Table 1. Overview of PAH variants and *in vitro* expression data.

N. Himmelreich, N. Shen, J.G. Okun, C. Thiel, G.F. Hoffmann, N. Blau, Relationship between genotype, phenylalanine hydroxylase expression and in vitro activity and metabolic phenotype in phenylketonuria Mol Genet Metab doi: 10.1016/j.ymgme.2018.06.011 (2018).

Variant	Aberration	APV	PAH activity (%)**					PAH protein (%)***	Reference
			COS*	*E. coli*	TNT-T7	HEK293	PRO system		
p.M1V	c.1A>G	0	2					2[1]	[1]
p.F39L	c.117C>G	1.5	73*	67	49	46		100[2.3.5]; 96[3]; 13[4]	[2-4] [5]
p.G46S	c.136G>A	1.5		0 62	32			3[4]; 100[2.3]	[2, 6]
p.A47V	c.140C>T	10.0	123*	40	13			100[2.3]	[2] [5]
p.L48S	c.143T>C	2.4	47	0		39		93[3]; 12[4]; 100[5]	[3, 4, 7, 8]
p.D59Y	c.175G>T	0	9* 92					100	[9] [5]
p.T63P	c.187A>C	5.0		43	12			100[2.3]	[2]
p.I65T	c.194T>C	1.4	26 27 33 48	60	29			100[2.3.5]; 25[1]; 22[2]; 14[4]	[2, 3, 7-9] [1, 4, 10-12]
p.I65S	c.194T>G	2.5	32*						[5]
p.R68G	c.202A>G	5.6	40* 100					100[1]	[13] [5]
p.R68S	c.204A>T	7.0	98 25*	76	28			100[2.1]	[2, 13] [5]
p.E76G	c.227A>G	6.3	24* 47 88					100[1]	[9, 14] [5]
p.S87R	c.259A>C	10.0		82	25			100[2.3]	[2]
p.T92I	c.275C>T	10.0	76					91	[15]
p.I95del	c.284_286del	1.7				27			[16]
p.A104D	c.311C>A	6.7	77*	67	27	26		100[3.5]; 20[4]; 72[3]	[2, 8, 15, 17] [5]
p.P122Q	c.365C>A	0	22					27[1]	[9]
p.D143G	c.428A>G	5.0	52 98*			33		100[4]	[18] [5]
p.R155H	c.464G>A	8.8	21*						[5]
p.R158Q	c.473G>A	0.2	5 9 10 29	2	9			100[1]; 35[1]	[2, 7, 9, 12, 19, 20]
p.F161S	c.482T>C	2.5	7					17[1]	[21]
p.R176L	c.527G>T	8.8	35*	21	42			100[3]	[2] This study
p.E178G	c.533A>G	7.8	31						[7]
p.E178K	c.533A>G	-						70[4]	[22]

p.V190A	c.569T>C	6.6	40*						[5]
p.P211T	c.631C>A	9.3	72					63[1]	[15]
p.L212P	c.635T>C	0	17						[23]
p.G218V	c.653G>T	4.5	15 25* 101	2	63			100[3.1]	[2, 9, 15] This study
p.Q226K	c.676C>A							1[6]	[24]
p.V230I	c.688G>A	10.0		52	63			100[3]	[2]
p.R241C	c.721C>T	9.5	25 57*						[25] [5]
p.R241H	c.722G>A	5.0	23						[15]
p.R243*	c.727C>T	0	0	0				0[1]; 100[2]	[19, 26]
p.R243Q	c.728G>A	0.5	6* 9 10 18					10[1]; 9[1]; 57[4]	[9, 22, 27, 28] [5]
p.P244L	c.731C>T	0	21* 68 70					100[1]	[9, 29] [5]
p.V245M	c.733G>A	10.0						59[4]	[22]
p.V245E	c.734T>A	0		100	7			100	[2]
p.V245A	c.734T>C	10.0	50 51	62				100[2.3]	[2, 7]
p.G247V	c.740G>T	0.9	4					56[1]	[21]
p.L249F	c.745C>T	5.6	51						[23]
p.L249P	c.746T>C	0	7						[23]
p.S250F	c.749C>T	-						45[4]	[22]
p.R252W	c.754C>T	0	0 15*	0	0			0[1]; 100[2]	[9, 26, 30-32] [5]
p.R252Q	c.755G>A	0	24			2 3		2[4]; 100[3]	[28, 33]
p.L255S	c.764T>C	0			1	2		11[4]; 100[3]	[33]
p.A259T	c.775G>A	1		0	8	3		2; 100[3.2]	[26, 33]
p.A259V	c.776C>T	0	0	0	3	3			[15, 26, 33]
p.R261Q	c.781C>T	3.9	23* 39						[7] [5]
p.R261Q	c.782G>A	3.9	23* 27 30 43 47	52	48		28	22[2]; 20[1]; 30[1]; 100[2]	[2, 9, 15, 19, 26] [10-12, 20] [5]
p.R261P	c.782G>C	2.4	10						[23]
p.R270K	c.809G>A	0	11				2		[11, 23, 34]
p.G272*	c.814G>T	0	0	0					[18, 35]
p.Y277D	c.829T>G	1.1	0					99[1]	[9]
p.T278I	c.833C>T	0	1						[36]
p.E280K	c.838G>A	0.1	2 11*	0; 10	6			100[2]; 0[1]; 2[1]	[2, 9, 19, 26] [5]
p.P281L	c.842C>T	0	0	1	3			0[1]	[2, 31, 37]

