr132)[3,5]
- **p.F205L** (**mature Phe176**)[2,5]
- p.M210V (mature Met181)[2]
- p.W214R (mature Trp185)[5]
- p.L216F, p.L216S (mature Leu187)[2,5]
- p.Y243S (mature Tyr214)[3]
- **p.R253G** (**mature Arg224**)[4]
- p.R263G (mature Arg234)[3,5]
- p.S390KextX32 (mature Ser361)[1,3]
- p.X391FextX33 (MedLink Neurology)[6]

[1]Narisawa *et al.* (1992) *J Nutr Sci Vitaminol*, PMID: 1297818; [2]Brown (2014) *JIMD* **37**:577-585; [3]van Dongen et al (2015) *JIMD Rep* **15**:13-27; [4]Pavlu-Pereira et al. (2021) Biochimie 183:78-88; [5]Ducich *et al.* (2022) *JIMD* **45**:557-570; [6]Bedoyan (2024) MedLink Neurology

## E1 = αβα'β'




Pavlu-Pereira et al. (2021) Biochimie 183:78-88

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# PDCD Guidelines Project

- An initiative between myself and Dr. Shamima Rahman from UK
- Team includes:
  - 15 investigators (8 European and 7 USA)
  - 2 early career representatives ( 1 European and 1 USA)
  - 4 experienced ketogenic dietitians (2 European and 2 USA)
  - Individual single representation from 3 PDCD family groups
    - The Elizabeth Watt PDCD Research Fund, USA
    - The Freya Foundation, UK
    - Hope for PDCD, USA
  - Group of 24 with almost equal gender representation; 13 F and 11 M
  - A family group member (rotating basis) will have tiebreaker voting privilege if needed
- Monthly virtual meeting, with first started on October 14, 2024
- One in-person meeting in either USA or UK; TBD
  - Support by SSIEM and philanthropy funds
- Project to be reported in *Journal of Inherited Metabolic Disease* (*JIMD*)

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# Current Active PDCD Investigations at UPMC

- NAMDC **PDCD Natural History Study** (since 2014; >115 subjects enrolled) – NIH U54 grant. **ClinicalTrials ID: NCT03056794**, active recruiting (virtual and in person)
- **C7 (triheptanoin) for primary-specific PDCD** (Enrollment goal: 6 affected on KD) – Ultragenyx-sponsored. **ClinicalTrials ID: NCT06340685**, active recruiting (limited to Pittsburgh site)
- **Trial of DCA in PDCD (Phase III)** - Site and Core Investigator. **ClinicalTrials ID : NCT02616484**, active not recruiting
- **fNIRS for PDCD** (Enrollment plan: 9 affected and 6 age-matched controls) – NIH NAMDC pilot grant. Active recruiting (limited to Pittsburgh site)
- **Advanced genetics for disorders of pyruvate metabolism** – NIH U54 grants (2014 and 2019 cycles). Ongoing
- **NBS for PDCD** – NIH NAMDC Project grant (since 2019) – NIH U54 grant. Ongoing
- **Protein-specific target-based small molecule therapeutics for PDCD** – Philanthropy support. Ongoing

# 2019-24 NAMDC PDCD Natural History Study Questionnaire

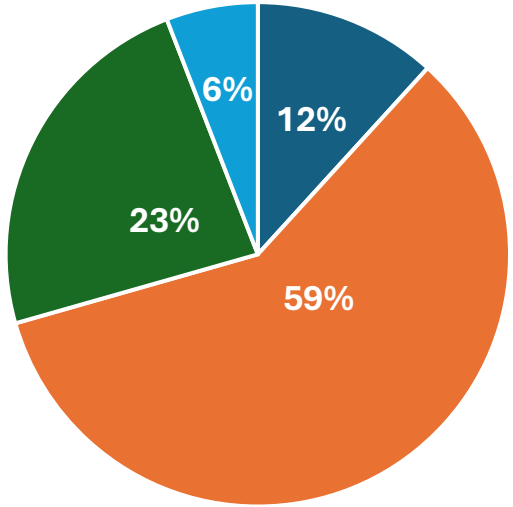
**- Total 68 questions (with sometimes multiple sub-questions), total 17 pages**

**- Also available online through a weblink when subjects are enrolled in Study**

**- What is on the PDCD NH Study Questionnaire?**

- **Demographics (8 questions)**
- **Disease/Growth-Development/Behavior-Mood/Neurologic-Seizure/ Other Clinical Phenotypic Details (40 questions)**
  - Disease Information Form (**3 questions**)
  - Growth and Development Form (**5 questions**)
  - Behavior and Mood Form (**3 questions**)
  - Neurological Symptoms Data From (**12 questions**)
  - Seizure Symptoms Data Form (**5 questions**)
  - Seizure Medication Record Form (**2 questions**)
  - Historical Data Form (other Clinical Phenotypic Details) (**10 questions**)
- **Diet/Medications/Supplements (16 questions)**
  - Diet Review (**6 questions**)
  - Thiamine Treatment Record Form (**2 questions**)
  - General Medications (**1 question** – multiple options)
  - Vitamins, Supplements, and Mixtures (“Cocktails”) (**7 questions**)
- **Family History (4 questions)**

## CATEGORY

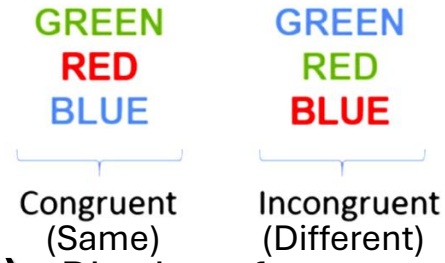


# Functional near-infrared spectroscopy (fNIRS)

- Used in several inborn errors of metabolism (IEM) such as
  - Ornithine transcarbamylase deficiency (**OTCD, a urea cycle disorder**)[1-3]
  - Glucose transporter type 1 deficiency syndrome (Glut1DS)[4]
  - Maple syrup urine disease (MSUD)[5]
- Used to
  - explore **hemodynamic changes** and cerebrovascular disease in the frequency range of cerebral autoregulation (0.001-0.03 Hz).
  - investigate **neuronal activation and brain functional connectivity** (FC) in autism spectrum disorder (ASD), ADHD, stroke, trauma, OTCD, MSUD, Glut1DS, etc…
- **To date, fNIRS has not been used in patients with PDCD**
- fNIRS could potentially be used in other mitochondrial disorders with neurocognitive dysfunction
  - Potential outcome measure for use in pre- and post-therapeutics

[1]Anderson *et al.* (2020) *Front. Neurol.* **11**:809, [2]Anderson el *et al.* (2020) *MGM*, **129**:207-12, [3]Gropman *et al.* (2013) *Hum. Brain Mapp.*, **34**:753-61, [4]Gropman *et al.*, unpublished data, and [5]Khaksari *et al.*, unpublished data

# Tasks to Complete

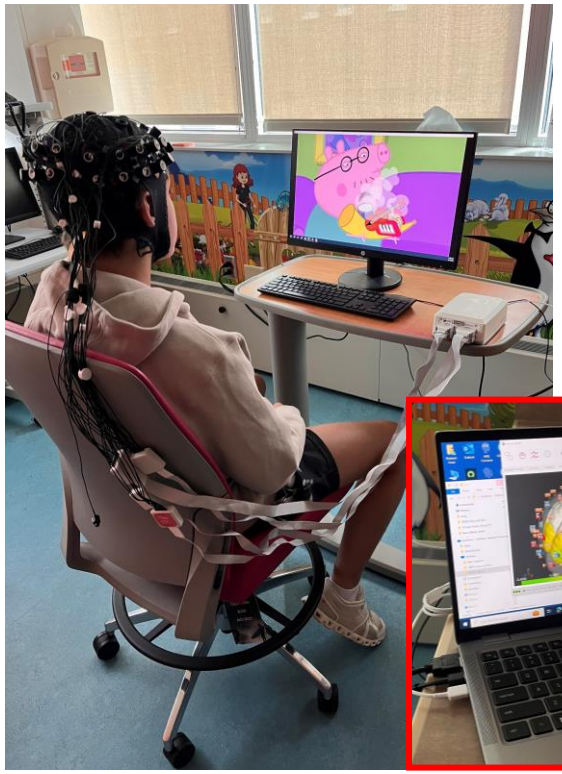



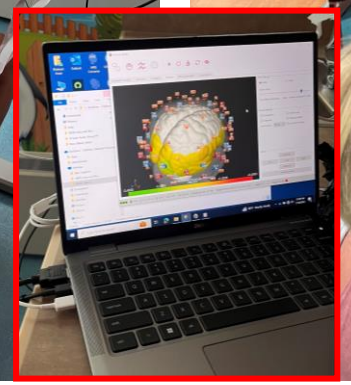

- **Stroop task (executive processing abilities).** Blocks of congruent and incongruent are displayed on the screen with rest blocks in between. This task is repeated 3 times with a rest period in between blocks. **For individuals ≥5 years old;** *normative average % correct by 5 years of age, 95% and 90% for congruent and incongruent calls, respectively (Ikeda et al. 2014 J. Psych.)*


