Visions & Reflections

Functional polymorphisms of the brain serotonin synthesizing enzyme tryptophan hydroxylase-2

X. Zhang, J.-M. Beaulieu, R. R. Gainetdinov and M. G. Caron*

Department of Cell Biology, and Center for Models of Human Disease, Institute for Genome Sciences and Policy, Box 3287, Duke University Medical Center, Durham, North Carolina 27710 (USA), Fax: +1 919 681 8641, e-mail: caron002@mc.duke.edu

Abstract. Many neuropsychiatric disorders are considered to be related to the dysregulation of brain serotonergic neurotransmission. Tryptophan hydroxylase-2 (TPH2) is the neuronal-specific enzyme that controls brain serotonin synthesis. There is growing genetic evidence for the possible involvement of *TPH2* in serotonin-related neuropsychiatric disorders; however, the degree of genetic variation in *TPH2* and, in particular, its possible functional consequences remain unknown. In this short review, we will summarize some recent findings with respect to the functional analysis of *TPH2*.

Key words. Brain serotonin; tryptophan hydroxylase-2 (TPH2); single nucleotide polymorphism (SNP); congenic mice; PC12 cells.

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has been implicated in various physiological functions in both peripheral and central nervous systems (CNS). Many neuropsychiatric disorders, including depression [1–4], schizophrenia [4–6], aggression and suicidal behavior [1, 6], attention-deficit/hyperactivity disorder (ADHD) [7–9], obsessive-compulsive disorder [10] and autism [11] have been suggested to be related to the dysregulation of brain serotonergic neurotransmission.
Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in 5-HT synthesis and belongs to the superfamily of aromatic amino acid hydroxylase, which also includes tyrosine hydroxylase (TH) and phenylalanine hydroxylase (PAH). TPH, TH and PAH share considerable structural similarity and require the same co-factor (Fe^{2+}) and co-substrate (tetrahydrobiopterin, BH4) for function [12]. While TH and PAH are each encoded by a single gene, there has been evidence suggesting that two genes may encode *TPH* [13, 14]. The recent identification of a *TPH2* gene [15] has demonstrated that this is indeed the case. The well-characterized TPH1 was identified 4 decades ago [16] and has recently been shown to be essentially peripheral, being expressed predominantly in the pineal gland and enterochromaffin cells of the gut [15, 17–19]. In contrast to the results of RNase protection assay [15] and *in situ* hybridization study [19], Zill et al. [20] recently reported the expression of *TPH1* messenger RNA (mRNA) in several brain regions in post-mortem human brains. However, further studies may be needed to examine whether such *TPH1* expression represents the pineal projections in these brain regions [21], as serotonin N-acetyltransferase (AANAT), the rate-limiting enzyme in melatonin synthesis, has been shown to be expressed also in certain brain regions [21]. *TPH2*, however, is expressed predominantly in serotonergic neurons of the raphe nuclei [15, 19] and in the peripheral myenteric neurons in the gut [18]. To date, *TPH2* has been identified and cloned in human, mice, rat, chicken, zebrafish, tora-

fugu and fruit fly (gene accession numbers: NM_173353 [human], NM_173391 [mouse], NM_173839 [rat], NM_001001301 [chicken], NM_214795 [zebrafish], AY616189 [torafugu]) [15, 22, 23]. This paper will summarize some recent findings about *TPH2*, and focus on the functional analysis of *TPH2* and its potential contribution to the dysregulation of brain serotonin homeostasis associated with the pathophysiology of neuropsychiatric disorders.

