Variation in Diagnostic Care and Disease Classification for PAH Deficiency: Initial Findings From the Canadian Inherited Metabolic Diseases Research Network (CIMDRN)

Pranesh Chakraborty[a], Kylie Tingley[b], Mike Kowalski[a], Monica Lamoureux[a], Beth K. Potter[b], Michael Pugliese[b], Doug Coyle[b], Kumanan Wilson[b], Valerie Austin[c], Catherine Brunel[d], Daniela Buhas[e], Maggie Chapman[f], Alicia K.J. Chan[g], Sarah Dyack[f], Annette Feigenbaum[c], Michael Geraghty[a], Alette Giezen[h], Jane Gillis[h], Sharan Goobie[i], Shailly Jain[g], Mariya Kozenko[j], Erica Langley[a], Julian Little[b], Jennifer MacKenzie[k], Bruno Maranda[l], Aizeddin Mhanni[m], Grant Mitchell[d], John J. Mitchell[e], Laura Nagy[c], Amy Pender[j], Murray Potter[j], Chitra Prasad[i], Suzanne Ratko[j], Ramona Salvarinova[h], Andreas Schulze[c], Komudi Siriwardena[g], Neal Sondheimer[c], Rebecca Sparkes[n], Sylvia Stockler[h], Yannis Trakadis[e], Lesley Turner[o], Keiko Ueda[h], Clara Van Karnebeek[h], Hilary Vallance[h], Anthony Vandersteen[f], Jagdeep Walia[k], Brenda J. Wilson[b], Nataliya Yuskiv[h], and Jonathan B. Kronick[c]

[a]Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada, [b]University of Ottawa, Ottawa, Ontario, Canada, [c]University of Toronto/Hospital for Sick Children, Toronto, Ontario, [d]CHU Ste-Justine, Montreal, Quebec, Canada, [e]Montreal Children's Hospital, Montreal, Quebec, Canada, [f]Dalhousie University, Halifax, Nova Scotia, Canada, [g]University of Alberta, Edmonton, Alberta, Canada, [h]BC Children's Hospital, Vancouver, British Columbia, Canada, [i]Western University, London, Ontario, Canada, [j]McMaster University, Hamilton, Ontario, Canada, [k]Queen's University, Kingston, Ontario, Canada, [l]University of Sherbrooke, Sherbrooke, Quebec, Canada, [m]University of Manitoba, Winnipeg, Manitoba, Canada, [n]Alberta Children's Hospital, Calgary, Alberta, Canada, [o]Memorial University, St John's, NFLD, Canada

Background & Objectives

- **Care during the diagnostic period for phenylalanine hydroxylase (PAH) deficiency may include:**
  - Monitoring of initial/untreated blood phenylalanine during the diagnostic period through repeated testing
  - Evaluating response to biopterin load testing
  - Molecular genetic testing for identifying pathogenic mutations in the PAH gene
  - Prescription of daily dietary phenylalanine intake and low or phenylalanine-free medical foods
- **There is uncertainty about classification of severity of PAH deficiency:**
  - How can diagnostic test results be used to classify disease according to severity: classical phenylketonuria (PKU), mild PKU, mild hyperphenylalaninemia (HPA)?
  - How well do different methods for classifying the severity of PAH deficiency agree with each other?
- **Study Objectives:**
  - To describe patterns of diagnostic care for children with PAH deficiency across Canada.
  - To investigate methods for assigning disease classification and to explore their level of agreement

Methods: CIMDRN Cohort

Sample Source:

- Children born between 2006 and 2015 and receiving care for an inherited metabolic disease at one of 13 CIMDRN centres

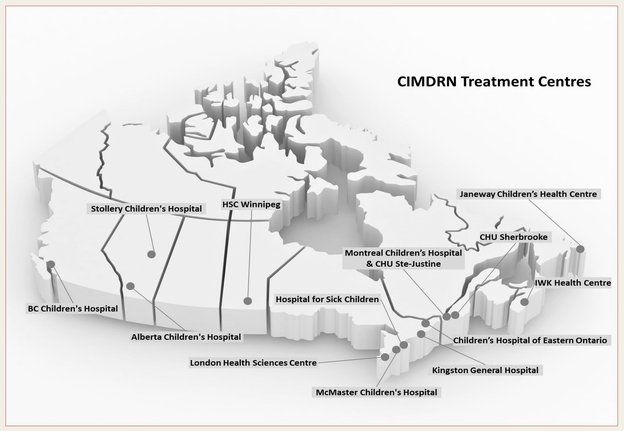

Figure 1. Treatment Centres Participating in CIMDRN

Methods: Clinical Data Collection & Analysis

Eligibility:

