Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2012 March ; 159B(2): 152–171. doi:10.1002/ajmg.b.32023.

Advances in Tryptophan Hydroxylase-2 Gene Expression Regulation: New Insights into Serotonin-Stress Interaction and Clinical Implications

Guo-Lin Chen^{1,*} and Gregory M. Miller¹

¹Harvard Medical School, New England Primate Research Center, Division of Neuroscience, One Pine Hill Drive, Southborough, MA 01772–9102, USA

Abstract

Serotonin (5-HT) modulates the stress response by interacting with the hormonal hypothalamic-pituitary-adrenal (HPA) axis and neuronal sympathetic nervous system (SNS). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in 5-HT biosynthesis, and the recent identification of a second, neuron-specific TPH isoform (TPH2) opened up a new area of research. While TPH2 genetic variance has been linked to numerous behavioral traits and disorders, findings on TPH2 gene expression have not only reinforced, but also provided new insights into, the long-recognized but not yet fully understood 5-HT-stress interaction. In this review, we summarize advances in *TPH2* expression regulation and its relevance to the stress response and clinical implications. Particularly, based on findings on rhesus monkey *TPH2* genetics and other relevant literature, we propose that: 1) upon activation of adrenal cortisol secretion, the cortisol surge induces *TPH2* expression and *de novo* 5-HT synthesis; 2) the induced 5-HT in turn inhibits cortisol secretion by modulating the adrenal sensitivity to ACTH via the suprachiasmatic nuclei (SCN)-SNS-adrenal system, such that it contributes to the feedback inhibition of cortisol production; 3) basal *TPH2* expression or 5-HT synthesis, as well as early-life experience, influence basal cortisol primarily via the hormonal HPA axis; and 4) 5' - and 3' -regulatory polymorphisms of *TPH2* may differentially influence the stress response, presumably due to their differential roles in gene expression regulation. Our increasing knowledge of *TPH2* expression regulation not only helps us better understand the 5-HT-stress interaction and the pathophysiology of neuropsychiatric disorders, but also provides new strategies for the treatment of stress-associated diseases.

Keywords

TPH2; stress response; glucocorticoids; genetics; epigenetics

Introduction

All living organisms must maintain a complex dynamic equilibrium, or homeostasis, which is constantly challenged by internal or external, physical or emotional adverse environmental forces termed stressors. Stress can be defined as a state of threatened homeostasis under which a repertoire of physiological and behavioral adaptive responses are triggered to re-establish homeostasis (Chrousos, 2009; Chrousos and Gold, 1992). In some sense, the adaptive response to stressors, or the stress response, can be considered as a crosstalk between the organism and the environmental stimuli, which renders the organism

*Correspondence to Tel: 508-786-3323; Fax: 508-624-8166; guo-lin_chen@hms.harvard.edu.

Conflict of Interest Statement Dr. Guo-Lin Chen and Dr. Gregory M. Miller do not have any conflict of interest to report.

to make adaptation to the ever-changing environment. For this reason, it can be readily inferred that the stress response is determined by the interplay between factors of the organism (internal) and of the environment (external). While the genetic predisposition and pathophysiological conditions of an individual have a great impact on the stress response, stressful events during early-life (prenatal or postnatal) program the plasticity of the stress response in adults through epigenetic mechanisms such as DNA methylation and histone modification (Liu et al., 1997; Weaver, 2007; Weaver, 2009; Weaver et al., 2004). Hence, the stress response represents an excellent example of gene-environment (G×E) interactions and early-life programming of phenotypic traits manifested in later life, which contribute to the development of most, if not all, somatic and mental disorders (Dempfle et al., 2008; Ober and Vercelli, 2011; Tsuang et al., 2004).

The stress response is mediated by the stress system, a complex neuroendocrine, cellular and molecular infrastructure that locates in both the central nervous system (CNS) and the periphery. The central components of the stress system include the corticotropin-releasing hormone (CRH)/arginine-vasopressin (AVP) and locus ceruleus–noradrenaline (LC-NA)/autonomic (sympathetic) neurons of the hypothalamus and brainstem, which regulate the peripheral activities of the hypothalamic–pituitary–adrenal (HPA) axis and the systemic/adrenomedullary sympathetic nervous systems (SNS), respectively. Activation of the HPA axis and SNS results in systemic elevations of glucocorticoids and catecholamines, respectively, which act in concert to restore homeostasis (Chrousos, 2000). Particularly, the neuroendocrine HPA axis has long been the focus of research. Upon stimulation by stressors, the paraventricular nucleus (PVN) of the hypothalamus releases CRH, which reaches the anterior pituitary gland where it stimulates release of adrenocorticotrophic hormone (ACTH). ACTH is then transported by the blood to the adrenal cortex, where it stimulates the secretion of glucocorticoids (primarily cortisol in primates and corticosterone in rodents), the final products of the HPA axis, which exert an extensive effect on a wide variety of biological processes including metabolism, cardiovascular function, the inflammatory/immune response, as well as behavior and cognition, primarily by altering the expression of an array of genes. Meanwhile, glucocorticoid exerts negative feedback on CRH and ACTH secretion, such that the magnitude and duration of stress response are finely tuned. The effects of glucocorticoid are mediated by two types of intracellular steroid receptors, the mineralocorticoid (MR) or type I and the glucocorticoid (GR) or type II, receptors, which differ in binding affinity to glucocorticoid and mediate distinct but complementary actions (Chrousos, 2009; Chrousos and Gold, 1992). Moreover, other than its activation of ACTH and subsequent glucocorticoid release, CRH is widely distributed in extrahypothalamic circuits of the CNS and directly acts as a neuroregulator to coordinate the complex neuroendocrine, autonomic and behavioral response to stress, with many behavioral aspects of the stress response, such as locomotor activity, food intake, sexual behavior, sleep, arousal, anxiety, learning and memory formation, being attributable to neuronal CRH projections to brain structures such as the amygdale, hippocampus, and various cortical areas (Arborelius et al., 1999; Bale and Vale, 2004; Jose Bonfiglio et al., 2011).

The stress response is also modulated by a number of neurotransmitters and neuropeptides that interact with the HPA axis and SNS. One of the neurotransmitters, serotonin (5-hydroxy-tryptamine, 5-HT) - a biogenic monoamine implicated in many brain functions, has been long recognized for its involvement in the stress response. Evidence supporting this notion includes, but is not limited to, the following findings: 1) terminals of 5-HT neurons in the midbrain raphe nuclei project to the PVN of hypothalamus as well as to other regions relevant to the stress response (Liposits et al., 1987; Meyer-Bernstein and Morin, 1996; Pickard and Rea, 1997; Yamakawa and Antle, 2010); 2) 5-HT regulates GR expression and stress hormone release, and it contributes to early-life programming of the plasticity of HPA

axis response to stress (Andrews and Matthews, 2004; Boisvert et al., 2011; Ho et al., 2007; Laplante et al., 2002); 3) stressors and stress hormones (e.g. CRH and glucocorticoids), in turn modulate the synthesis/turnover and release of 5-HT in the midbrain (Dinan, 1996; Lowry, 2002); and 4) dysfunction of 5-HT, as well as a concomitant abnormality of the HPA axis, are observed in numerous stress disorders such as depression and anxiety (Dinan, 1996; Lowry, 2002). Hence, the central 5-HT system is a major target for the treatment of stress disorders. For example, the selective serotonin reuptake inhibitors (SSRIs), which enhance 5-HT neurotransmission by blocking the serotonin transporter (5-HTT)-mediated reuptake of 5-HT into presynaptic neurons, are widely used for the treatment of depression and anxiety. It is noteworthy that the interaction between 5-HT and stress is rather complicated with conflicting findings having been reported, presumably due to the complexity of the 5-HT receptor system and its subregion-specific effects, as well as the wide spectrum of stressors (Keeney et al., 2006). Owing to the 5-HT-stress interaction, variation in 5-HT neurotransmission caused by genetic or epigenetic factors may lead to alteration in the stress response and thereby phenotypic traits, i.e., the altered stress response may link 5-HT dysfunction to phenotypic consequences. Indeed, it has been reported that HPA axis reactivity mediates the link between a functional 5-HTT promoter polymorphism (5-HTTLPR) and depression (Gotlib et al., 2008).

5-HT neurotransmission comprises multiple consecutive processes including synthesis, storage/release, signaling, reuptake and metabolism, of which the first step - synthesis - is a critical modulator of 5-HT neurotransmission. The synthesis of 5-HT is initiated by the hydroxylation of the essential amino-acid tryptophan to 5-hydroxytryptophan (5-HTP) which is further decarboxylated to 5-HT. Tryptophan hydroxylase (TPH) catalyzes the first, rate-limiting step of 5-HT synthesis. It had been thought that TPH is derived from a single gene (now referred to as *TPH1*) until it was observed that *tph1* knockout mice expresses normal amounts of 5-HT in the brain but not in the periphery. This observation led to the identification of a second, neuron-specific TPH isoform (TPH2) that is encoded by a gene distinct from *TPH1* (Cote et al., 2003; Walther and Bader, 2003; Walther et al., 2003; Zhang et al., 2004). While TPH1 is predominantly expressed in the periphery (especially in the enterochromaffin cells of the gut) and the pineal gland, TPH2 is primarily expressed in the brain and enteric neurons (McKinney et al., 2005; Walther and Bader, 2003; Walther et al., 2003; Zhang et al., 2004; Zill et al., 2004b). The human TPH1 and TPH2 proteins share an overall amino-acid sequence identity of 71% and differ in aspects such as the solubility and kinetic properties (McKinney et al., 2005; Walther and Bader, 2003).

As the rate-limiting enzyme for the synthesis of central 5-HT, TPH2 is a key player in the modulation of 5-HT neurotransmission and is thus a promising target for the therapeutic treatment of psychiatric disorders, just like the case for 5-HTT and monoamine oxidase A (MAOA, the major enzyme for the degradation of 5-HT). Hence, TPH2 is of both pathophysiological and pharmacological significance, and for this reason TPH2 has received much attention since its identification in 2003, with a rapid expansion of literature in the past few years. While numerous studies have linked TPH2 genetic variance to a wide variety of behavioral traits and disorders, a limited number of studies on TPH2 gene expression regulation have not only reinforced, but also provided new insights into, the reciprocal interaction between 5-HT and the stress response, as well as the control of the biological clock. In this review, we summarize recent advance in TPH2 research, with a focus on TPH2 gene expression regulation and its relevance to the stress response.

I. TPH2 gene expression and its relevance to the stress response

TPH2 gene has been cloned in many species including the mouse, rat, human, chimpanzee, and rhesus macaque. The human TPH2 gene locates on chromosome 12 and it comprises 11 exons and 10 introns spanning about 95 kb, and the encoded product – TPH2 protein –

contains 490 amino acids with a molecular weight of 56 kDa (Walther and Bader, 2003; Walther et al., 2003). Intriguingly, a growing body of evidence indicates that TPH2 gene expression is highly inducible and is closely related to the stress response.

1. Spatio-temporal expression of TPH2

1) TPH2 expression at different developmental stages: Gutknecht et al. (2009) examined tph1 and tph2 expression at both mRNA and protein levels in the whole mouse brain at different developmental stages from embryonic day 9.5 (E9.5) to postnatal day 23 (P23) as well as in 2 and 4 month old adult. They found that while tph1 expression in the brain was negligible, the earliest detectable tph2 expression commenced at E11.25, increased exponentially until the end of gestation and peaked at birth, then maintained at intermediate to high levels throughout postnatal development and adult life. In addition, an earlier study which examined mouse brainstem tph1 and tph2 expression at three developmental stages (P7, P21 and 2-month, representing early-, late-development and adult, respectively) revealed tph2 expression at all stages while tph1 expression at the late developmental stage only (Nakamura et al., 2006). In catfish, tph2 mRNA expression in the whole brain was evident from 5 days post hatch (dph), and increased during the period of gonadal sex differentiation, which begins around 40-50 dph (Raghuvver et al., 2011). Accordingly, TPH2 expression starts at an early developmental stage and is maintained across the lifespan.

2) Tissue distribution of TPH2 expression: The tissue distribution of TPH2 expression has been investigated at the mRNA and protein levels in different species. In their first identification of TPH2, Walther et al. (2003) employed the ribonuclease protection assay (RPA) to clarify tissue distribution of tph2 mRNA in mouse, showing that tph2 was exclusively expressed in the brain but not in peripheral tissues. By using the quantitative real-time PCR (qRT-PCR) assay, Gutknecht et al. (2009) confirmed the expression of mouse tph2 mRNA in numerous brain regions, including the raphe nuclei, cortex, striatum, hippocampus and cerebellum, with the highest expression in raphe nuclei where the majority of the 5-HT neurons reside. In rat, *in situ* hybridization (ISH) assay using tph2-specific oligoprobe revealed that tph2 mRNA is almost exclusively expressed in midbrain raphe nuclei (Malek et al., 2005; Patel et al., 2004). In human, postmortem analysis of brain tissues by quantitative real-time PCR also demonstrated that TPH2 mRNA expression is abundant in raphe nuclei or raphe nuclei-containing regions such as the pons and medulla oblongata, while it is detectable in a number of other regions including the cortex, hypothalamus, thalamus, hippocampus, amygdala and cerebellum (Bach-Mizrachi et al., 2006; De Luca et al., 2006; Gutknecht et al., 2009; Zill et al., 2007). At the protein level, Western blotting and immunocytochemistry/histochemistry assays using TPH2-specific antibodies have been performed to explore TPH2 expression in rodent and human tissues, and despite some discrepancy, these findings further supported abundant TPH2 expression in raphe nuclei as well as detectable TPH2 expression in other brain regions including the pituitary, hypothalamus, mesencephalic tegmentum, striatum, hippocampus and pineal gland (Clark et al., 2008; Gutknecht et al., 2009; Sakowski et al., 2006). In addition, high expression of tph2 mRNA and protein was observed in cell bodies of the rat ventral tegmental area (VTA), another site of 5-HT neurons besides raphe nuclei (Carkaci-Salli et al., 2011), suggesting that the wide detection of TPH2 in the brain may result from the presence of 5-HT neurons outside the raphe nuclei; however, it may also be attributable to the extensive projection of 5-HT fibers.

TPH2 expression was thought to be exclusively expressed in the brain but not periphery; however, it was reported in rodents that tph2 mRNA is expressed in enteric neurons of the gut, as well as in several specialized neuroepithelial cells including the taste receptor cells of

the taste buds (Ortiz-Alvarado et al., 2006), cholangiocytes of the bile duct (Omenetti et al., 2011), and the retinal pigment epithelium (Liang et al., 2004; Zmijewski et al., 2009). Interestingly, the catfish tph2 mRNA was detected not only in brain regions including the preoptic area-hypothalamus (POA-HYP) region, optic-cerebellum-thalamus (OCT) region, olfactory bulb, telencephalon and medulla oblongata, but also in numerous peripheral tissues, including muscle, gill, heart, kidney, liver, testis (but not ovary) and spleen (Raghuveer et al., 2011). Moreover, it was found in mouse that placental lactogens during pregnancy and high fat diet potentially induce tph2 mRNA expression in the islets of Langerhans (regions of the pancreas that contain the endocrine cells) and adipose tissues, respectively (Hageman et al., 2010; Schraenen et al., 2010). Taken together, these findings strongly suggest that although TPH2 was thought to be neuron-specific and is predominantly expressed in the brain, it may also be expressed or induced to express in peripheral tissues.

3) Daily rhythmicity of TPH2 expression: A notable feature of TPH2 expression is that it displays a circadian rhythmicity. By using the qRT-PCR assay, Liang et al. (2004) found that tph2 mRNA in rat retina, though expressed at a very low level, exhibits a significant variation during the light/dark (LD) cycle with a peak in the night. In accordance with this finding, by using the ISH assay, Malek et al. (2005) showed that tph2 mRNA was abundantly expressed in rat median raphe (MR) and dorsal raphe (DR) with a significant daily variation during the LD cycle, with the highest expression at Zeitgeber Time (ZT) 10 - 2 hours prior to the light-dark transition. Notably, this daily rhythmic tph2 mRNA expression was correlated with the previous report of circadian variations in TPH protein level and 5-HT synthesis in serotonergic neurons projecting to the hypothalamic suprachiasmatic nuclei (SCN) and the thalamic intergeniculate leaflets (IGL) – both are involved in the entrainment of the biological clock (Malek et al., 2005). Similarly, it was demonstrated that tph2 mRNA and TPH protein levels in dorsal and median raphe display daily fluctuations in hamsters held in long photoperiod (Nexon et al., 2009).

2. Relevance of TPH2 expression to the stress response—Since the HPA axis function is under the control of the biological clock, the rhythmic TPH2 gene expression in the retina and midbrain neurons projecting to the circadian system (SCN and IGL) suggests that TPH2 might be implicated in the stress response. Indeed, a close relationship between TPH2 expression and the stress response is supported by at least two lines of evidence: 1) TPH2 expression is modulated by stressors and stress hormones; and 2) altered TPH2 expression is linked to the stress response and stress-related disorders.

