

# The Developmental Basis of Epigenetic Regulation of *HTR2A* and Psychiatric Outcomes

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## ABSTRACT

The serotonin receptor 5-HT<sub>2A</sub> (encoded by *HTR2A*) is an important regulator of fetal brain development and adult cognitive function. Environmental signals that induce epigenetic changes of serotonin response genes, including *HTR2A*, have been implicated in adverse mental health outcomes. The objective of this perspective article is to address the medical implications of *HTR2A* epigenetic regulation, which has been associated with both infant neurobehavioral outcomes and adult mental health. Ongoing research has identified a region of the *HTR2A* promoter that has been associated with a number of medical outcomes in adults and infants, including bipolar disorder, schizophrenia, chronic fatigue syndrome, borderline personality disorder, suicidality, and neurobehavioral outcomes. Epigenetic regulation of *HTR2A* has been studied in several different types of tissues, including the placenta. The placenta is an important source of serotonin during fetal neurodevelopment, and placental epigenetic variation of *HTR2A* has been associated with infant neurobehavioral outcomes, which may represent the basis of adult mental health disorders. Further analysis is needed to identify intrinsic and extrinsic factors that modulate *HTR2A* methylation, and the mechanism by which this epigenetic variation influences fetal growth and leads to altered brain development, manifesting in psychiatric disorders. *J. Cell. Biochem.* 115: 2065–2072, 2014. © 2014 Wiley Periodicals, Inc.

**KEY WORDS:** DEVELOPMENTAL ORIGINS OF ADULT DISEASE; SEROTONIN; PSYCHIATRIC OUTCOMES; NEUROBEHAVIOR; EPIGENETICS; PLACENTA

The developmental origins of adult disease model (DoHAD) highlights the importance of the intrauterine developmental period in defining lifelong health including cognitive and mental health outcomes. Serotonin response genes, including the post-synaptic serotonin receptor *HTR2A*, can be epigenetically regulated through DNA methylation, and have been shown to influence infant brain development. Thus, it has been posited that *HTR2A* epigenetic regulation within key tissues during fetal development has the potential to influence life-long mental health. This prospective article will address the medical implications of *HTR2A* epigenetic regulation, which has been associated with infant neurobehavioral outcomes and adult mental health. The goal of this prospective article is to (1) provide an overview of mechanistic data regarding the importance of *HTR2A* and other serotonin response genes and infant growth and brain development, (2) highlight current research regarding *HTR2A* and medical outcomes, focusing on epigenetic

regulation of *HTR2A* on human psychiatric and neurobehavioral outcomes, and (3) discuss the implications of this research and important questions generated.

## THE SIGNIFICANCE OF PLACENTAL SEROTONIN DURING NEURODEVELOPMENT

The serotonin response pathway is one of the most well-studied pathways in psychology. In adults, serotonin is largely produced by intestinal enterochromaffin cells, and modulates human behavioral and neuropsychological processes [Berger et al., 2009]. There are many components of this serotonin response pathway, ranging from the enzymes that synthesize serotonin from its precursor tryptophan, to the genes that metabolize serotonin within the presynapse, the serotonin transporters, and pre- and post-synaptic serotonin

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receptors. These genes have the potential to be transcriptionally regulated, altering serotonin response [Deneris and Wyler, 2012], which has important consequences for physiological and behavioral processes. We performed a literature search of the 21 most important regulatory genes of the serotonin response pathway as highlighted by Deneris and Wyler [2012], and found a total of 3,229 articles published within from 10 years (2004–2014). This search only included human studies and excluded review articles. As shown in Figure 1, the majority of the studies focused on the serotonin transporter *SLC6A4* (N = 1474). Although there are 12 pre- and post-synaptic receptors highlighted in this search, the serotonin receptor *HTR2A* was much more extensively studied than any other receptor, with a total of 246 publications. Mechanistic data suggest that *HTR2A* expression in important developmental tissues such as the placenta may play an important role in infant neurodevelopment. Thus, we concentrate this review on the elucidating the relationship between *HTR2A* and mental health outcomes.

The developing serotonin response pathway plays a distinct role in fetal brain development. Serotonin influences the identity of

callosal projection neurons and shapes fetal brain circuits [Deneris and Wyler, 2012; Homberg et al., 2013]. Serotonin signaling is also intimately involved in the formation of the fetal HPA axis, the hormonal signaling pathway comprised of the hypothalamus, pituitary, and adrenal glands, which is responsible for responding to stress. Animal models have revealed the consequences of dysregulation of serotonin response pathways. The absence of serotonin during the development of the central nervous system caused severe brain abnormalities in mice [Chen et al., 2012; Migliarini et al., 2013], and reductions in perinatal serotonin concentration were associated with reduced anxiety-like behavior in adult rats [Blazevic et al., 2012]. Thus, changes to serotonin levels during pregnancy have the potential to influence the formation of brain circuits, leading to neurological and cognitive deficits that persist into adulthood, consistent with the developmental origins of adult disease model.

