Genotype-phenotype Associations Among Children with Phenylalanine Hydroxylase Deficiency in Canada: Preliminary Findings from the Canadian Inherited Metabolic Diseases Research Network (CIMDRN)

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Background & Objectives

- **Phenylalanine hydroxylase (PAH) deficiency is clinically heterogeneous**
  - The National Institutes of Health (NIH) has classified PAH deficiency into 5 categories of severity based on pre-treatment blood phenylalanine (phe) levels[1]:

PAH Deficiency Classifications	Blood Phe Level (µmol/L):
Classical phenylketonuria (PKU)	>1200
Moderate PKU	900-1200
Mild PKU	600-900
Mild hyperphenylalaninemia (HPA)-gray zone	360-600
Mild HPA-no treatment (NT)	120-360

  - Prediction of phenotypic characteristics for many *PAH* variants remains uncertain
- **Description of *PAH* variants and genotype-phenotype association analysis among children with PAH deficiency has not yet been studied in Canada**
  - Currently, more than 800 variant *PAH* alleles have been recorded worldwide[2]
  - It is important to update and extend PAH deficiency genotypic information
- **Study Objectives:**
  - To describe variants identified in the *PAH* gene among a cohort of Canadian children with PAH deficiency
  - To assess the genotype-phenotype associations of 112 PAH deficiency patients in Canada

Methods

Participants:

- Children born between 2006 and 2015
- Receiving care for an inherited metabolic disease at one of 13 participating Canadian Hereditary Metabolic Diseases Treatment Centres

Eligibility:

- Diagnosed with PAH deficiency
- Complete baseline data (demographic & diagnostic information)
- *PAH* genotype information identified (via molecular genetic testing or inference from sibling)
- At least 1 blood phe level measured in first 21 days of life

Data collection:

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

Measures:

- Max blood phe level in first 21 days of life:
  - Used to estimate pre-treatment blood phe levels and assign disease classification per NIH categories[1]
  - Sensitivity analysis conducted by comparing max blood phe in first year of life
- Prescribed dietary phe intake between age 2-3 years:
  - Used as a potential proxy for phe tolerance

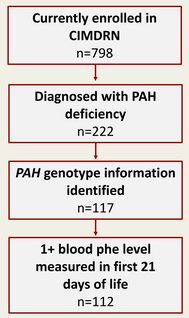

Figure 1. Participant Flow Diagram

Analysis:

- Descriptive analysis of children with PAH deficiency in Canada

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Database hosted at: CHEO RESEARCH INSTITUTE

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Results & Interpretation: Descriptive Analysis & Max Blood Phenylalanine Levels

Table 1. Participant characteristics in the present study compared with all PAH deficiency participants enrolled in CIMDRN

Variable	All CIMDRN Participants with PAH Deficiency (n=222)		PAH Deficiency Participants included in the present study (n=112)	
	n	%	n	%
Year of Birth				
2006	24	11%	9	8%
2007	21	9%	14	13%
2008	14	6%	7	6%
2009	22	10%	13	12%
2010	18	8%	5	4%
2011	19	9%	6	5%
2012	27	12%	13	12%
2013	22	10%	13	12%
2014	27	12%	17	15%
2015	28	13%	15	13%
Sex				
Male	115	52%	63	56%
Female	107	48%	49	44%
Centre				
Calgary	11	5%	8	7%
Edmonton	12	5%	< 5	< 5%
Halifax	9	4%	7	6%
Hamilton	20	9%	13	12%
Kingston	< 5	< 3%	< 5	< 5%
London	17	8%	12	11%
Montreal	15	7%	7	6%
Ottawa	14	6%	11	10%
Sherbrooke	6	3%	< 5	< 5%
St. John's	< 5	< 3%	< 5	< 5%
Toronto	66	30%	43	38%
Vancouver	37	17%	< 5	< 5%
Winnipeg	11	5%	< 5	< 5%

Cells with < 5 participants are suppressed for data privacy purposes.