1) Regulation of TPH2 expression by glucocorticoids and stressors: Glucocorticoids affect tph2 expression and 5-HT neurotransmission in both mice and rats, but the direction of effect seems opposite in the two species. In both ovariectomized female and intact male mice, repeated administration of the synthetic glucocorticoid dexamethasone induced a significant decrease in raphe tph2 expression at both mRNA and protein levels, and this effect was abolished by co-administration of mifepristone - an antagonist of GR (Clark et al., 2008; Clark et al., 2005). In agreement, adrenalectomy, which deprives glucocorticoid production, resulted in an elevation of tph2 mRNA levels in mouse raphe nuclei, and such effect was reversed by glucocorticoid replacement (Heydendaal and Jacobson, 2009), supporting down-regulation of tph2 expression by glucocorticoids in the mouse. In contrast, it was reported in rat that glucocorticoid induced tph2 mRNA expression (Malek et al., 2007) and enhanced 5-HT neurotransmission (Barr and Forster, 2011), while adrenalectomy led to a decrease of TPH level and 5-HT synthesis in the brain (Azmitia et al., 1993; De Kloet et al., 1982; Singh et al., 1990; Vanloon et al., 1981). More recently, Donner et al. (2011) reported that chronic administration of corticosterone induced tph2 mRNA expression in the DR of intact male rats during the animals' inactive light phase, such that

the diurnal pattern of tph2 expression was abolished. It is notable that a previous study using tph1-based primers and probes revealed a tissue-specific effect of glucocorticoid on rat tph mRNA expression (Clark and Russo, 1997). Accordingly, glucocorticoid regulation of TPH2 expression is species-dependent and may be tissue-specific.

In agreement with the glucocorticoid regulation of tph2 gene expression, the circadian rhythmic tph2 expression is dependent on the daily fluctuation of glucocorticoids. In both rat and hamster, adrenalectomy led to a complete suppression of the daily rhythmic tph2 mRNA expression in raphe nucleus, while restoration of glucocorticoid daily variations in adrenalectomized animals induced the tph2 mRNA rhythmic pattern *de novo* (Malek et al., 2007; Nexon et al., 2009). These findings, along with the previously reported correlation between rhythmic TPH protein level and 5-HT synthesis in neurons projecting to the SCN and IGL, strongly suggest that the fluctuation of TPH2 gene expression is closely related to the biological clock and the stress response.

TPH2 gene expression is also sensitive to stressful events or stressors (Brown et al., 2006; Gardner et al., 2009; Mueller and Bale, 2008; Rahman and Thomas, 2009), presumably due to the stress-induced alteration in glucocorticoid levels. For example, hypotensive haemorrhage triggers a significant increase of tph2 mRNA level in the rat caudal midline medulla (Brown et al., 2006), while exposure to hypoxia leads to a significant decline of tph2 mRNA and protein levels in the hypothalami of the Atlantic croaker (Rahman and Thomas, 2009). Interestingly, in conflict with the above-mentioned glucocorticoid inhibition of mouse tph2 expression, exposure of adult male mice to chronic variable stress elevates tph2 mRNA expression in both DR and MR (especially in MR which projects to the SCN) (McEuen et al., 2008). More recently, it has been documented that early-life experience (maternal separation) and adulthood stressful event (social defeat) interact to determine tph2 mRNA expression in the rat dorsal raphe nucleus (Gardner et al., 2009). In line with the glucocorticoid regulation of TPH2 expression, these findings reinforced the relevance of TPH2 gene expression to the stress response.

2) A link between altered TPH2 expression and the stress response: TPH2 is primarily expressed in the brain, which is not easily accessible, making it infeasible to measure TPH2 expression *in vivo* under different pathophysiological conditions. Fortunately, genetically manipulated animals as well as naturally occurring genetic polymorphisms provide an opportunity to examine the effect of altered TPH2 expression/function on phenotypic traits. In addition, postmortem analysis of brain tissues has also been performed to evaluate the link between TPH2 expression and behavioral traits; however, it should be pointed out that postmortem analysis has some limitations: 1) the circadian rhythm of TPH2 expression may considerably confound the findings; 2) it cannot reflect the dynamic TPH2 expression in response to stressors; and 3) it cannot address whether altered TPH2 expression is causative for or resulted from a specific pathophysiological condition.

a. Genetically manipulated animals: Tph2 knockout (-/-) mice, which lack 5-HT in the brain, can be born and survive but show growth retardation and increased lethality in early life, as well as altered autonomic control as indicated by abnormalities in sleep, respiration, body temperature, blood pressure and heart rate (Alenina et al., 2009). Tph2(-/-) female mice, despite being fertile and producing milk, exhibit impaired maternal care leading to poor survival of their pups (Alenina et al., 2009). In addition, it was reported in rat that overexpression or knockdown of tph2 affects anxiety behavior, which is related to the stress response, in an estrogen-dependent manner (Hiroi et al., 2011). These findings suggest that TPH2-derived 5-HT not only contributes to behavior regulation, but also is involved in the regulation of autonomic and metabolic pathways, of which the former is a critical nervous system that controls the stress response while the latter is a major target of stress hormones.

Moreover, Liu et al. (2011) reported that male *tph2*-KO mice lost sexual preference although they were not generally defective in olfaction or in pheromone sensing, suggesting that *tph2* gene expression may modulate mammalian sexual preference.

b. Naturally occurring genetic polymorphisms: Functional genetic polymorphisms that alter gene expression/function may serve as a natural loss- or gain-of-function model for the investigation of gene function. Basically, polymorphisms in the regulatory regions (e.g. promoter and 5'- or 3'-UTR) may transcriptionally or posttranscriptionally modulate gene expression, while polymorphisms in the coding region may change amino acid sequence and therefore function of the gene product, but may in some cases affect gene expression transcriptionally or posttranscriptionally (Chen and Miller, 2008; Griseri et al., 2011; Knight, 2005; Pastinen et al., 2006; Wang et al., 2005). In addition, intronic polymorphisms also have the potential to affect mRNA splicing and gene expression. Genetic polymorphisms in *TPH2* have been widely investigated in human, nonhuman primate and rodent, with numerous phenotypic traits or disorders having been linked to *TPH2* gene variation. In particular, a systematic analysis of *TPH2* genetics in the nonhuman primate not only provided unprecedented evidence for the effect of *TPH2* genetics and its interaction with early-life experience on the stress response, but also shed new light on the well-established 5-HT-stress interaction. Details of the genetic regulation of *TPH2* expression and its relevance to the stress response, with a focus on *TPH2* genetics in nonhuman primate, will be addressed hereinafter in Section II of this review.

c. Postmortem analysis of human brain tissues: Bach-Mizrachi et al. (2006) reported that *TPH2* mRNA expression in drug-free suicides is 33% higher in dorsal raphe nucleus (DRN) and 17% higher in the median raphe nucleus (MRN) as compared to matched nonpsychiatric controls, and later the same group found that *TPH2* mRNA expression is elevated at the neuronal level in the dorsal and median raphe nuclei of depressed suicides (Bach-Mizrachi et al., 2008). Similarly, Perroud et al. (2010) found that *TPH2* mRNA levels in the ventral prefrontal cortex (VPFC) are significantly higher in suicide completers than that in controls, and suggested that the elevated *TPH2* expression may represent an upregulatory homeostatic response to deficient brain 5-HT neurotransmission. In addition, Duncan et al. (2010) reported that the *TPH2* protein and 5-HT levels in sudden infant death syndrome (SIDS) cases were 22% and 26% lower, respectively, compared with age-adjusted controls.

II. Genetic regulation of *TPH2* expression

1. Nonhuman primate *TPH2* genetics

Nonhuman primates share high genetic, physiological and behavioral similarities with humans, and therefore have an advantage over rodents to model human pathophysiology, especially for neuropsychiatric disorders. Rhesus monkeys are the most widely used nonhuman primates in biomedical research. As for the rhesus monkey *TPH2* (*rhTPH2*), Chen et al. (2006; 2008; 2010b) identified and characterized a number of regulatory and coding polymorphisms that significantly affect gene expression *in vitro*, of which a single nucleotide polymorphism (SNP) - 2051A>C in the 3'-UTR is worthy of particular attention.

1) 2051A>C influences *in vitro* gene expression and *in vivo* cortisol

production—Regardless of the driving promoter or cell line examined, the 2051A>C SNP exerts a significant effect on gene expression as indicated by *in vitro* reporter assay, with the C allele exhibiting lower expression than the A allele. Mechanism(s) by which 2051A>C influences gene expression are yet to be determined; however, this SNP is predicted to change the secondary structure and free energy of *TPH2* mRNA, with the C allele eliminating a major stem-loop structure and possessing higher free energy (i.e. lower

stability), and indeed, preliminary data showed that the 2051C allele is associated with lower mRNA stability as compared with the 2051A allele (Chen et al., 2006; Chen et al., 2010b).

The *in vivo* effect of 2051A>C was evaluated by genotype-phenotype correlation analysis in a cohort of 32 male, Indian-origin rhesus monkeys that had been physiologically and behaviorally characterized. Interestingly, animals homozygous for the C allele (i.e. CC genotype) showed significantly higher cortisol levels in the early morning (8:30 – 9:00 h) and in response to ACTH challenge (especially at 30-min post ACTH administration) as compared with the A-allele carriers (AA/AC genotype), with about 47.9% variation of the morning cortisol level being explained by this SNP. Thus, the 2051C allele predictive of reduced TPH2 expression (i.e. *de novo* 5-HT synthesis) is linked to increased cortisol production (Chen et al., 2006). Considering the co-existence of 5-HT deficit and cortisol hypersecretion under numerous pathological conditions, these findings not only suggest a causative link between 2051A>C and cortisol production, but also indicate that TPH2 expression, which is modulated by glucocorticoid, in turn regulates glucocorticoid secretion, i.e., that there is a reciprocal interaction between TPH2 expression and the stress response, adding to the well recognized 5-HT-stress interaction. It is notable that 2051A>C affects cortisol level in the early morning (circadian-induced) and in response to ACTH challenge when a burst of cortisol secretion is expected, but not in the afternoon when low cortisol secretion is expected, suggesting that 2051A>C regulation of cortisol is dependent on the status of cortisol secretion.

While 2051A>C influences morning cortisol and cortisol response to ACTH challenge, it exerts no significant effect on the cerebrospinal fluid (CSF) CRH and plasma ACTH levels in the morning, suggesting the 2051A>C regulation of cortisol is unlikely mediated by alteration in CRH and ACTH via the hormonal HPA axis, but rather, may be attributable to altered adrenal response to ACTH (Chen et al., 2006; Chen et al., 2010a). In addition, the plasma ACTH level showed no significant AM-PM fluctuation in the cohort of 32 monkeys examined by Chen et al (2006). Accordingly, these findings suggest the disassociation of ACTH and cortisol, or ACTH-independent cortisol production, a phenomenon observed under many physiological and pathophysiological conditions (Bornstein et al., 2008). Since currently there is no evidence for TPH2 expression in adrenal gland, the ACTH-independent 2051A>C regulation of cortisol gives rise to a question - how does the genetically determined TPH2 expression (or 5-HT synthesis) in the brain affect adrenal cortisol production without activating the hormonal HPA axis? This question will be discussed hereinafter.

2) 2051A>C interacts with rhTPH2 5'-FR -1485(AT)_n and early-life experience to affect the stress response—Besides the 2051A>C, a number of polymorphisms in the rhTPH2 promoter (i.e. 5'-flanking region, 5'-FR) also affect gene expression and may thus affect 5-HT synthesis and the stress response (Chen et al., 2010b). In addition, it has been well-documented that early-life experience (e.g. maternal care) programs the plasticity of the HPA axis response to stress and interacts with genetic predisposition to determine the stress response as well as the development of phenotypic traits in late life. For this reason, Chen et al. (2010a; 2010b) examined whether or not 2051A>C interacts with rhTPH2 5'-FR polymorphisms and early-life experience to modulate the stress response. Notably, the 2051A>C regulation of cortisol induced by circadian and ACTH challenge was not obscured by either rhTPH2 promoter polymorphisms or rearing condition, while the 5'-FR -1485(AT)_n - a dinucleotide repeat polymorphism and rearing condition was linked to the PM cortisol and AM CRH levels, respectively. In addition, 2051A>C interacts with -1485(AT)_n and rearing condition to influence the HPA axis activity, especially the plasma ACTH level and the HPA negative feedback as indicated by the dexamethasone suppression test (DST) (Chen et al., 2010a).

In contrast to 2051A>C which affects cortisol production upon high cortisol secretion, the -1485(AT)n is associated with the PM cortisol level, with carriers of the (AT)6 allele (predictive of reduced TPH2 expression) showing lower PM cortisol than non-carriers of the (AT)6 allele. Hence, the 3'-UTR 2051A>C and 5'-FR -1485(AT)n are differentially associated with induced and basal cortisol production, respectively. In addition, 2051A>C and -1485(AT)n interact to influence the PM ACTH level as well as the nocturnal HPA negative feedback, with animals genotyped as CC/(AT)6-noncarrier showing the lowest PM ACTH level and the highest suppression of urinary cortisol excretion by dexamethasone in the night.

It has been established in rodents that maternal care may permanently influence the HPA axis response to stress, with pups lacking maternal licking/grooming exhibiting reduced hippocampal GR expression and therefore decreased HPA negative feedback and increased glucocorticoid production (Liu et al., 1997; Weaver, 2007; Weaver et al., 2004). In agreement, Chen et al. (2010a) demonstrated in rhesus monkeys that rearing condition significantly affects the CSF CRH production, with mother-reared (MR) monkeys (expected to express more GR) exhibiting markedly lower CRH levels than peer-reared (PR) monkeys (expected to express less GR). Interestingly, rearing condition exerts no significant effect on cortisol levels in the morning or in response to ACTH challenge, but does interact with 2051A>C to influence the PM cortisol as well as the ACTH and HPA negative feedback, with the 2051CC-PR and 2051AA/AC-MR monkeys having the lowest PM ACTH level and the highest HPA negative feedback, respectively (Chen et al., 2010a).

3) Mechanisms by which rhTPH2 polymorphisms and early-life experience modulate the stress response—It can be inferred that regulation of the stress response by rhTPH2 polymorphisms is mediated by alteration in 5-HT neurotransmission; however, as mentioned in the *Introduction*, the 5-HT-stress interaction is rather complicated and conflicting findings have been reported. Based on the findings of TPH2 genetic and rearing regulation of the stress response in rhesus monkey, as well as literature on the hormonal HPA axis and neuronal SNS pathways in the regulation of the stress response, we speculate that 2051A>C regulation of circadian-induced and ACTH-challenged cortisol production is primarily mediated by the neuronal regulation of the adrenal response to ACTH, while the -1485(AT)n and rearing modulation of the stress response is mainly mediated by the hormonal pathway.

a. Mechanism(s) for 2051A>C regulation of cortisol: involvement of 5-HT modulation of adrenal response to ACTH via the SCN-SNS system: Besides receiving hormonal regulation through ACTH stimulation, the adrenal gland is also innervated by preganglionic sympathetic fibers (or splanchnic nerve), with a growing body of evidence suggesting that the adrenal response to ACTH is controlled by the SCN-SNS system (Dickmeis, 2009). It has been recognized that light stimuli are the most important synchronizer or “zeitgeber” of circadian rhythms in mammals, allowing the adaptation of biological functions to the environmental day/night cycle. As a light-entrainable circadian oscillator, the SCN is critically important for the expression of behavioral and physiological circadian rhythms. In mammals, light signals received in retinal photoreceptor cells are converted into nerve pulses, which are then conveyed to the SCN by the retinal ganglion cells and entrain the circadian clock to environmental day/night cycles. It was recently reported in rodents that light induces the adrenal gland to secrete corticosterone via the SCN-SNS pathway without affecting ACTH secretion (Ishida et al., 2005), and that the adrenal splanchnic (i.e. SNS) innervation contributes to the diurnal rhythm of corticosterone release by modulating adrenal sensitivity to ACTH (Ulrich-Lai et al., 2006). Both SCN lesion and adenosympathetic nerve transection eliminate the light-induced corticosterone response and the diurnal rhythm of plasma corticosterone (Ishida et al., 2005; Ulrich-Lai et al., 2006).

Accordingly, these findings suggest that: 1) the neuronal SCN-SNS system controls the adrenal response to ACTH, which contributes to the daily fluctuation of glucocorticoid production; 2) the glucocorticoid surge induced by the night-day transition in the early morning, which is usually referred to as the cortisol awakening response (CAR) in human, is conferred primarily by light-induced neuronal modulation of the adrenal response to ACTH rather than by hormonal activation of the HPA axis; and 3) the SCN-SNS-adrenal pathway provides a mechanism for neuronal regulation of glucocorticoid without affecting the hormonal HPA axis.