During the period of fetal development, the placenta is an important regulatory organ that links the mother and fetus and regulates the fetal environment. Factors that may influence placental

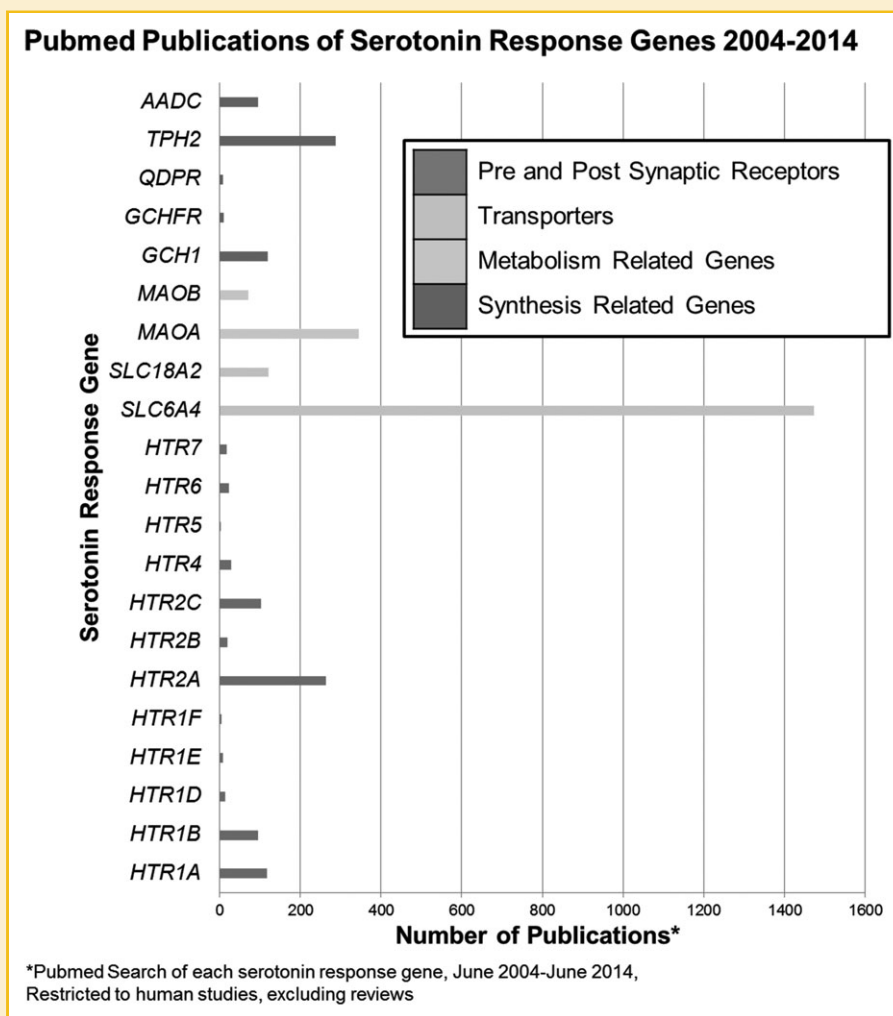


Fig. 1. Pubmed publications of human studies of genes involved in the regulation of the Serotonin response pathway (2004–2014, excluding reviews).

gene expression and subsequently placental physiology, such as epigenetic regulation, may modulate downstream outcomes such as infant growth and long-term health. The placental methylome is dynamic throughout gestation, and demonstrates DNA methylation patterning of partially methylated domains similar to the neuronal system [Schroeder et al., 2013]. This epigenome is modulated by genetic and environmental factors [Novakovic and Saffery, 2012], and has been associated with a number of human pregnancy outcomes, ranging from infant birth weight and physiological outcomes, as reviewed by Novakovic and Saffery [2012], to infant neurobehavior, as reviewed by Lesueur et al. [2014]. Variation within the placental epigenome may influence placental physiology and the environment of the developing infant. This may subsequently alter neurodevelopmental trajectories and have lifelong impacts on cognitive and mental health outcomes.

The placenta possesses many components of the serotonergic pathway, and there is a degree of serotonin signaling that occurs during pregnancy, with consequences for placental physiology and infant neurodevelopment. Infants develop serotonergic response pathways before they produce their own serotonin [Bonnin and Levitt, 2011]. During the prenatal period, the fetal brain is exposed to serotonin from the maternal environment [Davies et al., 1996]. This serotonin can cross the placental barrier, and is also actively synthesized from its precursor tryptophan within the placenta [Bonnin and Levitt, 2011]. In addition to producing serotonin, the placenta also expresses a number of components of the serotonin response pathway, including the primary serotonin receptor 5-HT<sub>2A</sub>. In the adult brain, 5-HT<sub>2A</sub> acts as a post-synaptic inhibitory receptor, but within the placenta it appears to play a mitogenic role by activating the JAK2/STAT pathway [Oufkir and Vaillancourt, 2011]. This activation of the placental 5-HT<sub>2A</sub> receptor has been predicted influence placental implantation. The placental environment plays a crucial role in neurodevelopment, with placental abnormalities linked to a number of developmental disorders [Redline, 2009]. Decreased levels of serotonin derived from placental tissue have been hypothesized to result in a hypo-serotonergic environment in the fetal forebrain, leading to mis-wiring of regions and an overgrowth of serotonergic fibers, manifesting in the autism phenotype, which is characterized by alterations in the prefrontal cortex and increased response to serotonin [Sato, 2013]. This elegant hypothesis has not been tested, and it is possible that the placental 5-HT<sub>2A</sub> receptor may also play a role in this signaling. Overall, it is clear that serotonergic tone within the placenta has important implications for infant neurodevelopment.

