



Short Communication

Genotypes of patients with phenylalanine hydroxylase deficiency in the Wisconsin Amish

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ABSTRACT

In the Plain Community, there is an increased frequency of genetic disorders including phenylalanine hydroxylase (PAH) deficiency. Common pathogenic variants have been observed due to founder effect and closed community. This study obtained genotypes of 12 Plain individuals with PAH deficiency, identified through newborn screen or diagnosed by symptomatic presentation, who are receiving medical care at the University of Wisconsin metabolic clinic. Genotype and phenotypic data were evaluated to characterize genotype-phenotype correlations. Results can inform the need for confirmatory testing for the disorder and provide a better understanding of the biochemical phenotype, which may help with management.

1. Introduction

Phenylalanine hydroxylase (PAH) deficiency, often referred to as Phenylketonuria (PKU) (OMIM #261600) is the most common inborn error of protein metabolism, affecting approximately 1/10,000 people of Northern European or East Asian descent [1]. The enzyme deficiency leads to a decreased conversion of phenylalanine (Phe) to tyrosine. Without treatment to reduce Phe levels, an individual with PAH deficiency can develop intellectual disability, seizures, and autism-like features. The mainstay of treatment is diet restriction to reduce Phe intake and medical formula to supply adequate protein. Identification most commonly occurs on newborn screen with an elevation in Phe on dried blood specimens.

In the Plain Community, including Amish and Mennonite populations, there is an increased frequency of genetic disorders due to founder effect and closed community. Several metabolic disorders, including PAH deficiency, are more common in the Plain Community compared with the general population. The exact incidence in the Plain Community is unknown, but it is estimated at 1/1000 people [2]. There are four reported common pathogenic variants in PAH identified in the Amish and Mennonite populations in Pennsylvania and Ohio: 284_286delTCA, c.782G > A, IVS10-11G > A, and IVS12 + 1G > A, but no allelic frequency for each pathogenic variant is not known [2,3]. These PAH pathogenic variants are also described in the general population [4–6].

In Wisconsin, the 2017 Amish population is estimated at 20,095 and is derived from Amish from both Pennsylvania and Ohio [7]. Improved

newborn screening rates within this population have allowed for early diagnosis and treatment of Amish individuals with PAH deficiency in Wisconsin. Increased knowledge of common genotypes in the Wisconsin community will help aid in diagnosis and management [8].

2. Materials and methods

The study was open to Plain community patients with PAH deficiency who are receiving care at the University of Wisconsin (UW) metabolic clinic. This study was approved by the UW Institutional Review Board. After appropriate consent, targeted Sanger sequencing was performed for the common Plain community pathogenic variants PAH 284_286delTCA (p.I94del), PAH c.782G > A (p.R261Q), PAH IVS10-11G > A, and PAH IVS12 + 1G > A). If 2 common pathogenic variants were not found, PAH gene was sequenced for other disease-causing pathogenic variants. Reference sequence NM_000277.2(PAH) was used for PAH for PAH valiant annotation.

A chart review of each patient was completed for Phe levels and estimated Phe tolerance. For individuals diagnosed in the neonatal period (Cases 1–7), a combination of breastmilk/standard infant formula and medical formula was initiated after abnormal newborn screen. The amount of medical formula was titrated based on frequent Phe levels (weekly to twice weekly depending on age). Phe tolerance was estimated based on approximate daily intake of breastmilk or standard infant formula. For cases 8–12 where clinical diagnosis occurred, all patients were on a casein free/gluten free diet to improve behavioral concerns at time of diagnosis. For these late diagnosed

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Table 1
Clinical, genotypic, phenotypic information on Amish individuals with phenylalanine hydroxylase deficiency in Wisconsin.

