Rationale and feasibility of newborn screening for pyruvate dehydrogenase complex (PDC) deficiencies

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Abstract

Pyruvate dehydrogenase complex (PDC) deficiency is a treatable neurometabolic mitochondrial disorder with high morbidity and mortality, and is currently not on the US Recommended Uniform Screening Panel (RUSP). Prompt and correct diagnosis of this disorder and early initiation of therapy (e.g., ketogenic diet) is critical for the long-term positive developmental and cognitive outcome of a child affected with primary-specific PDC deficiency (PDCD), most commonly due to *PDHA1* mutations.

We present a rationale for newborn screening (NBS) for PDC deficiency using alanine, proline and leucine, and their ratios as screening metrics. This approach makes use of exiting analytical approaches at state NBS laboratories. We have used plasma alanine, proline, and leucine data from affected subjects with pathogenic PDHA1 or PDHB mutations to show that the screening scheme using a combination of Pro:Leu, Ala:Leu, and Ala:Pro ratios are highly sensitive (**Fig. 1**) but likely not specific for identifying individuals at high risk for this disorder. This approach is also likely to identify other mitochondrial disorders and other causes of lactic acidosis in newborns. We have evaluated these amino acids and their ratios from dried blood spot (DBS) specimens of about 45,000 de-identified newborns screened through Ohio NBS laboratory (**Table 1** and **Fig. 2**) to predict the false positive rates given specific cut-offs, known frequency of mitochondrial disorders and the estimated incidence of PDCD. We discuss the sensitivity, specificity, and feasibility of a tier-based NBS approach to identifying newborns with primary-specific PDCD for early intervention.

This screening approach (**Fig. 3**) lays the foundation for a NBS protocol for identifying newborns at high risk for primary-specific PDCD who might benefit from early known and potential therapeutic intervention(s).

Introduction

1. Overall prevalence of mitochondrial disorders is 1 in 9,000 individuals
2. PDCD is the 2nd most common mitochondrial disorder
3. We estimate 1 in 50,000 to 75,000 births annually will have primary-specific PDCD and ~80% of them will likely be due to *PDHA1*