## ASSOCIATIONS BETWEEN *HTR2A* AND MEDICAL OUTCOMES

The serotonin receptor *HTR2A* plays an important role in mood regulation in adults, and serotonin signaling is also crucial during development. *HTR2A* genetic polymorphisms are associated with a number of psychiatric disorders, as reviewed by Serretti et al. [2007]. We analyzed the 264 articles published in the last 10 years that focused on human studies of the gene encoding serotonin receptor

5-HT<sub>2A</sub> (excluding review articles), and identified 183 studies that identified associations between this gene and medical outcomes, as shown in Figure 2. The majority of these studies involved schizophrenia and depression, with a particular focus on pharmacologic response to antipsychotics and antidepressants. There were only 20 studies that found associations with neurodevelopmental disorders, and most of these studies focused on common neurodevelopmental conditions including attention deficit disorder (ADHD) and autism. The developmental origins of adult disease theory suggests that a variety of psychological disorders may be traced back to events that occurred in utero. For example, schizophrenia has specifically been associated with in utero conditions ranging from birth month, to exposure to maternal stress, to maternal dietary insufficiency, as reviewed by Cannon et al. [2002]. These disorders are not well understood, and although not classified as neurodevelopmental disorders, may also be rooted to in utero distress, with detectable biological differences manifesting during infancy. There is a need for better understanding of how disorders associated with dysregulation of *HTR2A* alter brain function throughout life, as well as the development of better biomarkers of psychiatric diseases.

## EPIGENETIC REGULATION OF *HTR2A*

Epigenetic regulation during the in utero period has been suggested to be the mechanism by which fetal programming occurs. Epigenetic variation including DNA methylation, histone acetylation, and microRNA expression have important regulatory roles within the placenta, and have been associated with a number of developmental outcomes, as reviewed by Lesueur et al. [2014]. The most well-characterized epigenetic modifications in epidemiological studies is DNA methylation, which can alter the transcription or transcriptional potential of genes, and is usually associated with transcriptional repression. During fetal development, DNA methylation is erased then reset in a tissue specific manner, which is crucial for cell and tissue differentiation [Reik, 2007]. This reprogramming occurs during crucial windows of development during which the maternal environmental can influence the placental and fetal epigenome, and have lifelong impacts on fetal health. More research is needed to understand the mechanism by which these epigenetic changes occur as well as identify how epigenetic variation of specific genes influences fetal development, specifically neurodevelopment.

Epigenetic variation of the serotonin receptor *HTR2A* has been associated with psychological and medical outcomes in a limited number of studies. Of the 246 studies from 2004 to 2014 involving *HTR2A* (Fig. 2), only five of these studies analyzed the relationship between *HTR2A* epigenetic variation and medical outcomes (Table I). Epigenetic variation to the *HTR2A* promoter region in adults was associated with schizophrenia [Abdolmaleky et al., 2011; Ghadirvasfi et al., 2011], psychosis related suicide [Luca et al., 2009], borderline personality disorder [Dammann et al., 2011], and chronic fatigue syndrome [Falkenberg et al., 2011]. DNA methylation has been shown to be altered within specific CpG islands by environmental exposures in a tissue and age specific manner [Christensen et al., 2009], highlighting the need for sampling of DNA methylation in functional

### 2004-2014 Pubmed Publications involving *HTR2A* Stratified By Medical Outcome

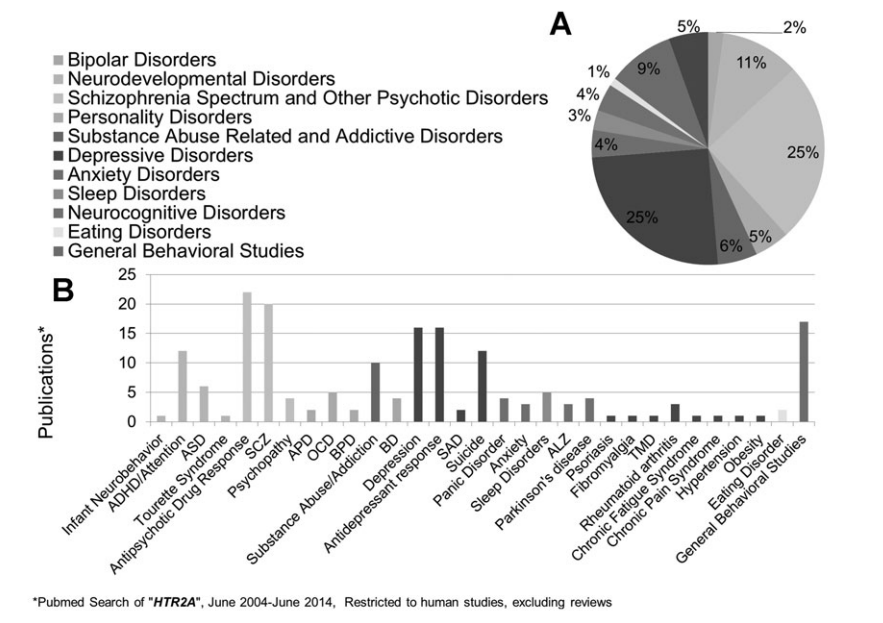


Fig. 2. Pubmed publications of human studies of *HTR2A* (2004–2014, excluding reviews) (A) stratified by DSMIV criteria, (B) with medical outcome of interest. ADHD, attention deficit hyperactivity disorder; ALZ, Alzheimer's; APD, antisocial personality disorder; ASD, autism spectrum disorder; BD, bipolar disorder; BPD, borderline personality disorder; CFS, chronic fatigue syndrome; OCD, obsessive compulsive disorder; SAD, seasonal affective disorder; SCZ, schizophrenia; TMD, temporomandibular disorder.

tissues. These studies examined DNA methylation in blood [Luca et al., 2009; Dammann et al., 2011; Falkenberg et al., 2011] or saliva [Abdolmaleky et al., 2011; Ghadirivasfi et al., 2011], which imposes a significant limitation to their interpretation as these are not the tissues generally considered to be producing or responding to this receptor and are not the most functionally relevant. However, similar patterns of DNA methylation were identified between saliva and the prefrontal

cortex in a limited study analyzing associations between schizophrenia and suicide [Abdolmaleky et al., 2011], which suggests that saliva may be used as an appropriate surrogate tissue.