It has been established that the SCN receives a dense serotonergic innervation arising from the midbrain raphe nuclei, and that 5-HT is involved in both photic and non-photic synchronization of the mammalian biological clock in the SCN, with abundant evidence implicating 5-HT as an inhibitory neurotransmitter that modulates the effects of light on circadian rhythms (Glass et al., 2003; Meyer-Bernstein and Morin, 1996; Meyer-Bernstein and Morin, 1999; Pickard and Rea, 1997; Yamakawa and Antle, 2010). Thus, 5-HT may exert an inhibitory effect on the light-induced morning cortisol surge, with high 5-HT input to the SCN rendering a low AM cortisol level, or *vice versa*. In agreement with this notion, rhesus monkeys homozygous for the 2051C allele (predictive of reduced TPH2 expression or *de novo* 5-HT synthesis) produced strikingly higher morning cortisol than 2051AA/AC monkeys (carriers of the 2051A allele predictive of high TPH2 expression) (Chen et al., 2006). This notion is also supported by the finding that 5-HT deficit and cortisol hypersecretion co-exist in numerous stress disorders (e.g. depression). In addition, findings from tph2-KO mice (Alenina et al., 2009) and the tryptophan depletion test (van Veen et al., 2009) also provided evidence for the 5-HT modulation of SNS. Thus, it can be inferred that 2051A>C regulation of cortisol in the rhesus monkey is primarily mediated by the midbrain-SCN-SNS-adrenal gland pathway; however, other mechanisms cannot be excluded because: 1) rhythmic TPH2 expression also exists in the retina where light signals are received and processed before being transferred to the SCN (Liang et al., 2004; Zmijewski et al., 2009); and 2) 5-HT fibers may directly innervate the adrenal gland (Fernandezvivero et al., 1993).

Since 2051A>C regulation of cortisol is dependent on the status of high cortisol secretion, it is tempting to speculate that: 1) upon activation of adrenal cortisol secretion, the burst of cortisol release induces TPH2 expression and *de novo* 5-HT synthesis; 2) the induced 5-HT synthesis in turn inhibits cortisol secretion by modulating adrenal sensitivity to ACTH via the SCN-SNS system, i.e., cortisol regulation of TPH2 expression may initiate a neuronal negative feedback for the control of cortisol secretion; and 3) 2051A>C affects cortisol-induced TPH2 expression and therefore 5-HT-mediated negative control of cortisol secretion. In support of these presumptions, 2051AA/AC monkeys (predictive of high TPH2 expression) showed significantly higher dexamethasone suppression of urinary free cortisol excretion as compared with 2051CC monkeys (predictive of low TPH2 expression), especially during the day-time with higher cortisol secretion as compared with night-time (Chen et al., 2006). In addition, there is a significant decline of cortisol from 15 to 30 min post ACTH-administration in 2051AA homozygotes, suggesting the existence of a negative feedback that commences as early as 15-30 minutes post ACTH-administration. By contrast, such decline is not observed in 2051CC homozygotes, presumably due to low TPH2 expression and deficient negative feedback (Chen et al., 2006). The time frame of the cortisol production profile post ACTH administration supports the notion that 2051A>C regulation of cortisol involves the relatively faster neuronal SNS rather than the slower hormonal HPA axis. In agreement, it has been reported that GR which plays critical roles in hormonal regulation of glucocorticoids is involved in delayed (4~12 h) but not fast negative feedback following administration of the synthetic glucocorticoid methyl-prednisolone in rats (Spiga et al., 2008). Accordingly, the higher circadian-induced and ACTH-challenged cortisol production in 2051CC monkeys is primarily caused by deficiency in 5-HT-mediated

negative feedback, which is mediated by the neuronal regulation of adrenal sensitivity to ACTH. Notably, the involvement of 5-HT in the negative feedback of cortisol secretion was also suggested by a recent study, which revealed that the 5-HTTLPR polymorphism predicts the waking cortisol level in young girls (Chen et al., 2009).

Molecular mechanism(s) underlying the 2051A>C regulation of glucocorticoid-induced TPH2 expression is still unknown; however, it may be relevant to mechanism(s) by which glucocorticoids modulate TPH2 expression. It is well recognized that the 3'-UTR modulates gene expression by post-transcriptional mechanism(s), such as mRNA stability, translocation and translation, and that glucocorticoids can modulate gene expression by posttranscriptional as well as transcriptional mechanisms. For example, it has been reported that glucocorticoids posttranscriptionally regulate the expression of an increasing number of genes, such as cyclin D3, surfactant protein-B (SP-B) and inducible nitric oxide synthase (iNOS), by affecting mRNA stability that involves the 3'-UTR (Garcia-Gras et al., 2000; Huang et al., 2008; Ozaki et al., 2010). Interestingly, glucocorticoid regulation of mRNA stability is not necessarily dependent on activated GR (Tillis et al., 2011). As for the 2051A>C in 3'-UTR of rh $TPH2$, preliminary data showed that it has a significant effect on mRNA stability (Chen et al., 2010b). Hence, it is very likely that glucocorticoids modulate $TPH2$ expression by affecting mRNA stability, which might be influenced by 2051A>C via a mechanism not necessarily dependent on GR. Nevertheless, this presumption needs to be verified by further studies.

b. Potential mechanism(s) by which rh $TPH2$ -1485(AT)n and rearing condition regulate cortisol: involvement of the hormonal HPA axis pathway:

It has been well established that early-life experience (e.g. maternal care) programs the plasticity of the HPA axis response to stress by epigenetic regulation of hippocampal GR expression, which in turn alters the negative feedback control of CRH and ACTH release and consequently glucocorticoid production. Thus, it can be readily inferred that early-life programming of the stress response involves the hormonal HPA axis pathway. In rhesus monkeys, early-life rearing condition exerts a significant effect on the morning CSF CRH level but not the circadian-induced or ACTH-challenged cortisol production (Chen et al., 2010a). This finding not only strengthens the involvement of the hormonal HPA axis in early-life programming of stress response plasticity, but also supports our above-mentioned presumption that circadian-induced or ACTH-challenged cortisol production is primarily modulated by the neuronal SNS rather than the hormonal HPA axis. However, since there is a weak tendency for mother-reared monkeys to have lower morning cortisol but higher ACTH-challenged cortisol than peer-reared monkeys, a minor, non-significant effect of rearing condition (or hormonal HPA axis) on the circadian-induced and ACTH-challenged cortisol production may exist (Chen et al., 2010a).

5-HT modulation of the stress response involves not only neuronal, but also hormonal mechanisms. It has been documented that 5-HT activates CRH expression in hypothalamic PVN (Boisvert et al., 2011; Heisler et al., 2007; Ho et al., 2007), while it induces hippocampal GR expression during specific time periods of development (Andrews and Matthews, 2004). In contrast to its negative effect on the neuronal SCN-SNS system, 5-HT likely exerts an overall excitatory effect on the hormonal HPA axis. While 2051A>C-affected TPH2 expression exerts a significant negative effect on the neuronal SNS, its excitatory effect on the hormonal HPA axis is negligible as indicated by a weak tendency for 2051CC homozygotes (predictive of low TPH2 expression) to have lower CRH than 2051AA/AC monkeys (Chen et al., 2010a). Like rearing condition, the -1485(AT)n in rh $TPH2$ 5'-FR shows no significant effect on circadian-induced or ACTH-challenged cortisol production, but is associated with PM cortisol levels and interacts with 2051A>C to influence PM ACTH levels, suggesting that in contrast to 2051A>C, -1485(AT)n influences

cortisol under basal or low cortisol secretion, presumably by affecting the hormonal HPA axis rather than the neuronal SNS. Mechanism(s) underlying the differential effect of rh *TPH2* 5' and 3' polymorphisms on the stress response have yet to be determined, but may involve the complexity of the 5-HT-stress interaction, the feature of *TPH2* gene expression, as well as the differential role of 5' and 3' regions in gene expression regulation. Moreover, considering the heterogeneity of 5-HT neurons, it is possible that glucocorticoids selectively and potentially modulate *TPH2* expression in specific subsets of 5-HT neurons that project to the SCN-SNS system but not in 5-HT neurons that innervate the hormonal HPA axis.

Both the 5'-FR and 3'-UTR play roles in the regulation of gene expression. While the 5'-FR modulates gene transcription, the 3'-UTR regulation of gene expression primarily involves post-transcriptional processes including mRNA stability, translocation and translation.

TPH2 exhibits a low level of basal (or constitutive) transcription, which might be influenced transcriptionally by 5'-FR polymorphism(s) or posttranscriptionally by 3'-UTR polymorphism; however, upon the stimulation by the circadian rhythm or stressors, *TPH2* transcription is induced considerably such that the influence of the 5'-FR polymorphism(s) on basal transcription is obscured unless the polymorphism *per se* affects the transcriptional activation, but instead post-transcriptional processes that might be affected by 3'-UTR polymorphism function as the major determinant of *TPH2* expression. In other words, functional 5'-FR and 3'-UTR polymorphisms may preferentially exert their effects under low (basal or constitutive) and high (induced) transcription of *TPH2*, respectively, which likely coincides with low and high status of cortisol secretion. In support of this presumption, Chen et al. (2008) demonstrated *in vitro* that h *TPH2* 5'-FR polymorphisms affect reporter gene expression in the presence of the repressive h *TPH2* 5'-UTR which renders a poor transcription, but such effect disappears when gene expression is dramatically increased by the deletion of 5'-UTR. In addition, preliminary data suggests that 2051A>C has a significant effect on mRNA stability (Chen et al., 2010b). Accordingly, it is very likely that basal 5-HT primarily modulates cortisol production by activating the hormonal HPA axis, whereas the cortisol-induced *de novo* 5-HT negatively regulates cortisol production via the neuronal SNS. And so, cortisol-induced *TPH2* expression or *de novo* 5-HT synthesis, which can be influenced by functional 3'-UTR polymorphism, may represent an alternative, neuronal mechanism for the intermediate negative feedback control of cortisol production. In this regard, the basal and induced cortisol production could be modulated by differential mechanisms, which may explain the differential association of AM and PM cortisol levels with personality traits of confidence and excitability, respectively, in rhesus monkeys (Capitanio et al., 2004). Moreover, the differential effects of rh *TPH2* 5' and 3' polymorphisms on cortisol production suggest that biological function of a regulatory polymorphism depends not only on whether it affects gene expression or not, but also on the mechanism(s) by which it affects gene expression.

2. Human *TPH2* genetics

There is one common SNP (1952G>A) in the 3'-UTR of h *TPH2* which parallels in position with 2051A>C in rh *TPH2*; however, this SNP does not affect gene expression *in vitro* (Chen et al., 2006). Instead, three common SNPs (-703G>T, -473T>A and 90A>G) in the 5' regulatory region of h *TPH2* exert a significant effect on gene expression *in vitro*, despite conflicting findings reported by different groups (Chen et al., 2008; Lin et al., 2007; Scheuch et al., 2007). While the functional significance of -473T>A was consistently demonstrated by all the studies, -703G>T was reported to be functional by Chen et al. (2008) and Lin et al. (2007), but not by Scheuch et al. (2007). The 90A>G in the 5'-UTR of *TPH2* was not examined by Lin et al. (2007) and was reported to exert no effect on gene expression by Scheuch et al. (2007); however, Chen et al. (2008) found this SNP exerts a significant effect on gene expression, in support of the critical role of the 5'-UTR in the

regulation of *TPH2* gene expression (Chen et al., 2008; Patel et al., 2007). Consistently, the 90A>G was later reported to be associated with TPH2 gene expression in lymphoblastoid cell lines derived from Prader-Willi syndrome subjects with differing TPH2 genotypes (Henkhaus et al., 2010). As for the underlying mechanisms, all three SNPs are predicted to alter the putative binding sites of specific transcription factors (TFs) (Chen et al., 2008). In particular, it was reported that both -703G>T and -473T>A alter the binding of the transcription factor POU3F2 to the *TPH2* promoter (Lin et al., 2007; Scheuch et al., 2007).

More recently, Zhang et al. (2010a) reported that a common SNP (G>A, rs1386493) in intron 5 of h*TPH2* decreases efficiency of normal RNA splicing and results in a truncated TPH2 protein (TPH2-TR) by alternative splicing. This TPH2-TR lacks TPH2 enzyme activity but dominant-negatively affects full-length TPH2 function, causing reduced 5-HT production (Zhang et al., 2010a).

3. Rodent TPH2 genetics

No functional polymorphism has been documented in the 5' - and 3' -regulatory regions of rodent *tph2*; however, a non-synonymous SNP – 1473C>G (447Pro>Arg), which results in reduced *tph2* activity and 5-HT synthesis but unchanged *tph2* gene expression, has been identified in inbred mouse strains (Zhang et al., 2004). It was determined that C57Bl/6 and 129X1/SvJ mice are homozygous for the 1473C allele, whereas DBA/2 and BALB/cJ mice are homozygous for the 1473G allele (Zhang et al., 2004); however, this 1473C>G polymorphism is in fact a rare mutation in wild mice with the frequency of the variant 1473G allele being lower than 0.5%, possibly due to the forces of natural selection (Osipova et al., 2010).

III. Epigenetic or environmental regulation of TPH2 expression

Epigenetics, which refers to heritable change in gene expression caused by mechanisms other than changes in DNA sequence, contributes to tissue-type and developmental stage specific gene expression. Epigenetic mechanisms encompass an array of modifications to DNA and chromatin (e.g. DNA methylation and histone acetylation, which may interact with other factors to change chromatin structure and the accessibility of DNA *cis*-elements to TFs), as well as non-coding RNA, alternative RNA splicing, and mRNA degradation. Epigenetic modifications are dynamic and can be changed by environmental factors including stressful events, especially at developing stages in early life, such that they constitute a molecular basis underlying G×E interactions (Bollati and Baccarelli, 2010; Feinberg, 2007; Jirtle and Skinner, 2007). TPH2 gene expression is tissue-specific and responsive to stressors, including adverse experience during early life and adulthood (Gardner et al., 2009; Mueller and Bale, 2008). Hence, epigenetic mechanisms may play important roles in the regulation of *TPH2* expression.

1. cis-acting elements involved in TPH2 gene expression

By using serial deletion analysis, Chen et al. (2008) localized the core promoter of h*TPH2* to the region between nt -107 and 7 (the transcription start site designated as 1), while Lenicov et al. (2007) revealed a minimal basal promoter of h*TPH2* between nt -88 and 1, which contains a calcium-responsive element that mediates cell-type specific induction of *TPH2* transcription by calcium mobilization. It is notable that the h*TPH2* 5'-UTR, which is of only 141-bp in length but enriched in GC contents (~60%), plays a critical role in the regulation of TPH2 gene expression, with the upstream segment (nt 8~53) repressing gene expression while the downstream portion (nt 61~141) harbors an asymmetric bidirectional promoter activity (Chen and Miller, 2009; Chen et al., 2008). In agreement, the upstream segment (nt 9~35) of h*TPH2* 5'-UTR contains a binding-motif for the neuron-restrictive silencer factor

(NRSF; also known as REST for repressor element-1 silencing transcription factor) which mediates transcriptional repression (Patel et al., 2007). Thus, it is likely that the 5'-UTR serves as an "on-off" switch for the regulation of *TPH2* expression.

The bidirectional promoter in the downstream segment of *TPH2* 5'-UTR may also regulate *TPH2* gene expression (Chen and Miller, 2009). Notably, it has been documented that a number of genes use promoters of different strength to direct tissue type and/or developmental stage specific expression (Davuluri et al., 2008), suggesting that the sense promoter in *TPH2* 5'-UTR, though much weaker than the normal *TPH2* promoter, may alternatively be used to transcribe *TPH2* mRNA variant(s) under specific conditions. Indeed, a variant of h*TPH2* mRNA (Accession number: AK094614 in Genbank and ENST00000266669 in Ensembl database) whose 5' terminal starts at nt 132 in the downstream 5'-UTR of h*TPH2*, is expressed in the brainstem, prefrontal cortex, hippocampus and amygdala (Haghighi et al., 2008; Ota et al., 2004). This *TPH2* mRNA variant is 2,992-bp long and comprises 6 or 7 exons, with the first 5 exons identical with the normal *TPH2* mRNA while the last 2 exons are derived from intron 5. Hence, this shortened mRNA variant results not only from transcriptional regulation through alternative promoter usage, but also from posttranscriptional regulation by alternative splicing, which is affected by an exon-intron boundary SNP (rs1386493) as mentioned above (Zhang et al., 2010a). As for the strong antisense promoter in *TPH2* 5'-UTR, it may transcribe an antisense non-coding RNA that partially overlaps the conventional *TPH2* mRNA, such that dsRNA might be formed to regulate *TPH2* gene expression. Nevertheless, the existence of natural antisense transcript(s) of *TPH2* is yet to be verified.