- **N-back task (working memory).** In the 0-back condition, the target letter is "X" (indicated with underline). In the 1- and 2-back conditions, the target letter is the same as the letter from 1- or 2-steps before in a given sequence, respectively. Each condition is repeated 8 times with a rest period in between conditions. **For individuals ≥5 years old;** *normative % completion of 1- and 2-back by 7 years of age is 98.7% and 77.8%, respectively [Pelegrina et al. (2015) J. Psych.].*

- **Video task (overall brain development with focus on language).** Video clips that contain communicative and non-communicative language (sounds without using real words). **For children <5 years old.**

# fNIRS for PDCD at UPMC Children's Hospital of Pittsburgh



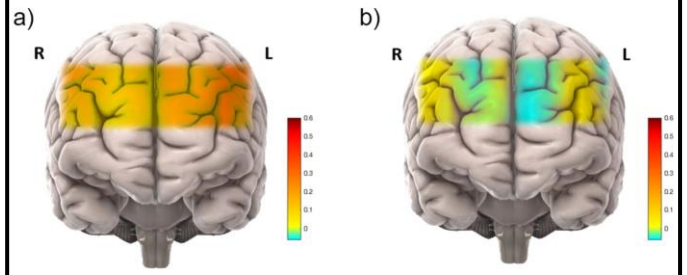


**Adult OTCD Study**

**Activation Maps**

**Stroop Executive Processing Test**

Anderson et al. (2020)
*Mol. Genet. Metab.*




**Control** **OTCD**

**Prefrontal Cortex**

# Newborn Screening (NBS)

March of Dimes

A few drops of blood from a heel stick allows hospitals to screen newborns for genetic conditions.

## Getting Answers

Where to turn if you need information or a specialist on genetic screening:

| Resource | Comment |
|---|---|
| **National Newborn Screening and Genetics Resource Center** genes-r-us.uthscsa.edu/ | Offers comprehensive source of information on screening. |
| **American College of Medical Genetics** www.acmg.net | Includes "ACT" sheets for doctors, with information on what doctors should to do when a baby screens positive for a rare condition; information on specialists and regional collaboratives. |
| **March of Dimes** www.marchofdimes.com/pnhec/ | Consumer-friendly reading material; includes video about screening. |

**NBS first developed in 1963 by Robert Guthrie for population-based testing of newborns for phenylketonuria (PKU).**

# Considerations for NBS programs synthesized from 40 years of use of the original Wilson and Jungner criteria (1968)

- The screening program should respond to a recognized need.
- The objectives of screening should be defined at the outset.
- There should be a defined target population.
- There should be scientific evidence of screening program effectiveness.
- The program should integrate education, testing, clinical services and program management.
- There should be quality assurance, with mechanisms to minimize potential risks of screening.
- The program should ensure informed choice, confidentiality and respect for autonomy.
- The program should promote equity and access to screening for the entire target population.
- Program evaluation should be planned from the outset.
- The overall benefits of screening should outweigh the harm.

Source: Andermann et al. (2008) and McCandless and Wright (2020)

# Estimated frequency of some IEMs among live births in USA

| | |
|---|---|
| Hemophilia A (males) | 1 in 10,000 |
| MCAD (all) | 1 in 14,000 |
| MCAD (Caucasians) | 1 in 10,000 |
| Phenylketonuria | 1 in 12,000 |
| Mucopolysaccharidoses (all types) | 1 in 25,000 |
| Glycogen storage disorders (all types) | 1 in 50,000 |
| Galactosemia (classical form) | 1 in 60,000 |
| Biotinidase | 1 in 60,000 |
| Chance of being struck by lightning in 80 yrs. | 1 in 10,000 |

**About 130,000 live newborns in Ohio annually**
**Therefore, we would expect about 9 newborns with MCAD (all) annually**

# Impact and Limitations of NBS

- It is a **SCREENING** test
- A screen positive result merits rapid follow-up for **DIAGNOSTIC** confirmatory testing
  - Biochemical, enzymatic, and/or molecular
- Does **NOT** catch every affected newborn (tests, cutoffs, and TAT) and **NOT** every newborn screened
  - Turn-around-time (TAT) ~7 days in various states
  - Prone to false negatives (FN) – a sensitivity question!
  - Religious objection and NBS not completed for other reasons
  - Birth certificates without matching NBS
- Threshold (cut-off) set to catch as many as possible
  - prone to false positives (FP) – a specificity question!
  - Importance of positive predictive value (PPV)
- **NOT** all disorders are represented on NBS
- Older children, adults, individuals **born in other countries** may or may not have had NBS

# Selection of Disorders for NBS

- **Disease characteristics for NBS consideration**
  - Disease incidence
  - Clinical manifestations of disease
  - Outcome of disease if not treated
- **Questions about feasibility of NBS**
  - How to screen
  - Cost of screening
  - Turnaround time for testing
- **Questions about treatment of diseases on NBS**
  - Availability of treatment
    - Value in screening if there is no effective treatment
  - Efficacy of treatment
- **ACMG (2006)**
  - Outlined a process of standardization of the NBS process
  - Recommended a uniform panel
  - **Primary** (Core) disorders that NBS should include for clear clinical consequences
  - **Secondary** disorders that are screened as a byproduct of screening the primary disorders and may have clinical consequences
- **Department of Health and Human Services maintains the Recommended Uniform Screening Panel (RUSP)**
  - 35 primary and 26 secondary disorders
  - https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp

# Adding a New Disorder – RUSP Nomination Process

1. **Section 1: Your Details**
   This section provides the contact information for the primary nominator. The full nomination package will request the contact information for the entire nominator team.
2. **Section 2: Preliminary Nomination Questions**
   This section consists of four preliminary nomination questions and provides a section for up to three supportive reference URLs for each question. If the reference does not have a URL and requires an attachment, please email achdnc@hrsa.gov.
   1. Question 1: Is there a newborn screening test available?
   2. Question 2: Is there an agreement about the case definition of the targeted condition and diagnostic confirmation after a positive newborn screen?
   3. Question 3: Is there a prospective population-based newborn screening project that has identified at least one infant with the condition?
   4. Question 4: Can identification of the targeted condition before clinical presentation allow provision of effective therapy and improve outcomes for screened infants?

# Adding a New Disorder - RUSP Nomination Process

**3. Nomination and Prioritization Workgroup**

- The Committee's Nomination and Prioritization (N&P) Workgroup reviews the preliminary nomination form and verifies that the nomination meets the four basic requirements needed for a condition to be considered for review. The Designated Federal Official (DFO) will communicate the N&P's findings to the Nominators.
- The N&P Workgroup compiles a summary for Committee consideration. The Committee decides if sufficient evidence is available, and votes to assign, or not assign, the nominated condition to the external Evidence-Based Review Group (ERG). Nominators whose conditions are not assigned to the ERG are provided with feedback.

**4. Evidence-Based Review Group (ERG)**

- The external ERG completes a systematic evidence-based review, provides updates, and presents a final report to the Committee on assigned conditions. Past ERG reports can be found on the Previously Nominated Conditions page.

**5. Committee Deliberations and Votes**

- The Committee discusses and deliberates on the evidence presented by the ERG. The Committee uses a decision matrix (PDF - 202 KB) and accompanying decision matrix guidance to guide their final decisions. Then the Committee votes to recommend or not recommend adding the nominated condition to the RUSP for consideration by the Secretary of Health and Human Services. Nominators whose conditions are not recommended for addition to the RUSP are provided with feedback.

**6. Final Decision**

- The **Secretary of Health and Human Services makes the final decision** on whether to add, or not add, a recommended condition to the RUSP. Committee recommendations and the Secretary's responses can be found on the Recommendations to HHS Secretary with Responses page.