These studies quantified methylation within the *HTR2A* promoter region up to 1,500 bp upstream (Fig. 3), and predominantly focused their analysis on three CpGs located 1,439, 1,420, and 1,224 bp upstream of the transcriptional start site. The CpG position at –1,439

TABLE I. Human Studies Involving Epigenetic Regulation of *HTR2A* and Medical Outcomes

Outcome of interest	Region of <i>HTR2A</i> studied	Significant finding	Ref.
SCZ and BD	Entire <i>HTR2A</i> promoter region (qMSP)	Hypermethylation at –1,438 (rs6311), hypomethylation at rs6313 of SCZ and BD vs. controls (PFC)	Abdolmaleky et al. [2011]
SCZ and BD	Entire <i>HTR2A</i> promoter region (qMSP)	Increased methylation of –1,439 (rs6311), –1,420 and –1,224 (saliva), decreased methylation of rs6313 (saliva) in SCZ, BD, and SCZ FDR vs. controls	Ghadirivasfi et al. [2011]
Suicidality in patients with major psychosis	Rs6313 (T102C) (MSRD)	Increased methylation at T102C (rs6313) associated with SHZ suicide attempters compared to SHZ non attempters (peripheral leukocytes)	Luca et al. [2009]
BPD	143 bp CpG island of <i>HTR2A</i> promoter region (47470800–47470140) (qMSP)	Increased <i>HTR2A</i> methylation in BPD patients vs. controls at 2 out of 8 CpGs examined (whole blood)	Dammann et al. [2011]
CFS	17 CpGs located –1500 bp upstream of TSS (qMSP)	Increased <i>HTR2A</i> expression in CFS cases, modulated by CpGs –1,438 (rs6311), –1,420, and –1,224 (PBMCs)	Falkenberg et al. [2011]
Infant neurobehavioral outcomes	CpGs located 1,439 (rs6311), 1,420 and 1,224 upstream of TSS (Pyrosequencing)	Mean methylation (placenta) positively associated with Infant attention score and negative associated with quality of movement	Paquette et al. [2013]

BD, bipolar disorder; BPD, borderline personality; CFS, chronic fatigue; FDR, first degree relatives disorder; MSRD, methylation sensitive restriction digest, PBMC, peripheral blood mononuclear cell syndrome; PFC, prefrontal cortex, qMSP, quantitative methylation-specific PCR; SCZ, schizophrenia; TSS, transcriptional start site.

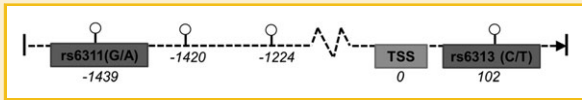


Fig. 3. Map of *HTR2A* promoter region, with CpGs of interest indicated by open circles.

is dependent on the genotype at rs6311, which is in linkage disequilibrium with rs6313, located 102 bp downstream of the transcriptional start site [Falkenberg et al., 2011], which was also analyzed [Luca et al., 2009; Abdolmaleky et al., 2011; Ghadirivasfi et al., 2011]. Based on a meta-analysis of several adult populations, rs6311 is associated with increased risk of schizophrenia and bipolar disorder in Caucasian populations [Gu et al., 2013]. Falkenberg et al. [2011] explored transcription factors bound to the promoter region, and the association between specific CpG sites and expression. They found that the methylation of a CpG located 1,224 bp upstream of the TSS altered the binding of transcription activator SP1. They developed a model in which the allele specific CpG located 1,439 bp upstream, as well as CpGs located 1,420 and 1,224 bp upstream modulated expression of *HTR2A* in PBMCs [Falkenberg et al., 2011]. These combined studies have revealed a specific epigenetic regulation of *HTR2A* expression by these 3 CpGs within the *HTR2A* promoter region and their association with medical outcomes. The mechanism by which these CpG sites control gene expression remains unclear, as well as the factors that contribute to this epigenetic variation within populations.

Fetal programming of *HTR2A* through epigenetic regulation may have long-term impacts on infant neurobehavioral health. Specifically, *HTR2A* has been associated with placental mitogenesis [Sonier et al., 2005], so DNA methylation of *HTR2A* on the placenta may alter fetal serotonin signaling and placental implantation and physiology, which can be linked to significant reproductive outcomes including fetal growth restriction and preeclampsia.