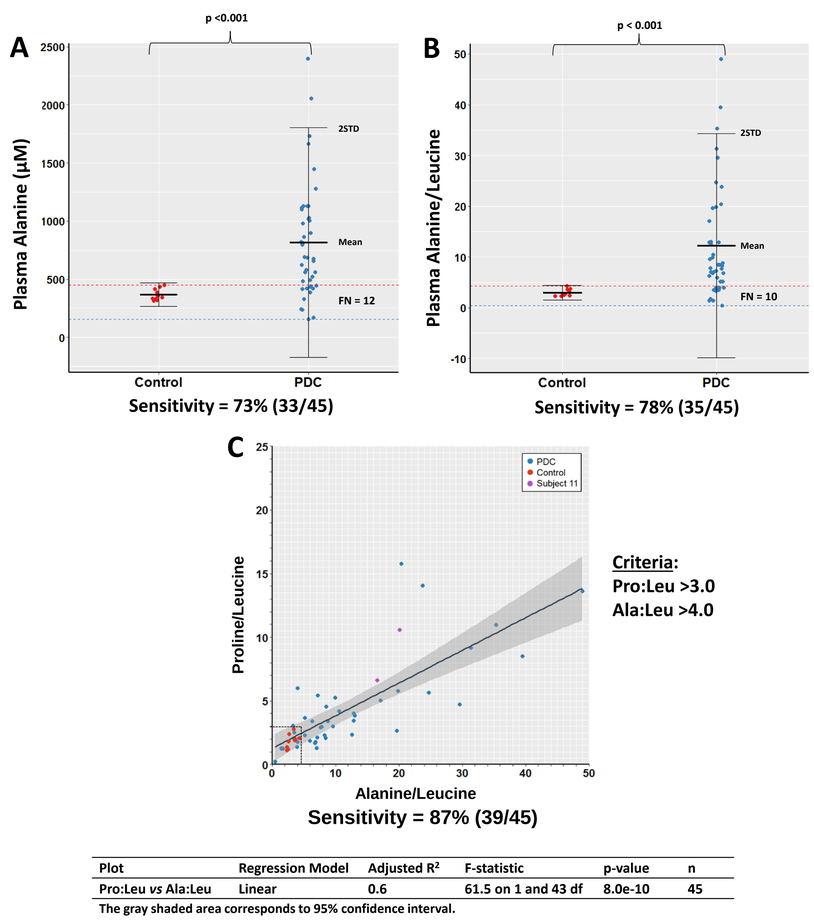
Premise for NBS

Early diagnosis of primary-specific PDCD and early intervention with ketogenic diet (KD) would lead to **improved**

1. **Developmental and cognitive outcome**
2. **Quality of life**
    1. Less seizures
    2. Less hospitalizations
    3. Longer survival

Elements of the pilot NBS protocol

1. Category of screened newborns for the pilot study
    - Full term
    - Normal birth weight
    - No perinatal complications or interventions needed
        - No TPN
        - No transfusions
2. Use existing analytical approaches in Ohio NBS Lab
    - *no change in instrumentation*
3. Data to collect from screened newborns (from DBS)
    - Alanine, Proline, and Leucine



Plot	Regression Model	Adjusted R^2	F-statistic	p-value	n
Pro:Leu *vs* Ala:Leu	Linear	0.6	61.5 on 1 and 43 df	8.0e-10	45

The gray shaded area corresponds to 95% confidence interval.

Fig. 1. Distribution of plasma (A) Ala and (B) Ala:Leu as well as plot and regression analysis of plasma (C) amino acid ratios (Pro:Leu and Ala:Leu) from PDCD subjects due to *PDHA1* (21) or *PDHB* (2) mutations (blue and purple dots) and normal controls (red dots). (A, B) P-values shown were obtained from two-sample t-test for un-pooled variances with one-tailed significance level of $p < 0.05$. (C) The gray shaded area corresponds to 95% confidence interval for the regression. FN, false negative.

Table 1. Alanine, proline, and leucine measurements on 44,699 de-identified specimens from the Ohio NBS program

	Alanine	Proline	Leucine	Ala:Leu	Pro:Leu
Mean	336	201	115	3.0	1.8
STD	91	48	28	0.8	0.4
Min	115	56	25	0.8	0.6
Max	1521	2115	488	14.3	21.5
99.50 %ile	642	371	211	5.7 (**181**)	3.3 (**224**)
99.90 %ile	765	455	275	6.7 (**45**)	4.1 (**45**)

Number in bracket (bold), correspond to the number of specimens exceeding the specified %ile for that ratio. Amino acids values in µM.

Between May and Sept 2018, de-identified limited data on ~40% (44,699/108,103) of screened Ohio newborns. 74 of 44,699 screened newborns exceeded the 99.90 %ile for either Ala:Leu >4.0 or Pro:Leu >3.0. 16 of 44,699 newborns exceeded the 99.90 %ile for both.

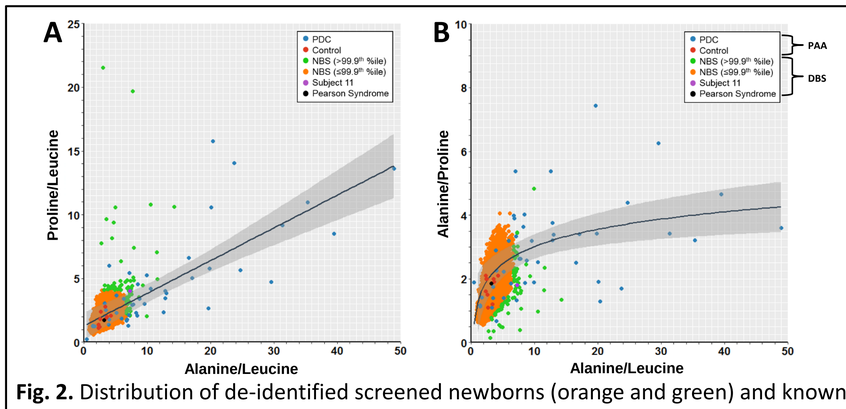


Fig. 2. Distribution of de-identified screened newborns (orange and green) and known older PDC deficient subjects (blue) and normal controls (red) on (A) Pro:Leu *vs* Ala:Leu and (B) Ala:Pro *vs* Ala:Leu plots. NBS results of **subject 11** with PDC deficiency due to *PDHA1* shown. The gray shaded areas correspond to 95% CI for the regression.