- **Just because a disease is approved by RUSP doesn’t mean it automatically gets put on State NBS panels.**

- **Even if a disease is not approved by RUSP it can still get placed on State NBS panels.**

# AA Ratios as Biomarkers for PDCD: Takeaways

- PDCD incidence:
  - **At least 1:40,000** live births annually in United States
- Ala/Leu, Ala/Lys, (Ala+Pro)/Leu, and (A+P)/(L+K) ratios and/or their combinations are **highly sensitive biomarkers (≥90%) for identifying individuals with PDCD**
  - **Specificity is 75-85%**
- **2nd tier molecular testing needed for higher specificity and to exclude other conditions (such as PMDs) not on NBS**
  - ***PDHA1* alone** (would pick up about 85% of PDCD cases)
  - **5 primary-specific PDCD genes** – *PDHA1*, *PDHB*, *DLAT*, *PDP1*, *PDHX* (would pick up about 90% of PDCD cases)

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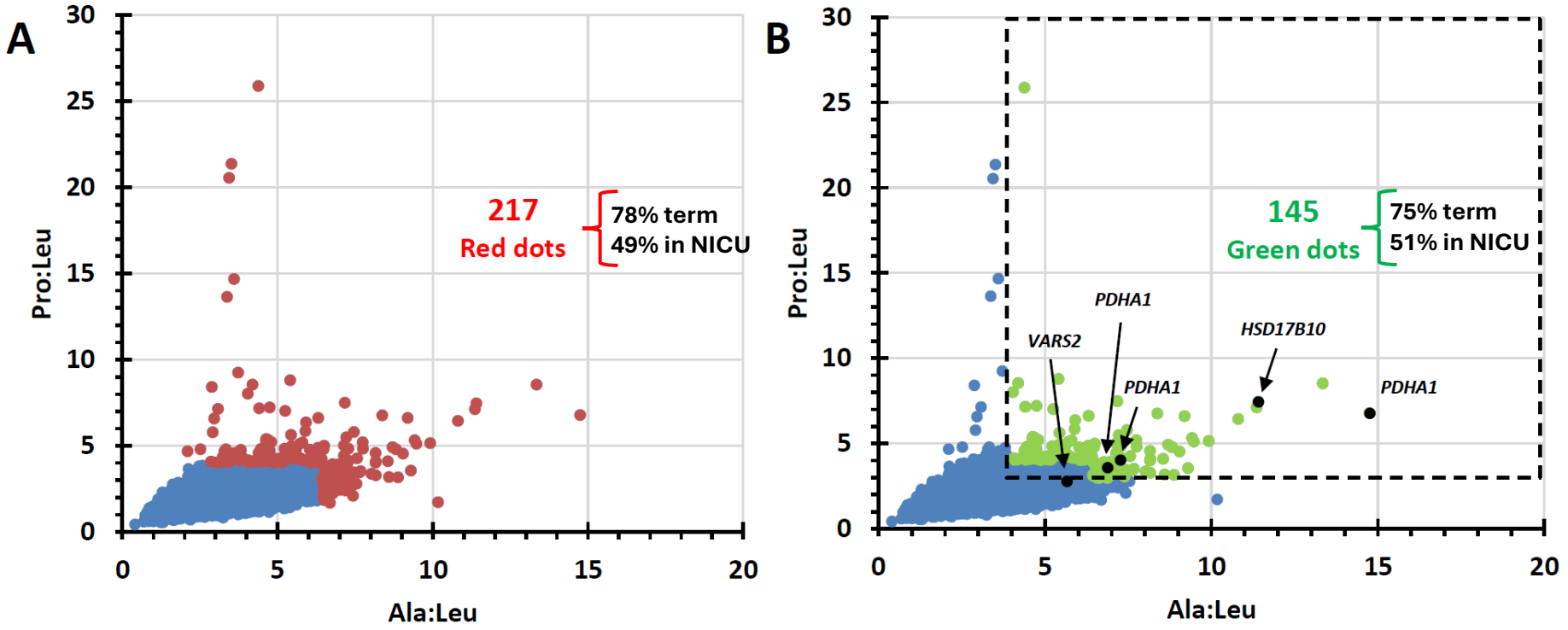
# NBS DBS Data[1]
## (Semi-Prospective Study)

**123,414 non-duplicate and data complete DBS specimens analyzed**

(91% of initial 136,282 specimens in a 12-month period; **Nov 15, 2018-Nov 14, 2019**)

≥99.9%ile for either Ala:Leu or Pro:Leu, 217 (**red**) vs ≥99.9%ile **+** Ala/Leu≥4.0 **AND** Pro/Leu≥3.0, 145




**Red dots** (217; **0.18% of all**) mean **NOT** statistically different than **green dots** (145; **0.12% of all**) mean for GA, BW, Ala, Pro, Ala:Leu and Pro:Leu

Valid to use either red or green dots for subsequent analyses

[1]Bedoyan *et al.* (2020) *JIMD Rep* **56**:70-81

RARE DISEASES
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# NOW ENROLLING

## Pilot Newborn Screening (NBS) Study for PDCD

The Center for Human Genetics at University Hospitals Cleveland Medical Center is currently enrolling patients to help us validate a new test to screen newborn babies for rare inherited disorders. **No clinic visits or new laboratory blood draws or clinical testing is necessary for participation**. This study will *only* involve the review of medical records and Ohio newborn screening (NBS) test results. To be eligible for this study, participants

- must have a diagnosis of one of the following:
  - **Pyruvate Dehydrogenase Complex Deficiency (PDCD)**
  - **Disorder of Pyruvate Metabolism (DPM)**
  - Any other **Mitochondrial Disorder**
  - An **Organic Acidemia** or **Fatty Acid Oxidation Disorder**
- must be born in Ohio on or after May 4th, 2018

For more information, please contact Genya Kisin at the Center for Human Genetics, 11100 Euclid Avenue, LKSD 1500, Cleveland, OH 44106

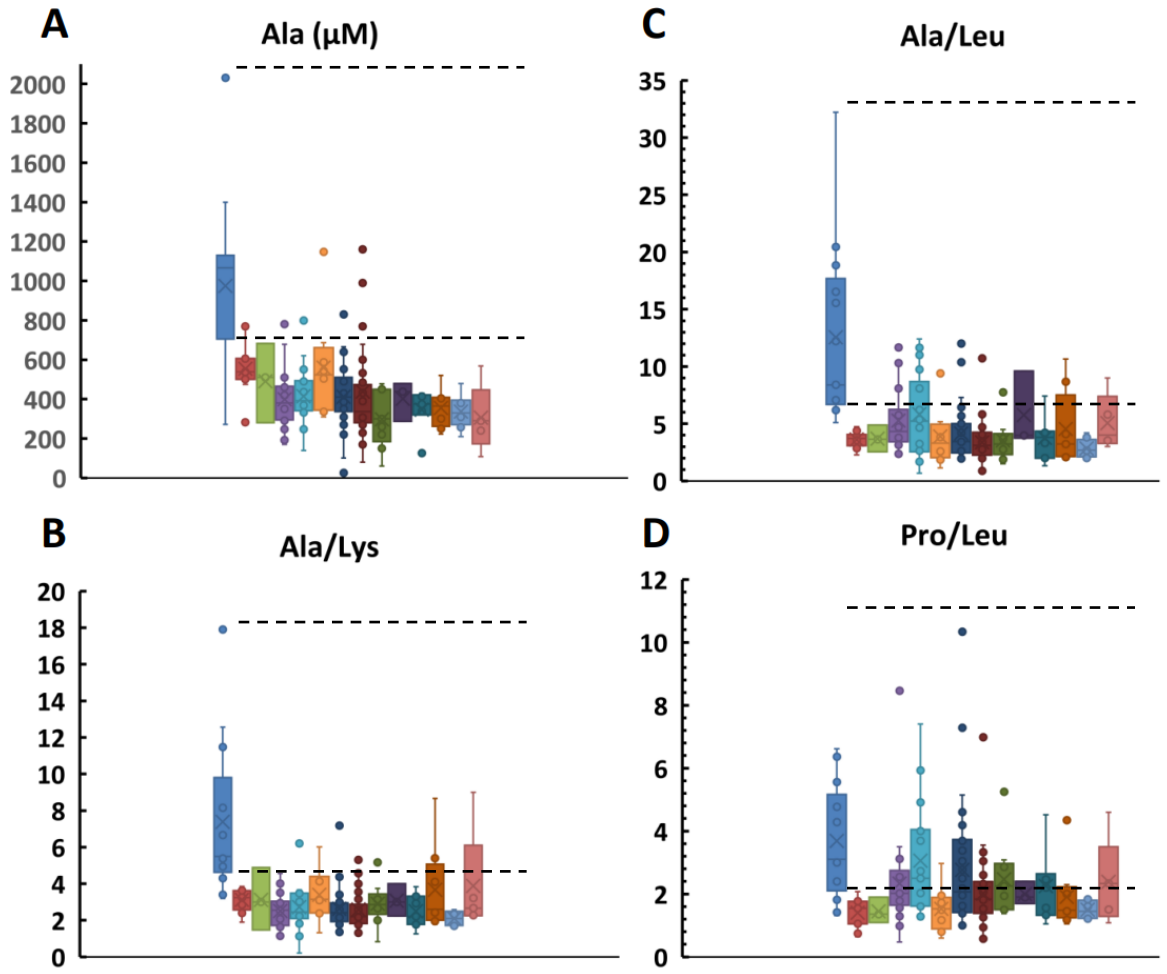
Phone: (216) 286-9202 or email: NBSPDCResearch@UHHospitals.org

