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Review:

Phenylketonuria: Genomic interaction and the phenotype

Reuben Matalon[1]*, Sankar Surendran[1], Stephen K. Tyring[2] and Kimberlee Michals-Matalon[1]

[1]Departments of Pediatrics, Childrens Hospital, The University of Texas Medical Branch, Galveston, TX 77555-0359, USA, e-mail: rmatalon@utmb.edu, telephone: +1 409 772 3466, fax: +1 409 772 9595 (*corresponding author), [2]Department of Dermatology, University of Texas Health Science Center, Houston, TX

ABSTRACT

Genome research is emerging as a new and important tool in biology used to obtain information on gene sequences, genomic interaction, and how genes work in concert to produce the final syndrome or phenotype. Defect in phenylalanine hydroxylase (PAH) gene result in Phenylketonuria (PKU). Molecular studies using the brain of the mouse model for PKU (PAH^{enu2}) showed altered expression of several genes including upregulation of orexin A and a low activity of branched chain aminotransferase. These studies suggest that a single gene (PAH) defect is associated with altered expression, transcription and translation of other genes. It is the combination of the primary gene defect, the altered expression of other genes, and the new metabolic environment that is created, which lead to the phenotype.

Keywords: phenylketonuria, phenylalanine hydroxylase, Pah^{enu2} mouse, proteome, metabolome

INTRODUCTION

Phenylketonuria (PKU) is a common inborn error in aminoacid metabolism, with an average of 1 in 8000 U.S. Caucasians (DiLella et al., 1986). Deficiency of phenylalanine hydroxylase (PAH) affects normal conversion of phenylalanine to tyrosine. A deficiency of the activity of PAH causes PKU, which if untreated is characterized by high blood phenylalanine (Phe) levels and mental retardation (Eisensmith et al., 1991).

Genome research has become an important new methodology used to investigate the complexity of the gene interaction (Lander et al., 2001; Venter et al., 2001). Functional genomics relies on determining changes in the levels of mRNA (the transcriptome), the levels of proteins or enzymes (the proteome), and the levels of metabolite concentrations resulting from such changes (the metabolome), affecting the function of a tissue or organ (Oliver, 1996). Deficiency of enzyme caused by mutations in the phenylalanine hydroxylase (PAH) gene (Antonarkis and McKusick, 2000; Jimenez-Sanchez et al., 2001; Pey et al., 2003) leads to PKU. The regulatory, catalytic, tetrameric and BH4 binding regions of the PAH gene and mutations affecting the gene are shown in **Figure 1**. The mechanism causing mental retardation and the entire spectrum of neuropathology seen in the disease is unclear and cannot be explained by only the primary enzyme defect.

