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# Le rôle de la sérotonine sur le développement de traits anxieux: une étude de trajectoire longitudinale

par

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Le rôle de la sérotonine sur le développement de traits anxieux: une étude de trajectoire longitudinale

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Résumé

Certains gènes, modulant la sérotonine (5-hydroxytryptamine, 5-HT), ont été associés aux tempéraments liés à l'anxiété. Une limitation dans la plupart de ces études est que les études sont de nature transversale et l'anxiété a été évaluée à un seul point dans le temps. De plus, seules quelques études ont été réalisées chez les enfants. Le but de la présente étude était d'étudier le rôle des gènes $HTR_{2A}$ et $TPH_2$ dans le développement des trajectoires d'anxiété durant l'enfance. Les associations entre ces gènes, ces trajectoires, le diagnostic d'anxiété à l'âge adulte et les différences entre les sexes ont été examinées dans l'Étude Longitudinale des Enfants de Maternelle au Québec, composée de 3185 enfants recrutés en 1986-1987. Leur anxiété a été cotée par leur professeur annuellement entre 6 et 12 ans. Ces cotes ont été modélisées en trajectoires comportementales. Les données genotypées de 5-HT, disponibles pour 1068 personnes, ont été analysées en utilisant les statistiques du Chi-carré, des régressions logistiques et des analyses de variance. Sur les 37 polymorphismes étudiés, plusieurs ont été associés à la trajectoire de forte anxiété, tels le 5-$HTR_{2A}$ (rs1328684, rs95534511, rs1745837, rs7984966, 7330636) et $TPH_2$ (rs11179050, rs11179052, rs1386498). Bien que les trajectoires d'anxiété en enfance n'aient pas prédit le diagnostic d'anxiété à 21 ans, les relations ont été trouvées entre ce diagnostic, $HTR_{2A}$ et les polymorphismes du nucléotide simple (PNS) de $TPH_2$. On remarque que les PNS associés à l'anxiété durant l'enfance et l'âge adulte ne sont pas les mêmes. La force d'association entre les gènes étudiés et l'anxiété diffère entre les garçons et les filles. Cette étude est la première à identifier une association entre les variantes

$TPH_2$, 5-$HTR_{2A}$ et les trajectoires d'anxiété en enfance. Les études futures devraient reproduire les résultats dans d'autres échantillons, enquêter sur l'interaction avec les facteurs de stress, et étudier la pertinence fonctionnelle de la PNS.

Mots- Clés : La sérotonine, $HTR_{2A}$, $TPH_2$, l'anxiété, la trajectoire

**SUMMARY**

A number of genes known to modulate serotonin (5-hydroxytryptamine, 5-HT) have been associated with anxiety-related temperaments. A limitation in most of these studies is that the studies are cross-sectional and anxiety has been measured at a single point in time. Furthermore, only a few studies have been done in children. The aim of the present study was to investigate the role of the $HTR_{2A}$ and $TPH_2$ gene in the development of trajectories of anxiety in childhood/ adolescence. Associations between these genes, anxiety trajectories in childhood and anxiety diagnoses in adulthood were also investigated. Finally, gender differences were explored. Research questions were investigated in the Quebec Longitudinal Study of Kindergarten Children, consisting of 3185 boys and girls, selected in 1986-1987. Children`s anxiety was rated by their teacher every year between the age of 6 and 12 years. The ratings were modeled into behavioral trajectories. 5-HT genotyping data were available for 1068 cohort members. Data were analyzed using Chi-square statistics, logistic regressions and ANOVAs. Out of 37 investigated polymorphisms, several polymorphisms, such as 5-$HTR_{2A}$ (rs1328684, rs95534511, rs1745837, rs7984966, 7330636) and $TPH_2$ (rs11179050, rs11179052, rs1386498) were associated with a high anxiety trajectory. Though trajectories of high anxiety in childhood did not predict an anxiety diagnosis at age 21, relationships were found between $HTR_{2A}$ and $TPH_2$ SNPs and anxiety diagnosis at age 21. We note that the SNPs associated with anxiety were different between adults and children. The strength of association between the investigated genes and anxiety differed between boys and girls. This

is the first study reporting an association with some $HTR_{2A}$ and $TPH_2$ variants and trajectories of anxiety in children. Future studies should replicate the findings in other samples, investigate the interaction with stressors, and study the functional relevance of the SNPs.

**Keywords:** Serotonin, $HTR_{2A}$, $TPH_2$, anxiety, trajectory

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**List of abbreviations**

5-HT: 5-hydroxytryptamine, serotonin

AADC: aromatic L-amino acid decarboxylase

DIS: Diagnostic Interview Schedule

DRN: dorsal raphe nuclei

ELEMQ: Étude Longitudinal des Enfants de Maternelle au Québec

GABA: gamma-aminobutyric acid

GAD: general anxiety disorders

$HTR_{2A}$: hydroxytryptamine receptor 2a

LD: linkage disequilibrium

MRI: magnetic resonance imaging

OCD: obsessive-compulsive disorder

PD: panic disorder

PET: positron emission tomography

PTSD: post-traumatic stress disorder

SAD: social anxiety disorder

SNP: single-nucleotide polymorphism

SSRI: selective serotonin reuptake inhibitor

TPH: tryptophan hydroxylase

To the bright memory of my

husband

**Acknowledgements**

I would like to dedicate my thesis in memory of my beloved husband Mehdi, who passed away before my final submission, his love and lifelong support has enabled me to achieve my goals and finish what I have started.