Participants with *PAH* genotype information:

- 50% of all PAH deficiency participants in CIMDRN cohort have *PAH* genotype information
  - Differences in proportion of participants with genotype information reflect the variation in availability and usage of molecular genetic testing across Canadian treatment centres and over time
  - Genotype information was inferred from sibling data for 9 participants

Max blood phenylalanine levels:

- 58% of participants had a max blood phe level ≥600 µmol/L in first 21 days of life
- 19% of max blood phe levels in first 21 days of life came from newborn screening test results, not diagnostic tests
- When comparing disease classification based on max blood phe level in first 21 days of life and max blood phe level in first year of life, <5 participants changed classifications

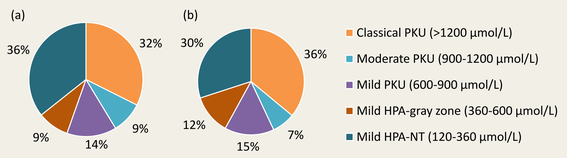

Figure 2. PAH Deficiency Classification[1] based on Max Blood Phe Level in first 21 days of life for (a) all PAH deficiency participants enrolled in CIMDRN, and (b) participants included in the present study

Results & Interpretation: *PAH* Genotypes Identified

Genotypes identified through molecular genetic testing:

- Alleles were classified according to their variant type noted in ClinVar[3] (as of April 28th, 2019)
- 80 unique alleles were identified
  - Most common variant type: Missense (n=49, 61%)
  - Most common alleles: **c.1222C>T** (n=30), **c.1315+1G>A** (n=20), **c.194T>C** (n=12)
- 93 unique genotypes identified
  - Most common genotype classification: Missense/Missense (n=45, 48%)
- 4 alleles were not reported in ClinVar[3] (as of April 28th, 2019)

Table 2. Unique genotypes identified in PAH deficiency cohort

Missense/Missense		Missense/Splice site	Missense/Deletion	Nonsense/Splice site
c.1049C>A/c.734T>C	c.1A>G/c.896T>G	c.1042C>G/c.1315+1G>A	c.194T>C/c.1355insA	c.1068C>A/c.1315+1G>A
c.1139C>T/c.453T>A	c.331C>T/c.734T>C	c.1066-11G>A/c.1222C>T	c.208_210delTCT/c.728G>A	c.727C>T/c.1066-11G>A
c.1157A>G/c.1139C>T	c.428A>G/c.1222C>T	c.1169A>G/c.1315+1G>A	c.47_48delCT/c.1222C>T	c.814G>T/c.1066-11G>A
c.117C>G/c.1222C>T	c.527G>A/c.782G>A	c.117C>G/c.1315+1G>A	c.688G>T/Exon 3 deletion	c.814G>T/c.1315+1G>A
c.117C>G/c.838G>A	c.653G>T/c.844G>A	c.1208C>T/IVS8-7A>G	c.734T>C/Deletion of at least 6 exons	
c.1208C>T/c.1222C>T	c.688G>A/c.1241A>G	c.1222C>T/c.1066-3C>T		**Splice site/Splice site**
c.1208C>T/c.1238G>C	c.721C>T/c.1172G>C	c.1222C>T/c.1315+1G>A		c.1315+1G>A/c.1315+1G>A
c.1222C>T/c.1162G>C	c.734T>C/c.782G>A	c.168+5G>C/c.1089G>C		c.1315+1G>A/c.842+5G>A
c.1222C>T/c.1222C>T	c.754C>T/c.898G>T	c.194T>C/c.1066-11G>A		
c.1222C>T/c.1241A>G	c.782G>A/c.1042C>G	c.204A>T/c.1315+1G>A	**Missense/Nonsense**	**2nd mutation unknown**
c.1241A>G/c.1241A>G	c.782G>A/c.1208C>T	c.353-6T>C/c.355C>T	c.1169A>G/c.331C>T	c.1169A>G/
c.136G>A/c.838G>A	c.782G>A/c.1222C>T	c.442-1G>A/c.782G>A	c.1222C>T/c.727C>T	c.1315+1G>A/
c.143T>C/c.1222C>T	c.809G>A/c.842C>T	c.464G>A/c.1066-11G>A	c.143T>C/c.913G>T	c.311C>A/
c.143T>C/c.143T>C	c.848T>A/c.1208C>T	c.464G>A/c.1315+1G>A	c.158G>A/c.498C>G	c.60+5G>T/
c.158G>A/c.964G>A	c.896T>G/c.1222C>T	c.722G>A/c.1066-11G>A	c.526C>T/c.1139C>T	c.838G>A/
c.194T>C/c.1139C>T	c.896T>G/c.898G>T	c.734T>C/c.1315+1G>A	c.721C>T/c.781C>T	c.847A>T/
c.194T>C/c.1157A>G	c.898G>T/c.1042C>G	c.745C>T/c.1315+1G>A	c.838G>A/c.1068C>G	
c.194T>C/c.1162G>A	c.898G>T/c.1045C>T	c.782G>A/c.1066-3C>T	c.916A>G/c.1068C>G	
c.194T>C/c.1208C>T	c.898G>T/c.1208C>T	c.842C>T/c.1315+1G>A		
c.194T>C/c.1243G>A	c.898G>T/c.1222C>T	c.842C>T/c.442-5C>G	**Nonsense/Nonsense**	**Alleles not found in ClinVar**
c.194T>C/c.665A>G	c.898G>T/c.916A>G		c.612T>G/c.727C>T	c.1045C>T
c.194T>C/c.896T>G	c.929C>T/c.929C>T			c.1355insA
c.1A>G/c.434A>T			**Deletion/Deletion**	c.353-6T>C
			c.47_48delCT/c.450insA	c.450insA
			c.664_665delGA/c.116_118delTCT	