TPH polymorphisms and neuropsychiatric disorders

TPH1 has long been considered the sole rate-limiting enzyme for the synthesis of 5-HT. Two common intronic (non-coding) single nucleotide polymorphisms (SNPs), A218C and A779C, in *TPH1* have been extensively studied for their possible associations with neuropsychiatric conditions, including suicidal behavior, unipolar depression, bipolar depression and schizophrenia with conflicting outcomes (reviewed in [1]). However, the recent discovery of the neuronal-specific *TPH2* [15] that is critical for brain serotonin synthesis [24] has redefined our perspective of the serotonergic systems. These findings have suggested that *TPH2*, rather than its peripheral counterpart (*TPH1*), should be investigated as a candidate gene for 5-HT-related neuropsychiatric conditions. In fact, a number of studies have already indicated the possible association between several *TPH2* polymorphisms and major depression [25–28], suicidal behavior [28, 29], ADHD [30, 31], autism [32], bipolar disorder [33] and obsessive-compulsive disorder [34]. In contrast, other studies have documented in different cohorts a lack of association between *TPH2* polymorphisms and suicidal behavior, schizophrenia, bipolar disorder or depression [35–37]. Discrepancies in such studies are difficult to reconcile, but not unexpected given the different cohorts used and perhaps the limitations in diagnostic criteria [38]. It is also noteworthy that most of the SNP in *TPH1* and *TPH2* identified in the above-mentioned studies are located in the introns or promoter region, thus corresponding to non-coding SNPs without clear functional consequences. These observations underscore the importance of determining the functional consequences of any given SNP in order to address whether such SNP(s) would affect *TPH2* gene expression (e.g., transcriptional regulation, splicing) and/or function (e.g., enzyme kinetics, protein folding, protein stability). As an example of the importance of the functional consequences of genetic variants, the polymorphism in the serotonin transporter (SERT) promoter region, which is associated with anxiety and depression [39–41], has been functionally characterized and shown to result in decreased transcriptional efficiencies.

Serotonin-related neuropsychiatric disorders are complex heterogeneous disorders with markedly different clinical profiles and responses to drugs [3, 4], and may also represent disorders with different endophenotypes [2]. Genetic, environmental as well as biochemical influences are thought to represent the contributing factors for these polygenic disorders which are associated with polymorphism(s) in several genes, including the serotonin transporter, serotonin receptors, the vesicular monoamine transporter, monoamine oxidases, as well as *TPH1* and *TPH2* [1–3]. Two hypotheses have been suggested to explain the genetic basis of polygenic disorders: the common disease/common variant (CD/CV) hypothesis, where disease susceptibility variants are common in the population, and the common disease/rare variant (CD/RV) hypothesis, with large numbers of rare variants at many loci [42]. The multiple functional polymorphisms in *PAH* (see below) and their association with phenylketonuria (PKU), a metabolic disease with an incidence of 1 per 15,000 in the United States [43] is one example supporting the CD/RV hypothesis. Similarly, more than 100 missense mutations in superoxide dismutase-1 (*SOD1*) have been identified in amyotrophic lateral sclerosis (ALS), a disorder with a frequency of 4–6 per 100,000, yet only 2–3% of ALS patients carry *SOD1* mutations [44]. With regard to the *TPH2* gene, our laboratory has recently identified a G1463A SNP in human *TPH2* resulting in a severe loss-of-function in TPH2, where a R441H mutation translates to an ~80% decrease in 5-HT production when assessed in cell culture systems [27]. The G1463A SNP was identified in a cohort of unipolar depression subjects who were resistant to various forms of pharmacological treatments. Among the 9 subjects carrying the G1463A SNP in a cohort of 87 subjects, 5 subjects were treated with electroconvulsive therapy (ECT), while only 16 subjects in this cohort received ECT treatment.