			1						
p.D282N	c.844G>A	0		1	2				[2]
p.I283F	c.847A>T	0.4		23	10				[2]
p.R297H	c.890G>A	9.8	39*						[5]
p.R408Q	c.890G>A	6.2	33 41* 55 84	9	0			70[1]; 91[1]	[2, 7, 9, 38, 39] [5]
p.F299C	c.896T>G	0.2	2	1				100[2]	[26, 40]
p.A300S	c.898G>T	8.9	32 65*						[7] [5]
p.I306V	c.916A>G	10.0	25*	12	39				[2] [5]
p.A309V	c.926C>T	3.3	12* 70					100[1]	[9] [5]
p.L311P	c.932T>C	0	0*					0[1]	[9, 41] [5]
p.A313T	c.937G>A	5.0	29*						[5]
p.A322G	c.965C>G	10.0	75					105[1]	[38]
p.L333F	c.997C>T	-	7						[15]
p.A342T	c.1024G>A	4.0		24	26			100[3]	[2]
p.L348V	c.1042C>G	3.6	25* 33 38	41		44			[2, 15, 40, 42] [5]
p.S349P	c.1045T>C	0	0	0	0			100[2]; 0[1]	[2, 43, 44]
IVS10-11G>A	c.1066-11G>A	0	0					100[1]	[9]
p.T380M	c.1139C>T	10.0	28						[23]
p.F39del	c.116_118del	1.3		83	20			100[2]	[2]
p.V388M	c.1162G>A	2.5	15 43 83*	23	41		27	100[1.3]; 96[1]; 22[2]	[2, 10, 11, 36, 42] [34, 45] [5]
p.E390G	c.1169A>G	7.6	54 62 70	75	85			100[2]	[2, 7, 12, 15]
p.A395P	c.1183G>C	0.9		15	16				[2]
p.A403V	c.1208C>T	9.4	32 33* 100					100[1]	[15, 46] [5]
p.R408W	c.1222C>T	0	0 1 2* 3 5		1			0[1]; 3[1]	[2, 7, 9, 12, 39, 47] [5]
p.R413S	c.1237C>A	5.0	34						[15]
p.R413P	c.1238G>C	0.1	2 11*					0[1]	[48] [5]
p.Y414C	c.1241A>G	5.0	28 50 80	38	42			100[2.3]; 50[1]; 84[1]	[2]
p.D415N	c.1243G>A	10.0	35*	72	114			100[2.3]	[2] [5]

p.Y417H	c.1249T>C	5.0	44*						[5]
p.Q419R	c.1256A>G	10.0				70			[49]
p.A434D	c.1301C>A	2.6	9*						[5]
IVS12+1G>A	c.1315+1G>A	0	0					0[1]	[50]

*This study; **Compared with the wild-type activity; **1 = COS; 2 = *E.coli*; 3 = TNT-T7; 4 = HEK293; 5= Yeast 6 = HepG2; 7 = PRO**.** APV: Allelic phenotype value (cPKU = 0 – 2.7; mPKU = 2.8 – 6.6; MHP = 6.7 – 10.0)

References

[1] S.W. John, C.R. Scriver, R. Laframboise, R. Rozen, In vitro and in vivo correlations for I65T and M1V mutations at the phenylalanine hydroxylase locus Hum Mutat 1 (1992) 147-153.

[2] T. Gjetting, M. Petersen, P. Guldberg, F. Guttler, In vitro expression of 34 naturally occurring mutant variants of phenylalanine hydroxylase: correlation with metabolic phenotypes and susceptibility toward protein aggregation Mol Genet Metab 72 (2001) 132-143.