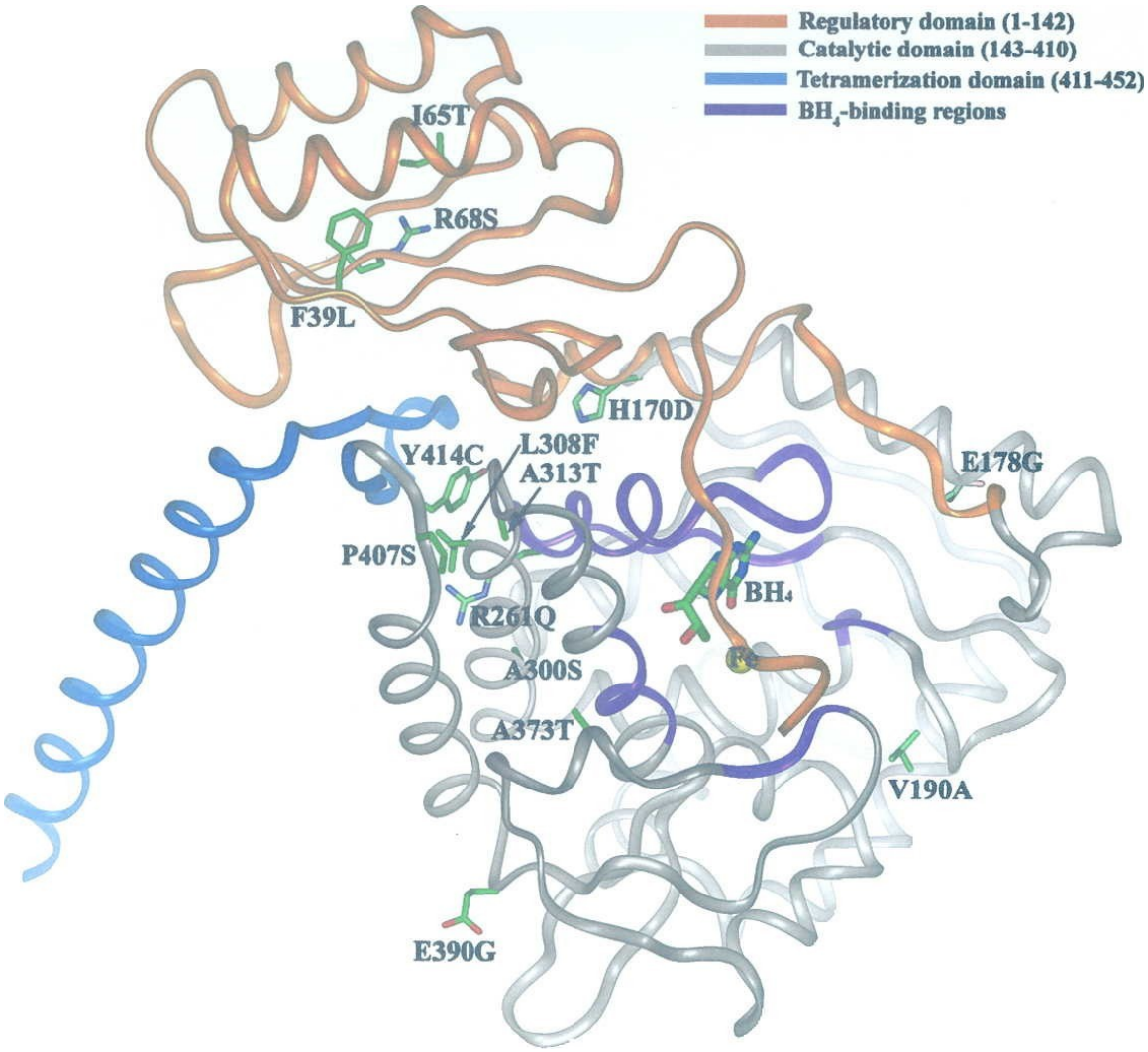

Fig 1: C-Alpha trace of a monomer of the composite model of phenylalanine hydroxylase (Matalon et al., 2004). Regulatory domain is colored in orange (residues 19-142), the catalytic domain in gray (residues 143-410), and the tetramerization domain (residues 411-452) in blue. Regions responsible for binding the BH_4 cofactor are shown in purple. BH_4 cofactor is shown together with the iron at the active site. Side chain of the residues found to be BH_4 responsive are shown mapped onto the structure.

Mouse models are being used to investigate the cascade of events in the hierarchy of gene expression involved in human diseases (McDonald et al., 1990; Shefer et al., 2000) since there is close homology of the human and mouse genomes (Gunter and Dhand, 2002; Madsen et al., 2001; Murphy et al., 2001; Nadeau 2002; Waterson et al., 2002). Altered gene expression, transcription, translation and metabolites in the brain of the mouse model for PKU (BTBR-Pah^{enu2}) is the subject of this study.

THE MOUSE MODELS FOR PKU

The mouse model for PKU (BTBR-Pah^{enu2}) was produced using the alkylating agent N-ethyl-N-nitrosourea (McDonald et al., 1990). The resulting PAH mutation was in the same locus observed in human patients. The mutation in the mouse caused a heritable inability to metabolize phenylalanine efficiently and the mouse model resembles very similar to human classical PKU in both initial genetic cause and ultimate phenotypic effects. The mouse leads to enzyme deficiency and increased blood and tissue levels of phenylalanine (Phe) as observed in the human counterpart (McDonald et al., 1990). The phenotype also has features of the human disease, the coat color is lighter than in the normal mouse and the PKU mice are

considered retarded. Therefore we studied genomic expression in the brain of the PKU mouse.