It would not have been possible to write this master thesis without the help and support of the kind people around me, to only some of whom it is possible to give particular mention here.

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The most special thanks go to my parents for giving me your unconditional support and love through all this long process to complete my thesis.

# Introduction

# 1. Anxiety

## 1.1 Anxiety trait versus anxiety disorders

Anxiety and fear are human emotions that have a protective role in order to avoid situations which cause injury, pain or even death (Moffitt, 2005; Rex, 2010). Although anxiety is a biologically normal and adaptive condition, it could be pathological and interfere with the capability to successfully manage numerous challenges and/or stressful events, and even alter the body condition (Steimer, 2002). Acute or immediate levels of anxiety could be defined as state anxiety; whereas trait anxiety is a long-term anxiety response predisposition to events (Perez-Edgar & Fox, 2005). Certain personality traits, especially high levels of neuroticism (a tendency to experience negative emotions, thereby having a greater chance of feeling anxious, sad and angry in normal situations) is an important risk factor for the development of an anxiety disorder (Hettema, Neale, Myers, Prescott, & Kendler, 2006).

The development of human anxiety disorders is based on three interacting sets of vulnerability factors: 1) a generalized psychological vulnerability: largely resulting from early life experiences 2) a specific psychological vulnerability: mainly focused on specific events or situations and 3) a generalized biological vulnerability: based on genetic origins (Barlow, Grenyer, & Ilkiw-Lavalle, 2000; Steimer, 2002).

According to the Diagnostic and Statistical Manual of Mental Disorders (fourth edition, test revision) (DSM-IV-TR) anxiety disorders are divided into general anxiety disorders (GAD), social anxiety disorder (SAD), panic disorder

(PD), specific and social phobias, obsessive-compulsive disorders (OCD) and post-traumatic stress disorder (PTSD) (American Psychiatric & American Psychiatric Association. Task Force on, 2000).

Anxiety-related behaviors seem to be controlled by the distribution and interconnection of neural circuits in the brain (Lowry & Hale, 2010). A variety of neuroendocrine, neurotransmitter and neuroanatomical alterations could occur in anxiety disorders (Martin, Ressler, Binder, & Nemeroff, 2009). In the following sections of my thesis, the neural and anatomical systems involved in modulating anxiety will be briefly discussed.

**1.2 Epidemiology of anxiety**

Anxiety disorders are among the most prevalent psychiatric disorders with an approximate lifetime prevalence of 28.8% and an approximate 12-month prevalence of 18.1% in the general population (Kessler, Berglund, et al., 2005; Kessler, Chiu, Demler, Merikangas, & Walters, 2005). Epidemiological studies demonstrated that females are more likely to develop PD (7.7%) and GAD (6.6%) than men (2.9% and 3.6 %, respectively). The prevalence of PTSD is also higher in females than in males (12.5% vs. 6.2%)(Kessler et al.,1994). This thesis focuses on GAD and PD.

1.2.1 General Anxiety Disorders

According to the national comorbidity survey (NCS) in United States, the lifetime prevalence of GAD is approximately 5.7 % (Kessler, Berglund, et al., 2005). According to the DSM-IV-TR (American Psychiatric & American Psychiatric Association. Task Force on, 2000) GAD is characterized by excessive

anxiety and worry, occurring more days than not for a period at least 6 months, about a number of events or activities and the person usually finds it difficult to control the worry (American Psychiatric & American Psychiatric Association. Task Force on, 2000). There are six symptoms for GAD: restlessness, being easily fatigued, irritability, muscle tension, difficulty concentrating and sleep disturbance (American Psychiatric & American Psychiatric Association. Task Force on, 2000). In order to meet the formal diagnostic criteria, at least three of these symptoms need to be present and they must affect social or occupational functioning. Most of the people with GAD report that they have felt anxious and nervous all their life (American Psychiatric & American Psychiatric Association. Task Force on, 2000). More than half of individuals presenting for treatment report onset in childhood and onset in adulthood (American Psychiatric & American Psychiatric Association. Task Force on, 2000). GAD has a chronic but fluctuating course and often worsens during periods of stress (American Psychiatric & American Psychiatric Association. Task Force on, 2000).