University Hospitals Cleveland Medical Center

School of Medicine Case Western Reserve University

Rainbow Babies & Children's Hospital

## Retrospective Study of 11 Years of UPMC Data



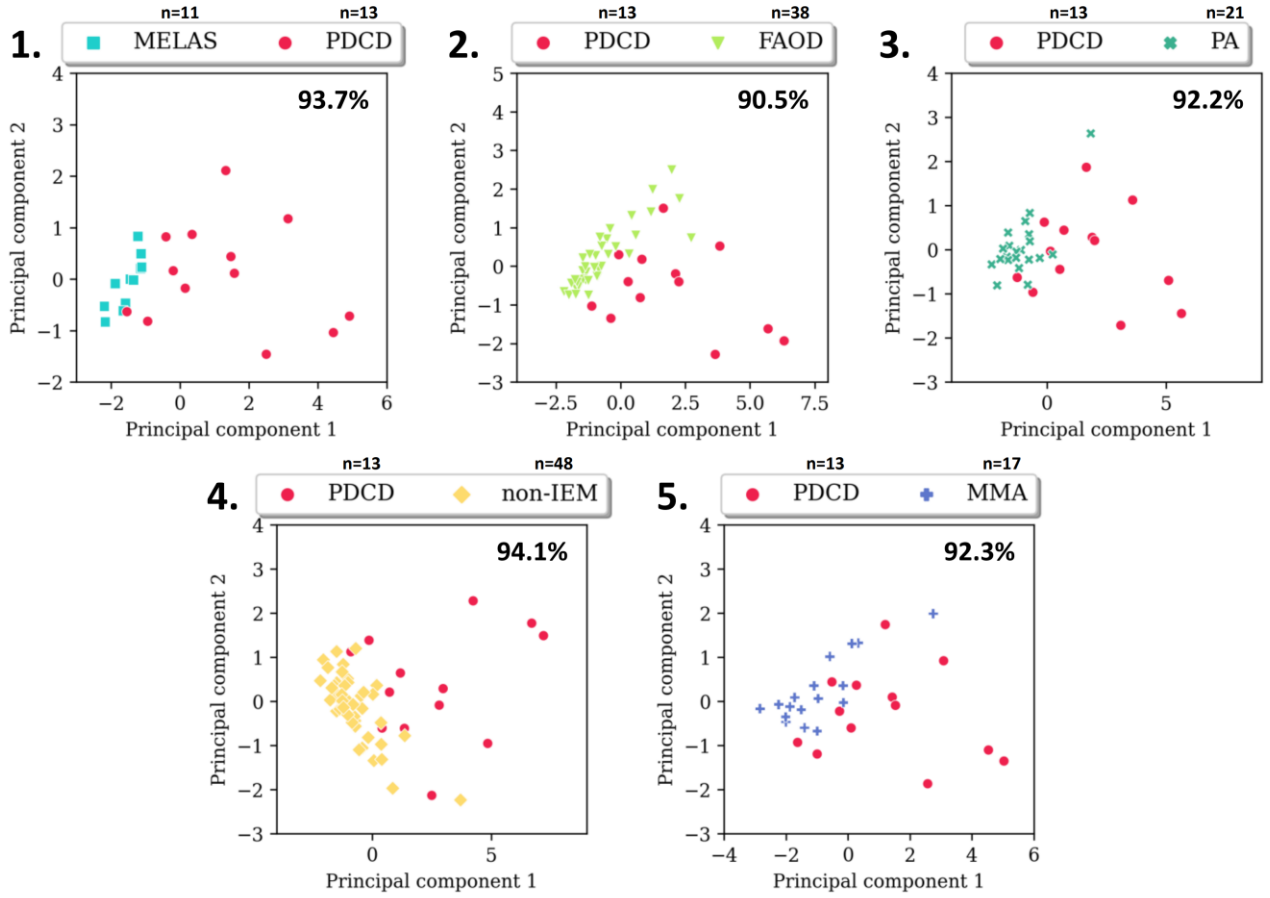

**n=201**

- 13 PDCD
- 11 MELAS
- 3 MERRF
- 21 PA
- 18 MMA
- 8 Leigh syn
- 39 FAOD
- 48 non-IEM
- 9 Barth syn
- 3 Classic GAL
- 7 TYR1
- 8 Other CM mito IEM
- 8 TGV
- 5 RTA/CKD

| | Ala/Leu | Pro/Leu | Ala/Lys | Pro/Lys | (Ala+Pro)/(Leu+Lys) | Cit/Leu | Cit/Lys |
|---|---|---|---|---|---|---|---|
| Ala/Leu | 1 | | | | | | |
| Pro/Leu | 0.760159 | 1 | | | | | |
| Ala/Lys | 0.900278 | 0.559598 | 1 | | | | |
| Pro/Lys | 0.726704 | 0.772676 | 0.788739 | 1 | | | |
| (Ala+Pro)/(Leu+Lys) | 0.906637 | 0.673643 | 0.871250 | 0.816506 | 1 | | |
| Cit/Leu | 0.231832 | 0.536538 | 0.034028 | 0.240664 | 0.048611 | 1 | |
| Cit/Lys | -0.058400 | 0.122083 | 0.131461 | 0.360781 | -0.085516 | 0.572284 | 1 |

Verma *et al.* (2023) *Mol Genet Genomic Med* – PMID:37688338

# Principal Component Analysis (PCA) using the 5 Relevant AA ratios for each Condition Pair (n>11 for each condition)




**Two principal components explaining >90% of overall data variance for each condition pairs**

Verma *et al.* (2023) *Mol Genet Genomic Med* – PMID:37688338

# Continuation: Collaborations and Initiatives for PDCD NBS Prospects

- Austin **Larson**, Colorado Children's Hospital
  - NBS DBS (with Ala and Pro analytes) with concurrent first PAA data
- Patricia **Hall**, Laboratory of Genetics and Genomics, Mayo Clinic
  - CLIR – 5 million reference samples; 20,000 diagnosed cases
    - Looking into Ala and Pro analytes
    - Working plan to resolve Lys from Gln on DBS by LC-MS/MS
- Steve **Dobrowolski**, Director Biochem. Lab, Children's of Pittsburgh
  - Prospective study of patients ordered plasma amino acids (PAA) testing
- Jirair **Bedoyan, APHL National NBS Webinar talk about PDCD**
  - July 30, 2024