TPH2 mutations

To date, over 500 SNPs have been identified in the *TPH2* gene among different species, including more than 300 SNPs in human (http://www.ncbi.nlm.nih.gov). Among these SNPs, there are 6 coding non-synonymous SNPs (L36P, P206S, A328V, R441H, D479E in humans and P447R in mice) and 3 coding synonymous SNPs (P312P, L327L, A375A in humans) [24, 27, 45]. Although the coding sequence represent only ~1.5% of the *TPH2* gene (93, 600 bp), the prevalence of SNPs (9 to this point) in coding regions of *TPH2* is reminiscent of the genetic variants in the *PAH* gene [46, 47]. At present, ~500 mutations, including more than 300 missense mutations, have been identified in patients with symptoms ranging from mild hyperphenylalaninemia to severe PKU [46, 47]. Importantly, the amino acids in PAH whose mutations either cause protein misfolding or affect substrate and/or BH4

binding [46, 48] are identical in TPH2 (fig. 1). As an example, the R441H mutation in TPH2 identified in the unipolar major depression patients that severely impairs 5-HT production when expressed in cell culture systems [27] is identical to Arg408 in PAH whose mutation (R408W) is the most prevalent and severe pathogenic mutation in PAH [43, 46, 47] Based on the considerable structural and sequence similarities between TPH2 and PAH [12], it is likely that additional rare functional mutations in *TPH2* will be identified, and that the presence of multiple susceptibility genes and/or multiple mutations in a single gene may contribute to the complex polygenic nature and wide range of clinical profiles of 5-HT-related neuropsychiatric conditions. It is also possible that the existence of distinct SNPs in *TPH2* with different degrees of *TPH2* dysfunction could contribute to the development of phenotypes associated with different neuropsychiatric conditions. Therefore, functional PAH mutations could provide an important roadmap for understanding the potential mechanisms of TPH2 dysfunction.

Mutations identified in *TPH2* may generate missense and nonsense mutations (coding mutations), splicing variants (intronic mutations) or other regulatory variants to affect gene function. Therefore, in order to develop a better understanding of the relationships between mutations and functions, it is important to functionally characterize *TPH2* mutations and correlate them to 5-HT-related neuropsychiatric disorders. Clearly, a straightforward approach is to using *TPH2* complementary DNA (cDNA) to assess 5-HT production of coding non-synonymous SNPs in *TPH2*, whose potential molecular mechanisms could be predicted based on previous studies of loss-of-function mutations in PAH. Non-coding SNPs of *TPH2* (i.e., SNPs in promoter regions, untranslated regions, introns) may potentially regulate *TPH2* gene functions by transcriptional regulation and splicing. It should also be mentioned that, while coding synonymous SNPs are typically called 'silent mutations', more and more evidence indicates that some of these 'silent mutations' may play an important role in regulating premRNA splicing as a mechanism of gene regulation [49]. For example, an exon-skipping mutation caused by a coding synonymous SNP in *PAH* was characterized from a previously categorized 'silent mutation' [50]. Thus, it is necessary to perform detailed studies of *TPH2* gene function using both cDNA and genomic DNA.

PC12 cells as a model system for characterizing TPH2

Although expression of recombinant protein in *Escherichia coli* is indispensable and has been widely used to obtain purified TPH1 [51, 52], TPH2 [53] and PAH [46] in large quantity for further detailed structural and func-
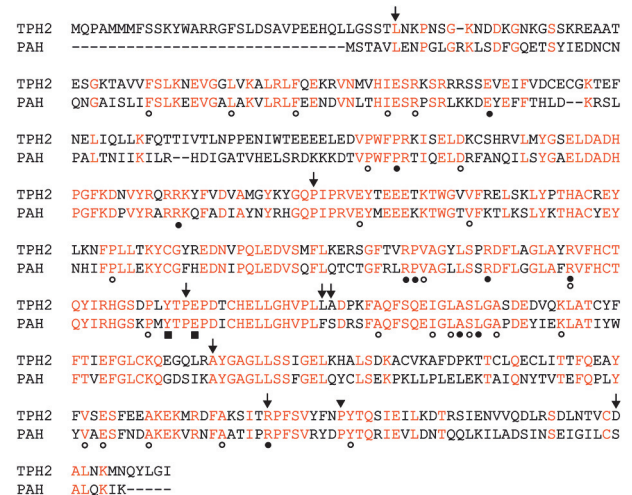