### 1.2.2 Panic Disorder

The lifetime prevalence of PD is estimated to be 4.7% in the United States (Kessler, Chiu, et al., 2005). According to the DSM-IV-TR, one-year prevalence rates are approximately 1% and 2 % (American Psychiatric & American Psychiatric Association. Task Force on, 2000). PD implicates recurrent, unexpected panic attacks followed by at least one month of persistent concern about having another panic attack, worry about the consequences of the panic attacks and/or significant change in behavior related to the attacks (American

Psychiatric & American Psychiatric Association. Task Force on, 2000). The symptomology associated with this disorder includes shortness of breath, feeling of choking, palpitation, sweating, trembling or shaking, a feeling of losing control or going crazy and fear of dying. PD is associated with the discrete period of intense fear in the absence of real danger and having at least four of these symptoms (American Psychiatric & American Psychiatric Association. Task Force on, 2000).The age of onset lies between late teenage years and the mid-30s, with the first peak in late adolescence and a second smaller peak in the mid-30s. However, the age of onset could differ considerably, from the beginning of childhood until after age 45 (American Psychiatric & American Psychiatric Association. Task Force on, 2000).

**1.3 Heritability of anxiety disorders**

Anxiety disorders have been shown to be heritable. Family studies compare the phenotype prevalence among affected members of family with unaffected members (Na, Kang, Lee, & Yu, 2011). Several family studies have demonstrated an increasing risk for PD (5.7%-17.3%) between affected individuals' relatives and unaffected relatives (Goldstein et al., 1994; Horwath et al., 1995; Maier, Lichtermann, Minges, Oehrlein, & Franke, 1993; Noyes et al., 1986). A meta-analysis confirmed a heritability across GAD, OCD, PD and phobias in a moderate range (30%-40%) (Hettema, Neale, & Kendler, 2001). Moreover, in a large population-based female adult twins sample, comparing monozygotic with dizygotic twins, Kendler et al. estimated the heritability at approximately 30-40% for both GAD, PD and phobias (agoraphobia, social

phobia, situational phobia and simple phobia) (Kendler, Neale, Kessler, Heath, & Eaves, 1992, 1993). These results were confirmed in a later study, showing similar estimations of hereditability for men in GAD and PD (Scherrer et al., 2000). A recent adult twin-study analysis, consisting of more than 5,000 twins, estimated a heritability of 28% for PD (Hettema, Prescott, Myers, Neale, & Kendler, 2005).

Finally, a high familial and genetic connection has been demonstrated for anxiety-related personality traits (Smoller et al., 2001; Weissman et al., 2000). Thus, genetic factors clearly contribute to the pathogenesis of anxiety disorders.

**1.4 Development of Anxiety; role of childhood factors**

Longitudinal epidemiological studies have shown that high and persisting levels of anxiety or depressive disorders in childhood or early adolescence are strong predictors of anxiety diagnosis in adulthood (Beesdo, Knappe, & Pine, 2009; Bittner et al., 2007; Pine, Cohen, Gurley, Brook, & Ma, 1998). For instance, in an epidemiological study, Pine et al. selected a sample of 776 young people who received DSM-based psychiatric assessment between1983-1992. They showed that an anxiety disorder during adolescence predicted recurrent anxiety disorders in early adulthood (Pine et al., 1998).

While anxiety in childhood could predict anxiety disorders in adulthood, other longitudinal studies have shown that *a lack of anxiety* (fearlessness) could be a predictor of aggressive problems later in life. Specifically, fearlessness at age 3 was associated with aggression problems at age 11 (Raine, Reynolds, Venables, Mednick, & Farrington, 1998) and the children from that longitudinal study who

later became criminals showed significantly reduced fear conditioning in adulthood (Gao, Raine, Venables, Dawson, & Mednick, 2010). An animal study investigated underlying genetic mechanisms of a lack of fear. By globally disrupting the function of the $HTR_{2a}$ receptor Weisstaub et al. demonstrated an association between fearlessness behaviors and 5-$HTR_{2A}$ polymorphisms in mice (for a description of this gene in greater detail- see page 33) (Weisstaub et al., 2006). No other animal studies or any human studies investigated the genetic underpinnings of fearlessness and especially not in the context as predictor of future problems, such as aggression, and thus the relevance for humans is not known. However, the above mentioned studies suggest the importance of measuring both high and low anxiety at an early age and to identify genetic mechanisms, since both sides of the spectrum may predict future maladaptive outcome.

**1.5 Neuroanatomy of anxiety**

Anxiety has a complex function which does not depend on specific brain areas executing a unique function, so the interaction of several brain regions has been proposed to be responsible for the symptoms (Martin et al., 2009). The imbalance of activity in the "emotion center" of the brain is thought to be responsible for the symptoms of anxiety disorders (Martin et al., 2009). The limbic system and associated paralimbic brain structures are known to be a set of brain regions related to emotional-processing and involved in the mediation of fear(Martin et al., 2009). In a mixed sample of anxiety disorder patients (obsessive-compulsive disorder, simple phobia or posttraumatic stress disorder)

(using PET), it was shown that elements of the paralimbic belt together with right inferior frontal cortex and subcortical nuclei mediate symptoms across different anxiety disorders (S.L. Rauch, C.R. Savage, N.M. Alpert, A.J. Fischman, & M.A. Jenike, 1997). The hippocampus, amygdala, and limbic cortex are the most important brain regions modulating anxiety.