- Diagnosed with phenylalanine hydroxylase deficiency
- Complete baseline data (demographic and diagnostic information)

Data Collection:

- Database hosted on REDCap (Research Electronic Data Capture)
- Medical chart-abstracted data

Analysis:

- Descriptive analysis of diagnostic practices by centre
- Associations between methods of assigning disease severity

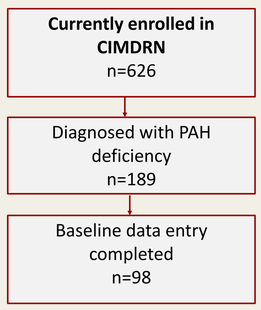

Figure 2. Participant Flow Diagram

Results & Interpretation: Diagnostic Care

Diagnostic Tests Performed Across Centres:

- There was variation in routinely reported diagnostic tests by centre, which likely reflects incomplete reporting of essential diagnostic tests and true variation in diagnostic practices between centres and over time.

Diagnostic laboratory tests	Practice Variation in Diagnostic Work-Up for PAH deficiency				
	Centre A	Centre B	Centre C	Other Centres	All Centres (Total)
Phenylalanine (Phe)	91%	100%	100%	71%	91%
Tyrosine (Tyr) or Phe/Tyr ratio	88%	100%	100%	71%	90%
Biopterin loading test	56%	62%	0%	10%	45%
Pterins (neopterin/biopterin)	50%	97%	50%	48%	68%
DHPR enzyme activity	59%	97%	50%	29%	67%
Molecular genetic testing	0%	67%	17%	29%	34%

N = 98. Centres labelled to preserve anonymity.

Age at Diagnosis:

- Overall, the median age of diagnosis was 10 days (range = 2 - 230 days)
- There were some outlying cases with unusually late diagnoses

PAH Deficiency Diagnosis	PAH Deficiency Diagnosis as Noted in Patient Charts and Age at Diagnosis		
	Frequency	Percent	Mean Age at Diagnosis* in Days (SD)
Not specified/Other	10	10%	11 (4)
Mild HPA	24	24%	41 (58)
Mild PKU	15	15%	13 (16)
Classical PKU	49	50%	12 (10)

N = 93. 5 cases missing date of diagnosis * Age of diagnosis defined as difference between date of birth and date of diagnosis. Date of diagnosis defined as date upon which investigation results confirming the diagnosis become available.

Results & Interpretation: Max Blood Phenylalanine Levels

Observed Trends:

- > 60% had a max phe level >= 600 umol/L during the diagnostic period
- 28% of max phe values came from Newborn Screening, not diagnostic tests
- Participants with lower phe tended to have their max level recorded from Newborn Screening, not from diagnostic tests (numbers too small to report)

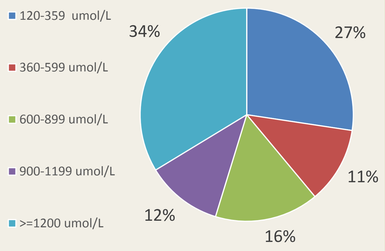

Figure 3. PAH Deficiency Severity Based on Maximum Blood Phe Measurement During the Diagnostic Period. N = 95. Three cases missing blood phe measurements.

Assignment of Disease Classification:

- Disease severity noted in patient charts did not always align with categorization based on maximum blood phenylalanine during the diagnostic period

Diagnosis Noted in Patient Charts	PAH Deficiency Diagnosis as Noted in Patient Charts by Maximum Blood Phenylalanine Measurement During the Diagnostic Period.			
	<600 umol/L	=> 600 umol/L	<1200 umol/L	=>1200 umol/L
Mild PKU, Mild HPA, Not Specified/Other	67%	33%	94%	6%
Classical PKU	9%	91%	37%	63%

N = 95.Three cases missing blood phenylalanine measurements.