Case	Presentation	Family state of origin	Phe on NBS (mg/dl)	Age at NBS	Average Phe level (mg/dl)			Estimated average Phe tolerance (mg/day)		Current estimated Phe tolerance (mg/day)	Genotype	Phenotype
					0–6 months	6–12 months	0–6 months	6–12 months	Mutation 1			
1	Newborn screen ^a	NA	15.8	6 days	4.9	3.6	257	233	At 8 years old, 250–300 mg	c.1066-11G > A	c.1066-11G > A	Classical
2	Family history ^a	NA	5.0	5 days	4.0	2.1	196	180	At 4 years old, 250–300 mg	c.1066-11G > A	c.1066-11G > A	Classical
3	Newborn screen	IN/VA	18	19 days	4.0	4	333	462	At 5 years old, 300–325 mg	c.1066-11G > A	c.168 + 5G > C	Classical
4	Newborn screen	PA/PA	3.2	48 h	2.5	2.4	419	868	At 4 years old, 1550 mg	c.284_286delTCA	c.284_286delTCA	Mild
5	Newborn screen ^b	OH/IN	7.8	73 h	4.8	3.2	191	218	At 5 years old, 300–350 mg	c.1066-11G > A	c.1066-11G > A	Classical
6	Family history ^b	OH/IN	5.3	25 h	3.5	4.7	172	194	At 4 years old, 300–350 mg	c.1066-11G > A	c.1066-11G > A	Classical
7	Family history ^b	OH/IN	7.6	39 h	3.5	NA ¹	208	NA ¹	At 20 months old, 350 mg PHE	c.1066-11G > A	c.1066-11G > A	Classical
8	Clinical presentation ^c		16.3	20 years	NA	NA	NA	NA	At 21 years old, 300–400 mg	c.1066-11G > A	c.1066-11G > A	Moderate - Classical
9	Clinical presentation ^a		12.9	19 years	NA	NA	NA	NA	At 20 years old, 300–400 mg	c.1066-11G > A	c.1066-11G > A	Moderate - Classical
10	Clinical presentation ^c		15.3	18 years	NA	NA	NA	NA	at 19 years old, 300–400 mg	c.1066-11G > A	c.1066-11G > A	Moderate - Classical
11	Clinical presentation ^c		14.0	11 years	NA	NA	NA	NA	at 12 years old, 300–400 mg	c.1066-11G > A	c.1066-11G > A	Moderate - Classical
12	Clinical presentation ^c		15.7	9 years	NA	NA	NA	NA	at 10 years old, 300–400 mg	c.1066-11G > A	c.1066-11G > A	Moderate - Classical

N/A – Not available 1. Below 1 year of age at the time of data collection. States – IN – Indiana, OH – Ohio, PA – Pennsylvania, VA – Virginia. Sibships – a. Family #1 b. Family #2 c. Family #3.

individuals, initiation of Phe restricted diet was done in a stepwise fashion (formula first, then gradual restriction of whole protein).

Currently all individuals are on the simplified PKU diet counting grams of protein instead of milligrams of Phe. Fruits and vegetables with less than 0.75 mg Phe per gram are considered free and do not need to be counted. Daily Phe tolerance is estimated from the protein goal (50 mg Phe per 1 g protein) plus assumed 100 mg Phe from uncounted fruits and veggies. With Amish patients, there is limited communication with families which may impact estimation.

3. Results

Twelve patients were identified and participated in this study. Results are summarized in Table 1. Presentations included detection by newborn screen without family history of PAH deficiency, newborn screen with family history, and clinical diagnosis. Diagnosis was based on dried blood spot Phe levels of greater than 2.5 mg/dl in the newborn period or greater than 1.5 mg/dl outside the newborn period. Cases 1–7 were diagnosed by newborn screening with Cases 2, 6, and 7 a family history was known. The 7 patients identified by newborn screen and/or family history in the newborn period demonstrate normal growth and development. Clinical diagnosis of PAH deficiency was initially suspected in Case 12 due to intellectual disability, seizures, autism, decrease pigmentation, and a history of no newborn screen. All symptomatic siblings had moderate to severe intellectual disability and autistic like behaviors. Due to cultural norms, none have undergone formal evaluation for IQ or formal diagnosis of autism. Subsequent testing of the four older siblings (Cases 8–11) was completed due to their similar features, confirming diagnoses of PAH deficiency.

For Cases 1–7, all infant average Phe levels were within the treatment range of 2–6 mg/dl. Variable Phe tolerances were observed between patients, but most patients were relatively consistent over time with the exception of cases 3 and 4 whose Phe tolerance increased 28% and 52%, respectively, during the first year of life. No patient has been treated on sapropterin.

For Cases 8–12, most recent Phe levels in all five patients were below 6 mg/dl. Parents have not provided diet records, but dietary recommendations and verbal report implies these patients would fit with a classical phenotype.

Genotype was completed on all twelve individuals. All but one allele (Case 3) had pathogenic variants identified that were of the 4 pathogenic variants common in the Plain community. Sequencing of the PAH gene in Case 3 showed a previously described pathogenic variant [9,10,11].

For phenotype classification, current dietary Phe tolerance was used to subdivide to classic, moderate, mild and mild hyperphenylalaninemia [12]. All cases but case #4 were moderate to classical. For case #4, estimated Phe would place the individual as benign, but he requires treatment to maintain levels less than 6 mg/dl. Therefore, he was categorized as mild PAH deficiency.