### 1.5.1 Hippocampus

The hippocampus has an important role in memory consolidation. The hippocampus is partly regulated by the hypothalamus-pituitary-adrenal (HPA) axis, and has an inhibitory control over this system (Martin et al., 2009). Hippocampal volume and neurogenesis involved in stress and volume changes were shown to be related to anxiety disorders (Bonne et al., 2008; Gorman & Docherty, 2010; Villarreal et al., 2002). A meta-analysis revealed a significantly reduced hippocampal volume (bilateral) in adults diagnosed with PTSD compared with healthy controls (Kitayama, Vaccarino, Kutner, Weiss, & Bremner, 2005).

### 1.5.2 The amygdala

The amygdala is formed of multiple subnuclei, where each nucleus has a separate pattern of afferent and efferent projections (Martin et al., 2009). This complex structure is involved in the expression of fear and in fear conditioning, environment-specific defensive behaviors (Etkin, 2010). Also, the amygdala plays a role in the realization of emotional and fear-related memories (Martin et al., 2009). Particularly, basolateral complex and the central nucleus are involved in anxiety (Heimer, Harlan, Alheid, Garcia, & de Olmos, 1997). Using Magnetic Resonance Imaging (MRI), MacMillan et al. showed an association between

increases in ratio of the left and right regions of amygdala: hippocampal volume and severity of anxiety (MacMillan et al., 2003). Another MRI study found that right and total amygdala volumes were significantly larger in participants with GAD compared to healthy controls (De Bellis et al., 2000).

1.5.3 Limbic cortex

The limbic cortex, which includes the insular cortex and cingulate cortex, processes internal bodily state and pain-related sensory and affective information, motivation and mood (Etkin, 2010). Rauch et al. measured relative regional cerebral blood flow (rCBF) by Positron Emission Tomography (PET) in 23 participants suffering from anxiety disorders (OCD, simple phobia, PTSD) (S. L. Rauch, C. R. Savage, N. M. Alpert, A. J. Fischman, & M. A. Jenike, 1997). They showed increased activation in response to disorder relevant emotional stimuli (relative to neutral stimuli) in different regions of the brain, including the bilateral insular cortex. In their previous study, they had found bilateral insula activation for both simple phobia and PTSD (Rauch et al., 1995; Rauch et al., 1996) bilateral anterior cingulate activation for simple phobia and PTSD and only left anterior cingulate activation for patients with OCD (Rauch et al., 1994). Thus, these findings suggest an implication of the limbic cortex in a variety of anxiety disorders.

**1.6 Neurotransmitters mediating anxiety**

Neurotransmitters are chemical messengers involved in the transmission of a nerve impulse from one cell to another. They are formed inside the presynaptic neuron in vesicles (small membrane-bound sacks). After activating a

neuron, by an exocytosis process these vesicles release their content in the synapse. The most important neurotransmitter systems that are implicated in anxiety include the GABAergic system, the noradrenergic system and the serotonergic system (Rex, 2010).

1.6.1 Gamma-amino butyric acid

Amino butyric acid (GABA) is the most abundant inhibitory neurotransmitter in the brain (Steimer, 2002) which tends to be deficient in anxiety disorders. There are 3 GABA receptor subtypes, $GABA_A$, $GABA_B$ and $GABA_C$ (Bormann, 2000). The $GABA_A$ receptor is known to have a major role in anxiety, epilepsy, alcoholism and other psychiatric disorders (Smith, 2001). $GABA_A$ receptors contain 5 protein subunits ($\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha_4$, $\alpha_6$) which their distribution is heterogeneous within regions of the brain. Receptor subunit configuration determines the receptor`s sensitivity to different ligands and its diversity of function (Lydiard, 2003). The $GABA_A$ receptor is often the target of pharmacologic agents, including the neurosteroids and benzodiazepines. The relevance of the GABA system for anxiety is widely supported by pharmacological studies, showing that medication that impact GABA receptors system (i.e. GABA $_A$ receptors), such as benzodiazepines, are effective in the treatment of anxiety (Nemeroff, 2003; Steimer, 2002). Moreover, a number of studies have demonstrated that, compared to healthy controls, patients with anxiety disorders (panic disorder, PTSD and GAD) have lower benzodiazepine binding in various brain regions as assessed by several researches(J. D. Bremner, Innis, Southwick, et al., 2000; Goddard et al., 2001; Malizia et al., 1998; Tiihonen

et al., 1997). Also other GABAergic agents, have been evaluated for therapeutic options (Nemeroff, 2003). Hence, GABA system is an important target in the treatment of anxiety disorders.

1.6.2 Noradrenalin

The neurotransmitter noradrenalin is closely involved in a range of psychological processes. Consistent with this, alterations in this neurotransmitter system have been implicated in a range of psychiatric disorders, including anxiety. Preclinical studies in rats have shown associations between anxiety and high levels of noradrenalin in several brain regions, including the hypothalamus and amygdala (Steimer, 2002). Moreover, Bremner et al., showed that, yohimbine administration (which is an $\alpha_2$-antagonist) that increases brain noradrenalin release and results in symptoms of PTSD and fear and anxiety in PTSD patients) was decreased in prefrontal, temporal, orbitofrontal, and parietal cortex in patients with Vietnam combat-related PTSD, compared to healthy controls (Bremner et al., 1997). This suggests that yohimbine administration in normal controls may be associated with lower levels of norepinephrine release in central brain regions than in patients with PTSD. Thus, noradrenaline appears to play an important role in the etiology and treatment of anxiety disorders.