1. Anticipate 5 **green** dots will have a mitochondrial disorder
2. 1-2 would have PDCD (**purple** dot falls among the **green** dots group)
3. 93% (69/74) likely false positive; could reduce this by 1/2 if cutoff set at 99.99 %ile
4. Who are the false positives? Would milder PDCD be FN and likely not be picked up?

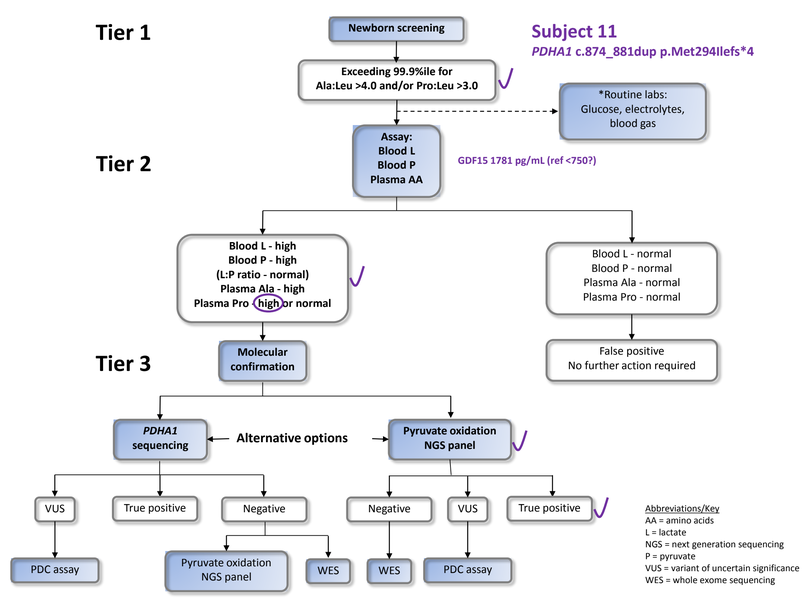


Fig. 3. Proposed algorithm for newborn screening for PDCD. Actions are shown in shaded boxes, while results are in un-shaded boxes. **Subject 11** is a 1 month-old female infant referred to our institution with lactic acidosis, congenital brain anomalies and hypotonia, advanced through this algorithm, diagnosed with PDCD due to a novel pathogenic *PDHA1* variant, and started on a ketogenic diet.

Takeaways

1. Ala:Leu and Pro:Leu combined data provide better sensitivity than Ala, Ala:Leu, or Ala:Lys (data not shown) alone. Ala:Pro with Ala:Leu data is informative and as sensitive.
2. For about 140,000 newborns screened annually in Ohio, we would expect **about 230 Ohio newborns annually will meet the criteria of both Ala:Leu >4.0 and/or Pro:Leu >3.0 at >99.90 %ile cutoff**.
3. Given the estimated incidence for primary-specific PDCD, **expect to identify 2 to 3 PDCD cases annually in Ohio.**
4. Get less false positives (50%), if those exceeding 99.99 %ile analyzed
5. A newborn screening algorithm for PDCD is proposed (**Fig. 3**)

Benefits and Feasibility of NBS for PDCD

Benefits:

1. Shorten time for diagnosis
2. Early intervention with KD or other therapies
3. Improve developmental and cognitive outcome
4. Improve quality of life; less seizures, less hospitalizations, and longer survival

Feasibility:

1. Use existing instrumentation and analytical approaches
2. Simple and sensitive approach
3. False positive and false negative rates yet to be determined
4. Funding – NIH RDCRN NAMDC U54 renewal grant (?)

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 or Clay Ferren at the Center for Human Genetics, 11100 Euclid Avenue, LKSD 1500, Cleveland, OH 44106

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