4. Discussion

PAH deficiency appears to be more common in the Plain community as estimated in Ohio at 1/1000 people. In Wisconsin, with an estimated Amish population of 20,000, 14 individuals with PAH deficiency are followed, many are school aged or younger. There are challenges to treatment in this population and increased knowledge of the genotype and its corresponding phenotype can help with diagnosis as well as management.

Frequently, the identification of PAH deficiency is made by newborn screen. In a community where cost is a large factor in follow-up of healthcare, the use of genotype could allow for molecular confirmation of PAH deficiency diagnosis in a more time- and cost-efficient manner. In the setting of a newborn screen, common pathogenic variants testing could be completed at the newborn screen lab when an elevated Phe is

detected in a patient that is identified as part of the Plain community. Two identified disease-causing pathogenic variants would confirm diagnosis, eliminating the need for bipterin studies, saving patient cost and allowing for more timely diagnosis. If two pathogenic variants are not identified, then bipterin testing would be required.

The genotypes of Wisconsin Amish patients included two mutations commonly found in the Plain community: c.1066-11G > A and 284_286delTCA. 284_286delTCA has a reported biochemical phenotype of less elevated Phe levels over the first year of life with increasing levels after this time (personal communication – Dr. Holmes Morton 6/11/16). Case 4's (homozygous for this genotype) initial biochemical phenotype fit with hyper-phe and treatment was not required to maintain Phe levels in the treatment range (2–6 mg/dl) during the first year of life. With age, Phe tolerance declined and formula was initiated between 12 and 18 months to maintain Phe levels in the treatment range. Currently, Case 4 is on the simplified PKU diet, receives 25 g whole protein/day, and does not demonstrate a classical presentation. Knowledge of this milder phenotype would help avoid false assumption of benign hyper-phe particularly in a community where follow-up to medical care can be challenging. Medical formula can be initiated during the first year to avoid later formula intolerance. This mutation, with its milder phenotype and presumed residual enzyme activity, would likely also be responsive to sapropterin [13].

The pathogenic variant, c.1066-11G > A, is associated with a classical PAH deficiency phenotype [13]. This more severe biochemical phenotype was demonstrated in all homozygous patients. Case 1 did appear to have a high tolerance in the first few months of life, possibly related to history of prematurity necessitating catch-up growth (increase Phe need), and breastfeeding (there is less Phe per ounce of breast milk than standard infant formula). In the literature, this genotype is not responsive to sapropterin [13]. The third pathogenic variants, a splice site pathogenic variants c.168 + 5G > C, found in Case 3 has not been previously described in the Plain community. It was initially described in German and Polish individuals with PAH deficiency, where the Plain community originates, but has been reported in other populations as well. While this pathogenic variants c.168 + 5G > C, is predicted to cause a classical PAH deficiency phenotype, there are reports of the pathogenic variants in combination with other mutations (p.Glu178Gly or p.Arg395Gly) presenting as hyper-phe or mild PAH deficiency [10,11,14]. One report showed 50% of patients with this pathogenic variants responded to sapropterin although it is unclear if patients were homozygotes for the pathogenic variant or compound heterozygotes [15]. Case 3 is a compound heterozygote for the c.1066-11A > G pathogenic variant (classical pathogenic variant) and this pathogenic variant. Phe tolerance is approximately twice that of those homozygous for the c.1066-11A > G pathogenic variant.

This study is limited by its small size. Further study of the genotype and phenotype in the Plain community are needed to verify our observation and to see if other genotypes exist in this closed community. The genotype would aid in diagnosis and also help the clinician predict

phenotype and expectations for level of intervention needed for adequate treatment and possible sapropterin responsiveness.