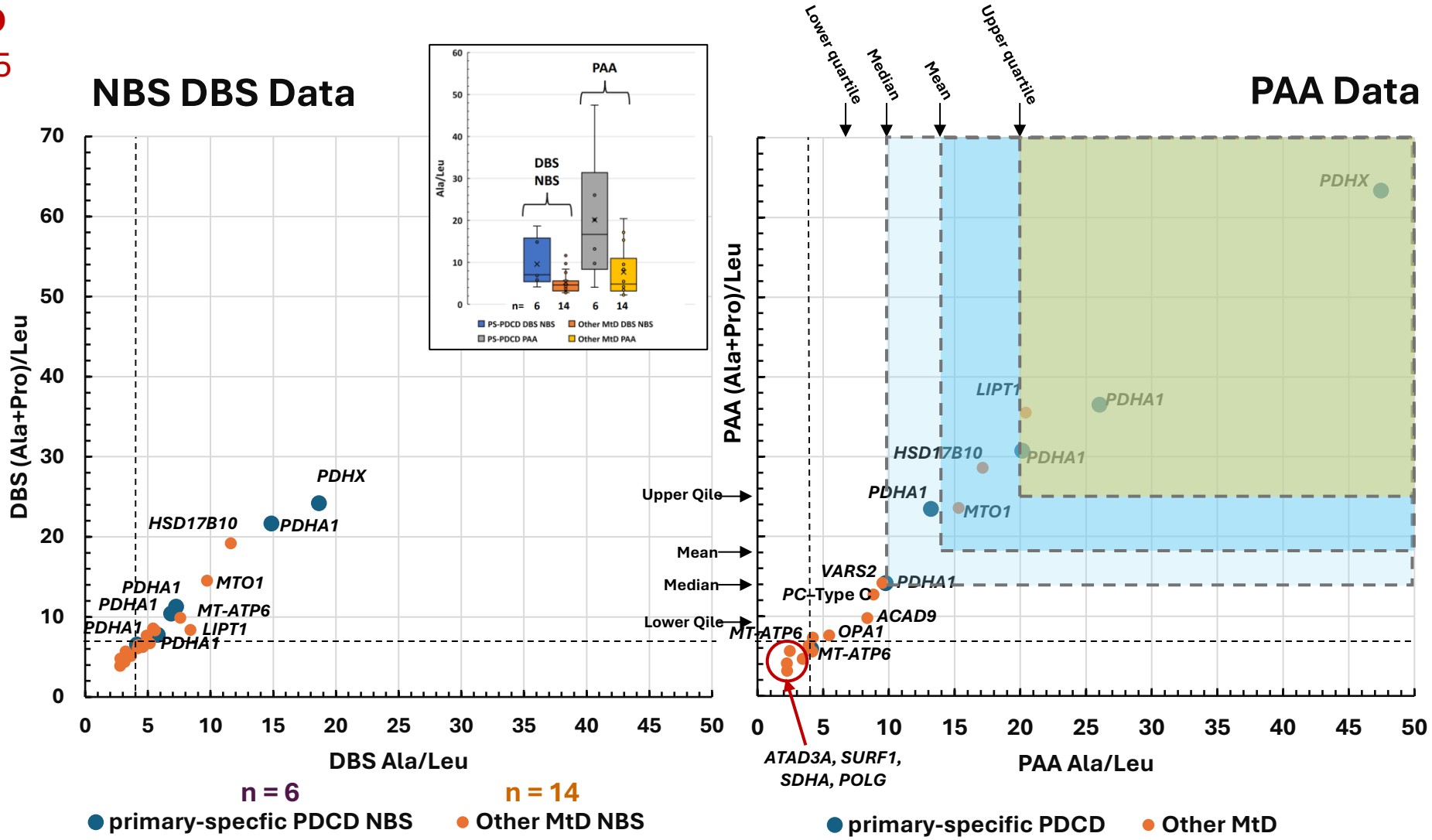
# Ohio and Colorado Data

**Cutoff for PDCD**

Ala/Leu ≥4.0 **AND**
(Ala+Pro)/Leu ≥6.5

| GENE | n |
|---|---|
| *PDHA1* | 5 |
| *PDHX* | 1 |
| | 6 |

| Other MtDs | |
|---|---|
| *LIPT1* | 1 |
| *PC* | 1 |
| *ACAD9* | 1 |
| *SURF1* | 1 |
| *POLG* | 1 |
| *SDHA1* | 1 |
| *ATAD3A* | 1 |
| *OPA1* | 1 |
| *PPA2* | 1 |
| *HSD17B10* | 1 |
| *VARS2* | 1 |
| *MTO1* | 1 |
| *MT-ATP6* | 2 |
| | 14 |




**Unpublished data**

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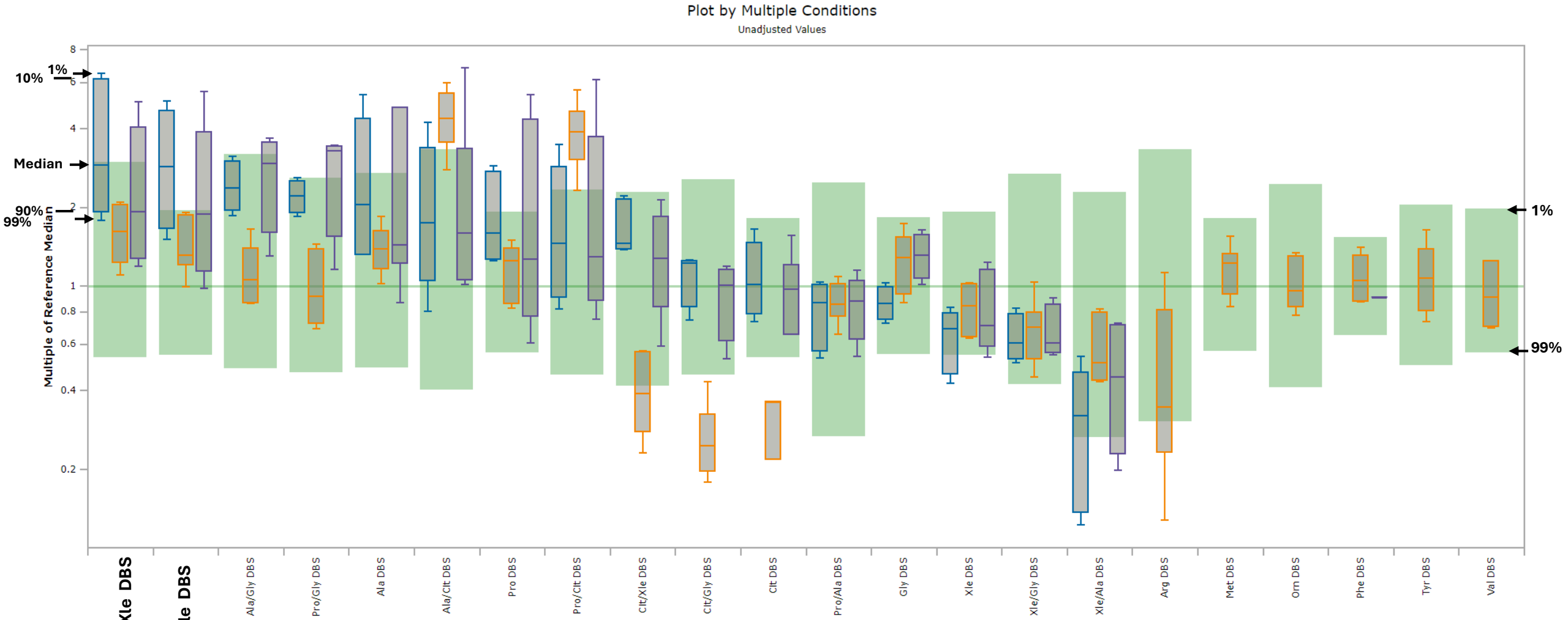
# NBS DBS PDCD and Other MtDs from OH and CO data, and MT-ATP6 in CLIR