**2. Serotonin**

The neurotransmitter serotonin controls anxiety with different distinct mechanisms. For instance, the ascending serotonin pathway (originated from dorsal raphe nuclei-DRN) facilitates conditioned fear, while the DRN-periventricular pathway inhibits reaction to danger (Steimer, 2002). Serotonin is

the most widely investigated neurotransmitter in relation to mental disorders. Moreover, serotoninergic antidepressants, in particular Selective Serotonin Reuptake Inhibitors (SSRIs), are the most widely prescribed medication for internalizing disorders, including anxiety disorders. For the purpose of the present thesis, this neurotransmitter system will be described in greater detail in the next section.

## 2.1 The serotonergic system

### 2.1.1 The anatomy of the serotonergic system

Serotonin (or 5-hydroxitryptamine, 5-HT) is a key neurotransmitter which is developed and expressed early in humans (Whitaker-Azmitia, 2010). The location of serotonergic cell bodies in the brain is limited to the raphe nuclei, caudal to the isthmus, but serotonergic fibers project to most parts of the brain. Raphe nuclei are located near the midline of the brainstem with its entire rostro-caudal extension (Hornung, 2003). There are two groups of serotonergic neurons: superior group (superior brain stem group) and inferior group (inferior brain stem group). The superior raphe group is mainly divided into caudal linear nucleus, dorsal raphe nuclei and median raphe nucleus. The caudal linear nucleus is the most rostral nucleus of the raphe nuclei containing 5-HT-synthesizing neurons, while the largest population of serotonergic neurons in human is located in the dorsal raphe (Hornung, 2003). Most of the serotonergic fibers in the superior group of raphe nuclei project to the forebrain (Tork, 1990). Also, the dorsal raphe nucleus and caudal linear nucleus (rostral division of superior group), project to

the basal ganglia, cerebral cortex and median raphe nuclei (caudal division), innervates the amygdala (Jacobs & Azmitia, 1992).

The inferior raphe group is divided into nucleus raphe magnus, nucleus raphe obscurus, and nucleus raphe pallidus, all of which project to the spinal cord. In the central nervous system, the inferior parts contain only 15% of the total 5-HT production (Jacobs & Azmitia, 1992).

2.1.2 Serotonin synthesis and turnover

The physiological effects of 5-HT are mediated by a variety of proteins that regulate its synthesis, release, reuptake and degradation. 5-HT is a biogenic monoamine transmitter which contains only one ethylamine linked to an aromatic ring system (Hasegawa & Nakamura, 2010). In serotonergic cells, 5-HT is derived from tryptophan, an essential amino acid, which is actively transported into brain (Hasegawa & Nakamura, 2010). 5-HT cannot pass the brain barrier by itself, but its precursor L-tryptophan can pass by a facilitated diffusion process, through the competition between free tryptophan and large neutral amino acids (Buckworth & Dishman, 2002). 5-HT is synthesized by a short metabolic pathway consisting of two enzymes: 1) tryptophan hydroxylase (TPH) and 2) aromatic- L-amino acid decarboxylase (AADC) (Hasegawa & Nakamura, 2010). Tryptophan hydroxylase is a biopterin-dependent monooxygenase (Kaufman, 1962) which is a rate-limiting step in the pathway (Fitzpatrick, 1999; Grahame-Smith, 1964; Ruddick et al., 2006). Tryptophan hydroxylase exists in two isoforms: $TPH_1$and $TPH_2$. The AADC catalyzes the decarboxylation of 5-hydroxy-L-tryptophan, yielding in 5-HT producing cells (Hasegawa & Nakamura, 2010; Voltattorni,

Minelli, & Turano, 1971) (Figure 1). The synthesis of 5-HT is dependent on the tryptophan plasma concentration. A tryptophan-free diet causes a decrease in tryptophan's level in plasma and brain which in turn leads to a decrease in concentration of 5-HT (Biggio, Fadda, Fanni, Tagliamonte, & Gessa, 1974; Booij, Van der Does, & Riedel, 2003). Finally, a decrease in extracellular 5-HT will have consequences on neural activity (Feenstra & van der Plasse, 2010).

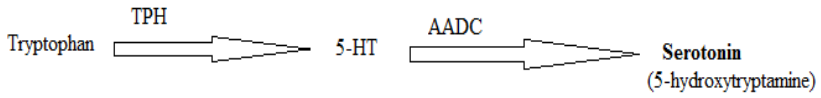


**Figure 1:** Biosynthesis of 5-HT.

After synthesizing, 5-HT is stored in vesicular pools or is released to the extracellular space and be transferred to the receptors (Figure 2).