2. Epigenetic markers involved in *TPH2* expression

The *TPH2* promoter contains no CpG island but a number of scattered CpG sites, and an enriched signal of DNA hypomethylation at the 5'-UTR locus is indicated by the UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>) (Fig.1A and 1B). In particular, a putative motif for the CCCTC-binding factor (CTCF) – the only known chromatin insulator protein in mammals – locates in the upstream segment (nt 26~51) of *TPH2* 5'-UTR, and it overlaps with the NRSF motif and harbors a conserved CpG site. Indeed, enriched signals of CTCF and NRSF at the *TPH2* 5'-UTR locus are shown by the UCSC Genome Browser. CTCF contributes to multiple aspects of epigenetics and it only binds to unmethylated DNA sequence (Filippova, 2008; Phillips and Corces, 2009). Also, it has been reported that methylation of a single CpG site affects gene expression (Ceccarelli et al., 2011; Sohn et al., 2010; Zhang et al., 2010b). Hence, CTCF may interact with DNA methylation to influence *TPH2* expression. Additional evidence for the involvement of CTCF in *TPH2* expression includes: 1) the chromatin state at the *TPH2* 5'-UTR locus was determined as "insulator" in almost all cell lines as shown by the UCSC genome browser; and 2) the locus of *TPH2* and its neighboring *TBC1D15* gene (ubiquitously expressed) display an enriched signal of H3K27me3 (indicator of heterochromatin) and H3K36me3 (indicator of euchromatin), respectively (Fig.1B). In addition, the *TPH2* 5'-UTR locus shows almost no signal of H3K27Ac and H3K4Me1 that are indicative of active transcription, while it exhibits enriched signals for TF binding (by ChIP-seq), nucleosome positioning and DNase I hypersensitivity, providing further evidence for epigenetic regulation of *TPH2* expression (Fig.1B). Taken together, we hypothesize that the 5'-UTR is a major locus for the epigenetic regulation of *TPH2* expression, consistent with its repression of gene expression *in vitro* (Chen et al., 2008), and that this region may function as an "on-off" switch for *TPH2* expression.

Alternative splicing and RNA editing post- or co-transcriptionally regulate gene expression and are major sources for protein diversity in higher eukaryotes. Particularly, alternative splicing, which is regulated by the interplay between *trans*-acting splicing regulators and *cis*-

regulatory elements, is now estimated to affect more than 90% of human genes and plays critical roles in cell differentiation, development and disease (Han et al., 2011; Luco et al., 2011; Wang and Burge, 2008; Witten and Ule, 2011). Besides the above-mentioned 2,292-bp TPH2 mRNA variant, Grohmann et al. (2010) identified another human TPH2 mRNA variant caused by the usage of an alternative splice site at the end of exon 3, resulting in an insertion of 6-bp and 2-AA in the mRNA and protein sequence, respectively, and kinetic studies showed that the protein product of this TPH2 variant shows higher enzymatic activity than the normal TPH2 protein. Meanwhile, the same authors found that the pre-mRNAs of both normal and alternatively spliced variants are dynamically RNA-edited in a mutually exclusive manner, and they further showed evidence that deregulated alternative splicing and RNA editing is involved in the etiology of psychiatric diseases, such as suicidal behavior (Grohmann et al., 2010). In addition, *tph2* mRNA variants with different sizes of the 3'-UTR have been identified in rats (Abumaria et al., 2008). With the existence of multiple TPH2 mRNA variants and their tissue-specific expression, one should take caution in interpreting TPH2 expression measured by real-time PCR, which may not exactly reflect the expression of the normal TPH2 mRNA, i.e., previous findings on TPH2 expression based on real-time PCR results only should be reconsidered.

3. Environmental factors affecting TPH2 gene expression

TPH2 gene expression is responsive to a diversity of environmental factors, including stressors or stressful events (internal or external), endogenous hormones or growth factors, as well as diet and exogenous drugs or chemicals.

1) Stressors or stressful events—As mentioned above in Section I, *tph2* expression is influenced by hypotensive haemorrhage (Brown et al., 2006), exposure to hypoxia (Rahman and Thomas, 2009), as well as adverse early-life experience and adulthood stressful event (Gardner et al., 2009; Mueller and Bale, 2008). Particularly, Gardner et al. (2009) demonstrated that adult rats that had been exposed to neonatal handling as pups exhibit decreased *tph2* mRNA expression in the dorsal raphe nucleus/ventrolateral periaqueductal gray region (DRVL/VLPAG). In addition, treatment of neonatal mice with lipopolysaccharide (LPS), which represents an immune challenge, increases *tph2* mRNA expression in the DRVL/VLPAG specifically at P14, but not at P17, 21, or 28 (Sidor et al., 2010). However, it was reported in rats that *tph1* but not *tph2* mRNA expression is up-regulated by daily restraint stress for 1 week (Abumaria et al., 2008), while conflicting findings were reported by the same group regarding the effect of forced swim exposure on *tph2* expression in rat brainstem (Shishkina et al., 2011; Shishkina et al., 2008).

2) Endogenous hormones or growth factors—Besides glucocorticoids, a sex-related hormone, estrogen, which exhibits an antidepressant-like effect (Estrada-Camarena et al., 2010), has been repeatedly demonstrated to affect TPH2 expression (Bethea et al., 2011; Charoenphandhu et al., 2011; Donner and Handa, 2009; Hiroi et al., 2006; Pandaranandaka et al., 2009; Sanchez et al., 2005), despite a report of no detectable effect of estrogen on *tph2* mRNA expression in mouse DRN (Clark et al., 2005). Specifically, studies in rhesus monkeys showed that treatment with estrogen (alone or plus progesterone) for 1 month significantly induces TPH2 mRNA expression in the raphe region as measured by ISH and qRT-PCR (Sanchez et al., 2005), while long-term ovariectomy decreases overall TPH2 expression as well as the detectable number of 5-HT neurons (Bethea et al., 2011). Similarly, Hiroi et al. (2006) revealed that estrogen increases *tph2* mRNA expression in distinct subregions (mid-ventromedial and caudal of DRN as well as caudal MRN) of rat midbrain raphe nucleus, and that *tph2* mRNA in caudal and rostral dorsomedial DRN is differentially associated with lower and higher anxiety-like behavior, respectively. It was later demonstrated that estrogen-induced *tph2* expression in rat DRN is mediated by the

estrogen receptor beta (Donner and Handa, 2009). Notably, Pandaranandaka et al. (2009) reported that ovariectomized rats show increased TPH protein in the midbrain while exogenous estrogen replacement enhances 5-HT turnover, suggesting that exogenous and endogenous estrogen may have opposite effects on TPH2 expression, which may account for the differential effects of exogenous (anxiolytic) and endogenous (panicolytic) estrogen on anxiety.

It was recently reported that methyl-testosterone significantly increases tph2 mRNA expression in the brain of male catfish (Raghuveer et al., 2011), while placental lactogens markedly induce TPH2 expression in a subset of mouse beta cells during pregnancy (Schraenen et al., 2010). In addition, the transforming growth factor beta 1 (TGF- β 1) released from liver myofibroblasts represses TPH2 expression in cholangiocytes as a paracrine modulation of 5-HT synthesis in biliary remodeling in adults (Omenetti et al., 2011). Moreover, an early study reported that chemically-induced hypothyroidism significantly decreases TPH activity and overall 5-HT content in the midbrain of newborn rat pups (Rastogi and Singhal, 1978), and it was later proposed that thyroid hormone modulation of 5-HT contributes to the early-life programming of GR expression and HPA axis plasticity (Meaney et al., 2000), suggesting that thyroid hormone may also influence TPH2 expression.

3) Diet and exogenous drugs or chemicals—High-fat diet (HFD) potently induces tph2 mRNA expression in mouse adipose tissues (Hageman et al., 2010), while maternal consumption of HFD during pregnancy leads to a significant increase of TPH2 expression in the rostral raphe of nonhuman primate offspring (Sullivan et al., 2010). In addition, McNamara et al. (2009) revealed that omega-3 fatty acid deficiency during perinatal development reduces midbrain tph2 mRNA expression in adult female rats.

TPH2 expression is also sensitive to an increasing number of exogenous drugs or chemicals, including the antidepressants fluoxetine (Di Lieto et al., 2007; Dygalo et al., 2006; Shishkina et al., 2011; Shishkina et al., 2007), imipramine (Heydendael and Jacobson, 2009), lithium (Scheuch et al., 2010), as well as the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) (Bonkale and Austin, 2008). Notably, imipramine regulation of TPH2 expression is dependent on the glucocorticoid status (Heydendael and Jacobson, 2009). In addition, the Bacopa monniera leaf extract, a traditional Indian herbal medicine that has an antidepressant effect (Sairam et al., 2002), up-regulates tph2 expression in rat hindbrain (Charles et al., 2011), while Wuzhuyutang (Evodiae prescription) - a traditional Chinese herbal medicine for the treatment of migraine - influences TPH2 promoter activity in PC12 cells (Wang et al., 2009).

IV. Clinical implications of TPH2 gene expression regulation

TPH2 plays a critical role in the regulation of 5-HT neurotransmission and is closely related to the stress response, which is involved in the pathophysiology of a wide spectrum of somatic and mental disorders. TPH2 gene expression is highly inducible and is under the control of genetic and environmental factors, consistent with its role in the stress response and its sensitivity to gene-environment interactions. Since the 5-HT system is a major target for the therapeutic treatment of stress disorders, it is anticipated that TPH2 represents a promising target for the development of new therapeutic strategies. Accordingly, TPH2 gene expression regulation is of both pathophysiological and pharmacological significance.

1. Pathophysiological significance of TPH2 gene expression/function

Chen et al. (2010a; 2010b) examined the association of rhTPH2 genetic variance with aggression and self-injurious behavior. They found that the 2051A>C interacts with rearing

condition to affect aggression, with 2051CC/mother-reared monkeys showing the lowest aggressive threat (Chen et al., 2010a). As for SIB, monkeys were categorized as self-wounder (SW) or non-wounder (NW) groups based on their veterinary records, of which SW had experienced one or more instances of self-inflicted wounding serious enough to require veterinary treatment. Meanwhile, self-biting rates were measured by systematic observations based on the median split of the self-biting distribution. Half of the animals were identified as high-frequency biters (HFB) while the other half of animals were low-frequency biters (LFB). Chen et al. (2010b) found that a dinucleotide (TA) indel polymorphism (-1325Ins>Del) in rh *TPH2* 5'-FR is significantly associated with self-wounding but not self-biting, while a 159-bp indel polymorphism (2128S>L) in rh *TPH2* 3'-UTR is linked to self-biting but not self-wounding. Accordingly, distribution of 5'-FR haplotypes differs significantly between the two self-wounding groups (SW and NW), while distribution of 3'-UTR haplotypes differed significantly between the two self-biting groups (HFB and LFB). In addition, the 3'-UTR 2051A>C exerts a significant main effect on self-biting rate, while rh *TPH2* 5'-FR polymorphisms did not. Hence, in accordance with their differential effects on the stress response, rh *TPH2* 5'-FR and 3'-UTR polymorphisms appear to be differentially associated with SIB. Notably, HFB and LFB differed significantly in cortisol response to ACTH which is influenced by 2051A>C, while SW and NW differed in HPA negative feedback during night-time (low cortisol secretion, more likely influenced by rh *TPH2* 5'-FR polymorphisms). Again, these findings, in line with the differential association of AM and PM cortisol with personality traits in rhesus monkeys (Capitanio et al., 2004), support the differential modulation of basal and induced cortisol production. Thus, as is the case for 5-HTTLPR (Gotlib et al., 2008), the stress response underlies the association between *TPH2* genetic variance and the two aspects of SIB. In addition, another group found that 2051A>C may affect the risk proclivity in rhesus monkey (Watson et al., 2009). Moreover, in chimpanzee - another nonhuman primate, a functional non-synonymous SNP (Q468R) in *TPH2* has been associated with the personality trait neuroticism (Hong et al., 2011).

In humans, while a limited number of postmortem studies reported altered TPH2 expression in suicidality (Bach-Mizrachi et al., 2008; Bach-Mizrachi et al., 2006; Perroud et al., 2010) and SIDS (Duncan et al., 2010), numerous association studies have linked h *TPH2* genetic variance to a wide spectrum of endophenotypes, behavioral traits and neuropsychiatric diseases, despite the inconsistent findings in some cases possibly due to the G×E interaction. Briefly, a number of h *TPH2* polymorphisms (or haplotypes), especially the promoter -703G>T which affects gene expression *in vitro* (Chen et al., 2008; Lin et al., 2007), have been associated with amygdala responsiveness (Brown et al., 2005; Canli et al., 2005; Furmark et al., 2008; Furmark et al., 2009; Lee and Ham, 2008), amygdalar and hippocampal volumes (Inoue et al., 2010), cognitive and emotional processing (Armbruster et al., 2010; Canli et al., 2008; Dykens et al., 2011; Gong et al., 2011; Herrmann et al., 2007; Leppänen et al., 2011; Osinsky et al., 2009; Reuter et al., 2008; Reuter et al., 2007c), decision-making and risk-taking behaviors (Jollant et al., 2007; Juhasz et al., 2009; Stoltenberg and Vandever, 2010), personality traits (e.g. impulsivity, harm avoidance and anger-related trait) (Gutknecht et al., 2007; Reuter et al., 2007b; Stoltenberg et al., 2006; Yang et al., 2010) and occurrence of personality disorders (Jacob et al., 2010; Mercedes Perez-Rodriguez et al., 2010), aggression (Mercedes Perez-Rodriguez et al., 2010; Oades et al., 2008), major depression (Haghighi et al., 2008; Lin et al., 2009; Siddheshwar et al., 2009; Tsai et al., 2009; Van den Bogaert et al., 2006; Zhou et al., 2005; Zill et al., 2004a), affective disorders (Cichon et al., 2008; Harvey et al., 2004; Lin et al., 2007; Lopez et al., 2007; Roche and McKeon, 2009), suicidality (de Lara et al., 2007; Fudalej et al., 2010; Fudalej et al., 2009; Jollant et al., 2007; Ke et al., 2006; Lopez et al., 2007; Yoon and Kim, 2009; Zhang et al., 2010c; Zill et al., 2004c; Zupanc et al., 2011), panic disorder (Kim et al., 2009; Maron et al., 2007; Maron et al., 2008), attention deficit hyperactivity disorder

(ADHD) (Lasky-Su et al., 2008; McKinney et al., 2008; Sheehan et al., 2005; Walitza et al., 2005), early onset obsessive-compulsive disorder (Moessner et al., 2006), migraine without aura (Jung et al., 2010), Tourette syndrome (Moessner et al., 2007), severity of schizophrenia (Zhang et al., 2011), anxiety during alcohol detoxification outcome (Serretti et al., 2009), drug addiction (Nielsen et al., 2008; Reuter et al., 2007a), and chronic fatigue syndrome (Goertzel et al., 2006; Smith et al., 2006). In addition, h*TPH2* genetic variance was also reportedly associated with somatic symptoms (Holliday et al., 2010) and eating disorders such as hyperphagia (Dykens et al., 2011), anorexia nervosa and self-induced vomiting (Slof-Op 't Landt et al., 2011), as well as the susceptibility to coronary artery lesions in children with Kawasaki disease (Park et al., 2010).

The mouse *tph2* C1473G polymorphism has been associated with aggressive and depressive-like behaviors (Kulikov et al., 2005; Osipova et al., 2009), as well as the severity of hypoxic pulmonary hypertension (Izikki et al., 2007). In addition, as mentioned above, studies using genetically manipulated mice and rats suggested the involvement of *tph2* gene expression in growth and autonomic control of biological processes (e.g. sleep, respiration, body temperature, blood pressure and heart rate), as well as maternal care behavior, depressive-like behaviors and sexual preference (Alenina et al., 2009; Hiroi et al., 2011; Liu et al., 2011). More recently, increased baseline *tph2* expression was observed in high novelty-seeking rats compared with low novelty-seeking rats (Kerman et al., 2011).

2. Pharmacological significance of TPH2 gene expression

The pharmacological significance of TPH2 lies in that: 1) TPH2 is a promising target for the development of new therapeutic strategies in neuropsychiatric disorders; and 2) TPH2 gene expression or function affects drug response. As a number of therapeutic compounds targeting 5-HTT and MAOA are widely used for the treatment of neuropsychiatric diseases, TPH2 represents a promising therapeutic target for the manipulation of 5-HT neurotransmission. TPH2 is highly inducible, making it possible for us to manipulate its expression by appropriate strategies. As mentioned above, TPH2 gene expression is influenced by a number of hormones and exogenous drugs such as the antidepressants fluoxetine (Di Lieto et al., 2007; Dygalo et al., 2006; Shishkina et al., 2011; Shishkina et al., 2007), imipramine (Heydendael and Jacobson, 2009), lithium (Scheuch et al., 2010), as well as the drug of abuse 3,4-methylenedioxy-methamphetamine (MDMA, "ecstasy") (Bonkale and Austin, 2008). Hence, modulation of TPH2 expression or function may contribute to the therapeutic or side effects of such drugs. In this regard, h*TPH2* genetic variance has been linked to the antidepressant response to SSRIs (Peters et al., 2004; Tsai et al., 2009; Tzvetkov et al., 2008), the antipsychotic-induced adverse effect (Al-Janabi et al., 2009), as well as electroconvulsive therapy (Anttila et al., 2009). Similarly, it has been reported that up-regulation of *tph2* mRNA in the rat brain by chronic fluoxetine treatment correlates with its antidepressant effect (Shishkina et al., 2007), while the mouse *tph2* C1473G polymorphism was linked to the antidepressant response to citalopram and paroxetine treatment (Cervo et al., 2005; Jiao et al., 2011; Kulikov et al., 2011).