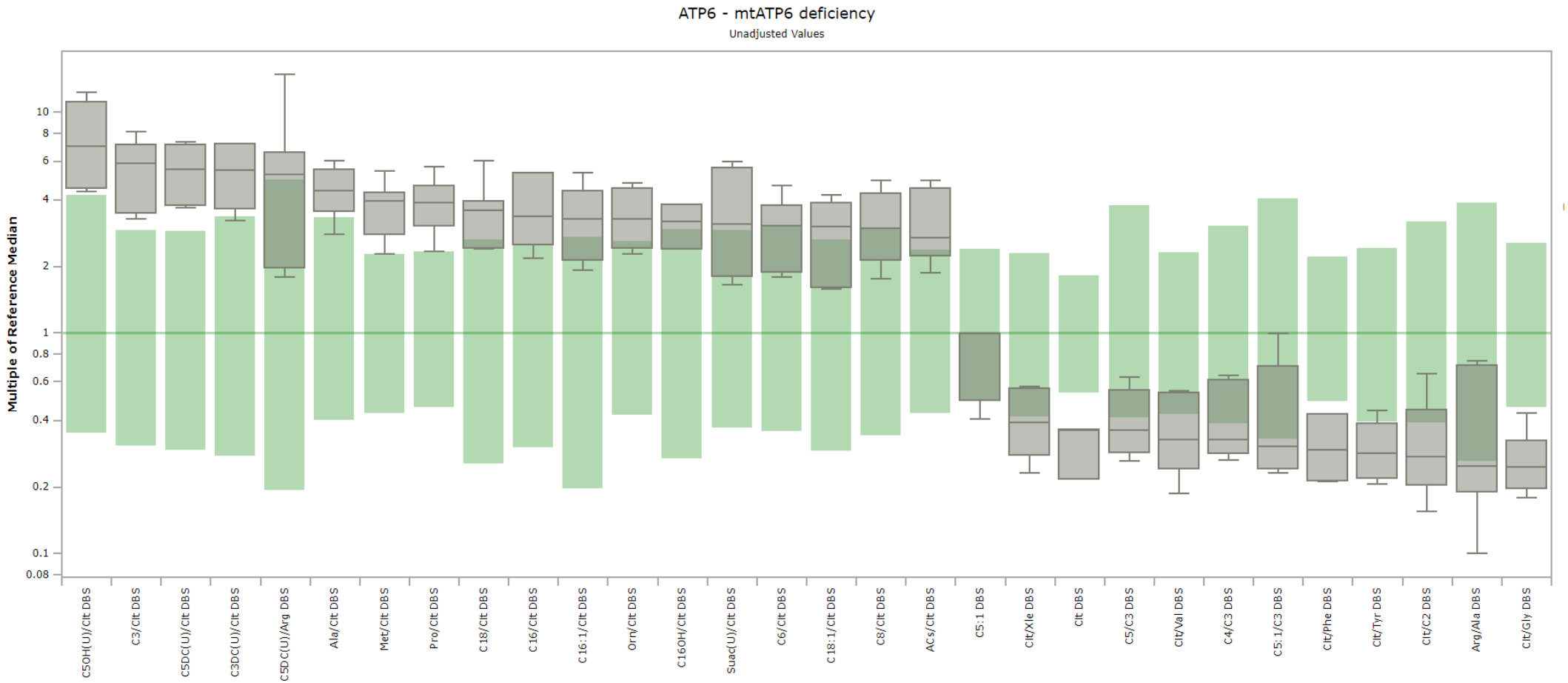

**Unpublished data**

MAYO CLINIC

Children's Hospital Colorado

UPMC CHILDREN'S HOSPITAL OF PITTSBURGH

# MT-ATP6 deficiency cases in CLIR



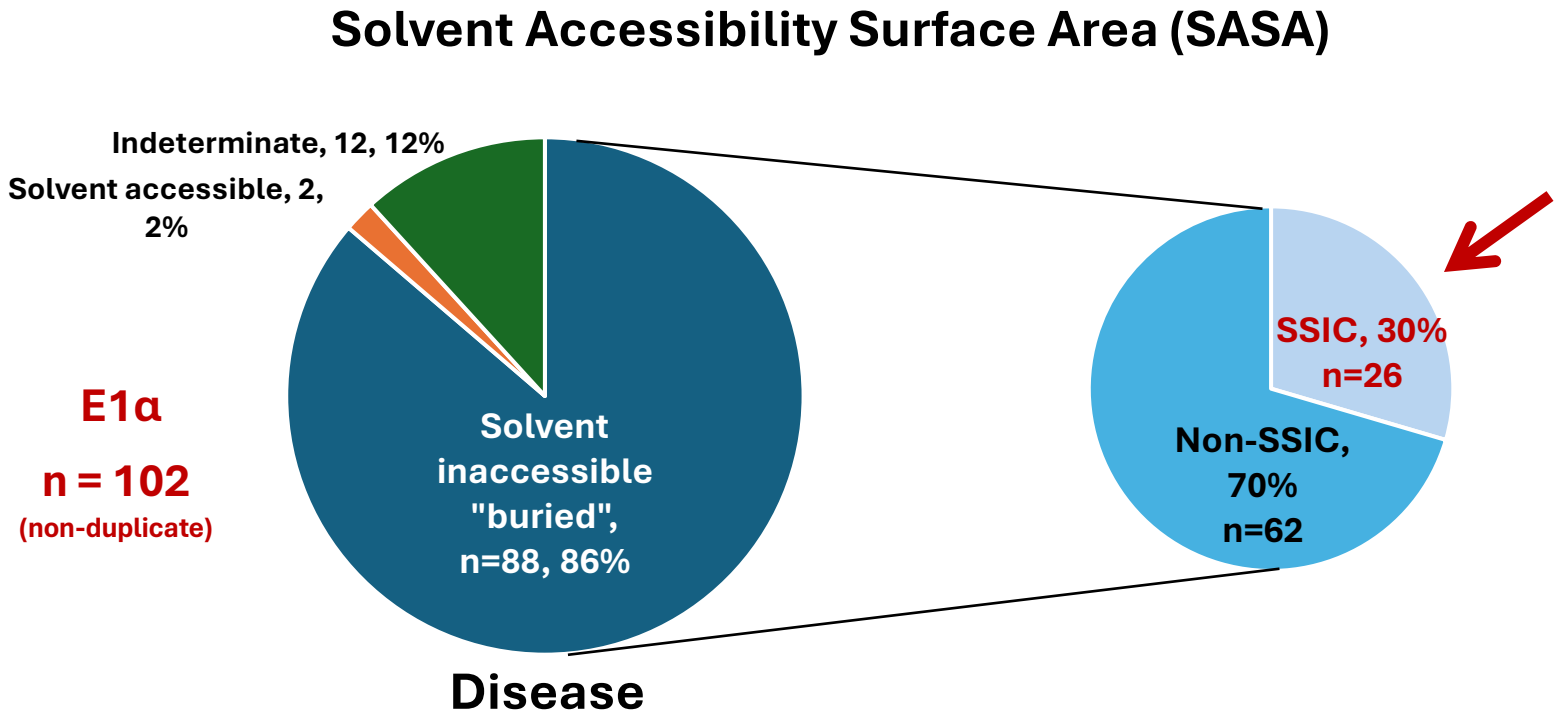

**Unpublished data**

# Protein-Specific Target-Based Small Molecule Therapeutics for PDCD

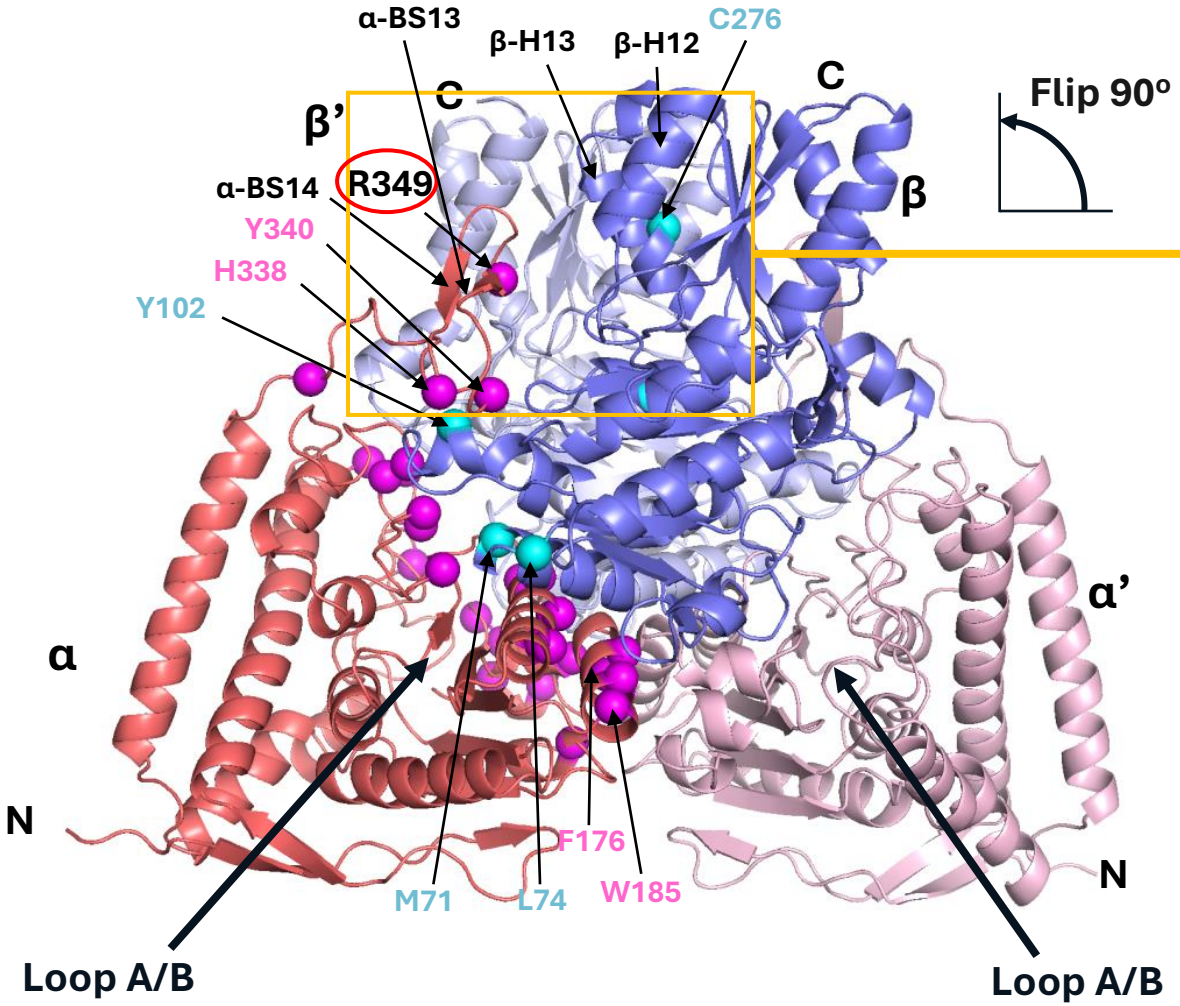

Carnegie Mellon University

UPMC | CHILDREN'S HOSPITAL OF PITTSBURGH

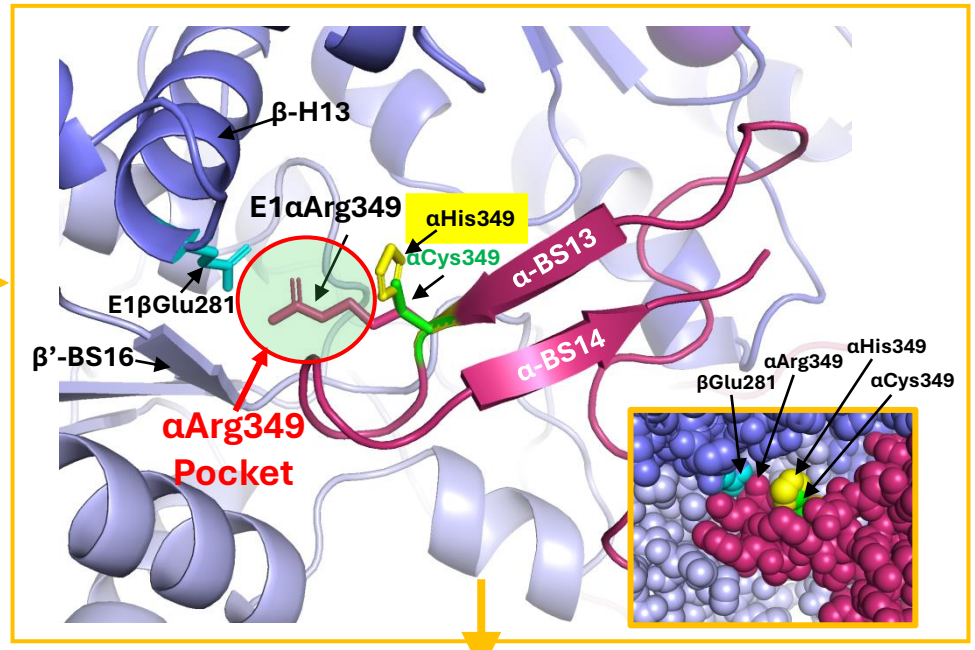