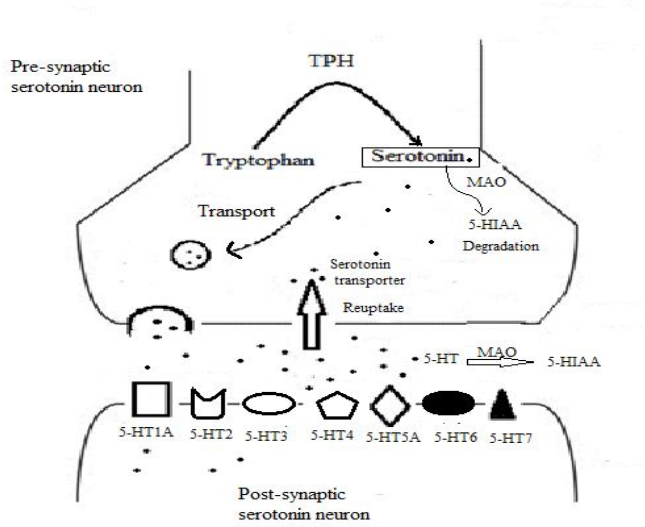


**Figure 2:** Schematic illustration of serotonergic synapse. After tryptophan is converted to 5-HT, 5-HT is packaged into storage vesicles or removed from the synaptic cleft by the by the 5-HT transporter (SERT). Monoamine oxidase (MAO) converted 5-HT to 5-hydroxyindoleacetic acid (5-HIAA). Inter-synaptic 5-HT stimulates its post synaptic receptors ($5\text{-HT}_{1\text{-}7}$). Figure based on the Handbook of behavioral neurobiology of 5-HT (Müller & Jacobs, 2009).

2.1.3 Serotonin receptors

5-HT receptors are distributed in the brain and body in many cell types. There are at least 14 distinct receptors that are classified into seven groups, $5\text{-HT}_{1\text{-}7}$ that are mediating 5-HT signaling (Figure 2) (Barnes & Sharp, 1999; Hoyer, Hannon, & Martin, 2002). 5-HT receptors are all coupled G-proteins (guanine nucleotide-binding protein), except for $5\text{-HT}_3$ receptor which is a ligand-gated ion channel.

**Table-1** Summary of different subtypes of 5-HT receptors with their distributions and effectors mechanisms (Siegel et al., 1999).

| Receptor | Human locus | Distribution | Effector mechanism |
|---|---|---|---|
| 5-$HT_{1A}$ | 5q11.2–13 | Hippocampus, amygdala, septum, entorhinal cortex, hypothalamus, raphe nuclei | Inhibition of adenylyl cyclase, opening of $K^+$ channels |
| 5-$HT_{2A}$ | 13q14–21 | Claustrum, cerebral cortex, olfactory tubercle, striatum, nucleus accumbens | Stimulation of phosphoinositide -specific phospholipase C, closing of $K^+$ channels |
| 5-$HT_{2B}$ | 2q36.3–37.1 | ? [b] | Stimulation of phosphoinositide -specific phospholipase C |
| 5-$HT_{2C}$ | Xq24 | Choroid plexus, globus pallidus, cerebral cortex, pallidus,hypothalamus, septum, substantia nigra, spinal cord | Stimulation of phosphoinositide - specific phospholipase C |
| 5-$HT_3$ | ? | Hippocampus, entorhinal cortex, amygdala, nucleus accumbens, solitary tract nerve, trigeminal nerve, motor nucleus of the dorsal vagal nerve, area postrema, spinal cord | Ligand-gated cation channel |
| 5-$HT_4$ | ? | Hippocampus, striatum, olfactory tubercle, substantia nigra | Stimulation of adenylyl cyclase |
| 5-$ht_{5A}$ | 7q36 | ? | Inhibition of adenylyl cyclase |
| 5-$HT_{5B}$ | 2q11–13 | ? | ? |
| 5-$ht_6$ | ? | ? | Stimulation of adenylyl cyclase |
| 5-$HT_7$ | 10q23.3–24.3 | Cerebral cortex, septum, thalamus, hypothalamus, amygdala, superior colliculus | Stimulation of adenylyl cyclase |

a) Lower-case appellations are used in some cases because the functions mediated by these receptors in intact tissue are presently unknown.

b) Question marks mean that presently no studies have examined this, and thus the locus or distribution is unknown

## 2.1.4 The serotonin transporter

The 5-HT transporter is a monoamine integral membrane protein (known as the SERT). In the brain, the highest concentration of 5-HT transporter is in the midbrain, thalamus and basal ganglia, while the lowest concentration is in the cortex (Frankle et al., 2006; Houle, Ginovart, Hussey, Meyer, & Wilson, 2000). The function of the 5-HT transporter is to move 5-HT from the synaptic space back into presynaptic neurons to be re-used again (Figure 2). This protein regulates serotonergic system and its receptors by modulating extracellular 5-HT concentration (Murphy, Lerner, Rudnick, & Lesch, 2004). The 5-HT transporters carry the preponderance of extracellular 5-HT back into the serotonergic neuron. These transports are dependent on extracellular concentration of $Na^{+}/CL^{-}$ (Figure 3).