References

- [1] D.S. Regier, C.L. Greene, Phenylalanine hydroxylase deficiency, 2000 Jan 10, Updated 2017 Jan 5, in: M.P. Adam, H.H. Ardinger, R.A. Pagon, et al. (Eds.), GeneReviews® [Internet], University of Washington, Seattle, WA, 1993–2017 Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1504/>.
- [2] H. Wang, L. Nye, E. Puffenberger, H. Morton, Phenylalanine hydroxylase deficiency exhibits mutation heterogeneity in two large old order Amish settlements, *Am. J. Med. Genet.* 143A (2007) 1938–1940.
- [3] E.G. Puffenberger, Genetic heritage of older order Mennonites of southeastern Pennsylvania, *Am. J. Med. Genet. Part C* 121C (2003) 18–31.
- [4] V. Abadie, S. Lyonnet, N. Maurin, M. Berthelon, C. Caillaud, F. Giraud, J.F. Mateei, J. Rey, F. Rey, A. Munnich, CpG dinucleotides are mutation hot spots in phenylketonuria, *Genomics* 5 (4) (1989) 936–939.
- [5] C. Caillaud, S. Lyonnet, F. Rey, D. Melle, T. Frebourg, M. Berthelon, L. Vilarinho, R. Vaz Osorio, J. Rey, A. Munnich, A 3-base pair in-frame deletion of the phenylalanine hydroxylase gene results in a kinetic variant of phenylketonuria, *J. Biol. Chem.* 5 (4) (2010) 9351–9354.
- [6] B. Dworniczak, C. Aulehla-Scholz, L. Kalavdjieva, K. Bartholome, K. Grudka, J. Horst, Aberrant splicing of phenylalanine hydroxylase mRNA: the major cause of phenylketonuria in parts of southern European, *Genomics* 11 (2) (1991) 242–246.
- [7] Amish Population, Young Center for Anabaptist and Pietist Studies, Elizabethtown College, 2017, <http://groups.etsu.edu/amishstudies/statistics/population-2017/>, Accessed date: 26 September 2017.
- [8] K.M. Camp, M.A. Parisi, P.B. Acosta, G.T. Berry, D.A. Bilder, N. Blau, O.A. Bodamer, J.P. Brosco, C.S. Brown, A.B. Burlina, B.K. Burton, C.S. Chang, P.M. Coates, A.C. Cunningham, S.F. Dobrowolski, J.H. Ferguson, T.D. Franklin, D.M. Frazier, D.K. Grange, C.L. Greene, S.C. Groft, C.O. Harding, R.R. Howell, K.L. Huntington, H.D. Hyatt-Knorr, I.P. Jevaji, H.L. Levy, U. Lichter-Konecki, M.L. Lindgren, M.A. Lloyd-Puryear, K. Matalon, A. MacDonald, M.L. McPheeters, J.J. Mitchell, S. Mofidi, K.D. Moseley, C.M. Mueller, A.E. Mulberg, L.S. Nerurkar, B.N. Ogata, A.R. Pariser, S. Prasad, G. Pridjian, S.A. Rasmussen, U.M. Reddy, F.J. Rohr, R.H. Singh, S.M. Sirrs, S.E. Stremer, D.A. Tagle, S.M. Thompson, T.K. Urv, J.R. Utz, F. van Spronsen, J. Vockley, S.E. Waitsbren, L.S. Weglicki, D.A. White, C.B. Whitley, B.S. Wilfond, S. Yannicelli, J.M. Young, Phenylalanine hydroxylase deficiency: diagnosis and management guideline, *Genet. Med.* 16 (2) (2014) 188–200.
- [9] S. Wettstein, J. Underhaug, B. Perez, B.D. Marsden, W.W. Yue, A. Martinez, N. Blau, Linking genotypes database with locus-specific database and genotype–phenotype correlation in phenylketonuria, *Eur. J. Hum. Genet.* 23 (2015) 302–309.
- [10] M. Zygulska, A. Eigel, J.J. Pietrzyk, J. Horst, Phenylalanine hydroxylase gene: a novel splice mutation in intron 2 in two German and Polish families with severe phenylketonuria, *Hum. Mutat.* 2 (3) (1993) 238–239.
- [11] T. Georgiou, G. Ho, M. Vogazianos, M. Dionysiou, A. Nicolau, G. Chappa, P. Nicolaides, G. Stylianidou, J. Christodoulou, A. Drosiotou, The spectrum of mutations identified in Cypriot patients with phenylalanine hydroxylase deficiency detected through neonatal screening, *Clin. Biochem.* 45 (2012) 588–592.
- [12] P. Guldberg, F. Rey, J. Zschocke, V. Romano, B. François, L. Michiels, K. Ullrich, G.F. Hoffmann, P. Burgard, H. Schmidt, C. Meli, E. Riva, I. Dianzani, A. Ponzone, J. Rey, F.A. Güttler, European Zschockemulticenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype, *Am. J. Hum. Genet.* 63 (1) (1998) 71–79.
- [13] N. Blau, H. Erlandsen, The metabolic and molecular bases of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency, *Mol. Genet. Metab.* 82 (2004) 101–111.
- [14] BIOPKU, www.biopku.org, Accessed date: 18 September 2017.
- [15] A. Al-Shamsi, J.L. Hertecant, S. Al-Hamad, A.K. Souid, F. Al-Jasmi, Mutation spectrum and birth prevalence of inborn errors of metabolism among Emiratis, *Sultan Qaboos Univ. Med. J.* 14 (1) (2014) 42–49.