Figure 1. Sequence alignment of TPH2 and PAH in human. Coding synonymous and coding non-synonymous mutations in human TPH2 are shown with arrows. The coding non-synonymous mutation in mouse Tph2 is shown with an arrowhead. PAH mutations that affect substrate binding, protein folding and BH4 binding are indicated with filled boxes, closed circles and open circles, respectively (gene accession numbers: NM_173353 [TPH2], NM_000277 [PAH]).

tional analysis, mammalian cell culture systems can provide conditions for protein expression which are closer to the *in vivo* environment. We have established the pheochromocytoma PC12 cells as a model system to study 5-HT production [24, 27]. PC12 cells are neuroendocrine cells that endogenously synthesize dopamine and norepinephrine, but not 5-HT [54], indicating that PC12 cells possess essential elements (e.g., BH4 and aromatic amino acid decarboxylase [AADC]) for 5-HT synthesis and are capable of synthesizing 5-HT when *TPH2* is exogenously expressed. Moreover, the presence of endogenous dopamine in PC12 cells, which is easily detectable along with 5-HT, provides an ideal internal control for quantification. Walther et al. [15] have previously expressed *TPH2* in COS7 cells, which do not express AADC, and measured 5-HTP production as an approach to effectively monitor the rate of TPH2 synthesis. Regulation in 5-HT production (e.g., phosphorylation [53]) and interaction between TPH2 and accessory proteins (e.g., 14-3-3 protein [53]) can also be investigated in mammalian cell culture systems. TPH2, as well as other members of the superfamily of aromatic amino acid hydroxylases, forms homotetramers [12]. Previous studies using recombinant TPH1 [51] and PAH [47] revealed a tendency of the purified proteins to form aberrant oligomers and aggregates, a phenomenon which may or may not occur *in vivo*. Therefore, expression of *TPH2* in mammalian cell systems can provide a rapid functional analysis mimicking the *in vivo* condition to identify potential mutation(s) that affect protein folding and stability *in vivo*.

Animal models for studying 5-HT-related behaviors

It has been noted for many years that there are substantial behavioral differences among different inbred mouse strains, particularly in behavioral assessments using the tail suspension test, forced swim test, alcohol preference and aggression [55–58]. However, the underlying mechanism governing these differences has been unknown. We recently identified a functional (C1473G) SNP in mouse *Tph2* that exhibited an ~55% reduction in Tph2 activity when expressed in PC12 cells [24]. Importantly, BALB/cJ and DBA/2 inbred mice carrying the homozygous 1473G allele exhibited an ~50% decrease in the rate of brain 5-HT synthesis and tissue content as compared with C57Bl/6 and 129X1/SvJ inbred mice carrying the homozygous 1473C allele [24]. These results provide potential molecular and genetic explanations for the behavioral differences among these strains of mice. Interestingly, (C1473G) functional polymorphism in mouse *Tph2* gene was recently associated with differences in aggressive behavior [59], pre-mRNA editing of the 5-HT2C receptor [60] and responsiveness to selective serotonin reuptake inhibitor (SSRI) [61, 62], but not to certain aspects of impulsive behaviors [63]. These studies have independently confirmed the homozygous 1473C allele in C57Bl/6, 129X1/SvJ strains and the homozygous 1473G allele in BALB/cJ, DBA/2 strains. In addition, it has been shown that other inbred strains carry either the homozygous 1473C allele (ARK/J, C3H/HeJ, CBA/Lac, DD/He, PT/Y, YT/Y) or the homozygous 1473G allele (A/He, A/J, CBA/Ca, CC57BR/Mv) [59, 62, 63]. It should be emphasized that the behavioral studies mentioned above have used these different inbred mouse strains for comparison; thus the influence of potential variations in other genes cannot be ruled out. Therefore, to precisely address the role of functional polymorphism(s) in behavioral studies, it is imperative to generate congenic mice carrying either the 1473C or 1473G allele using a backcross breeding strategy (fig. 2). Congenic mice carrying 1473C/C, 1473C/G or 1473G/G alleles will enable assessment of the role of brain 5-HT while minimizing the potential contribution of other modifier genes. An alternative strategy would be to generate *Tph2* knock-out mice or knock-in mice carrying functional mutations of *TPH2*. Such transgenic mice, along with the congenic mice described above, may recapitulate behavioral manifestations of 5-HT-related neuropsychiatric conditions and provide valuable tools to test therapeutic pharmacological interventions.