[3] P.J. Waters, M.A. Parniak, B.R. Akerman, A.O. Jones, C.R. Scriver, Missense mutations in the phenylalanine hydroxylase gene (PAH) can cause accelerated proteolytic turnover of PAH enzyme: a mechanism underlying phenylketonuria J Inherit Metab Dis 22 (1999) 208-212.

[4] P.J. Waters, M.A. Parniak, B.R. Akerman, C.R. Scriver, Characterization of phenylketonuria missense substitutions, distant from the phenylalanine hydroxylase active site, illustrates a paradigm for mechanism and potential modulation of phenotype Mol Genet Metab 69 (2000) 101-110.

[5] N. Himmelreich, N. Shen, J.G. Okun, C. Thiel, G.F. Hoffmann, N. Blau, Relationship between genotype, phenylalanine hydroxylase expression and in vitro activity and metabolic phenotype in phenylketonuria Mol Genet Metab doi: 10.1016/j.ymgme.2018.06.011 (2018).

[6] H.G. Eiken, P.M. Knappskog, J. Apold, T. Flatmark, PKU mutation G46S is associated with increased aggregation and degradation of the phenylalanine hydroxylase enzyme Hum Mutat 7 (1996) 228-238.

[7] N. Shen, C. Heintz, C. Thiel, J.G. Okun, G.F. Hoffmann, N. Blau, Co-expression of phenylalanine hydroxylase variants and effects of interallelic complementation on in vitro enzyme activity and genotype-phenotype correlation Mol Genet Metab 117 (2015) 328–335.

[8] P.J. Waters, C.R. Scriver, M.A. Parniak, Homomeric and heteromeric interactions between wild-type and mutant Phenylalanine hydroxylase subunits: Evaluation of two-hybrid approaches for functional analysis of mutations causing hyperphenylalaninemia Mol Genet Metab 73 (2001) 230-238.

[9] A.L. Pey, L.R. Desviat, A. Gamez, M. Ugarte, B. Perez, Phenylketonuria: genotype-phenotype correlations based on expression analysis of structural and functional mutations in PAH Hum Mutat 21 (2003) 370-378.

[10] P. Leandro, I. Rivera, M.C. Lechner, I.T. de Almeida, D. Konecki, The V388M mutation results in a kinetic variant form of phenylalanine hydroxylase Mol Genet Metab 69 (2000) 204-212.

[11] J. Leandro, C. Nascimento, I.T. de Almeida, P. Leandro, Co-expression of different subunits of human phenylalanine hydroxylase: evidence of negative interallelic complementation Biochim Biophys Acta 1762 (2006) 544-550.

[12] C. Heintz, H. Troxler, A. Martinez, B. Thony, N. Blau, Quantification of phenylalanine hydroxylase activity by isotope-dilution liquid chromatography-electrospray ionization tandem mass spectrometry Mol Genet Metab 105 (2012) 559-565.

[13] C. Zekanowski, B. Perez, L.R. Desviat, W. Wiszniewski, M. Ugarte, In vitro expression analysis of R68G and R68S mutations in phenylalanine hydroxylase gene Acta Biochim Pol 47 (2000) 365-369.

[14] K.J. Chen, H.K. Chao, K.J. Hsiao, T.S. Su, Identification and characterization of a novel liver-specific enhancer of the human phenylalanine hydroxylase gene Hum Genet 110 (2002) 235-243.

[15] C.R. Scriver, P.J. Waters, C. Sarkissian, S. Ryan, L. Prevost, D. Cote, J. Novak, S. Teebi, P.M. Nowacki, PAHdb: a locus-specific knowledgebase Hum Mutat 15 (2000) 99-104.