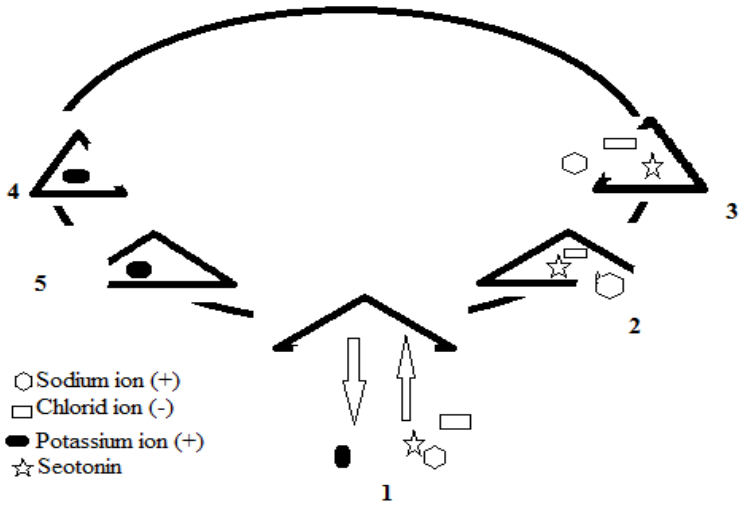

**Figure 3:** Schematic illustration of the 5-HT transporter. First, sodium binds to 5-HT and both of them bind to the transporter followed by a chloride ion. Then, neurotransmitters and ions release into the cytoplasm of the neuron. The reorientation of the carrier is occurring by binding intracellular potassium into the transporter and releasing potassium outside the cell. Figure based on (Cao, Li, Mager, & Lester, 1998).

2.1.5 Serotonin and behavior

Both animal and human studies have shown that 5-HT plays a significant role in a wide range of psychiatric disorders such as anxiety, aggression, suicide, as well as in certain personality traits, such as neuroticism. Fourteen distinct receptors mediate signalization of 5-HT (Barnes & Sharp, 1999; Bockaert, Claeysen, Dumuis, & Marin, 2010; Hoyer et al., 2002). Alteration of gene expression in these proteins in the brain could have important consequences for 5-HT synaptic availability or signaling in different receptors (O'Leary & Cryan, 2010). Thus, these changes in gene expression, in turn influence the serotonergic system, which may affect behaviors (O'Leary & Cryan, 2010).

In the past decade, numerous studies have tested associations between 5-HT genes and anxiety traits or anxiety disorders. One of the first studies investigating the link between 5-HT genes and behavior showed an association between the short variant of the 5-hydroxytryptamine-linked polymorphic region (5-HTTLPR) and anxiety-related personality traits (neuroticism) (Lesch et al., 1996). Since that time, numerous 5-HT genes have been associated with anxiety, though results are not entirely consistent. Among the many genes that have been

identified and investigated in association with behaviors, of particular interest are 5-hydroxytryptamine receptor 2A ($HTR_{2A}$) and Tryptophan hydroxylase-2 ($TPH_2$) polymorphisms. That is because $HTR_{2A}$ and $TPH_2$ are molecules strongly involved in 5-HT regulation that in turn influence the production of 5-HT in the brain. These genes and their associations with behaviors are described and investigated in greater detail in this thesis.

**2.2 $HTR_{2A}$ and anxiety**

The 5-$HTR_{2A}$ gene is located on chromosome 13q14-q21 in humans and consists of three exons separated by two introns (Chen, Yang, Grimsby, & Shih, 1992). A number of single nucleotide polymorphisms have been characterized in this gene, including T102C and –1438A/G in the promoter region (Li, Duan, & He, 2006).

Wesstaub et al. generated mice that were genetically modified with global disruption of $5HTR_{2A}$ signaling capacity ($htr_{2a}^{-/-}$ mice). In these mice, anxiety traits decreased, while after some restoration mainly in layer V of the cortex for 5-$HTR_{2A}$, the anxiety phenotype has been reversed (Weisstaub et al., 2006). This study suggests that $5HTR_{2A}$ plays an important role in the modulation of conflict anxiety. Also, 5-$HTR_{2A}$ has been shown to be involved in the pathophysiology of anxiety disorders in dogs; dogs with anxiety disorders had Lower 5-$HT_{2A}$ receptor binding index in the left and right frontal cortices than normally-behaving dogs (Vermeire et al., 2009).

In humans, one study demonstrated an association between PD symptom severity and the number of T alleles in $HTR_{2A}$ polymorphisms which 15

polymorphic SNPs of the $HTR_{2A}$ gene were associated with clinical diagnosis of PD and severity of PD (Unschuld et al., 2007).

Another study found an association between the promoter region of $HTR_{2A}$ (1438 G/A, rs6311) and OCD symptomatology (Walitza et al., 2011). Specifically, within the OCD patient group, the A allele was associated with higher scores on the Y-BOCS scores and a 2.5 year earlier age of onset. No other human studies were found that investigated the association between the 5-$HT_{2A}$ gene and anxiety. With regard to other psychiatric disorders such as schizophrenia, attention deficit hyperactivity disorder, suicide, eating disorders, and Alzheimer's disease, the association between the $HTR_{2A}$ gene and disease have been inconsistent (Norton & Owen, 2005).