Genotypes within each PAH deficiency classification:

- 7 genotypes were found in more than 1 disease classification

Prescribed dietary phe intake:

- 30 participants did not have a metabolic clinic visit between age 2-3 years or were under 2 years old by end of study period
- 22% of included participants were prescribed an unrestricted diet at age 2-3 years
  - 76% of those prescribed an unrestricted diet were classified with Mild HPA-NT
- As expected, our data suggests that there is an inverse relationship between max blood phe in the first 21 days of life and prescribed dietary phe intake

Results & Interpretation: Genotype-phenotype Associations

Table 3. Phenotypic characteristics and associated genotypes for PAH deficiency participants (n=112)

PAH Deficiency Classification based on Max Blood Phe Level in First 21 Days of Life[1]	Median Max Blood Phe Level in First 21 Days of Life in umol/L, N=112 (IQR^)	Median Prescribed Dietary Phe at Age 2-3 Years in mg/kg/day, N=78* (IQR^)	Participants on an Unrestricted Diet at Age 2-3 Years	Genotypes		
Classical PKU						
>2100 umol/L (n=6)	2922	21.35	0	c.612T>G/c.1222C>T c.1068C>A/c.1315+1G>A c.1066-11G>A/c.1222C>T	c.838G>A/c.1068C>G c.1222C>T/c.1315+1G>A[a] c.1222C>T/c.1222C>T	c.1222C>T/c.1222C>T[b] c.47_48delCT/c.1222C>T c.809G>A/c.842C>T c.664_665delGA/c.116_118delTCT
1801-2100 umol/L (n=14)	1932 (1877-1994)	22.24 (21.24-23.12)	0	c.896T>G/c.1222C>T c.838G>A/c.1222C>T[b] c.136G>A/c.838G>A	c.442-1G>A/c.782G>A c.1315+1G>A/c.1A>G/c.896T>G c.60+5G>T/c.1066-11G>A	c.47_48delCT/c.450insA c.814G>T/c.1066-11G>A c.1315+1G>A/c.842+5G>A
1501-1800 umol/L (n=8)	1592	21.94	0	c.916A>G/c.1068C>G c.814G>T/c.1315+1G>A c.1042C>G/c.1315+1G>A	c.1315+1G>A/c.1315+1G>A[c] c.428A>G/c.1222C>T[e] c.117C>G/c.1315+1G>A	c.194T>C/c.1066-11G>A[d] c.782G>A/c.1222C>T c.842C>T/c.1315+1G>A
1201-1500 umol/L (n=12)	1429 (1297-1484)	16.84 (14.12-28.56)	0	c.204A>T/c.1315+1G>A c.117C>G/c.1222C>T c.745C>T/c.1315+1G>A	c.194T>C/c.896T>G c.1222C>T/c.1315+1G>A[a]	c.194T>C/c.1066-11G>A[d]
Moderate PKU						
900-1200 umol/L (n=8)	1068	22.92	0	c.1222C>T/c.1222C>T[b] c.847A>T/	c.1222C>T/c.1241A>G[f] c.1222C>T/c.1066-3C>T[h]	c.194T>C/c.1157A>G[g] c.782G>A/c.1042C>G
Mild PKU						
751-900 umol/L (n=7)	871	34.29	<5	c.1222C>T/c.1241A>G[f] c.311C>A/	c.721C>T/c.781C>T c.1315+1G>A/c.1315+1G>A[c]	c.194T>C/c.1157A>G[g] c.653G>T/c.844G>A