# About 30% of buried non-duplicate disease-causing missense variants (DMVs) of *PDHA1* are involved in Subunit-Subunit Interface Contact (SSIC)[1]

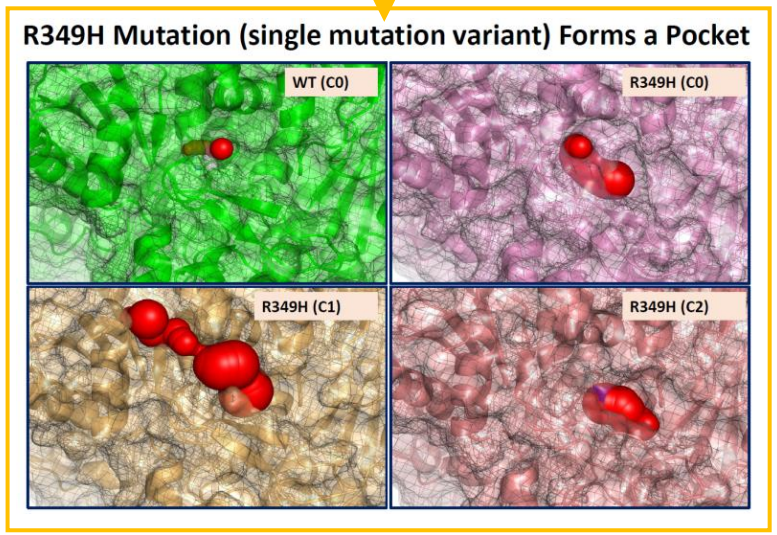



[1]Ducich *et al.* (2022) *JIMD* **45**:557-570




Flip 90°




**Mature protein numbering of residues**

Ducich *et al.* (2022) *JIMD* 45:557-70

**Note: *PDHA1* p.R378C (aka mature R349C)**

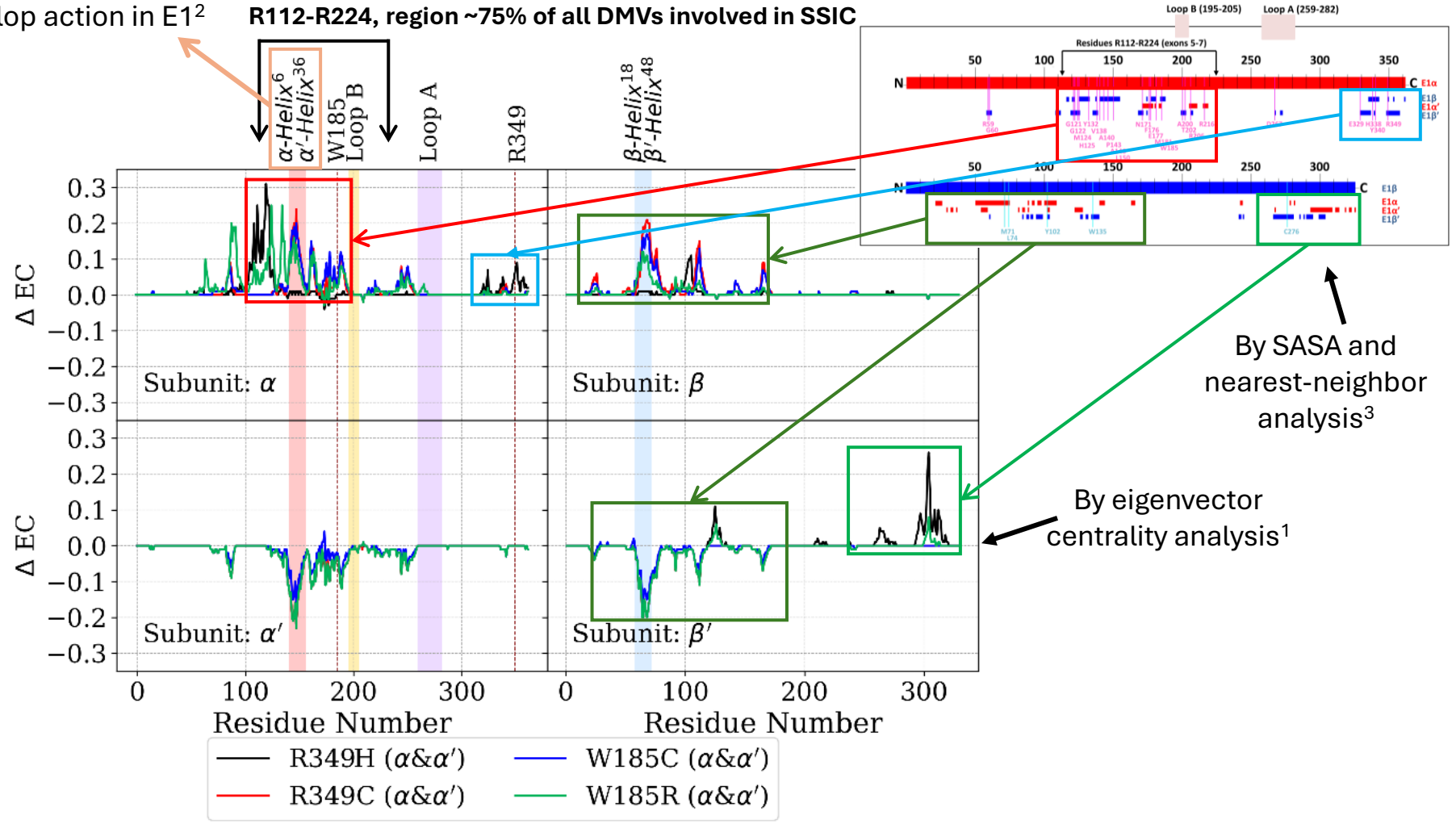
# Communication (cooperative) network in E1 altered by DMVs[1]