Epigenetic variation of the mean of the CpG sites located 1,420 and 1,224 bp upstream of the transcriptional start site have been associated with infant neurobehavioral outcomes using the Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale (NNS)

[Paquette et al., 2013]. This validated series of assessments has established predictive value for medical and behavioral problems at age 4 ½ [Liu et al., 2010]. The CpG sites from this analysis have been associated with chronic fatigue syndrome as well as schizophrenia in adults (Fig. 3), further validating the influence of this region on medical outcomes. Mean methylation at these CpG sites was associated with increased infant attention score, which characterizes the infants ability to follow auditory and visual stimuli, and attention is generally associated with enhanced development [Liu et al., 2010]. However, a high infant attention score may represent a maladaptive response to the environment, manifesting later in life as increased anxiety and difficulty focusing on a task [Talge et al., 2007]. Thus, the association observed in this population of low risk, health infants may represent an adaptive response to the maternal environment, in concurrence with the predictive response model, but it is unclear if there is a tipping point in which this response may become inappropriate. This study also identified a negative relationship between *HTR2A* mean promoter methylation and infant quality of movement scores, which characterizes infant's smoothness of motor control, and may predict non-optimal motor development later in life [Liu et al., 2010]. These results suggest that *HTR2A* methylation may influence cognitive and motor control regions of the brain differently, manifesting as differences in association with these NNS scores.

This study demonstrated a degree of variation of *HTR2A* DNA methylation present at birth using the placenta as a relevant biomarker of the fetal environment, and suggests that *HTR2A* epigenetic regulation may influence expression within the placenta, with long-term impacts on infant neurobehavior. The source of this variation, and the mechanism by which it influences neurological outcomes remain unclear, but since 5-HT<sub>2A</sub> receptor stimulation can induce placental mitogenesis [Oufkir and Vaillancourt, 2011], increased placental methylation may influence 5-HT<sub>2A</sub> expression within the placenta, modulating placental growth and physiology (see Fig. 4). The placenta plays a crucial role during development, and placental physiology is associated with a number of infant health outcomes, including neurodevelopmental outcomes [Baschat, 2004]. As previously discussed, serotonin is crucial to the fetal brain and central nervous system during development, and

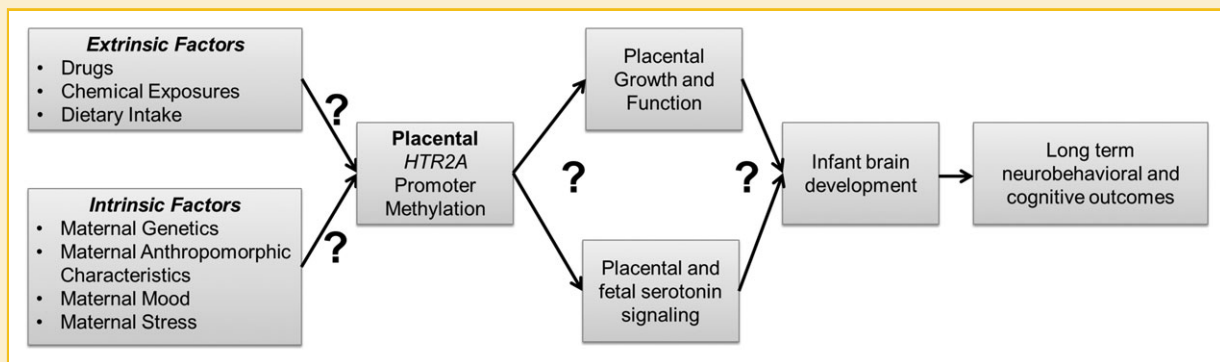


Fig. 4. Flow chart of the hypothetical mechanism by which placental *HTR2A* promoter methylation may influence neurobehavioral outcomes. More research is needed to identify intrinsic and extrinsic factors that influence methylation, the mechanism by which they alter *HTR2A* promoter methylation, and the mechanism by which this altered methylation influences placental growth and function and fetal serotonin signaling.

guides the formation of fetal brain circuits. The main source of serotonin during early fetal development is through the placenta [Bonnin and Levitt, 2011]. We hypothesize that *HTR2A* regulation with the placenta may play a larger, unknown role in placental serotonergic tone, and influence the timing and amount of serotonin reaching the fetal brain during these crucial developmental periods, thus influencing infant neurodevelopment (Fig. 4). These hypotheses require further examination in animal models in order to understand the mechanistic basis, as well as the long-term implications for psychiatric disease.

## LIMITATIONS OF CURRENT APPROACHES

Epigenetic regulation of *HTR2A* contributes to psychiatric outcomes, which may manifest during fetal development. More research is needed to (1) examine the mechanisms by which epigenetic changes within the placenta occur, (2) understand how these epigenetic changes influence neurodevelopment, and (3) identify factors that induce this epigenetic regulation within the placenta. Our current understanding of these mechanisms is hampered by a number of experimental and technical challenges, which will require interdisciplinary collaboration to overcome.

The placenta is an important regulator of the fetal environment and plays a significant role in shaping infant health outcomes [Godfrey, 2002]. There is an extensive body of work reviewing placental epigenetics in human studies [Gabory et al., 2013], but we have limited understanding of the molecular mechanism that underlie these interactions. The placenta is a major source of fetal serotonin early in development [Bonnin and Levitt, 2011], when the fetal brain is maturing. It remains unclear how changes in serotonin signaling change the physiology of the placenta in a manner that alters neurodevelopment, if the 5-HT<sub>2A</sub> receptors within the placenta alter the active conversion of serotonin, and the degree of influence of placental serotonin signaling on fetal brain development.