Estrogen modulation of TPH2 expression may contribute to sex differences in TPH2 expression or 5-HT levels and the prevalence of stress disorders, as well as to the pathogenesis of postmenopausal symptoms in women. For example, while the extracellular 5-HT levels in rat medial prefrontal cortex exhibits a sex-specific (males>females) 24-h profile (Jitsuki et al., 2009), a sex-related difference in *tph2* expression (males>females) was recently reported in mice and catfish (Raghuvier et al., 2011; Renoir et al., 2011). So, it is not surprising that estrogen or gender may moderate the association between TPH2 or 5-HTT genetics and phenotypic traits (Armbruster et al., 2010; Bogdan et al., 2011; Chen et al., 2009; Kim et al., 2009; Stoltenberg and Vandever, 2010; Utge et al., 2010; Wankerl et al., 2010), and that the effects of overexpression or knockdown of rat *tph2* on anxiety behavior

are dependent on estrogen (Hiroi et al., 2011). Estrogen has been suggested to modulate anxiety levels throughout a woman's life, with postmenopausal, hypoestrogenic or bilateral oophorectomized individuals showing a greater risk of developing anxious disorders such as depression and anxiety (Arpels, 1996; Bromberger et al., 2001; Colenda et al., 2010; Rocca et al., 2008). Since 5-HT deficiency and cortisol hypersecretion are presented in most depression patients, the antidepressant-like action of estrogen is presumably mediated, at least in part, by its up-regulation of TPH2 expression (Charoenphandhu et al., 2011; Pandaranandaka et al., 2009).

It is noteworthy that drugs targeting TPH are currently under development and have been recently tested in rodent for the treatment of osteoporosis (Inose et al., 2011; Karsenty and Yadav, 2011; Yadav et al., 2010). Osteoporosis is a disease of low bone mass most often caused by an increase in bone resorption that is not sufficiently compensated for by a corresponding increase in bone formation. Since gut-derived 5-HT inhibits bone formation, inhibition of its biosynthesis may treat osteoporosis through an anabolic mechanism (i.e. by increasing bone formation). LP533401, a small molecule that inhibits human TPH1 and TPH2 with similar potency ($K_i \sim 0.7 \mu\text{M}$) *in vitro*, can selectively lower 5-HT levels in the gut without affecting brain 5-HT levels, likely because it does not cross the blood-brain barrier. Oral administration of this small molecule once daily for up to six weeks acts prophylactically or therapeutically, in a dose-dependent manner, to treat osteoporosis in ovariectomized rodents because of an isolated increase in bone formation (Yadav et al., 2010). So, it is possible that LP533401 or other drugs targeting TPH2 may be efficacious in the treatment of neuropsychiatric disorders.

V. Future directions

Since its identification in 2003 by Walther group, *TPH2* has received much attention with a rapid expansion of literature in the past few years. Considering the critical role of TPH2 in the control of 5-HT neurotransmission and its clinical significance, we anticipate that TPH2 will continue to be the focus of research in the near future, particularly in the following directions: 1) mechanisms (especially epigenetics and G×E interaction) underlying the regulation of TPH2 gene expression; 2) the involvement of TPH2 gene expression, as well as the potential coordination between TPH1 and TPH2, in the regulation of the biological clock and the stress response; 3) the development of TPH2 as a therapeutic target. Undoubtedly, findings of these studies will not only help to better understand the pathophysiology of a wide spectrum of behavioral traits and neuropsychiatric disorders, but also lead to the introduction of new therapeutic strategies for the treatment of such diseases.

Acknowledgments

This paper was supported by DA030177 (GMM), DA025697 (GMM) and RR00168 (NEPRC). We thank the New England Primate Research Center Primate Genetics Core for bioinformatics support.

References

- Abumaria N, Ribic A, Anacker C, Fuchs E, Flügge G. Stress Upregulates TPH1 but not TPH2 mRNA in the Rat Dorsal Raphe Nucleus: Identification of Two TPH2 mRNA Splice Variants. *Cellular and Molecular Neurobiology*. 2008; 28(3):331–342. [PubMed: 18197473]
- Al-Janabi I, Arranz MJ, Blakemore AIF, Saiz PA, Susce MT, Glaser PEA, Clark D, de Leon J. Association study of serotonergic gene variants with antipsychotic-induced adverse reactions. *Psychiatric Genetics*. 2009; 19(6):305–311. [PubMed: 19829168]
- Alenina N, Kikic D, Todiras M, Mosienko V, Qadri F, Plehm R, Boyç P, Vilianovitch L, Sohr R, Tenner K, et al. Growth retardation and altered autonomic control in mice lacking brain serotonin. *Proceedings of the National Academy of Sciences*. 2009; 106(25):10332–10337.

- Andrews MH, Matthews SG. Programming of the Hypothalamo–Pituitary–Adrenal Axis: Serotonergic Involvement. *Stress*. 2004; 7(1):15–27. [PubMed: 15204029]
- Anttila S, Viikki M, Huuhka K, Huuhka M, Huhtala H, Rontu R, Lehtimäki T, Leinonen E. TPH2 polymorphisms may modify clinical picture in treatment-resistant depression. *Neuroscience Letters*. 2009; 464(1):43–46. [PubMed: 19679166]
- Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *Journal of Endocrinology*. 1999; 160(1):1–12. [PubMed: 9854171]
- Armbruster D, Mueller A, Strobel A, Kirschbaum C, Lesch K-P, Brocke B. Influence of functional tryptophan hydroxylase 2 gene variation and sex on the startle response in children, young adults, and older adults. *Biological Psychology*. 2010; 83(3):214–221. [PubMed: 20064585]
- Arpels JC. The female brain hypoestrogenic continuum from the premenstrual syndrome to menopause - A hypothesis and review of supporting data. *Journal of Reproductive Medicine*. 1996; 41(9):633–639. [PubMed: 8887186]
- Azmitia E, Liao B, Chen Y. Increase of tryptophan hydroxylase enzyme protein by dexamethasone in adrenalectomized rat midbrain. *The Journal of Neuroscience*. 1993; 13(12):5041–5055. [PubMed: 8254360]
- Bach-Mizrahi H, Dunderwood M, Tin A, Pellis S, Mann JJ, Arango V. Elevated expression of tryptophan hydroxylase-2 mRNA at the neuronal level in the dorsal and median raphe nuclei of depressed suicides. *Molecular Psychiatry*. 2008; 13(5):507–513. [PubMed: 18180753]
- Bach-Mizrahi H, Underwood MD, Kassir SA, Bakalian MJ, Sibille E, Tamir H, Mann JJ, Arango V. Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median raphe nuclei: Major depression and suicide. *Neuropsychopharmacology*. 2006; 31(4):814–824. [PubMed: 16192985]
- Bale TL, Vale WW. CRF and CRF receptors: Role in stress responsivity and other behaviors. *Annual Review of Pharmacology and Toxicology*. 2004; 44:525–557.
- Barr JL, Forster GL. Serotonergic neurotransmission in the ventral hippocampus is enhanced by corticosterone and altered by chronic amphetamine treatment. *Neuroscience*. 2011; 182:105–114. [PubMed: 21420472]
- Bethea CL, Smith AW, Centeno ML, Reddy AP. Long-term ovariectomy decreases serotonin neuron number and gene expression in free ranging macaques. *Neuroscience*. 2011; 192:675–88. [PubMed: 21763405]
- Bogdan R, Fitzgibbon H, Woolverton WL, Bethea CL, Iyo AH, Stockmeier CA, Kyle PB, Austin MC. 5-HTTLPR genotype and gender, but not chronic fluoxetine administration, are associated with cortical TREK1 protein expression in rhesus macaques. *Neuroscience Letters*. 2011 (0).
- Boisvert JP, Boschuetz TJ, Resch JM, Mueller CR, Choi S. Serotonin mediated changes in corticotropin releasing factor mRNA expression and feeding behavior isolated to the hypothalamic paraventricular nuclei. *Neuroscience Letters*. 2011; 498(3):213–217. [PubMed: 21600959]
- Bollati V, Baccarelli A. Environmental epigenetics. *Heredity*. 2010; 105(1):105–112. [PubMed: 20179736]
- Bonkale WL, Austin MC. 3,4-methylenedioxymethamphetamine induces differential regulation of tryptophan hydroxylase 2 protein and mRNA levels in the rat dorsal raphe nucleus. *Neuroscience*. 2008; 155(1):270–276. [PubMed: 18515011]
- Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends in Endocrinology & Metabolism*. 2008; 19(5):175–180. [PubMed: 18394919]
- Bromberger JT, Meyer PM, Kravitz HM, Sommer B, Cordal A, Powell L, Ganz PA, Sutton-Tyrrell K. Psychologic Distress and Natural Menopause: A Multiethnic Community Study. *Am J Public Health*. 2001; 91(9):1435–1442. [PubMed: 11527777]
- Brown HJ, Henderson LA, Keay KA. Hypotensive but not normotensive haemorrhage increases tryptophan hydroxylase-2 mRNA in caudal midline medulla. *Neuroscience Letters*. 2006; 398(3):314–318. [PubMed: 16483717]

- Brown SM, Peet E, Manuck SB, Williamson DE, Dahl RE, Ferrell RE, Hariri AR. A regulatory variant of the human tryptophan hydroxylase-2 gene biases amygdala reactivity. *Molecular Psychiatry*. 2005; 10(9):884–888. [PubMed: 16044172]
- Canli T, Congdon E, Constable RT, Lesch KP. Additive effects of serotonin transporter and tryptophan hydroxylase-2 gene variation on neural correlates of affective processing. *Biological Psychology*. 2008; 79(1):118–125. [PubMed: 18314252]
- Canli T, Congdon E, Gutknecht L, Constable RT, Lesch KP. Amygdala responsiveness is modulated by tryptophan hydroxylase-2 gene variation. *Journal of Neural Transmission*. 2005; 112(11):1479–1485. [PubMed: 16245070]
- Capitanio JP, Mendoza SP, Bentson KL. Personality characteristics and basal cortisol concentrations in adult male rhesus macaques (*Macaca mulatta*). *Psychoneuroendocrinology*. 2004; 29(10):1300–1308. [PubMed: 15288709]
- Carkaci-Salli N, Salli U, Kuntz-Melcavage KL, Pennock MM, Ozgen H, Tekin I, Freeman WM, Vrana KE. TPH2 in the ventral tegmental area of the male rat brain. *Brain Research Bulletin*. 2011; 84(6):376–380. [PubMed: 21272616]
- Ceccarelli V, Racanicchi S, Martelli MP, Nocentini G, Fettucciari K, Riccardi C, Marconi P, Di Nardo P, Grignani F, Binaglia L, et al. Eicosapentaenoic Acid Demethylates a Single CpG That Mediates Expression of Tumor Suppressor CCAAT/Enhancer-binding Protein δ in U937 Leukemia Cells. *Journal of Biological Chemistry*. 2011; 286(31):27092–27102. [PubMed: 21659508]
- Cervo L, Canetta A, Calcagno E, Burbassi S, Sacchetti G, Caccia S, Fracasso C, Albani D, Forloni G, Invernizzi RW. Genotype-dependent activity of tryptophan hydroxylase-2 determines the response to citalopram in a mouse model of depression. *Journal of Neuroscience*. 2005; 25(36):8165–8172. [PubMed: 16148224]
- Charles PD, Ambigapathy G, Geraldine P, Akbarsha MA, Rajan KE. *Bacopa monniera* leaf extract up-regulates tryptophan hydroxylase (TPH2) and serotonin transporter (SERT) expression: Implications in memory formation. *Journal of Ethnopharmacology*. 2011; 134(1):55–61. [PubMed: 21129470]
- Charoenphandhu J, Teerapornpuntakit J, Nuntapornsak A, Krishnamra N, Charoenphandhu N. Anxiety-like behaviors and expression of SERT and TPH in the dorsal raphe of estrogen- and fluoxetine-treated ovariectomized rats. *Pharmacology Biochemistry and Behavior*. 2011; 98(4):503–510.
- Chen G-L, Miller GM. 5'-Untranslated region of the tryptophan hydroxylase-2 gene harbors an asymmetric bidirectional promoter but not internal ribosome entry site in vitro. *Gene*. 2009; 435(1-2):53–62. [PubMed: 19344641]
- Chen GL, Miller GM. Rhesus monkey tryptophan hydroxylase-2 coding region haplotypes affect mRNA stability. *Neuroscience*. 2008; 155(2):485–491. [PubMed: 18593594]
- Chen GL, Novak MA, Hakim S, Xie Z, Miller GM. Tryptophan hydroxylase-2 gene polymorphisms in rhesus monkeys: association with hypothalamic-pituitary-adrenal axis function and in vitro gene expression. *Molecular Psychiatry*. 2006; 11(10):914–928. [PubMed: 16847459]
- Chen GL, Novak MA, Meyer JS, Kelly BJ, Vallender EJ, Miller GM. The effect of rearing experience and TPH2 genotype on HPA axis function and aggression in rhesus monkeys: A retrospective analysis. *Hormones and Behavior*. 2010a; 57(2):184–191. [PubMed: 19900455]
- Chen GL, Novak MA, Meyer JS, Kelly BJ, Vallender EJ, Miller GM. TPH2 5'- and 3'-regulatory polymorphisms are differentially associated with HPA axis function and self-injurious behavior in rhesus monkeys. *Genes Brain and Behavior*. 2010b; 9(3):335–347.
- Chen GL, Vallender EJ, Miller GM. Functional characterization of the human TPH2 5' regulatory region: untranslated region and polymorphisms modulate gene expression in vitro. *Human Genetics*. 2008; 122(6):645–657. [PubMed: 17972101]
- Chen MC, Joermann J, Hallmayer J, Gotlib IH. Serotonin transporter polymorphism predicts waking cortisol in young girls. *Psychoneuroendocrinology*. 2009; 34(5):681–686. [PubMed: 19128885]
- Chrousos GP. The Stress Response and Immune Function: Clinical Implications: The 1999 Novera H. Spector Lecture. *Annals of the New York Academy of Sciences* 917(NEUROIMMUNOMODULATION: PERSPECTIVES AT THE NEW MILLENNIUM). 2000:38–67.

- Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol*. 2009; 5(7):374–381. [PubMed: 19488073]
- Chrousos GP, Gold PW. The Concepts of Stress and Stress System Disorders - Overview of Physical and Behavioral Homeostasis. *Jama-Journal of the American Medical Association*. 1992; 267(9): 1244–1252.
- Cichon S, Winge I, Mattheisen M, Georgi A, Karpushova A, Freudenberg J, Freudenberg-Hua Y, Babadjanova G, Van Den Bogaert A, Abramova LI, et al. Brain-specific tryptophan hydroxylase 2 (TPH2): a functional Pro206Ser substitution and variation in the 5'-region are associated with bipolar affective disorder. *Human Molecular Genetics*. 2008; 17(1):87–97. [PubMed: 17905754]
- Clark JA, Flick RB, Pai LY, Szalayova I, Key S, Conley RK, Deutch AY, Hutson PH, Mezey E. Glucocorticoid modulation of tryptophan hydroxylase-2 protein in raphe nuclei and 5-hydroxytryptophan concentrations in frontal cortex of C57/BI6 mice. *Molecular Psychiatry*. 2008; 13(5):498–506. [PubMed: 17622221]
- Clark JA, Pai LY, Flick RB, Rohrer SP. Differential hormonal regulation of tryptophan hydroxylase-2 mRNA in the murine dorsal raphe nucleus. *Biological Psychiatry*. 2005; 57(8):943–946. [PubMed: 15820718]
- Clark MS, Russo AF. Tissue-specific glucocorticoid regulation of tryptophan hydroxylase mRNA levels. *Molecular Brain Research*. 1997; 48(2):346–354. [PubMed: 9332732]
- Colenda CC, Legault C, Rapp SR, DeBon MW, Hogan P, Wallace R, Hershey L, Ockene J, Whitmer R, Phillips LS, et al. Psychiatric Disorders and Cognitive Dysfunction Among Older, Postmenopausal Women: Results From the Women's Health Initiative Memory Study. *American Journal of Geriatric Psych*. 2010; 18(2):177–186. 10.1097/JGP.0b013e3181c65864.
- Cote F, Thevenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA, Bayard E, Hanoun N, Saurini F, Lechat P, et al. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(23):13525–13530. [PubMed: 14597720]
- Davuluri RV, Suzuki Y, Sugano S, Plass C, Huang THM. The functional consequences of alternative promoter use in mammalian genomes. *Trends in Genetics*. 2008; 24(4):167–177. [PubMed: 18329129]
- De Kloet ER, Kova'cs GL, Szabo' G, Telegdy G, Bohus B, Versteeg DHG. Decreased serotonin turnover in the dorsal hippocampus of rat brain shortly after adrenalectomy: selective normalization after corticosterone substitution. *Brain Research*. 1982; 239(2):659–663. [PubMed: 7093709]
- de Lara CL, Brezo J, Rouleau G, Lesage A, Dumont M, Alda M, Benkelfat C, Turecki G. Effect of tryptophan hydroxylase-2 gene variants on suicide risk in major depression. *Biological Psychiatry*. 2007; 62(1):72–80. [PubMed: 17217922]
- De Luca V, Likhodi O, Van Tol HHM, Kennedy JL, Wong AHC. Gene expression of tryptophan hydroxylase 2 in post-mortem brain of suicide subjects. *International Journal of Neuropsychopharmacology*. 2006; 9(1):21–25. [PubMed: 15941494]
- Dempfle A, Scherag A, Hein R, Beckmann L, Chang-Claude J, Schafer H. Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. *Eur J Hum Genet*. 2008; 16(10):1164–1172. [PubMed: 18523454]
- Di Lieto A, Leo D, Volpicelli F, di Porzio U, Colucci-D'Amato L. FLUOXETINE modifies the expression of serotonergic markers in a differentiation-dependent fashion in the mesencephalic neural cell line A1 mes c-myc. *Brain Research*. 2007; 1143:1–10. [PubMed: 17321503]
- Dickmeis T. Glucocorticoids and the circadian clock. *Journal of Endocrinology*. 2009; 200(1):3–22. [PubMed: 18971218]
- Dinan TG. Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. *Life Sciences*. 1996; 58(20):1683–1694. [PubMed: 8637392]
- Donner N, Handa RJ. Estrogen receptor beta regulates the expression of tryptophan-hydroxylase 2 mRNA within serotonergic neurons of the rat dorsal raphe nuclei. *Neuroscience*. 2009; 163(2): 705–718. [PubMed: 19559077]
- Donner NC, Montoya CD, Lukkes JL, Lowry CA. Chronic non-invasive corticosterone administration abolishes the diurnal pattern of tph2 expression. *Psychoneuroendocrinology*. 2011 (0).