Summary

Brain 5-HT plays an important role in regulating complex behaviors, and dysregulation of brain 5-HT homeostasis may contribute to many neuropsychiatric disorders. The
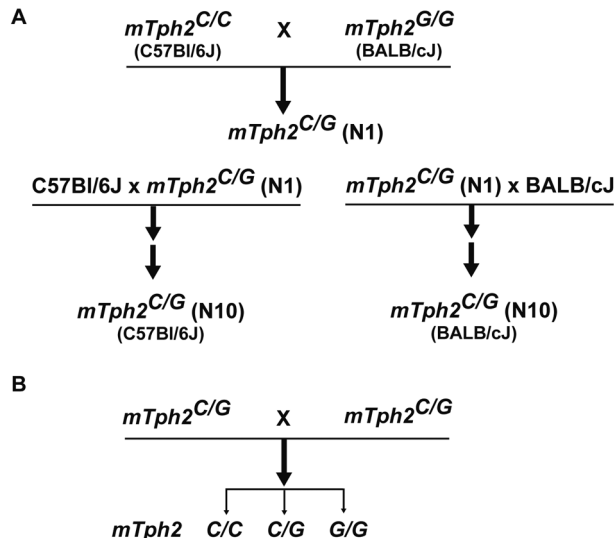

Figure 2. Strategy to generate congenic mouse lines carrying either 1473C or 1473G allele in *Tph2*. (*A*) Backcross breeding of C57Bl/6J carrying homozygous 1473C and BALB/cJ carrying homozygous 1473G in *Tph2*. (*B*) Using C57Bl/6J or BALB/cJ mice carrying heterozygous 1473C/G congenic mice to generate congenic mice carrying 1473C/C, 1473C/G and 1473G/G alleles in *Tph2*.

identification of the role of *TPH2* in brain 5-HT synthesis has opened a new area to explore the molecular and genetic mechanisms of 5-HT-related conditions. Functional and genetic analyses are two powerful, indispensable and complementary tools to address the role of *TPH2*. These studies should also pave the way for the development of animal models for behavioral and pharmacological studies. Therefore, characterization of *TPH2* and its functional SNPs may ultimately provide important insights into the pathophysiology of 5-HT-related neuropsychiatric disorders.

1 Arango V., Huang Y. Y., Underwood M. D. and Mann J. J. (2003) Genetics of the serotonergic system in suicidal behavior. J. Psychiatr. Res. **37:** 375–386

2 Hasler G., Drevets W. C., Manji H. K. and Charney D. S. (2004) Discovering endophenotypes for major depression. Neuropsychopharmacology **29:** 1765–1781

3 Lesch K. P. (2004) Gene-environment interaction and the genetics of depression. J. Psychiatry Neurosci. **29:** 174–184

4 Malhotra A. K., Murphy G. M. and Kennedy J. L. (2004) Pharmacogenetics of psychotropic drug response. Am. J. Psychiatry **161:** 780–796

5 Miyamoto S., LaMantia A. S, Duncan G. E., Sullivan P., Gilmore J. H. and Lieberman J. A. (2003) Recent advances in the neurobiology of schizophrenia. Mol. Interv. **3:** 27–39

6 Courtet P., Jollant F., Castelnau D., Buresi C. and Malafosse A. (2005) Suicidal behavior: relationship between phenotype and serotonergic genotype. Am. J. Med. Genet. **133C:** 25–33

7 Gainetdinov R. R., Wetsel W. C., Jones S. R., Levin E. D., Jaber M. and Caron M. G. (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science **283:** 397–401