Results & Interpretation: Biopterin Responsiveness

- Of those with reported results (N = 37), nearly 40% of cases were BH4-responsive
- Milder cases of PAH deficiency (as noted in patient charts and assigned using blood phenylalanine) tended to be reported as BH4-responsive although a small number of more severe cases were also reported as responsive

PAH Deficiency Diagnosis	Results of Biopterin Responsiveness Testing	
	Responder (>30% Decrease)	Non-Responder (<=30% Decrease)
All Diagnoses	38%	62%
Diagnosis Indicated in Patient Charts		
Mild PKU, Mild HPA, Not Specified, Other	83%	17%
Classical PKU	16%	84%
Maximum Blood Phe During Diagnostic Period		
120-599 umol/L	86%	14%
>=600 umol/L	27%	73%
Maximum Blood Phe During Diagnostic Period		
120-1199 umol/L	68%	32%
>=1200 umol/L	6%	94%

N = 37. Analysis includes participants for whom biopterin responsiveness was reported in the chart during the diagnostic period

Results & Interpretation: Molecular Genetic Testing

Alleles Identified Through Molecular Genetic Testing:

- Unique alleles collected from participants (N = 34) were classified according to their pathogenicity noted in ClinVar (as of April 18th, 2017)

PAH Allele Variants by Chart-Indicated PAH Deficiency Diagnosis for the CIMDRN Cohort				
Not Specified/Other	Mild HPA	Mild PKU	Classical PKU	
c.1222C>T	c.1208 C>T	c.117C>G	c.1068C>A	c.727C>T
c.1241A>G*	c.1238G>C	c.1222C>T	c.116_118delTCT	c.782G>A
c.194T>C	c.782G>A	c.1241A>G*	c.117C>G	c.809G>A
c.782G>A	c.442-5C>G	c.1315+1G>A	c.1222C>T	c.842C>T
c.844G>A	c.848T>A	c.143T>C	c.1315+1G>A	c.664_665delGA
c.1355insA		c.721C>T	c.143T>C	c.842+5G>A
c.653G>T		c.781C>T	c.194T>C	
		c.913G>T	c.208_210delTCT	
		c.929C>T	c.442-1G>A	

*Known pathogenic for both PKU and non-PKU HPA.

Pathogenic Likely Pathogenic Unclear/Unknown Pathogenicity

- Despite all participants having confirmed cases of PAH deficiency, not all of the identified PAH alleles were classified as "Pathogenic" by the ClinVar database
- 6 alleles classified as "Likely Pathogenic" by ClinVar and 3 alleles classified as "Unclear/Unknown Pathogenicity" by ClinVar have been confirmed as pathogenic by molecular genetic testing in our cohort
- Certain alleles were associated with a single PAH deficiency phenotype noted in patient charts while others were associated with multiple

Results & Interpretation: Initial Dietary Prescriptions

Dietary Interventions Within the First 30 Days of Life*:

- Median age at follow-up visit during first month of life, where initial dietary intervention was prescribed (N = 31), was 9 days (range: 4-25 days)
- Recommended diet composition commonly consisted of medical foods (87%) and/or natural food protein (58%)

Dietary Prescription	Dietary Intervention in the First 30 Days by PAH Disease Classifications				
	Receiving?	Diagnoses Noted in Patient Charts		Maximum Blood Phe During Diagnostic Period	
		Mild PKU, Mild HPA, Not Specified, Other % (Median Age)	Classical PKU % (Median Age)	120-1199 umol/L % (Median Age)	>=1200 umol/L % (Median Age)
Medical Food	Yes	62% (12 days)	96% (9 days)	79% (9 days)	94% (9 days)
	No	38% (7 days)	4% (9 days)	21% (7 days)	6% (9 days)
Food Protein	Yes	63% (12 days)	47% (9 days)	64% (12 days)	53% (9 days)
	No	37% (4 days)	53% (9 days)	36% (9 days)	47% (10 days)

N = 31. NIH: National Institutes of Health.

- Participants with milder cases of PAH deficiency tended to receive prescriptions less often and later than those with more severe cases.

*Data presented here are limited to the first follow-up visit in the first 30 days of life with an update to diet recommendations

Conclusions & Next Steps

- There appears to be variation in diagnostic and initial treatment timelines between participants with different PAH deficiency diagnoses which may reflect differences in diagnostic practices
- Assignment of disease severity using diagnostic testing requires complete reporting of tests that were conducted: difficult to distinguish between practice variation and reporting variation
- Assignment of disease severity using maximum blood phenylalanine levels did not always agree with diagnoses indicated in patient charts, suggesting either practice variation or presence of other factors affecting clinician-assigned diagnoses
- Diagnosis using routine molecular genetic testing requires a complete/accurate database of PAH allele pathogenicity
- Next steps: continued baseline data collection from the CIMDRN cohort and analysis of intervention data beyond first 30 days of life

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