- Duncan JR, Paterson DS, Hoffman JM, Mokler DJ, Borenstein NS, Belliveau RA, Krous HF, Haas EA, Stanley C, Nattie EE, et al. Brainstem Serotonergic Deficiency in Sudden Infant Death Syndrome. *Jama-Journal of the American Medical Association*. 2010; 303(5):430–437.
- Dygalo NN, Shishkina GT, Kahnina TS, Yudina AM, Ovchinnikova ES. Effect of repeated treatment with fluoxetine on tryptophan hydroxylase-2 gene expression in the rat brainstem. *Pharmacology Biochemistry and Behavior*. 2006; 85(1):220–227.
- Dykens EM, Roof E, Bittel D, Butler MG. TPH2 G/T polymorphism is associated with hyperphagia, IQ, and internalizing problems in Prader-Willi syndrome. *Journal of Child Psychology and Psychiatry*. 2011; 52(5):580–587. [PubMed: 21418060]
- Estrada-Camarena E, Lopez-Rubalcava C, Vega-Rivera N, Recamier-Carballo S, Fernandez-Guasti A. Antidepressant effects of estrogens: a basic approximation. *Behavioural Pharmacology*. 2010; 21(5-6):451–464. [PubMed: 20700047]
- Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature*. 2007; 447(7143):433–440. [PubMed: 17522677]
- Fernandezvivero J, Rodriguezsanchez F, Verastegui C, Moriano FC, Romero A, Decastro JM. Immunocytochemical Distribution of Serotonin and Neuropeptide-Y (Npy) in Mouse Adrenal-Gland. *Histology and Histopathology*. 1993; 8(3):509–520. [PubMed: 8358162]
- Filippova GN. Genetics and epigenetics of the multifunctional protein CTCF. *Current Topics in Developmental Biology*. 2008; Vol 80:337–360. [PubMed: 17950379]
- Fudalej S, Ilgen M, Fudalej M, Kostrzewa G, Barry K, Wojnar M, Krajewski P, Blow F, Ploski R. Association between Tryptophan Hydroxylase 2 Gene Polymorphism and Completed Suicide. *Suicide and Life-Threatening Behavior*. 2010; 40(6):553–560. [PubMed: 21198324]
- Fudalej S, Ilgen M, Fudalej M, Wojnar M, Matsumoto H, Barry KL, Ploski R, Blow FC. Clinical and Genetic Risk Factors for Suicide under the Influence of Alcohol in a Polish Sample. *Alcohol and Alcoholism*. 2009; 44(5):437–442. [PubMed: 19734157]
- Furmark T, Appel L, Henningsson S, Ahs F, Faria V, Linnman C, Pissioti A, Frans O, Bani M, Bettica P, et al. A Link between Serotonin-Related Gene Polymorphisms, Amygdala Activity, and Placebo-Induced Relief from Social Anxiety. *Journal of Neuroscience*. 2008; 28(49):13066–13074. [PubMed: 19052197]
- Furmark T, Henningsson S, Appel L, Ahs F, Linnman C, Pissioti A, Faria V, Orelund L, Bani M, Pich EM, et al. Genotype over-diagnosis in amygdala responsiveness: affective processing in social anxiety disorder. *Journal of Psychiatry & Neuroscience*. 2009; 34(1):30–40. [PubMed: 19125211]
- Garcia-Gras EA, Chi P, Thompson EA. Glucocorticoid-mediated Destabilization of Cyclin D3 mRNA Involves RNA-Protein Interactions in the 3'-Untranslated Region of the mRNA. *Journal of Biological Chemistry*. 2000; 275(29):22001–22008. [PubMed: 10896950]
- Gardner KL, Hale MW, Oldfield S, Lightman SL, Plotsky PM, Lowry CA. Adverse experience during early life and adulthood interact to elevate tph2 mRNA expression in serotonergic neurons within the dorsal raphe nucleus. *Neuroscience*. 2009; 163(4):991–1001. [PubMed: 19647049]
- Glass JD, Grossman GH, Farnbauch L, DiNardo L. Midbrain Raphe Modulation of Nonphotic Circadian Clock Resetting and 5-HT Release in the Mammalian Suprachiasmatic Nucleus. *The Journal of Neuroscience*. 2003; 23(20):7451–7460. [PubMed: 12930783]
- Goertzel BN, Pennachin C, Coelho LD, Gurbaxani B, Maloney EM, Jones JF. Combinations of single nucleotide polymorphisms in neuroendocrine effector and receptor genes predict chronic fatigue syndrome. *Pharmacogenomics*. 2006; 7(3):475–483. [PubMed: 16610957]
- Gong PY, Zhang FC, Ge WH, Lei X, Chi WY, Zhang KJ, Zheng AY, Gao XC. Association Analysis of TPH2, 5-HT2A, and 5-HT6 With Executive Function in a Young Chinese Han Population. *Journal of Neurogenetics*. 2011; 25(1-2):27–34. [PubMed: 21457069]
- Gotlib IH, Joormann J, Minor KL, Hallmayer J. HPA Axis Reactivity: A Mechanism Underlying the Associations Among 5-HTTLPR, Stress, and Depression. *Biological Psychiatry*. 2008; 63(9):847–851. [PubMed: 18005940]
- Griseri P, Bourcier C, Hieblot C, Essafi-Benkhadir K, Chamorey E, Touriol C, Pagès G. A synonymous polymorphism of the Tristetraprolin (TTP) gene, an AU-rich mRNA-binding protein, affects translation efficiency and response to Herceptin treatment in breast cancer patients. *Human Molecular Genetics*. 2011

- Grohmann M, Hammer P, Walther M, Paulmann N, Buettner A, Eisenmenger W, Baghai TC, Schuele C, Rupprecht R, Bader M, et al. Alternative Splicing and Extensive RNA Editing of Human TPH2 Transcripts. *PLoS One*. 2010; 5(1)
- Gutknecht L, Jacob C, Strobel A, Kriegebaum C, Muller J, Zeng Y, Markert C, Escher A, Wendland J, Reif A, et al. Tryptophan hydroxylase-2 gene variation influences personality traits and disorders related to emotional dysregulation. *International Journal of Neuropsychopharmacology*. 2007; 10(3):309–320. [PubMed: 17176492]
- Gutknecht L, Kriegebaum C, Waider J, Schmitt A, Lesch KP. Spatio-temporal expression of tryptophan hydroxylase isoforms in murine and human brain: Convergent data from Tph2 knockout mice. *European Neuropsychopharmacology*. 2009; 19(4):266–282. [PubMed: 19181488]
- Hageman RS, Wagener A, Hantschel C, Svenson KL, Churchill GA, Brockmann GA. High-fat diet leads to tissue-specific changes reflecting risk factors for diseases in DBA/2J mice. *Physiological Genomics*. 2010; 42(1):55–66. [PubMed: 20215417]
- Haghighi F, Bach-Mizrahi H, Huang YY, Arango V, Shi S, Dwork AJ, Rosoklija G, Sheng HT, Morozova I, Ju J, et al. Genetic architecture of the human tryptophan hydroxylase 2 Gene: existence of neural isoforms and relevance for major depression. *Mol Psychiatry*. 2008; 13(8): 813–820. [PubMed: 18180764]
- Han J, Xiong J, Wang D, Fu X-D. Pre-mRNA splicing: where and when in the nucleus. *Trends in Cell Biology*. 2011; 21(6):336–343. [PubMed: 21514162]
- Harvey M, Shink E, Tremblay M, Gagne B, Raymond C, Labbe M, Walther D, Bader M, Barden N. Investigation of the tryptophan hydroxylase 2 (TPH2) gene in bipolar affective disorders. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*. 2004; 130B(1):151–152.
- Heisler LK, Pronchuk N, Nonogaki K, Zhou L, Raber J, Tung L, Yeo GSH, O'Rahilly S, Colmers WF, Elmquist JK, et al. Serotonin Activates the Hypothalamic–Pituitary–Adrenal Axis via Serotonin 2C Receptor Stimulation. *The Journal of Neuroscience*. 2007; 27(26):6956–6964. [PubMed: 17596444]
- Henkhaus R, Bittel D, Butler M. TPH2 polymorphisms and expression in Prader-Willi syndrome subjects with differing genetic subtypes. *Journal of Neurodevelopmental Disorders*. 2010; 2(3): 144–148. [PubMed: 22127900]
- Herrmann MJ, Huter T, Muller F, Muhlberger A, Pauli P, Reif A, Renner T, Canli T, Fallgatter AJ, Lesch KP. Additive effects of serotonin transporter and tryptophan hydroxylase-2 gene variation on emotional processing. *Cerebral Cortex*. 2007; 17(5):1160–1163. [PubMed: 16801378]
- Heydendael W, Jacobson L. Glucocorticoid status affects antidepressant regulation of locus coeruleus tyrosine hydroxylase and dorsal raphe tryptophan hydroxylase gene expression. *Brain Research*. 2009; 1288:69–78. [PubMed: 19577549]
- Hiroi R, McDevitt RA, Morcos PA, Clark MS, Neumaier JF. Overexpression or knockdown of rat tryptophan hydroxylase-2 has opposing effects on anxiety behavior in an estrogen-dependent manner. *Neuroscience*. 2011; 176:120–131. [PubMed: 21182901]
- Hiroi R, McDevitt RA, Neumaier JF. Estrogen selectively increases tryptophan hydroxylase-2 mRNA expression in distinct subregions of rat midbrain raphe nucleus: Association between gene expression and anxiety behavior in the open field. *Biological Psychiatry*. 2006; 60(3):288–295. [PubMed: 16458260]
- Ho SSN, Chow BKC, Yung WH. Serotonin increases the excitability of the hypothalamic paraventricular nucleus magnocellular neurons. *European Journal of Neuroscience*. 2007; 25(10): 2991–3000. [PubMed: 17561813]
- Holliday KL, Macfarlane GJ, Nicholl BI, Creed F, Thomson W, McBeth J. Genetic variation in neuroendocrine genes associates with somatic symptoms in the general population: Results from the EPIFUND study. *Journal of Psychosomatic Research*. 2010; 68(5):469–474. [PubMed: 20403506]
- Hong K-W, Weiss A, Morimura N, Udono T, Hayasaka I, Humle T, Murayama Y, Ito Si, Inoue-Murayama M. Polymorphism of the Tryptophan Hydroxylase 2 (TPH2) Gene Is Associated with Chimpanzee Neuroticism. *PLoS ONE*. 2011; 6(7):e22144. [PubMed: 21765945]

- Huang HW, Bi W, Jenkins GN, Alcorn JL. Glucocorticoid Regulation of Human Pulmonary Surfactant Protein-B mRNA Stability Involves the 3'-Untranslated Region. *Am J Respir Cell Mol Biol*. 2008; 38(4):473–482. [PubMed: 18006875]
- Inose H, Zhou B, Yadav VK, Guo XE, Karsenty G, Ducy P. Efficacy of Serotonin Inhibition in Mouse Models of Bone Loss. *Journal of Bone and Mineral Research*. 2011; 26(9):2002–2011. [PubMed: 21608033]
- Inoue H, Yamasue H, Tochigi M, Takei K, Suga M, Abe O, Yamada H, Rogers MA, Aoki S, Sasaki T, et al. Effect of tryptophan hydroxylase-2 gene variants on amygdalar and hippocampal volumes. *Brain Research*. 2010; 1331:51–57. [PubMed: 20331984]
- Ishida A, Mutoh T, Ueyama T, Bando H, Masubuchi S, Nakahara D, Tsujimoto G, Okamura H. Light activates the adrenal gland: Timing of gene expression and glucocorticoid release. *Cell Metabolism*. 2005; 2(5):297–307. [PubMed: 16271530]
- Izikki M, Hanoun N, Marcos E, Savale L, Barlier-Mur AM, Saurini F, Eddahibi S, Hamon M, Adnot S. Tryptophan hydroxylase 1 knockout and tryptophan hydroxylase 2 polymorphism: effects on hypoxic pulmonary hypertension in mice. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2007; 293(4):L1045–L1052. [PubMed: 17675372]
- Jacob C, Nguyen T, Dempfle A, Heine M, Windemuth-Kieselbach C, Baumann K, Jacob F, Precht J, Wittlich M, Herrmann M, et al. A gene–environment investigation on personality traits in two independent clinical sets of adult patients with personality disorder and attention deficit/hyperactive disorder. *European Archives of Psychiatry and Clinical Neuroscience*. 2010; 260(4):317–326. [PubMed: 19894072]
- Jiao J, Nitzke A, Doukas D, Seigle M, Dulawa S. Antidepressant response to chronic citalopram treatment in eight inbred mouse strains. *Psychopharmacology*. 2011; 213(2):509–520. [PubMed: 21181117]
- Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet*. 2007; 8(4):253–262. [PubMed: 17363974]
- Jitsuki S, Kimura F, Funabashi T, Takahashi T, Mitsushima D. Sex-specific 24-h profile of extracellular serotonin levels in the medial prefrontal cortex. *Brain Research*. 2009; 1260:30–37.
- Jollant F, Buresi C, Guillaume S, Jaussent I, Bellivier F, Leboyer M, Castelnau D, Malafosse A, Courtet P. The influence of four serotonin-related genes on decision-making in suicide attempters. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*. 2007; 144B(5):615–624.
- Jose Bonfiglio J, Inda C, Refojo D, Holsboer F, Arzt E, Silberstein S. The Corticotropin-Releasing Hormone Network and the Hypothalamic-Pituitary-Adrenal Axis: Molecular and Cellular Mechanisms Involved. *Neuroendocrinology*. 2011; 94(1):12–20. [PubMed: 21576930]
- Juhász G, Downey D, Hinest N, Thomas E, Chase D, Toth ZG, Lloyd-Williams K, Mekli K, Platt H, Payton A, et al. Risk-Taking Behavior in a Gambling Task Associated with Variations in the Tryptophan Hydroxylase 2 Gene: Relevance to Psychiatric Disorders. *Neuropsychopharmacology*. 2009
- Jung A, Hüge A, Kühlenbaumer G, Kempt S, Seehafer T, Evers S, Berger K, Marziniak M. Genetic TPH2 variants and the susceptibility for migraine: association of a TPH2 haplotype with migraine without aura. *Journal of Neural Transmission*. 2010; 117(11):1253–1260. [PubMed: 20740293]
- Karsenty G, Yadav VK. Regulation of Bone Mass by Serotonin: Molecular Biology and Therapeutic Implications. *Annual Review of Medicine*. 2011; 62(1):323–331.
- Ke L, Qi ZY, Ping Y, Ren CY. Effect of SNP at position 40237 in exon 7 of the TPH2 gene on susceptibility to suicide. *Brain Research*. 2006; 1122:24–26. [PubMed: 17011525]
- Keeney A, Jessop DS, Harbuz MS, Marsden CA, Hogg S, Blackburn-Munro RE. Differential Effects of Acute and Chronic Social Defeat Stress on Hypothalamic-Pituitary-Adrenal Axis Function and Hippocampal Serotonin Release in Mice. *Journal of Neuroendocrinology*. 2006; 18(5):330–338. [PubMed: 16629831]
- Kerman IA, Clinton SM, Bedrosian TA, Abraham AD, Rosenthal DT, Akil H, Watson SJ. High novelty-seeking predicts aggression and gene expression differences within defined serotonergic cell groups. *Brain Research*. 2011 (0).