EXPRESSION AND LEVELS OF OREXINS

The mutation of PAH in the mouse model leads to altered expression of the orexin gene, causing an increase in the level of mRNA and the levels of orexin. Orexins (hypocretins) are recently discovered neuropeptides located mainly in the lateral hypothalamus of the brain (Nambu et al., 1999; van den Pol, 1999). Orexin A (hypocretin 1) contains 33 amino acid residues and has a pyroglutamyl residue at the NH2 terminal and amide group at the COOH terminal. Orexin B (hypocretin 2) contains 28 amino acids and is 46% identical to orexin A (Sakurai et al., 1998). Both orexins are encoded by a single gene and the mRNA for the precursor peptide, prepro-orexin, encodes 131 amino acid polypeptide (Sakurai et al., 1998). Orexins regulates various functions including arousal and food intake (Chemelli et al., 1999; Lu et al., 2000). Orexin deficiency was discovered in narcoleptic dogs and was found in humans with narcolepsy following diencephalic stroke (Scammell et al., 2001). There has been no report of any disease with increased production of orexin.

The gene expression of the precursor for orexins, prepro-orexin, and the mRNA are upregulated in the PKU mouse brain (**Table 1**). The high levels of mRNA for prepro-orexin leads to increased orexin A levels in the PKU mouse brain (**Table 2**; Surendran et al., 2003, 2004). Phenylketonuria is the only disease showing higher level of brain orexin A. Since orexin is associated with wakefulness and increased activity and PKU patients are hyperactive, it is likely that orexin A level is affected in the brain of patient with PKU. Orexins have not been studied in PKU and this may be an area of novel neurochemical research. Plasma can also be used to study orexin levels, although the level of orexin in plasma is very low. Since orexin expression regulates sleep-wake cycle, a time dependent orexin expression study will be necessary.

Table 1: Abnormal gene expression and mRNA levels in the PKU mouse brain

A: Microarray expression analysis of the PKU mouse brain (Surendran et al. 2003, 2004)

Gene bank accession no.	Gene	Expression ratio (in fold)
AF 019566	Hypocretin	18.3↑
J00356	α –amylase-1 mRNA	6.4↑
M57647	Mast cell growth factor mRNA	2.1↑
X05862	H2B and H2A histone genes	4.2↓
X16995	Hormone receptor	7.6↓
X14836	Calcium/calmodulin dependent Protein kinase II α	2.5↓

B: Real time one step RT-PCR measurement of prepro-orexin mRNA in the PKU mouse Brain (n=7±S.E) normalized to 18S RNA and relative to mouse heart (fold changes)

Wild type	2.19± 0.22
PKU mouse	12.84± 1.76

Mast cell growth factor (Viskochil, 2003), Hormone receptor (Ariznavarreta et al., 2003) and α-amylase I (Callis and Ho, 1983; Takkinen et al., 1983) are involved in growth regulation. Calcium/calmodulin dependent protein kinase II α (Tombes et al., 2003) and H2B and H2A histone genes (Soto et al., 2004) regulate cell signal (**Table 1**). Normal expression of these genes were altered by PAH mutation in the mouse. How these genes actions are synchronized to cause pathophysiology seen in the mouse is to be studied.

Table 2: Levels of orexin A in the brain, heart and plasma of PKU and wild type mice (n=8±S.E; Surendran et al. 2003)

Region	Wild type (pg/mg protein)	PKU (pg/mg protein)
Cerebrum	689.37±40.73	2473.37±282.16
Hypothalamus	540.87±93.09	3490.62±177.66
Cerebellum	295.25±64.90	868.25±188.44
Brainstem	414.37±59.74	3346.5±177.66
Heart	276.75±21.78	696.37±57.24
Plasma	138.8±26.58	223.0±27.04

ALTERED ENZYME ACTIVITY IN THE PKU MOUSE BRAIN

The hyperphenylalaninemia in PKU affects the transport of large neutral amino acids (LNAA) to the brain (Matalon et al., 2002, 2003). The LNAA include phenylalanine, tyrosine, tryptophan, the branched amino acids: leucine, isoleucine, and valine among others. All LNAA use the same transporter to cross the blood brain barrier with phenylalanine having the highest affinity of binding to the transporter. Due to the high levels of Phe in the blood of patients with PKU, the entry of other LNAA to the brain is impeded (Matalon et al., 2002, 2003). We examined branched chain aminotransferase activity (BCAT), which is substrate dependent, in the brain of the PKU mouse. The enzyme BCAT catalyzes amino transferase for leucine, isoleucine, and valine, yielding the common intermediates acetyl-CoA and succinyl-CoA. Because of the low levels of branched chain amino acids in the PKU mouse brain, we examined the BCAT activity and indeed it was decreased. The activity of BCAT in the brain of the mouse model for PKU was 0.049±0.010 mU/mg protein compared to the wild type, 0.126±0.003.