8 Quist J. F. and Kennedy J. L. (2001) Genetics of childhood disorders: XXIII. ADHD, Part **7:** The serotonin system. J. Am. Acad. Child Adolesc. Psychiatry **40:** 253–256

9 Bobb A. J, Castellanos F. X., Addington A. M and Rapoport J. L. (2005) Molecular genetic studies of ADHD: 1991 to 2004. Am. J. Med. Genet. **132B:** 109–125

10 Blier P. and Abbott F. V. (2001) Putative mechanisms of action of antidepressant drugs in affective and anxiety disorders and pain. J. Psychiatry Neurosci. **26:** 37–43

11 Veenstra-VanderWeele J. and Cook E. H. (2004) Molecular genetics of autism spectrum disorder. Mol. Psychiatry **9:** 819–832

12 Fitzpatrick P. F. (1999) Tetrahydropterin-dependent amino acid hydroxylases. Annu. Rev. Biochem. **68:** 355–381

13 Kim K. S., Wessel T. C., Stone D. M., Carver C. H., Joh T. H. and Park D. H. (1991) Molecular cloning and characterization of cDNA encoding tryptophan hydroxylase from rat central serotonergic neurons. Brain Res. Mol. Brain Res. **9:** 277–283

14 Haycock J. W., Kumer S. C., Lewis D. A., Vrana K. E. and Stockmeier C. A. (2002) A monoclonal antibody to tryptophan hydroxylase: applications and identification of the epitope. J. Neurosci. Methods **114:** 205–212

15 Walther D. J., Peter J. U., Bashammakh S., Hortnagl H., Voits M., Fink H. et al. (2003) Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science **299:** 76

16 Grahame-Smith D. G. (1964) Tryptophan hydroxylation in brain. Biochem. Biophys. Res. Commun. **16:** 586–592

17 Yu P. L., Fujimura M., Okumiya K., Kinoshita M., Hasegawa H. and Fujimiya M. (1999) Immunohistochemical localization of tryptophan hydroxylase in the human and rat gastrointestinal tracts. J. Comp. Neurol. **411:** 654–665

18 Cote F., Thevenot E., Fligny C., Fromes Y., Darmon M., Ripoche M. A. et al. (2003) Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. Proc. Natl. Acad. Sci. USA **100:** 13525–13530

19 Patel P. D., Pontrello C. and Burke S. (2004) Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. Biol. Psychiatry **55:** 428–433

20 Zill P., Buttner A., Eisenmenger W., Moller H. J., Ackenheil M. and Bondy B. (2005) Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: a post-mortem study. J. Psychiatr. Res. [doi:10.1016/j.jpsychires.2005.05.004]

21 Coon S. L., Mazuruk K., Bernard M., Roseboom P. H., Klein D. C. and Rodriguez I. R. (1996) The human serotonin N-acetyltransferase (EC 2.3.1.87) gene (AANAT): structure, chromosomal localization and tissue expression. Genomics **34:** 76–84

22 Teraoka H., Russell C., Regan J., Chandrasekhar A., Concha M. L., Yokoyama R. et al. (2004) Hedgehog and Fgf signaling pathways regulate the development of tphR-expressing serotonergic raphe neurons in zebrafish embryos. J. Neurobiol. **60:** 275–288

23 Coleman C. M. and Neckameyer W. S. (2005) serotonin synthesis by two distinct enzymes in *Drosophila melanogaster*. Arch. Insect Biochem. Physiol. **59:** 12–31

24 Zhang X., Beaulieu J. M., Sotnikova T. D., Gainetdinov R. R. and Caron M. G. (2004) Tryptophan hydroxylase-2 controls brain serotonin synthesis. Science **305:** 217

25 Peters E. J., Slager S. L., McGrath P. J., Knowles J. A. and Hamilton S. P. (2004) Investigation of serotonin-related genes in antidepressant response. Mol. Psychiatry **9:** 879–889