- Kim Y-K, Lee H-J, Yang J-C, Hwang J-A, Yoon H-K. A Tryptophan Hydroxylase 2 Gene Polymorphism is Associated with Panic Disorder. *Behavior Genetics*. 2009; 39(2):170–175. [PubMed: 19132526]
- Knight JC. Regulatory polymorphisms underlying complex disease traits. *Journal of Molecular Medicine*. 2005; 83(2):97–109. [PubMed: 15592805]
- Kulikov AV, Osipova DV, Naumenko VS, Popova NK. Association between Tph2 gene polymorphism, brain tryptophan hydroxylase activity and aggressiveness in mouse strains. *Genes, Brain and Behavior*. 2005; 4(8):482–485.
- Kulikov AV, Tikhonova MA, Osipova DV, Kulikov VA, Popova NK. Association between tryptophan hydroxylase-2 genotype and the antidepressant effect of citalopram and paroxetine on immobility time in the forced swim test in mice. *Pharmacology Biochemistry and Behavior*. 2011; 99(4):683–687.
- Laplanche P, Diorio J, Meaney MJ. Serotonin regulates hippocampal glucocorticoid receptor expression via a 5-HT7 receptor. *Developmental Brain Research*. 2002; 139(2):199–203. [PubMed: 12480134]
- Lasky-Su J, Neale BM, Franke B, Anney RJL, Zhou KX, Maller JB, Vasquez AA, Chen W, Asherson P, Buitelaar J, et al. Genome-Wide Association Scan of Quantitative Traits for Attention Deficit Hyperactivity Disorder Identifies Novel Associations and Confirms Candidate Gene Associations. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*. 2008; 147B(8):1345–1354.
- Lee BT, Ham BJ. Serotonergic genes and amygdala activity in response to negative affective facial stimuli in Korean women. *Genes Brain and Behavior*. 2008; 7(8):899–905.
- Lenicov FR, Lemonde S, Czesak M, Mosher TM, Albert PR. Cell-type specific induction of tryptophan hydroxylase-2 transcription by calcium mobilization. *Journal of Neurochemistry*. 2007; 103(5):2047–2057. [PubMed: 17868301]
- Leppänen JM, Peltola MJ, Puura K, Mäntymä M, Mononen N, Lehtimäki T. Serotonin and early cognitive development: variation in the tryptophan hydroxylase 2 gene is associated with visual attention in 7-month-old infants. *Journal of Child Psychology and Psychiatry*. 2011 no-no.
- Liang J, Wessel JH, Iuvone PM, Tosini G, Fukuhara C. Diurnal rhythms of tryptophan hydroxylase 1 and 2 mRNA expression in the rat retina. *Neuroreport*. 2004; 15(9):1497–1500. [PubMed: 15194882]
- Lin Y-M, Ko H-C, Chang F-M, Yeh T-L, Sun H. Population-specific functional variant of the TPH2 gene 2755C>A polymorphism contributes risk association to major depression and anxiety in Chinese peripartum women. *Archives of Women's Mental Health*. 2009
- Lin YMJ, Chao SC, Chen TM, Lai TJ, Chen JS, Sun HS. Association of functional polymorphisms of the human tryptophan hydroxylase 2 gene with risk for bipolar disorder in han chinese. *Archives of General Psychiatry*. 2007; 64(9):1015–1024. [PubMed: 17768266]
- Liposits Z, Phelix C, Paull WK. SYNAPTIC INTERACTION OF SEROTONERGIC AXONS AND CORTICOTROPIN RELEASING-FACTOR (CRF) SYNTHESIZING NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OF THE RAT - A LIGHT AND ELECTRON-MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. *Histochemistry*. 1987; 86(6):541–549. [PubMed: 3497137]
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997; 277(5332):1659–1662. [PubMed: 9287218]
- Liu Y, Jiang Ya, Si Y, Kim J-Y, Chen Z-F, Rao Y. Molecular regulation of sexual preference revealed by genetic studies of 5-HT in the brains of male mice. *Nature*. 2011; 472(7341):95–99. [PubMed: 21441904]
- Lopez VA, Detera-Wadleigh S, Cardona I, Kassem L, McMahon FJ. Nested association between genetic variation in tryptophan hydroxylase II, bipolar affective disorder, and suicide attempts. *Biological Psychiatry*. 2007; 61(2):181–186. [PubMed: 16806105]
- Lowry CA. Functional subsets of serotonergic neurones: Implications for control of the hypothalamic-pituitary-adrenal axis. *Journal of Neuroendocrinology*. 2002; 14(11):911–923. [PubMed: 12421345]

- Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T. Epigenetics in Alternative Pre-mRNA Splicing. *Cell*. 2011; 144(1):16–26. [PubMed: 21215366]
- Malek ZS, Dardente H, Pevet P, Raison S. Tissue-specific expression of tryptophan hydroxylase mRNAs in the rat midbrain: anatomical evidence and daily profiles. *European Journal of Neuroscience*. 2005; 22(4):895–901. [PubMed: 16115212]
- Malek ZS, Sage D, Pevet P, Raison S. Daily rhythm of tryptophan hydroxylase-2 messenger ribonucleic acid within raphe neurons is induced by corticoid daily surge and modulated by enhanced locomotor activity. *Endocrinology*. 2007; 148(11):5165–5172. [PubMed: 17595225]
- Maron E, Toru I, Must A, Tasa G, Toover E, Vasar V, Lang A, Shlik J. Association study of tryptophan hydroxylase 2 gene polymorphisms in panic disorder. *Neuroscience Letters*. 2007; 411(3):180–184. [PubMed: 17123728]
- Maron E, Toru I, Tasa G, Must A, Toover E, Lang A, Vasar V, Shlik J. Association testing of panic disorder candidate genes using CCK-4 challenge in healthy volunteers. *Neuroscience Letters*. 2008; 446(2-3):88–92. [PubMed: 18832011]
- McEuen JG, Beck SG, Bale TL. Failure to Mount Adaptive Responses to Stress Results in Dysregulation and Cell Death in the Midbrain Raphe. *The Journal of Neuroscience*. 2008; 28(33):8169–8177. [PubMed: 18701679]
- McKinney J, Johansson S, Halmoy A, Dramsdahl M, Winge I, Knappskog PM, Haavik J. A loss-of-function mutation in tryptophan hydroxylase 2 segregating with attention-deficit/hyperactivity disorder. *Mol Psychiatry*. 2008; 13(4):365–367. [PubMed: 18347598]
- McKinney J, Knappskog PM, Haavik J. Different properties of the central and peripheral forms of human tryptophan hydroxylase. *Journal of Neurochemistry*. 2005; 92(2):311–320. [PubMed: 15663479]
- McNamara RK, Able J, Liu Y, Jandacek R, Rider T, Tso P, Lipton JW. Omega-3 fatty acid deficiency during perinatal development increases serotonin turnover in the prefrontal cortex and decreases midbrain tryptophan hydroxylase-2 expression in adult female rats: Dissociation from estrogenic effects. *Journal of Psychiatric Research*. 2009; 43(6):656–663. [PubMed: 18986658]
- Meaney MJ, Diorio J, Francis D, Weaver S, Yau J, Chapman K, Seckl JR. Postnatal Handling Increases the Expression of cAMP-Inducible Transcription Factors in the Rat Hippocampus: The Effects of Thyroid Hormones and Serotonin. *The Journal of Neuroscience*. 2000; 20(10):3926–3935. [PubMed: 10804232]
- Mercedes Perez-Rodriguez M, Weinstein S, New AS, Bevilacqua L, Yuan Q, Zhou Z, Hodgkinson C, Goodman M, Koenigsberg HW, Goldman D, et al. Tryptophan-hydroxylase 2 haplotype association with borderline personality disorder and aggression in a sample of patients with personality disorders and healthy controls. *Journal of Psychiatric Research*. 2010; 44(15):1075–1081. [PubMed: 20451217]
- Meyer-Bernstein E, Morin L. Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *The Journal of Neuroscience*. 1996; 16(6):2097–2111. [PubMed: 8604054]
- Meyer-Bernstein EL, Morin LP. Electrical stimulation of the median or dorsal raphe nuclei reduces light-induced FOS protein in the suprachiasmatic nucleus and causes circadian activity rhythm phase shifts. *Neuroscience*. 1999; 92(1):267–279. [PubMed: 10392849]
- Moessner R, Walitza S, Geller F, Scherag A, Gutknecht L, Jacob C, Bogusch L, Remschmidt H, Simons M, Herpertz-Dahlmann B, et al. Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in children and adolescents with obsessive-compulsive disorder. *International Journal of Neuropsychopharmacology*. 2006; 9(4):437–442. [PubMed: 16146581]
- Mossner R, Muller-Vahl KR, Doring N, Stuhmann M. Role of the novel tryptophan hydroxylase-2 gene in Tourette syndrome. *Mol Psychiatry*. 2007; 12(7):617–619. [PubMed: 17592484]
- Mueller BR, Bale TL. Sex-Specific Programming of Offspring Emotionality after Stress Early in Pregnancy. *J Neurosci*. 2008; 28(36):9055–9065. [PubMed: 18768700]
- Nakamura K, Sugawara Y, Sawabe K, Ohashi A, Tsurui H, Xiu Y, Ohtsuji M, Lin QS, Nishimura H, Hasegawa H, et al. Late developmental stage-specific role of tryptophan hydroxylase 1 in brain serotonin levels. *Journal of Neuroscience*. 2006; 26(2):530–534. [PubMed: 16407550]

- Nexon L, Poirel V-J, Clesse D, Pévet P, Raison S. Complex regional influence of photoperiod on the nycthemeral functioning of the dorsal and median raphe serotonergic system in the Syrian hamster. *European Journal of Neuroscience*. 2009; 30(9):1790–1801. [PubMed: 19863652]
- Nielsen DA, Barral S, Proudnikov D, Kellogg S, Ho A, Ott J, Kreek MJ. TPH2 and TPH1: Association of variants and interactions with heroin addiction. *Behavior Genetics*. 2008; 38(2):133–150. [PubMed: 18181017]
- Oades RD, Lasky-Su J, Christiansen H, Faraone SV, Sonuga-Barke EJS, Banaschewski T, Chen W, Anney RJL, Buitelaar JK, Ebstein RP, et al. The influence of serotonin- and other genes on impulsive behavioral aggression and cognitive impulsivity in children with attention-deficit/hyperactivity disorder (ADHD): Findings from a family-based association test (FBAT) analysis. *Behavioral and Brain Functions*. 2008; 4
- Ober C, Vercelli D. Gene–environment interactions in human disease: nuisance or opportunity? *Trends in Genetics*. 2011; 27(3):107–115. [PubMed: 21216485]
- Omenetti A, Yang L, Gainetdinov RR, Guy CD, Choi SS, Chen W, Caron MG, Diehl AM. Paracrine modulation of cholangiocyte serotonin synthesis orchestrates biliary remodeling in adults. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2011; 300(2):G303–G315. [PubMed: 21071507]
- Ortiz-Alvarado R, Guzman-Quevedo O, Mercado-Camargo R, Haertle T, Vignes C, Bolanos-Jimenez F. Expression of tryptophan hydroxylase in developing mouse taste papillae. *Febs Letters*. 2006; 580(22):5371–5376. [PubMed: 16989820]
- Osinsky R, Schmitz A, Alexander N, Kuepper Y, Kozyra E, Hennig J. TPH2 gene variation and conflict processing in a cognitive and an emotional Stroop task. *Behavioural Brain Research*. 2009; 198(2):404–410. [PubMed: 19071164]
- Osipova DV, Kulikov AV, Mekada K, Yoshiki A, Moshkin MP, Kotenkova EV, Popova NK. Distribution of the C1473G polymorphism in tryptophan hydroxylase 2 gene in laboratory and wild mice. *Genes, Brain and Behavior*. 2010; 9(5):537–543.
- Osipova DV, Kulikov AV, Popova NK. C1473G polymorphism in mouse tph2 gene is linked to tryptophan hydroxylase-2 activity in the brain, intermale aggression, and depressive-like behavior in the forced swim test. *Journal of Neuroscience Research*. 2009; 87(5):1168–1174. [PubMed: 19006079]
- Ota T, Suzuki Y, Nishikawa T, Otsuki T, Sugiyama T, Irie R, Wakamatsu A, Hayashi K, Sato H, Nagai K, et al. Complete sequencing and characterization of 21,243 full-length human cDNAs. *Nat Genet*. 2004; 36(1):40–45. [PubMed: 14702039]
- Ozaki T, Habara K, Matsui K, Kaibori M, Kwon A-H, Ito S, Nishizawa M, Okumura T. DEXAMETHASONE INHIBITS THE INDUCTION OF iNOS GENE EXPRESSION THROUGH DESTABILIZATION OF ITS mRNA IN PROINFLAMMATORY CYTOKINE-STIMULATED HEPATOCYTES. *Shock*. 2010; 33(1):64–69. 10.1097/SHK.0b013e3181a7fd74. [PubMed: 19373131]
- Pandaranandaka J, Poonyachoti S, Kalandakanond-Thongsong S. Differential effects of exogenous and endogenous estrogen on anxiety as measured by elevated T-maze in relation to the serotonergic system. *Behavioural Brain Research*. 2009; 198(1):142–148. [PubMed: 19046994]
- Park SW, Ban JY, Yoon KL, Kim HJ, Chung JY, Yi JW, Lee BJ, Chung J-H. Involvement of tryptophan hydroxylase 2 (TPH2) gene polymorphisms in susceptibility to coronary artery lesions in Korean children with Kawasaki disease. *European Journal of Pediatrics*. 2010; 169(4):457–461. [PubMed: 19763617]
- Pastinen T, Ge B, Hudson TJ. Influence of human genome polymorphism on gene expression. *Human Molecular Genetics*. 2006; 15(suppl 1):R9–R16. [PubMed: 16651375]
- Patel PD, Bochar DA, Turner DL, Meng F, Mueller HM, Pontrello CG. Regulation of tryptophan hydroxylase-2 gene expression by a bipartite RE-1 silencer of Transcription/Neuron restrictive silencing factor (REST/NRSF) binding motif. *Journal of Biological Chemistry*. 2007; 282(37):26717–26724. [PubMed: 17613521]
- Patel PD, Pontrello C, Burke S. Robust and tissue-specific expression of TPH2 versus TPH1 in rat raphe and pineal gland. *Biological Psychiatry*. 2004; 55(4):428–433. [PubMed: 14960297]

- Perroud N, Neidhart E, Petit B, Vessaz M, Laforge T, Relecom C, Harpe RL, Malafosse A, Guipponi M. Simultaneous analysis of serotonin transporter, tryptophan hydroxylase 1 and 2 gene expression in the ventral prefrontal cortex of suicide victims. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2010 9999(9999):n/a.
- Peters EJ, Slager SL, McGrath PJ, Knowles JA, Hamilton SP. Investigation of serotonin-related genes in antidepressant response. *Molecular Psychiatry*. 2004; 9(9):879–889. [PubMed: 15052272]
- Phillips JE, Corces VG. CTCF: Master Weaver of the Genome. *Cell*. 2009; 137(7):1194–1211. [PubMed: 19563753]
- Pickard GE, Rea MA. Serotonergic innervation of the hypothalamic suprachiasmatic nucleus and photic regulation of circadian rhythms. *Biology of the Cell*. 1997; 89(8):513–523. [PubMed: 9618901]
- Raghuvver K, Sudhakumari CC, Senthikumar B, Kagawa H, Dutta-Gupta A, Nagahama Y. Gender differences in tryptophan hydroxylase-2 mRNA, serotonin, and 5-hydroxytryptophan levels in the brain of catfish, *Clarias gariepinus*, during sex differentiation. *General and Comparative Endocrinology*. 2011; 171(1):94–104. [PubMed: 21156177]
- Rahman MS, Thomas P. Molecular Cloning, Characterization and Expression of Two Tryptophan Hydroxylase (Tph-1 and Tph-2) Genes in the Hypothalamus of Atlantic Croaker: Down-Regulation after Chronic Exposure to Hypoxia. *Neuroscience*. 2009; 158(2):751–765. [PubMed: 19015006]
- Rastogi RB, Singhal RL. EVIDENCE FOR ROLE OF ADRENOCORTICAL HORMONES IN REGULATION OF NORADRENALINE AND DOPAMINE METABOLISM IN CERTAIN BRAIN AREAS. *British Journal of Pharmacology*. 1978; 62(1):131–136. [PubMed: 23193]
- Renoir T, Zajac MS, Du X, Pang TY, Leang L, Chevarin C, Lanfumey L, Hannan AJ. Sexually Dimorphic Serotonergic Dysfunction in a Mouse Model of Huntington's Disease and Depression. *PLoS One*. 2011; 6(7):e22133. [PubMed: 21760962]
- Reuter M, Esslinger C, Montag C, Lis S, Gallhofer B, Kirsch P. A functional variant of the tryptophan hydroxylase 2 gene impacts working memory: A genetic imaging study. *Biological Psychology*. 2008; 79(1):111–117. [PubMed: 18234415]
- Reuter M, Hennig J, Amelang M, Montag C, Korkut T, Hueweler A, Sturmer T. The role of the TPH1 and TPH2 genes for nicotine dependence: A genetic association study in two different age cohorts. *Neuropsychobiology*. 2007a; 56(1):47–54. [PubMed: 17986837]
- Reuter M, Kuepper Y, Hennig J. Association between a polymorphism in the promoter region of the TPH2 gene and the personality trait of harm avoidance. *International Journal of Neuropsychopharmacology*. 2007b; 10(3):401–404. [PubMed: 17176491]
- Reuter M, Ott U, Vaitl D, Hennig J. Impaired executive control is associated with a variation in the promoter region of the tryptophan hydroxylase 2 gene. *Journal of Cognitive Neuroscience*. 2007c; 19(3):401–408. [PubMed: 17335389]
- Rocca WA, Grossardt BR, Geda YE, Gostout BS, Bower JH, Maraganore DM, de Andrade M, Melton LJ. Long-term risk of depressive and anxiety symptoms after early bilateral oophorectomy. *Menopause*. 2008; 15(6):1050–1059. 10.1097/gme.0b013e318174f155. [PubMed: 18724263]
- Roche S, McKeon P. Support for tryptophan hydroxylase-2 as a susceptibility gene for bipolar affective disorder. *Psychiatric Genetics*. 2009; 19(3):142–146. [PubMed: 19352219]
- Sairam K, Dorababu M, Goel RK, Bhattacharya SK. Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. *Phytomedicine*. 2002; 9(3):207–211. [PubMed: 12046860]
- Sakowski SA, Geddes TJ, Thomas DM, Levi E, Hatfield JS, Kuhn DM. Differential tissue distribution of tryptophan hydroxylase isoforms 1 and 2 as revealed with monospecific antibodies. *Brain Research*. 2006; 1085:11–18. [PubMed: 16581041]
- Sanchez RL, Reddy AP, Centeno ML, Henderson JA, Bethea CL. A second tryptophan hydroxylase isoform, TPH-2 mRNA, is increased by ovarian steroids in the raphe region of macaques. *Molecular Brain Research*. 2005; 135(1-2):194–203. [PubMed: 15857682]
- Scheuch K, Hölte M, Budde H, Lautenschlager M, Heinz A, Ahnert-Hilger G, Priller J. Lithium modulates tryptophan hydroxylase 2 gene expression and serotonin release in primary cultures of serotonergic raphe neurons. *Brain Research*. 2010; 1307:14–21. [PubMed: 19840776]