The high level of gene expression for orexin, the increased level of orexin, and the lower activity of BCAT in the PKU mouse model are examples of changes not directly related to the primary gene defect in PKU. These changes are responsible for a new biochemical environment for the brain. The low tyrosine level, caused by high level of blood Phe, decreases catecholamine synthesis in the PKU mouse brain (Pascucci et al., 2002; Puglisi-Allegra et al., 2000; **Table 3**) as observed in human PKU (Curtis et al., 1972; McKean, 1972; Partridge, 1998). These metabolic changes result in a new metabolome, which is the PKU phenotype that emerges. The quantitative expression of altered genes in the PKU mouse brain shows genomic interaction, which is likely similar to the human with PKU.

Table 3: PAH gene mutation alters metabolites in the brain of mouse model for PKU (Pulgi-Allegra et al. 2000)

Brain regions	5-HT (ng/g wet weight)		NE (ng/g wet weight)		DA (ng/g wet weight)	
	Control	PKU	Control	PKU	Control	PKU
Prefrontal cortex	851±33	234±19	495±13	252±12	117±8	42±5
Cingulate cortex	515±34	123±16	421±36	140±13	289±50	200±45
Amygdala	1073±64	369±53	196±24	100±7	358±46	126±21
Hippocampus	1255±77	299±21	556±14	237±17	112±21	101±19
Nucleus accumbens	1515±57	918±35	662±67	836±62	9766±433	6213±450
Caudate putamen	719±21	382±19	137±7	134±7	13074±369	9565±524

5-HT - 5-hydroxytryptamine (serotonin), NE – norepinephrine, DA - dopamine

CONCLUSION

Functional genomic research yields new information on gene interaction and how the hierarchy of genes responds to the change in the levels of various proteins, so a new proteome is produced. Subsequently, the change of the proteome alters the normal metabolic environment of the metabolome, which also exerts an effect on gene expression and protein function.

REFERENCES

Antonarakis S, McKusick VA. OMIM passes the 1000 disease gene mark. *Nature Genet* 2000;25:11

Ariznaverreta C, Castillo C, Segovia G, Mora F, Azcoitia I, Tresgurres JA. Growth hormone and aging. *Homo* 2003;54:132-141

Callis J, Ho TH. Multiple molecular forms of the gibberellin induced alpha-amylase from the aleurone layers of barley seeds. *Arch Biochem Biophys* 1983;224:224-234

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong X, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knock out mice: molecular genetics of sleep regulation. *Cell* 1999;98:437-451

Curtis HC, Baerlocker K, Vollmin JA. Pathogenesis of phenylketonuria: inhibition of DOPA and catecholamine synthesis in patients with phenylketonuria. *Clin Chim Acta* 1972;42:235-239

DiLella AG, Kwok SCM, Ledley FD, Marvit J, Woo SLC. Molecular structure and polymorphic

map of the human phenylalanine hydroxylase gene. *Biochemistry* 1986;25:743-749

Eisensmith RC, Woo SLC. Phenylketonuria and the phenylalanine hydroxylase gene. *Mol Biol Med* 1991;8:3-18

Gunter C, Dhand R. Human biology by proxy. *Nature* 2002;420:509

Jimenez-Sanchez G, Childs B, Valle D. Human disease genes. *Nature* 2001;409:853-855

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwain J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, Harris K, Headford A, Howland J, Kann L, et al. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860-921

Lu XY, Bagnol D, Burke S, Akil H, Watson SJ. Differential distribution of OX1 and OX2 orexin/hypocretin receptor mRNA in the brain upon fasting. *Horm Behav* 2000;37:335-344

Madsen O, Scally M, Douady CJ, Kao DJ, DeBry RW, Adkins R, Amrine HM, Stanhope MJ, de Jong WW, Springer MS. Parallel adaptive radiations in two major clades of placental mammals. *Nature* 2001;409:610-614

Matalon R, Surendran S, Matalon KM, Quast M, Wei J, Ezell E, Szucs, S. Large neutral aminoacids (LNAA) and brain phenylalanine (Phe) in mice model for Phenylketonuria (PKU). *J Inherit Metab Dis* 2002;25: Suppl. 1, 14

Matalon R, Surendran S, Matalon KM, Quast M, Wei J, Ezell E, Szucs S. Future role of large neutral aminoacid in the transport of phenylalanine into the brain of mice with PKU. *Pediatrics* 2003;112:1570-1574