Our ability to study the underlying mechanisms governing placental serotonin signaling is limited due to the unique nature of the human placenta. There are significant interspecies differences in placental physiology, which creates challenges to studying the placenta using animal models [Carter, 2007]. Due to ethical issues and risks associated with intrauterine tissue sampling, human studies of the placenta are largely limited to samples collected at the end of successful pregnancy. Limited studies have profiled placental methylation in tissues from early gestation [Novakovic et al., 2011], and identified that this relationship changes over the course of gestation. In addition, studies examining methylation of placental tissue are confounded by cellular heterogeneity, as DNA methylation profiling is highly tissue specific. One potential solution to this problem is to adjust for cellular heterogeneity in genome-scale studies of placenta and other relevant tissues, using novel methodology which can account for this confounding [Houseman et al., 2014]. In vitro studies analyzing underlying molecular mechanisms must consider species and cell type differences in placental methylation and physiology.

Studies of *HTR2A* methylation have identified an epigenetic regulatory region within the promoter that is associated with

cognitive and neurological outcomes, ranging from early behavioral outcomes to diseases that manifest later in life such as schizophrenia and chronic fatigue syndrome. Based on the developmental origins of adult disease theory, we suspect that epigenetic variation adults associated with psychiatric outcomes may reflect epigenetic variation that occurred during early development. Genetic variation in conjunction with DNA methylation is associated with these medical outcomes, revealing a complex interplay between environmental factors and genetics that may contribute to predisposition to disease types. There are a number of intrinsic and extrinsic factors that have been shown to induce DNA methylation of other genes, and we suspect that these factors may also induce changes in DNA methylation of *HTR2A* (Fig. 4).

*HTR2A* methylation was associated with neurobehavioral outcomes in infants from low risk, non-pathological pregnancies, which are generalizable to the population as a whole. This study identified a high degree in variability of methylation of the *HTR2A* promoter region, but from this study it is unclear what is causing this epigenetic variation. Intrinsic factors including maternal genetics [Devlin et al., 2010], maternal stress and mood [Kinsella and Monk, 2009], and maternal anthropomorphic characteristics [Ornoy, 2011] have been associated with infant neurobehavioral outcomes and later life health in adults. Maternal mood has been shown to induce changes in methylation of other serotonin response genes, such as *SLC6A4* [Devlin et al., 2010]. Thus, we encourage further study in more “at risk” populations or with more complete assessments of psycho-social factors during pregnancy to identify how these intrinsic factors may influence *HTR2A* promoter methylation to modulate infant neurobehavioral outcomes.

Extrinsic factors have also been shown to influence long-term mental health, possibly modulated through promoter methylation. Infant neurobehavior has been associated with pharmaceutical and recreational drugs [Lester et al., 2002], chemical exposures [Grandjean et al., 2012], maternal socioeconomic status [DiPietro et al., 1998], and maternal diet [Shah and Sachdev, 2004]. One potential candidate modulator of these outcomes is maternal selective serotonin reuptake inhibitors (SSRIs), which are prescribed to pregnant women for a number of mood related disorders. SSRIs have been shown to cross the placental barrier to influence infant developmental outcomes [Velasquez et al., 2013]. SSRIs are associated with changes in promoter DNA methylation within maternal peripheral leukocytes as well as fetal umbilical leukocytes [Devlin et al., 2010], suggesting that SSRIs may induce epigenetic changes in developing infants. SSRI use has also been associated with a number of infant neurological outcomes [Velasquez et al., 2013], including cognitive outcomes in older infants [Skurtveit et al., 2014]. More research is needed to identify other extrinsic factors that influence methylation of *HTR2A* and the mechanism by which this occurs.

## CONCLUSIONS AND FUTURE DIRECTIONS

The 5-HT<sub>2A</sub> receptor has been implicated in a number of psychological and cognitive outcomes, including neurobehavioral outcomes that occur early in life. Neurobehavioral disorders are

extremely prevalent in the U.S. population, and place a large burden on society [Kessler et al., 2005]. In particular, autism spectrum disorders are increasing in prevalence, but the biological mechanisms underpinning these disorders remain unclear. Current treatment for neurobehavioral disorders is hampered by a lack of well-understood molecular mechanisms, as well as an absence of valid biomarkers of disease [Merikangas and Risch, 2003]. Epigenetic regulation has arisen as a potential way to explain psychiatric disorders, including affective disorder [McGowan and Kato, 2008]. *HTR2A* epigenetic regulation has been suggested as a candidate biomarker for schizophrenia [Ghadirivasfi et al., 2011], and it may be an ideal biomarker for other neurobehavioral disorders.

Previous studies have studied *HTR2A* epigenetic regulation in saliva or brain samples, and are limited by the difficulty of identifying an easily accessible and relevant tissue. Placental gene expression has been used as a diagnostic tool for diseases such as Down syndrome [Pennings et al., 2009], and there is a growing interest in using the placental methylation as a biomarker of the fetal environment [Novakovic and Saffery, 2012]. In addition, the placenta is a known source of fetal serotonin during development, suggesting it is a functionally relevant tissue of study. Thus, *HTR2A* placental methylation could serve as a useful biomarker of future adverse neurological outcomes. More research is necessary to identify clinical relevant measures of *HTR2A* methylation by examining more at risk populations, as well as to identify more long-term outcomes associated with *HTR2A* promoter methylation.