[16] 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 266 (1991) 9351-9354.
[17] P.I. Waters, A.S. Hewson, C.R. Scriver, E.P. Treacy, A. Martinez, P.M. Knappskog, M.A. Parniak, Comparative analysis of phenylalanine hydroxylase A104D mutant, associated with variant phenylketonuria, and wild-type enzyme Biochem Soc Trans 25 (1997) 362S.
[18] P.M. Knappskog, H.G. Eiken, A. Martinez, O. Bruland, J. Apold, T. Flatmark, PKU mutation (D143G) associated with an apparent high residual enzyme activity: expression of a kinetic variant form of phenylalanine hydroxylase in three different systems Hum Mutat 8 (1996) 236-246.
[19] Y. Okano, R.C. Eisensmith, F. Güttler, U. Lichter-Konecki, D.S. Konecki, F.K. Trefz, M. Dasovich, T. Wang, K. Henriksen, H. Lou, et al., Molecular basis of phenotypic heterogeneity in phenylketonuria N Engl J Med 324 (1991) 1232-1238.
[20] Y. Okano, T. Wang, R.C. Eisensmith, F. Guttler, S.L. Woo, Recurrent mutation in the human phenylalanine hydroxylase gene American journal of human genetics 46 (1990) 919-924.
[21] J. Li, R.C. Eisensmith, T. Wang, W.H. Lo, S.Z. Huang, Y.T. Zeng, L.F. Yuan, S.R. Liu, S.L. Woo, Identification of three novel missense PKU mutations among Chinese Genomics 13 (1992) 894-895.
[22] Y. Zong, N. Liu, S. Ma, Y. Bai, F. Guan, X. Kong, Three novel variants (p.Glu178Lys, p.Val245Met, p.Ser250Phe) of the phenylalanine hydroxylase (PAH) gene impair protein expression and function in vitro Gene DOI: 10.1016/j.gene.2018.03.078 (2018).
[23] R. Trunzo, R. Santacroce, N. Shen, S. Jung-Klawitter, A. Leccese, G. De Girolamo, M. Margaglione, N. Blau, In vitro residual activity of phenylalanine hydroxylase variants and correlation with metabolic phenotypes in PKU Gene 594 (2016) 138-143.
[24] K. Klaassen, M. Djordjevic, A. Skakic, L.R. Desviat, S. Pavlovic, B. Perez, M. Stojiljkovic, Functional Characterization of Novel Phenylalanine Hydroxylase p.Gln226Lys Mutation Revealed Its Non-responsiveness to Tetrahydrobiopterin Treatment in Hepatoma Cellular Model Biochem Genet (2018).
[25] Y. Okano, Y. Hase, H. Shintaku, K. Araki, J. Furuyama, T. Oura, G. Isshiki, Molecular characterization of phenylketonuric mutations in Japanese by analysis of phenylalanine hydroxylase mRNA from lymphoblasts Hum Mol Genet 3 (1994) 659.
[26] P.M. Knappskog, H.G. Eiken, A. Martinez, S. Olafsdottir, J. Haavik, T. Flatmark, J. Apold, Expression of wild type and mutant form of human phenylalanine hydroxylase in E. coli Adv Exp Med Biol 338 (1993) 59-62.
[27] T. Wang, Y. Okano, R.C. Eisensmith, W.H. Lo, S.Z. Huang, Y.T. Zeng, L.F. Yuan, S.R. Liu, S.L. Woo, Missense mutations prevalent in Orientals with phenylketonuria: molecular characterization and clinical implications Genomics 10 (1991) 449-456.
[28] M. Zhang, K. Hsiao, T. Su, H. Chao, R. Chen, X. Gu, Two novel mutations in phenylalanine hydroxylase gene and in vitro expression analysis on mutation Arg252Gln Chin Med Sci J 12 (1997) 22-25.
[29] B. Perez, L.R. Desviat, M. Ugarte, Expression analysis of mutation P244L, which causes mild hyperphenylalaninemia Hum Mutat 5 (1995) 188-190.
[30] T. Gjetting, A. Romstad, J. Haavik, P.M. Knappskog, A.X. Acosta, W.A. Silva, Jr., M.A. Zago, P. Guldberg, F. Guttler, A phenylalanine hydroxylase amino acid polymorphism with implications for molecular diagnostics Mol Genet Metab 73 (2001) 280-284.
[31] Y. Okano, T. Wang, R.C. Eisensmith, R. Longhi, E. Riva, M. Giovannini, R. Cerone, C. Romano, S.L. Woo, Phenylketonuria missense mutations in the Mediterranean Genomics 9 (1991) 96-103.
[32] V. Romano, G. Anello, S. Kaufman, Genotype-phenotype relationship in PAH deficiency J Inher Metab Dis 21 (1998) 3.
[33] E. Bjorgo, P.M. Knappskog, A. Martinez, R.C. Stevens, T. Flatmark, Partial characterization and three-dimensional-structural localization of eight mutations in exon 7 of the human phenylalanine hydroxylase gene associated with phenylketonuria Eur J Biochem 257 (1998) 1-10.