Matalon R, Koch R, Michals-Matalon K, Moseley K, Surendran S, Tyring S, Erlandsen H, Gamez A, Stevens RC, Romstad A, Moller LB, Guttler F. Biopterin responsive phenylalanine hydroxylase deficiency. *Genet Med* 2004;6:27-32

McDonald JD, Bode VC, Dove WF, Shedlovsky A. Pah^{hph}-5: A mouse mutant deficient in phenylalanine hydroxylase. *Proc Natl Acad Sci USA* 1990;87:1965-1967

McKean CM. The effects of high phenylalanine concentrations on serotonin and catecholamine metabolism in the human brain. *Brain Res* 1972;47:469-476

Murphy WJ, Eizirik E, Johnson WE, Zhang YP, Ryder OA, O'Brien SJ. Molecular phylogenetics and the origins of placental mammals. *Nature* 2001;409:614-618

Nadeau JH. Single nucleotide polymorphisms: tackling complexity. *Nature* 2002;420:517-518

Nambu T, Sakurai T, Mizukami K, Hosoy Y, Yanagisawa M, Goto K. Distribution of orexin neurons in the adult rat brain. *Brain Res* 1999;827:243-260

Oliver SG. From DNA sequence to biological function. *Nature* 1996;379:601-603

Partridge WM. Blood-brain barrier carrier-mediated transport and brain metabolism of aminoacids. *Neurochem Res* 1998;23:635-644

Pascucci T, Ventura R, Puglisi-Allegra S, Cabib S. Deficits in brain serotonin in a genetic mouse model of phenylketonuria. *Neuroreport* 2002;13:2561-2564

Pey AL, Desviat LR, Gamez A, Ugarte M, Perez B. Phenylketonuria: genotype-pheonotype correlations based on expression analysis of structural and functional mutations in PAH. *Hum Mutat* 2003;21:370-378

Puglisi-Allegra S, Cabib S, Pascucci T, Ventura R, Cali F, Romano V. Dramatic brain aminergic deficit in a genetic mouse model of phenylketonuria. *Neuroreport* 2000;11:1361-1364

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H. Orexins and orexin receptors: a family of hypothalamus neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;92:573-585

Scammell TE, Nishino S, Mignot E, Saper CB. Narcolepsy and low CSF orexin (hypocretin) concentration after a diencephalic stroke. *Neurology* 2001;56:1751-1753

Shefer S, Tint GS, Jean-Guillaume D, Daikhin E, Kendler A, Nguyen LE, Yudkoff M, Dyer CA. Is there a relationship between 3-hydroxy-3-methylglutaryl coenzyme A reductase activity

and forebrain pathology in the PKU mouse. *J Neurosci Res* 2000;61:549-563

Soto M, Iborra S, Quijada L, Folgueira C, Alonso C, Requena JM. Cell-cycle dependent translation of histone mRNAs is the key control point for regulation of histone biosynthesis in Leishmania infantum. *Biochem J* 2004 (Epub)

Surendran S, Campbell GA, Tyring SK, Matalon K, McDonald D, Matalon R. High levels of orexin A in the brain of the mouse model for phenylalanine: possible role of orexin A in hyperactivity seen in children with PKU. *Neurochem Res* 2003;28:1891-1894

Surendran S, Rady PL, Szucs S, Michals-Matalon K, Tyring SK, Matalon R. High level of orexin A observed in the phenylketonuria mouse brain is due to the abnormal expression of prepro-orexin. *Biochem Biophys Res Commun* 2004;317:522-526

Takkinen K, Petterson RF, Kalkkinen N, Palva I, Soderlund H, Kaariainen L. Aminoacid sequence of alpha-amylase from Bacillus amyolliquefaciens deduced from the nucleotide sequence of the clone gene. *J. Biol. Chem.* 1983;258:1007-1013

Tombes RM, Faison MO, Turbeville JM. Organization and evolution of multifunctional Ca(2+)/CaM-dependent protein kinase genes. *Gene* 2003;322:17-31

van den Pol AN. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. *J Neurosci* 1999;19:3171-3182

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GC, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, et al. The sequence of the human genome. *Science* 2001;291:1304-1351

Viskochil DH. It takes two to tango: mast cell and Schwann cell interactions in neurofibromas. J Clin Invest 2003;112:1036-1042

Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, Agarwala R, Ainscough R, Alexanderson M, An P, Antonarakis SE, Attwood J, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002;420:520-562