600-750 umol/L (n=10)	692 (647-720)	24.45 (23.03-25.27)	0	c.194T>C/c.1355insA c.208_210delTCT/c.728G>A c.194T>C/c.1162G>A c.143T>C/c.143T>C	c.929C>T/c.929C>T c.143T>C/c.913G>T c.782G>A/c.1066-3C>T	c.117C>G/c.838G>A c.1222C>T/c.1066-3C>T[h] c.428A>G/c.1222C>T[e]
Mild HPA-gray zone						
360-600 umol/L (n=13)	455 (418-493)	27.98 (13.89-29.75)	5	c.1222C>T/c.1162G>C c.1208C>T/c.1238G>C c.722G>A/c.1066-11G>A c.848T>A/c.1208C>T c.194T>C/c.665A>G	c.1208C>T/IVS8-7A>G c.1169A>G/c.1315+1G>A[j] c.1208C>T/c.1222C>T c.1169A>G/	c.1169A>G/c.331C>T c.1241A>G/c.1241A>G c.1222C>T/c.1241A>G[f] c.143T>C/c.1222C>T
Mild HPA-NT						
120-360 umol/L (n=34)	209 (175-249)	N/A	19	c.158G>A/c.964G>A c.734T>C/c.1315+1G>A c.168+5G>C/c.1089G>C c.158G>A/c.498C>G c.464G>A/c.1315+1G>A c.1157A>G/c.1139C>T c.898G>T/c.1045C>T c.734T>C/c.782G>A c.527G>A/c.782G>A c.688G>A/c.1241A>G	c.1049C>A/c.734T>C c.1139C>T/c.453T>A c.464G>A/c.1066-11G>A c.842C>T/c.442-5C>G c.688G>T/Exon 3 deletion c.1A>G/c.434A>T c.353-6T>C/c.355C>T c.896T>G/c.898G>T c.782G>A/c.1208C>T c.331C>T/c.734T>C c.734T>C/Deletion of at least 6 exons	c.526C>T/c.1139C>T c.898G>T/c.1042C>G c.194T>C/c.1139C>T c.721C>T/c.1172G>C c.1169A>G/c.1315+1G>A[j] c.754C>T/c.898G>T c.194T>C/c.1243G>A c.898G>T/c.916A>G c.194T>C/c.1208C>T c.898G>T/c.1222C>T c.898G>T/c.1208C>T

*N=78; 30 participants did not have a clinic visit at age 2-3 years, 4 participants were not prescribed dietary phe between age 2-3 years.
^Interquartile range (IQR) only reported for cells with ≥10 participants.
Visits between age 2-3 years that had unknown prescribed dietary phe and/or weight (n=12) were excluded from Median Prescribed Dietary Phe analysis.
27 participants had a natural protein prescription – converted to phe prescription using 1g natural protein = 50mg phe, and then added 4.5mg/kg/day (50% of standard deviation for phe tolerance at age 2)[4].

Black colour = once case variant
Same colour (other than black) = same variant
Same superscript = same genotype

Conclusions & Next Steps

- Better understanding of the genotype-phenotype relationship among children with PAH deficiency can inform expectations about disease severity and care needs, and may lead to improved management
- **Next steps:**
  - Harmonizing the collection and interpretation of data on prescribed dietary phe intake as a proxy indicator of phe tolerance
  - Genotype-phenotype analysis for BH4 responsiveness

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