- Scheuch K, Lautenschlager M, Grohmann M, Stahlberg S, Kirchheiner J, Zill P, Heinz A, Walther DJ, Priller J. Characterization of a functional promoter polymorphism of the human tryptophan hydroxylase 2 gene in serotonergic raphe neurons. *Biological Psychiatry*. 2007; 62(11):1288–1294. [PubMed: 17568567]
- Schraenen A, Lemaire K, de Faudeur G, Hendrickx N, Granvik M, Van Lommel L, Mallet J, Vodjdani G, Gilon P, Binart N, et al. Placental lactogens induce serotonin biosynthesis in a subset of mouse beta cells during pregnancy. *Diabetologia*. 2010; 53(12):2589–2599. [PubMed: 20938637]
- Serretti A, Liappas I, Mandelli L, Albani D, Forloni G, Malitas P, Piperi C, Politis A, Tzavellas EO, Papadopoulou-Daifoti Z, et al. TPH2 gene variants and anxiety during alcohol detoxification outcome. *Psychiatry Research*. 2009; 167(1-2):106–114. [PubMed: 19361870]
- Sheehan K, Lowe N, Kirley A, Mullins C, Fitzgerald M, Gill M, Hawi Z. Tryptophan hydroxylase 2 (TPH2) gene variants associated with ADHD. *Molecular Psychiatry*. 2005; 10(10):944–949. [PubMed: 15940290]
- Shishkina GT, Kalinina TS, Berezova IV, Dygalo NN. Stress-induced activation of the brainstem Bcl-xL gene expression in rats treated with fluoxetine: Correlations with serotonin metabolism and depressive-like behavior. *Neuropharmacology*. 2011 (0).
- Shishkina GT, Kalinina TS, Dygalo NN. Up-regulation of tryptophan hydroxylase-2 mRNA in the rat brain by chronic fluoxetine treatment correlates with its antidepressant effect. *Neuroscience*. 2007; 150(2):404–412. [PubMed: 17950541]
- Shishkina GT, Kalinina TS, Dygalo NN. Serotonergic Changes Produced by Repeated Exposure to Forced Swimming. *Annals of the New York Academy of Sciences*. 2008; 1148(1):148–153. [PubMed: 19120103]
- Siddheshwar U, Pia S, Timo P, Anu L, Erkki K, Sami P, Emma N, Tarja P-H, Tiina P. A population-based association study of candidate genes for depression and sleep disturbance. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2009 9999(9999):n/a.
- Sidor MM, Amath A, MacQueen G, Foster JA. A developmental characterization of mesolimbocortical serotonergic gene expression changes following early immune challenge. *Neuroscience*. 2010; 171(3):734–746. [PubMed: 20816924]
- Singh VB, Corley KC, Phan T-H, Boadle-Biber MC. Increases in the activity of tryptophan hydroxylase from rat cortex and midbrain in response to acute or repeated sound stress are blocked by adrenalectomy and restored by dexamethasone treatment. *Brain Research*. 1990; 516(1):66–76. [PubMed: 2364282]
- Slof-Op 't Landt MCT, Meulenberg I, Bartels M, Suchiman E, Middeldorp CM, Houwing-Duistermaat JJ, van Trier J, Onkenhout EJ, Vink JM, van Beijsterveldt CEM, et al. Association study in eating disorders: TPH2 associates with anorexia nervosa and self-induced vomiting. *Genes, Brain and Behavior*. 2011; 10(2):236–243.
- Smith AK, White PD, Aslakson E, Vollmer-Conna U, Rajeevan MS. Polymorphisms in genes regulating the HPA axis associated with empirically delineated classes of unexplained chronic fatigue. *Pharmacogenomics*. 2006; 7(3):387–394. [PubMed: 16610949]
- Sohn BH, Park IY, Lee JJ, Yang SJ, Jang YJ, Park KC, Kim DJ, Lee DC, Sohn HA, Kim TW, et al. Functional Switching of TGF- β 1 Signaling in Liver Cancer via Epigenetic Modulation of a Single CpG Site in TTP Promoter. *Gastroenterology*. 2010; 138(5):1898–1908. e12. [PubMed: 20038433]
- Spiga F, Harrison LR, Wood SA, MacSweeney CP, Thomson FJ, Craighead M, Grassie M, Lightman SL. Effect of the glucocorticoid receptor antagonist Org 34850 on fast and delayed feedback of corticosterone release. *Journal of Endocrinology*. 2008; 196(2):323–330. [PubMed: 18252955]
- Stoltenberg SF, Glass JM, Chermack ST, Flynn HA, Li S, Weston ME, Burmeister M. Possible association between response inhibition and a variant in the brain-expressed tryptophan hydroxylase-2 gene. *Psychiatric Genetics*. 2006; 16(1):35–38. [PubMed: 16395128]
- Stoltenberg SF, Vandever JM. Gender moderates the association between 5-HTTLPR and decision-making under ambiguity but not under risk. *Neuropharmacology*. 2010; 58(2):423–8. [PubMed: 19781560]
- Sullivan EL, Grayson B, Takahashi D, Robertson N, Maier A, Bethea CL, Smith MS, Coleman K, Grove KL. Chronic Consumption of a High-Fat Diet during Pregnancy Causes Perturbations in

the Serotonergic System and Increased Anxiety-Like Behavior in Nonhuman Primate Offspring. *Journal of Neuroscience*. 2010; 30(10):3826–3830. [PubMed: 20220017]

- Tillis CC, Huang HW, Bi W, Pan S, Bruce SR, Alcorn JL. Glucocorticoid regulation of human pulmonary surfactant protein-B (SP-B) mRNA stability is independent of activated glucocorticoid receptor. *American Journal of Physiology - Lung Cellular and Molecular Physiology*. 2011; 300(6):L940–L950. [PubMed: 21398497]
- Tsai S-J, Hong C-J, Liou Y-J, Yu YWY, Chen T-J, Hou S-J, Yen F-C. Tryptophan hydroxylase 2 gene is associated with major depression and antidepressant treatment response. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2009; 33(4):637–641. [PubMed: 19272410]
- Tsuang MT, Bar JL, Stone WS, Faraone SV. Gene-environment interactions in mental disorders. *World Psychiatry*. 2004; 3(2):73–83. [PubMed: 16633461]
- Tzvetkov MV, Brockmoller J, Roots I, Kirchheiner J. Common genetic variations in human brain-specific tryptophan hydroxylase-2 and response to antidepressant treatment. *Pharmacogenetics and Genomics*. 2008; 18(6):495–506. [PubMed: 18496129]
- Ulrich-Lai YM, Arnhold MM, Engeland WC. Adrenal splanchnic innervation contributes to the diurnal rhythm of plasma corticosterone in rats by modulating adrenal sensitivity to ACTH. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 2006; 290(4):R1128–R1135.
- Utge S, Soronen P, Partonen T, Loukola A, Kronholm E, Pirkola S, Nyman E, Porkka-Heiskanen T, Paunio T. A population-based association study of candidate genes for depression and sleep disturbance. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2010; 153B(2):468–476.
- Van den Bogaert A, Slegers K, De Zutter S, Heyrman L, Norrback KF, Adolfsson R, Van Broeckhoven C, Del-Favero J. Association of brain-specific tryptophan hydroxylase, TPH2, with unipolar and bipolar disorder in a Northern Swedish, isolated population. *Archives of General Psychiatry*. 2006; 63(10):1103–1110. [PubMed: 17015812]
- van Veen JF, van Vliet IM, de Rijk RH, van Pelt J, Mertens B, Fekkes D, Zitman FG. Tryptophan depletion affects the autonomic stress response in generalized social anxiety disorder. *Psychoneuroendocrinology*. 2009; 34(10):1590–1594. [PubMed: 19523772]
- Vanloon GR, Shum A, Sole MJ. DECREASED BRAIN-SEROTONIN TURNOVER AFTER SHORT-TERM (2-HOUR) ADRENALECTOMY IN RATS - A COMPARISON OF 4 TURNOVER METHODS. *Endocrinology*. 1981; 108(4):1392–1402. [PubMed: 6258905]
- Walitz S, Renner TJ, Dempfle A, Konrad K, Wewetzer C, Halbach A, Herpertz-Dahlmann B, Remschmidt H, Smidt J, Linder M, et al. Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in attention-deficit/hyperactivity disorder. *Molecular Psychiatry*. 2005; 10(12):1126–1132. [PubMed: 16116490]
- Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. *Biochemical Pharmacology*. 2003; 66(9):1673–1680. [PubMed: 14563478]
- Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science*. 2003; 299(5603):76–76. [PubMed: 12511643]
- Wang X, Tomso DJ, Liu X, Bell DA. Single nucleotide polymorphism in transcriptional regulatory regions and expression of environmentally responsive genes. *Toxicology and Applied Pharmacology*. 2005; 207(2, Supplement):84–90. [PubMed: 16002116]
- Wang Y, Lei F, Wang X, Hu J, Zhan H, Xing D, Du L, Wang YG, Lei F, Wang XK, et al. Regulatory effects of Wuzhuyutang (Evodiae prescription) and its consisting herbs on TPH2 promoter. *China Journal of Chinese Materia Medica*. 2009; 34(17):2261–2264. [PubMed: 19943499]
- Wang Z, Burge CB. Splicing regulation: From a parts list of regulatory elements to an integrated splicing code. *RNA*. 2008; 14(5):802–813. [PubMed: 18369186]
- Wankerl M, Zyriax B-C, Bondy B, Hinkelmann K, Windler E, Otte C. Serotonin transporter gene-linked polymorphic region (5-HTTLPR) and diurnal cortisol: A sex by genotype interaction. *Biological Psychology*. 2010; 85(2):344–346. [PubMed: 20637828]
- Watson KK, Ghodasra JH, Platt ML. Serotonin Transporter Genotype Modulates Social Reward and Punishment in Rhesus Macaques. *PLoS One*. 2009; 4(1):e4156. [PubMed: 19142220]

- Weaver ICG. Epigenetic Programming by Maternal Behavior and Pharmacological Intervention. Nature Versus Nurture: Let's Call The Whole Thing Off. *Epigenetics*. 2007; 2(1):22–28.
- Weaver ICG. Epigenetic effects of glucocorticoids. *Seminars in Fetal & Neonatal Medicine*. 2009; 14(3):143–150. [PubMed: 19217839]
- Weaver ICG, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl, Dymov S, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nature Neuroscience*. 2004; 7(8): 847–854.
- Witten JT, Ule J. Understanding splicing regulation through RNA splicing maps. *Trends in Genetics*. 2011; 27(3):89–97. [PubMed: 21232811]
- Yadav VK, Balaji S, Suresh PS, Liu XS, Lu X, Li Z, Guo XE, Mann JJ, Balapure AK, Gershon MD, et al. Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. *Nat Med*. 2010; 16(3):308–312. [PubMed: 20139991]
- Yamakawa GR, Antle MC. Phenotype and function of raphe projections to the suprachiasmatic nucleus. *European Journal of Neuroscience*. 2010; 31(11):1974–1983. [PubMed: 20604802]
- Yang J, Lee MS, Lee SH, Lee BC, Kim SH, Joe SH, Jung IK, Choi IG, Ham BJ. Association between Tryptophan Hydroxylase 2 Polymorphism and Anger-Related Personality Traits among Young Korean Women. *Neuropsychobiology*. 2010; 62(3):158–163. [PubMed: 20628266]
- Yoon HK, Kim YK. TPH2-703G/T SNP may have important effect on susceptibility to suicidal behavior in major depression. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 2009; 33(3):403–409. [PubMed: 19162119]
- Zhang C, Li Z, Shao Y, Xie B, Du Y, Fang Y, Yu S. Association Study of Tryptophan Hydroxylase-2 Gene in Schizophrenia and Its Clinical Features in Chinese Han Population. *Journal of Molecular Neuroscience*. 2011; 43(3):406–411. [PubMed: 20938755]
- Zhang X, Nicholls PJ, Laje G, Sotnikova TD, Gainetdinov RR, Albert PR, Rajkowska G, Stockmeier CA, Speer MC, Steffens DC, et al. A functional alternative splicing mutation in human tryptophan hydroxylase-2. *Mol Psychiatry*. 2010a
- Zhang X, Wu M, Xiao H, Lee M-T, Levin L, Leung Y-K, Ho S-M. Methylation of a single intronic CpG mediates expression silencing of the PMP24 gene in prostate cancer. *The Prostate*. 2010b; 70(7):765–776. [PubMed: 20054818]
- Zhang XD, Beaulieu JM, Sotnikova TD, Gainetdinov RR, Caron MG. Tryptophan hydroxylase-2 controls brain serotonin synthesis. *Science*. 2004; 305(5681):217–217. [PubMed: 15247473]
- Zhang YQ, Zhang CS, Yuan GZ, Yao JJ, Cheng ZH, Liu CJ, Liu QH, Wan GR, Shi GZ, Cheng YR, et al. Effect of Tryptophan Hydroxylase-2 rs7305115 SNP on suicide attempts risk in major depression. *Behavioral and Brain Functions*. 2010c; 6
- Zhou ZF, Roy A, Lipsky R, Kuchipudi K, Zhu GS, Taubman J, Enoch MA, Virkkunen M, Goldman D. Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression, and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. *Archives of General Psychiatry*. 2005; 62(10):1109–1118. [PubMed: 16203956]
- Zill P, Baghai TC, Zwanzger P, Schule C, Eser D, Rupprecht R, Moller HJ, Bondy B, Ackenheil M. SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. *Mol Psychiatry*. 2004a; 9(11):1030–1036. [PubMed: 15124006]
- Zill P, Buettner A, Eisenmenger W, Moeller H-J, Ackenheil M, Bondy B. Analysis of tryptophan hydroxylase I and II mRNA expression in the human brain: A post-mortem study. *Journal of Psychiatric Research*. 2007; 41(1-2):168–173. [PubMed: 16023677]
- Zill P, Buttner A, Eisenmenger W, Bondy B, Ackenheil M. Regional mRNA expression of a second tryptophan hydroxylase isoform in postmortem tissue samples of two human brains. *European Neuropsychopharmacology*. 2004b; 14(4):282–284. [PubMed: 15163437]
- Zill P, Buttner A, Eisenmenger W, Moller HJ, Bondy B, Ackenheil M. Single nucleotide polymorphism and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene in suicide victims. *Biological Psychiatry*. 2004c; 56(8):581–586. [PubMed: 15476687]
- Zmijewski MA, Sweatman TW, Slominski AT. The melatonin-producing system is fully functional in retinal pigment epithelium (ARPE-19). *Molecular and Cellular Endocrinology*. 2009; 307(1-2): 211–216. [PubMed: 19409957]

Zupanc T, Pregelj P, Tomori M, Komel R, Paska AV. TPH2 polymorphisms and alcohol-related suicide. *Neuroscience Letters*. 2011; 490(1):78–81. [PubMed: 21182896]

FIG.1.

Putative TF binding motifs and CpG sites in the upstream segment of human, macaca and mouse *TPH2* 5'-UTR (A) and epigenetic tracks at the h*TPH2* locus (B). Nucleotides are numbered relative to the transcription start site (TSS, +1), the symbols "+" in the parenthesis represents the sense strand for the TF binding sites. The epigenetic tracks are obtained from the UCSC Genome Browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>). MRE: methylation-sensitive restriction digestion. In both lymphoblastoid GM12878 and erythroleukemia K562 cell lines, enriched signals of DNA methylation, transcription factor binding (e.g. CTCF and NRSF), nucleosome positioning and DNase I hypersensitivity are presented at the locus of h*TPH2* 5'-UTR, at which the chromatin state is characterized as "insulator" in most cell lines except human embryonic stem cells.