26 Zill P., Baghai T. C., Zwanzger P., Schule C., Eser D., Rupprecht R. et al. (2004) SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. Mol. Psychiatry **9:** 1030–1036

27 Zhang X., Gainetdinov R. R., Beaulieu J. M., Sotnikova T. D., Burch L. H., Williams R. B. et al. (2005) Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. Neuron **45:** 11–16

28 Zhou Z., Roy A., Lipsky R., Kuchipudi K., Zhu G., Taubman J. et al. (2005) Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. Arch. Gen. Psychiatry **62:** 1109–1118

29 Zill P., Buttner A., Eisenmenger W., Moller H. J., Bondy B. and Ackenheil M. (2004) Single nucleotide polymorphism and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene in suicide victims. Biol. Psychiatry **56:** 581–586

30 Sheehan K., Lowe N., Kirley A., Mullins C., Fitzgerald M., Gill M. et al. (2005) Tryptophan hydroxylase 2 (TPH2) gene variants associated with ADHD. Mol. Psychiatry **10:** 944–949

31 Walitza S., Renner T. J., Dempfle A, Konrad K, Wewetzer C, Halbach A. et al. (2005) Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in attention-deficit/hyperactivity disorder. Mol. Psychiatry [doi:10.1038/sj.mp.4001734]

32 Coon H., Dunn D., Lainhart J., Miller J., Hamil C., Battaglia A. et al. (2005) Possible association between autism and variants in the brain-expressed tryptophan hydroxylase gene (TPH2). Am. J. Med. Genet. **135B:** 42–46

33 Harvey M., Shink E., Tremblay M., Gagne B., Raymond C., Labbe M. et al. (2004) Support for the involvement of TPH2 gene in affective disorders. Mol. Psychiatry **9:** 980–981

34 Mossner R., Walitza S., Geller F., Scherag A., Gutknecht L., Jacob C. et al. (2005) Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in children and adolescents with obsessive-compulsive disorder. Int. J. Neuropsychopharmacol. [doi:10.107/sia61145705005997]

35 De Luca V., Mueller D. J., Tharmalingam S., King N. and Kennedy J. L. (2004) Analysis of the novel TPH2 gene in bipolar disorder and suicidality. Mol. Psychiatry **9:** 896–897

36 De Luca V., Voineskos D., Wong G. W., Shinkai T., Rothe C., Strauss J. et al. (2005) Promoter polymorphism of second tryptophan hydroxylase isoform (TPH2) in schizophrenia and suicidality. Psychiatry Res. **134:** 195–198

37 Garriock H. A., Allen J. J., Delgado P., Nahaz Z., Kling M. A., Carpenter L. et al. (2005) Lack of association of TPH2 exon XI polymorphisms with major depression and treatment resistance. Mol. Psychiatry **10:** 976–977

38 Krishnan K. R. (2005) Psychiatric disease in the genomic era: rational approach. Mol. Psychiatry **10:** 978–984

39 Lesch K. P., Bengel D., Heils A., Sabol S. Z., Greenberg B. D., Petri S. et al. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science **274:** 1527–1531

40 Caspi A., Sugden K., Moffitt T. E., Taylor A., Craig I. W. and Harrington H. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science **301:** 386–389

41 Pezawas L., Meyer-Lindenberg A., Drabant E. M., Verchinski B. A., Munoz K. E., Kolachana B. S. et al. (2005) 5-HTTLPR polymorphism impacts human cingulate- amygdala interactions: a genetic susceptibility mechanism for depression. Nat. Neurosci. **8:** 828–834

42 Wright A., Charlesworth B., Rudan I., Carothers A. and Campbell H. (2003) A polygenic basis for late-onset disease. Trends Genet. **19:** 97–106

43 Scriver C. R., Hurtubise M., Konecki D., Phommarinh M., Prevost L., Erlandsen, H. et al. (2003) PAHdb **2003:** what a locus-specific knowledgebase can do. Hum. Mutat. **21:** 333–344