## DMVs >15Å away from the active site triggers dynamic and SSIC changes

leading to known reduced PDC activity and disease

Critical role in flip-flop action in E1[2]

**R112-R224, region ~75% of all DMVs involved in SSIC**




By SASA and nearest-neighbor analysis[3]

By eigenvector centrality analysis[1]

[1]Gokcan et al. (2022) *JCIM* **62**:3463-75; [2]Ciszak et al., (2003) *JBC* **278**:21240–6; [3]Ducich *et al.* (2022) *JIMD* **45**:557-70

# Pharmacological chaperoning protein-specific target-based small molecule approach: A model for other NAD-dependent dehydrogenase complexes implicated in human disease

- **Phase I (Computational)**
  - Virtual screening ~200,000 CNS-targeting small molecules using ML/DL
  - Docking of ligand to E1α R349C "pocket" and staying bound
  - Molecular dynamics simulations
  - Identifying hit and lead ligands
- **Phase II (*In vitro* experimental)**
  - Testing permeability *in vitro* – PAMPA and Caco-2 assays
  - Tagging ligands for mitochondrial matrix targeting and enrichment
  - *In vitro* testing of lead ligands on affected human fibroblasts (both sexes)
    - Partial or full restoration of 1) protein subunit stability, 2) cellular/mitochondrial bioenergetics, and 3) PDC activity on cells grown with glucose as energy source
- **Phase III (Animal model)**
  - Preclinical work using mouse models with *PDHA1* p.R378C
  - Testing safety, tolerability, efficacy, and pharmacokinetics

# Four Small Molecules Targeting E1α R349C

| Ligand | MW (Da) | Theoretical binding energy ΔG in kcal/mol (Kd) | | Permeability | |
|---|---|---|---|---|---|
| | | α pocket | α' pocket | PAMPA Mean $P_e$ nm/s | Caco-2 Mean $P_{app}$ $10^{-6}$ cm/s (A to B/B to A) |
| **Lig80** | 399.4 | ND | -4.0 (1.2 mM) | 150 **(high)** | 7.25/11.40 **(high)** |
| **Lig95** | 384.4 | -18.4 ($3.2 \times 10^{-5}$ nM) | -8.5 (0.6 μM) | 0.13 **(low-moderate)** | 0.01/17.1 **(low)** |
| **Lig58** | 376.8 | ND | -4.9 (0.3 mM) | 3.99 **(low-moderate)** | 0.72/0.99 **(moderate)** |
| **Lig66** | 325.0 | -3.7 (1.9 mM) | ND | ND | ND |

MW, molecular weight; ND, not done.

**Tagged ligands (red) for mitochondrial matrix targeting with ~300-fold enrichment.**

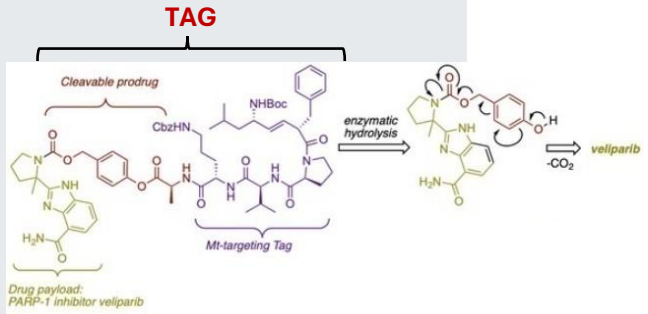
**PAMPA/Caco2 assays for Lig80, Lig95 and Lig58 first**

**PAMPA permeability: Low to Moderate: Pe < 15.0 nm/s; High: Pe ≥ 15.0 nm/s**

**Caco-2 permeability: Moderate: 0.60 < Papp < 6.00 (× $10^{-6}$ cm/s); High: Papp ≥ 6.00 (×$10^{-6}$ cm/s)**

Conversion ΔG to Kd: https://www.novoprolabs.com/tools/deltag2kd

Kd quantitative relationship with ΔG (molar Gibbs free energy): **ΔG=RTlnKd at 298K (25℃)**




**Unpublished data**

# Acknowledgments


- CIDEM/UHCMC
  - Douglas Kerr
  - Charles Hoppel
  - George Grahame
- CHG/UHCMC
  - Suzanne DeBrosse
  - Lori-Anne Schillaci
  - Edwin Ferren (fellow)
  - Genya Kisin
  - Rhonda Jones
- UPitt/UPMC CHP
  - Jerry Vockley
  - Steven Dobrowolski
  - Al-Walid Mohsen
  - Kaitlyn Bloom
  - Bianca Seminotti (fellow)
  - Lorna Cropcho
  - Jennifer Baker
  - Danielle Black
  - April Lehman (fellow)
  - Robert James Hannan
- CWRU/WV-SOM (med students)
  - Kirkland Wilson (CWRU-SOM)
  - Kelsey Murphy (CWRU-SOM)
  - Ha Kyung Shin (CWRU-SOM)
  - Nicole Ducich (CWRU-SOM)
  - Anisha Verma (WV-SOM)
- Ohio NBS Lab
  - Rosemary Hage (Director; CDC)
  - Sharon Linard
- CWRU Genomics Core
  - Alexander Miron
  - Simone Edelheit
- Collaborators
  - CWRU-Pharmacology – Jason Mears
  - Boston Children's Hosp. – Gerard Berry
  - Children's Hosp. Colorado – Johan Van Hove
  - Univ. Amsterdam – Ronald Wanders
  - Akron Children's Hosp. – Bruce Cohen
  - Mayo Clinic – Patricia Hall
  - Colorado Children's Hospital – Austin Larson
    - Emily Shelkowitz (Seattle Children's)
    - Molly Crenshaw (fellow)
  - Nationwide Children's Hospital
    - Mari Mori
    - Kandamurugu Manickam
    - Dennis Bartholomew
  - Carnegie Mellon Univ. – Computational Chemistry
    - Olexandr Isayev
    - Hatice Gokcan
    - Polina Avdiunina (grad student)
  - Univ. of Pittsburgh (UPitt)
    - Peter Wipf – Chemistry
    - Matt LaPorte – Chemistry

## Financial Support

- CHG Pilot Grant support (2013)
- CTSC CWRU Award (2014)
- NIH RDCRN U54 NAMDC Projects 2014-19 & 2019-24
- 2020 & 2023 UMDF/NAMDC Gateway to Mitochondrial Medicine Summer Grants to Nikki Ducich ('20), Anisha Verma ('23), Bianca Seminotti ('24)
- NAMDC/UMDF Pilot Grant (2023)
- Ultragenyx (2023)
- Elizabeth Watt PDCD Research Fund – 2022 & 2023 Philanthropy



Douglas Kerr


Jerry Vockley


Anisha Verma


April Lehman


Ha Kyung "Kris" Shin


Nicole Ducich


Robert James Hannan


Kaitlyn Bloom


Peter Wipf


Jason Mears


Matt LaPorte


Oles Isayev


Hatice Gokcan


Polina Avdiunina