Well-defined biomarkers of early life neurobehavioral outcomes are needed because it is crucial to identify infants at risk for cognitive and behavioral deficits early in development, where pharmacological and cognitive interventions have been proven to be more effective. It is also important to be able to quantitatively validate the efficacy of these interventions. The brain epigenome exhibits a degree of plasticity throughout life that can be modulated by environmental cues and parental effects, as reviewed by Caldji et al. [2011]. This suggests that cognitive therapies may be associated with measurable epigenetic changes within tissues. In addition, pharmacologic agents that induce epigenetic changes have been proposed for the treatment of certain cognitive disorders [Peedicayil, 2014]. For example, valproic acid, which inhibits histone deacetylation and acts as an epigenetic modulator, is used to treat a number of psychiatric disorders, including bipolar disorder and schizophrenia [Haddad et al., 2009]. The limitation to current epigenetic therapies is that they induce epigenetic changes in a nonspecific manner, which can limit efficacy and cause increased side effects. Before these epigenetic biomarkers can be used for therapeutic applications, it is necessary to identify valid genomic locations. A series of studies in unrelated populations have revealed a region of the *HTR2A* promoter that is associated with psychiatric outcomes in adults and infants, and may represent one such key region of epigenetic regulation.

## REFERENCES

Abdolmaleky HM, Yaqubi S, Papageorgis P, Lambert AW, Ozturk S, Sivaraman V, Thiagalingam S. 2011. Epigenetic dysregulation of *HTR2A* in the brain of patients with schizophrenia and bipolar disorder. *Schizophrenia Res* 129(2–3):183–190.

Baschat DAA. 2004. Fetal responses to placental insufficiency: An update. *BJOG* 111(10):1031–1041.

Berger M, Gray JA, Roth BL. 2009. The expanded biology of serotonin. *Annu Rev Med* 60:355–366.

Blazevic S, Colic L, Culig L, Hranilovic D. 2012. Anxiety-like behavior and cognitive flexibility in adult rats perinatally exposed to increased serotonin concentrations. *Behav Brain Res* 230(1):175–181.

Bonnin A, Levitt P. 2011. Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. *Neuroscience* 197:1–7.

Caldji C, Hellstrom IC, Zhang TY, Diorio J, Meaney MJ. 2011. Environmental regulation of the neural epigenome. *FEBS Lett* 585(13):2049–2058.

Cannon M, Jones PB, Murray RM. 2002. Obstetric complications and schizophrenia: Historical and meta-analytic review. *Am J Psychiat* 159(7):1080–1092.

Carter AM. 2007. Animal models of human placentation—A review. *Placenta* 28(Supplement A):S41–S47.

Chen A, Kelley LDS, Janušonis S. 2012. Effects of prenatal stress and monoaminergic perturbations on the expression of serotonin 5-HT<sub>4</sub> and adrenergic  $\beta_2$  receptors in the embryonic mouse telencephalon. *Brain Res* 1459(0):27–34.

Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, et al. 2009. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG Island context. *PLoS Genet* 5(8):e1000602.

Dammann G, Teschler S, Haag T, Altmuller F, Tuzcek F, Dammann RH. 2011. Increased DNA methylation of neuropsychiatric genes occurs in borderline personality disorder. *Epigenetics* 6(12):1454–1462.

Davies K, Richardson G, Akmentin W, Acuff V, Fenstermacher J. 1996. The Microarchitecture of Cerebral Vessels. In: Couraud P-O Scherman D, editors *Biology and physiology of the blood-brain barrier*. US: Springer. pp 83–91.

Deneris ES, Wyler SC. 2012. Serotonergic transcriptional networks and potential importance to mental health. *Nat Neurosci* 15(4):519–527.

Devlin AM, Brain U, Austin J, Oberlander TF. 2010. Prenatal exposure to maternal depressed mood and the *MTHFR* C677T variant affect *SLC6A4* methylation in infants at birth. *PLoS ONE* 5(8):e12201.

DiPietro JA, Costigan KA, Shupe AK, Pressman EK, Johnson TR. 1998. Fetal neurobehavioral development: associations with socioeconomic class and fetal sex. *Dev Psychobiol* 33(1):79–91.

Falkenberg VR, Gurbaxani BM. 2011. Functional genomics of serotonin receptor 2A (*HTR2A*): Interaction of polymorphism, methylation, expression and disease association. *Neuromol Med* 13(1):66–76.

Gabory A, Roseboom TJ, Moore T, Moore LG, Junien C. 2013. Placental contribution to the origins of sexual dimorphism in health and diseases: Sex chromosomes and epigenetics. *Biol Sex Diff* 4(1):5.

Ghadirivasfi M, Nohesara S, Ahmadkhaniha H-R, Eskandari M-R, Mostafavi S, Thiagalingam S, et al. 2011. Hypomethylation of the serotonin receptor type-2A Gene (*HTR2A*) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. *Am J Med Genet B* 156(5):536–545.

Godfrey KM. 2002. The role of the placenta in fetal programming—A review. *Placenta* 23(Supplement A):S20–S27.

Grandjean P, Weihe P, Nielsen F, Heinzow B, Debes F, Budtz-Jørgensen E. 2012. Neurobehavioral deficits at age 7 years associated with prenatal exposure to toxicants from maternal seafood diet. *Neurotoxicol Teratol* 34(4):466–472.

Gu L, Long J, Yan Y, Chen Q, Pan R, Xie X, et al. 2013. *HTR2A*–1438A/G polymorphism influences the risk of schizophrenia but not bipolar disorder or major depressive disorder: A meta-analysis. *J Neurosci Res* 91(5):623–633.

Haddad PM, Das A, Ashfaq M, Wieck A. 2009. A review of valproate in psychiatric practice. *Expert Opin Drug Metab Toxicol* 5(5):539–551.