[34] P. Leandro, M.C. Lechner, I. Tavares de Almeida, D. Konecki, Glycerol increases the yield and activity of human phenylalanine hydroxylase mutant enzymes produced in a prokaryotic expression system Mol Genet Metab 73 (2001) 173-178.
[35] E. Svensson, U. von Dobeln, R.C. Eisensmith, L. Hagenfeldt, S.L. Woo, Relation between genotype and phenotype in Swedish phenylketonuria and hyperphenylalaninemia patients Eur J Pediatr 152 (1993) 132-139.
[36] Y. Okano, M. Asada, Y. Kang, Y. Nishi, Y. Hase, T. Oura, G. Isshiki, Molecular characterization of phenylketonuria in Japanese patients Hum Genet 103 (1998) 613-618.
[37] B. Dworniczak, K. Grudda, J. Stumper, K. Bartholome, C. Aulehla-Scholz, J. Horst, Phenylalanine hydroxylase gene: novel missense mutation in exon 7 causing severe phenylketonuria Genomics 9 (1991) 193-199.
[38] E. Svensson, R.C. Eisensmith, B. Dworniczak, U. von Dobeln, L. Hagenfeldt, J. Horst, S.L. Woo, Two missense mutations causing mild hyperphenylalaninemia associated with DNA haplotype 12 Hum Mutat 1 (1992) 129-137.
[39] E. Bjorgo, R.M. de Carvalho, T. Flatmark, A comparison of kinetic and regulatory properties of the tetrameric and dimeric forms of wild-type and Thr427-->Pro mutant human phenylalanine hydroxylase: contribution of the flexible hinge region Asp425-Gln429 to the tetramerization and cooperative substrate binding Eur J Biochem 268 (2001) 997-1005.
[40] R.C. Eisensmith, S.L. Woo, Molecular basis of phenylketonuria and related hyperphenylalaninemias: mutations and polymorphisms in the human phenylalanine hydroxylase gene Hum Mutat 1 (1992) 13-23.
[41] U. Lichter-Konecki, D.S. Konecki, A.G. DiLella, K. Brayton, J. Marvit, T.M. Hahn, F.K. Trefz, S.L. Woo, Phenylalanine hydroxylase deficiency caused by a single base substitution in an exon of the human phenylalanine hydroxylase gene Biochemistry 27 (1988) 2881-2885.
[42] A. Gamez, B. Perez, M. Ugarte, L.R. Desviat, Expression analysis of phenylketonuria mutations. Effect on folding and stability of the phenylalanine hydroxylase protein J Biol Chem 275 (2000) 29737-29742.
[43] P.M. Knappskog, H.G. Eiken, A. Martinez, T. Flatmark, J. Apold, The PKU mutation S349P causes complete loss of catalytic activity in the recombinant phenylalanine hydroxylase enzyme Hum Genet 95 (1995) 171-173.
[44] M. Weinstein, R.C. Eisensmith, V. Abadie, S. Avigad, S. Lyonnet, G. Schwartz, A. Munnich, S.L. Woo, Y. Shiloh, A missense mutation, S349P, completely inactivates phenylalanine hydroxylase in north African Jews with phenylketonuria Hum Genet 90 (1993) 645-649.
[45] L.R. Desviat, B. Perez, M. De Lucca, V. Cornejo, B. Schmidt, M. Ugarte, Evidence in Latin America of recurrence of V388M, a phenylketonuria mutation with high in vitro residual activity Am J Hum Genet 57 (1995) 337-342.
[46] L.R. Desviat, B. Perez, M. Ugarte, Molecular basis of non-PKU hyperphenylalaninaemia in Spain: prevalence of A403V, a mutation with high residual activity J Inherit Metab Dis 19 (1996) 227-230.
[47] A.G. DiLella, J. Marvit, K. Brayton, S.L. Woo, An amino-acid substitution involved in phenylketonuria is in linkage disequilibrium with DNA haplotype 2 Nature 327 (1987) 333-336.
[48] T. Wang, Y. Okano, R.C. Eisensmith, M.L. Harvey, W.H. Lo, S.Z. Huang, Y.T. Zeng, L.F. Yuan, J.I. Furuyama, T. Oura, a.l. et, Founder effect of a prevalent phenylketonuria mutation in the Oriental population Proc. Natl. Acad. Sci. U S A 88 (1991) 2146-2150.
[49] A. Daniele, G. Cardillo, C. Pennino, M.T. Carbone, D. Scognamiglio, L. Esposito, A. Correra, G. Castaldo, A. Zagari, F. Salvatore, Five human phenylalanine hydroxylase proteins identified in mild hyperphenylalaninemia patients are disease-causing variants Biochim Biophys Acta 1782 (2008) 378-384.
[50] J. Marvit, A.G. DiLella, K. Brayton, F.D. Ledley, K.J. Robson, S.L. Woo, GT to AT transition at a splice donor site causes skipping of the preceding exon in phenylketonuria Nucleic acids research 15 (1987) 5613-5628.