44 Bendotti C. and Carri M. T. (2004) Lessons from models of SOD1-linked familial ALS. Trends Mol. Med. **10:** 393–400

45 Breidenthal S. E., White D. J. and Glatt C. E. (2004) Identification of genetic variants in the neuronal form of tryptophan hydroxylase (TPH2). Psychiatr. Genet. **14:** 69–72

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

47 Waters P. J. (2003) How PAH gene mutations cause hyper-phenylalaninemia and why mechanism matters: insights from *in vitro* expression. Hum. Mutat. **21:** 357–369

48 Erlandsen H., Pey A. L., Gamez A., Perez B., Desviat L. R., Aguado C. et al. (2004) Correction of kinetic and stability defects by tetrahydrobiopterin in phenylketonuria patients with certain phenylalanine hydroxylase mutations. Proc. Natl. Acad. Sci. USA **101:** 16903–16908

49 Cartegni L., Chew S. L. and Krainer A. R. (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat. Rev. Genet. **3:** 285–298

50 Chao H.-K., Hsiao K.-J. and Su T.-S. (2001) A silent mutation induces exon skipping in the phenylalanine hydroxylase gene in phenylketonuria. Hum. Genet. **108:** 14–19

51 Yang X. J. and Kaufman S. (1994) High-level expression and deletion mutagenesis of human tryptophan hydroxylase. Proc. Natl. Acad. Sci. USA **91:** 6659–6663

52 Wang L., Erlandsen H., Haavik J., Knappskog P. M. and Stevens R. C. (2002) Three- dimensional structure of human tryptophan hydroxylase and its implications for the biosynthesis of the neurotransmitters serotonin and melatonin. Biochemistry **41:** 12569–12574

53 McKinney J., Knappskog P. M. and Haavik J. (2005) Different properties of the central and peripheral forms of human tryptophan hydroxylase. J. Neurochem. **92:** 311–320

54 Greene L. A. and Tischler A. S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. USA **73:** 2424–2428

55 Popova N. K., Nikulina E. M. and Kulikov A. V. (1993) Genetic analysis of different kinds of aggressive behavior. Behav. Genet. **23:** 491–497

56 Lucki I., Dalvi A. and Mayorga A. J. (2001) Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology **155:** 315–322

57 Ripoll N., David D. J., Dailly E., Hascoet M. and Bourin M. (2003) Antidepressant- like effects in various mice strains in the tail suspension test. Behav. Brain Res. **143:** 193–200

58 Wahlsten D., Metten P., Phillips T. J., Boehm S. L., Burkhart-Kasch S,. Dorow J. et al. (2003) Different data from different labs: lessons from studies of gene- environment interaction. J. Neurobiol. **54:** 283–311

59 Kulikov A. V., Osipova D. V., Naumenko V. S. and Popova N. K. (2005) Association between *Tph2* gene polymorphism, brain tryptophan hydroxylase activity and aggressiveness in mouse strains. Genes Brain Behav. **4:** 482–485

60 Englander M. T., Dulawa S. C., Bhansali P. and Schmauss C. (2005) How stress and fluoxetine modulate serotonin 2C receptor pre-mRNA editing. J. Neurosci. **25:** 648- 651

61 Cervo L., Canetta A., Calcagno, Burbassi S., Sacchetti G., Caccia S. et al. (2005) Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. J. Neurosci. **25:** 8165–8172

62 Crowley J. J, Blendy J. A. and Lucki I. (2005) Strain-dependent antidepressant-like effects of citalopram in the mouse tail suspension test. Psychopharmacology **183:** 257–264

63 Isles A. R., Hathway G. J., Humby T., de la Riva C., Kendrick K. M. and Wilkinson L. S. (2005) An mTph2 SNP gives rise to alterations in extracellular 5-HT levels, but not in performance on a delayed-reinforcement task. Eur. J. Neurosci. **22:** 997–1000