- Homberg JR, Kolk SM, Schubert D. 2013. Editorial perspective of the research topic "Deciphering serotonin's role in neurodevelopment". *Front Cell Neurosci* 7:212.
- Houseman EA, Molitor J, Marsit CJ. 2014. Reference-free cell mixture adjustments in analysis of DNA methylation data. *Bioinformatics* 30(10):1431–1439.
- Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. 2005. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62(6):617–627.
- Kinsella MT, Monk C. 2009. Impact of maternal stress, depression and anxiety on fetal neurobehavioral development. *Clin Obstet Gynecol* 52(3):425–440.
- Lesseur C, Paquette AG, Marsit CJ. 2014. Epigenetic regulation of infant neurobehavioral outcomes. *Med Epigenetics* 2(2):71–79.
- Lester BM, Tronick EZ, LaGasse L, Seifer R, Bauer CR, Shankaran S, et al. 2002. The maternal lifestyle study: effects of substance exposure during pregnancy on neurodevelopmental outcome in 1-month-old infants. *Pediatrics* 110(6):1182–1192.
- Liu J, Bann C, Lester B, Tronick E, Das A, Lagasse L, et al. 2010. Neonatal neurobehavior predicts medical, behavioral outcome. *Pediatrics* 125(1):90–98.
- Luca VD, Viggiano E, Dhoot R, Kennedy JL, Wong AHC. 2009. Methylation and QTDT analysis of the 5-HT<sub>2A</sub> receptor 102C allele: Analysis of suicidality in major psychosis. *J Psychiatr Res* 43(5):532–537.
- McGowan PO, Kato T. 2008. Epigenetics in mood disorders. *Environ Health Prevent Med* 13(1):16–24.
- Merikangas KR, Risch N. 2003. Will the genomics revolution revolutionize psychiatry? *Am J Psychiatr* 160(4):625–635.
- Migliarini S, Pacini G, Pelosi B, Lunardi G, Pasqualetti M. 2013. Lack of brain serotonin affects postnatal development and serotonergic neuronal circuitry formation. *Mol Psychiatry* 18(10):1106–1118.
- Novakovic B, Saffery R. 2012. The ever growing complexity of placental epigenetics—Role in adverse pregnancy outcomes and fetal programming. *Placenta* 33(12):959–970.
- Novakovic B, Yuen RK, Gordon L, Penaherrera MS, Sharkey A, Moffett A, et al. 2011. Evidence for widespread changes in promoter methylation profile in human placenta in response to increasing gestational age, environmental/stochastic factors. *BMC Genomics* 12:529.
- Ornoy A. 2011. Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth restriction and macrosomia. *Reprod Toxicol* 32(2):205–212.
- Oufkir T, Vaillancourt C. 2011. Phosphorylation of JAK2 by serotonin 5-HT<sub>2A</sub> receptor activates both STAT3 and ERK1/2 pathways and increases growth of JEG-3 human placental choriocarcinoma cell. *Placenta* 32(12):1033–1040.
- Paquette AG, Lesseur C, Armstrong DA, Koestler DC, Appleton AA, Lester BM, et al. 2013. Placental HTR2A methylation is associated with infant neurobehavioral outcomes. *Epigenetics* 8(8):796–801.
- Peedicayil J. 2014. Epigenetic drugs in cognitive disorders. *Curr Pharm Des* 20(11):1840–1846.
- Pennings JLA, Koster MPH, Rodenburg W, Schielen PCJI, de Vries A. 2009. Discovery of novel serum biomarkers for prenatal Down syndrome screening by integrative data mining. *PLoS ONE* 4(11):e8010.
- Redline RW. 2009. Disorders of placental circulation and the fetal brain. *Clin Perinatol* 36(3):549–559.
- Reik W. 2007. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* 447(7143):425–432.
- Sato K. 2013. Placenta-derived hypo-serotonin situations in the developing forebrain cause autism. *Med Hypotheses* 80(4):368–372.
- Schroeder DI, Blair JD, Lott P, Yu HOK, Hong D, Cray F, et al. 2013. The human placenta methylome. *Proc Natl Acad Sci* 110(15):6037–6042.
- Serretti A, Drago A, De Ronchi D. 2007. HTR2A gene variants and psychiatric disorders: A review of current literature and selection of SNPs for future studies. *Curr Med Chem* 14(19):2053–2069.
- Shah D, Sachdev HPS. 2004. Maternal micronutrients and fetal outcome. *Indian J Pediatr* 71(11):985–990.
- Skurtveit S, Selmer R, Roth C, Hernandez-Diaz S, Handal M. 2014. Prenatal exposure to antidepressants and language competence at age three: Results from a large population-based pregnancy cohort in Norway. *BJOG* DOI: 10.1111/1471-0528.12821.
- Sonier B, Lavigne C, Arseneault M, Ouellette R, Vaillancourt C. 2005. Expression of the 5-HT<sub>2A</sub> serotonergic receptor in human placenta and choriocarcinoma cells: Mitogenic implications of serotonin. *Placenta* 26(6):484–490.
- Talge NM, Neal C, Glover V, Early Stress, Translational Research and Prevention Science Network: Fetal and Neonatal Experience on Child and Adolescent Mental Health. 2007. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry* 48(3-4):245–261.
- Velasquez JC, Goeden N, Bonnin A. 2013. Placental serotonin: Implications for the developmental effects of SSRIs and maternal depression. *Front Cell Neurosci* 7:47.