**2.3 $TPH_2$ and anxiety**

Tryptophan hydroxylase (TPH) has two isoforms, $TPH_1$ and $TPH_2$. Whereas $TPH_1$ is found mainly expressed in the periphery, a second isoform, $TPH_2$, is exclusively found in the brain (Hasegawa & Nakamura, 2010). The $TPH_2$ gene is located on chromosome 12q21.1 and consists of 11 exons (Booij et al., 2011). A study in inbred mice demonstrated lower (50%-70%) 5-HT synthesis in the frontal cortex and stratum in homozygous carriers of the 1473G mutation comparing into homozygous mice carriers of the 1473C mutation (Zhang, Beaulieu, Sotnikova, Gainetdinov, & Caron, 2004). The relevance of this 1473G mutation for humans was supported by the initial observation of an association between the human analogue of this mutant and increased prevalence of depression (Zhang et al., 2004). However, in spite of these associations the

prevalence of this human mutant could not be detected in other human populations (see Booij et al., 2011 for an overview).

The relevance of other $TPH_2$ variants for *human* brain 5-HT synthesis was investigated in a recent study. Specifically, Booij et al. (Booij et al., 2011) investigated the possible role of intronic $TPH_2$ variants for human brain 5-HT synthesis in the orbitofrontal cortex, using in vivo positron emission tomography measures of 5-HT synthesis in 70 participants including 54 with no current mental disorder. They found dose-effect relationships for two variants which have previously been associated with suicide, demonstrating the relevance of the $TPH_2$ gene variation for 5-HT synthesis in the *human* brain.

Compared to the $5\text{-}HT_{2A}$ gene, much more studies are available that have examined the TPH2 gene in relation to mental health, including studies in anxiety disorder patients. A summary of these studies can be found in table 2 (Ottenhof, Ruhé, Lévesque, Booij, & TPH2 and psychiatric morbidity, Manuscript submitted). The $TPH_2$ gene has also been implicated as a candidate in other neuropsychiatric phenotypes including; suicidal behavior, depression and attention deficit/hyperactivity disorder (Ottenhof, Ruhé, Lévesque, & Booij, Manuscript submitted; Pavlov, Chistiakov, & Chekhonin, 2012).

**Table 2-1:** Summary of studies investigating the link between anxiety disorders (social and specific phobia, OCD, GAD, SAD, PD) and $TPH_2$ polymorphisms.

| **First author; year** | **Participants (male/female; mean age)** | **n** | **Measurements** | **SNP** | **Association** |
|---|---|---|---|---|---|
| Lin; 2009 | Han Chinese women (29.8 yrs[a]) with prepartum, postpartum and persistent depression, social and specific phobia, OCD, or generalized anxiety disorder diagnosed using SADS<br><br>Controls (women; 29.7 yrs) with no history of depression or anxiety | 117<br><br>83 | Patients (pts) vs controls<br><br>LD[b] | **C2755A**<br><br>**rs4570625**<br>**rs11178997**<br>**rs11178998**<br>**rs11179003**<br>**rs17110747** | Frequency of the AC genotype was higher in pts relative to controls<br>No sig. differences in genotype distribution in pts. relative to controls<br>Higher frequency of the rs4570625- rs11178997 - rs11178998-C2755A G-T-A-A haplotype in pts relative controls (though stricter permutation tests did not show significance)<br>Weak LD between C2755A and C10662T. LD between most of the adjacent markers. |
| Fur-mark; 2009 | Medication-free pts (14/20; 37.6±8.6 yrs) diagnosed with social anxiety disorder (SAD) (25 generalized, 9 non generalized) using SCID. 7 pts had a | 34 | Pts vs controls<br><br>fMRI responses to an emotional face- | **rs4570625** | No sig. differences in allele frequency in pts. relative controls<br>T allele carrying patients and controls (controls only at the uncorrected level) show a higher left amygdala response than GG homozygotes |

| | | | | | |
|---|---|---|---|---|---|
| | comorbid anxiety disorder: specific phobia (n=5), panic disorder and agoraphobia. Exclusion criteria: SAD treatment of social anxiety disorder in 6 months preceding the study, current serious or dominant psychiatric disorder other than SAD, chronic use of prescribed medication, alcohol or drug abuse, pregnancy, menopause, left handedness, previous PET examination and any somatic or neurologic disorder that could be expected to influence the outcome of the study. Healthy controls (9/9; 34.5±9.5 yrs). | 18 | processing task:<br>- pts vs controls<br>- subgroups of patients and controls with different genotypes | | T allele carrying patients show a higher left amygdala response than GG homozygote controls<br><br>Carrying the T allele was a significant predictor of reactivity in the left amygdala in the whole sample and in SAD patients |

| | | | | | |
|---|---|---|---|---|---|
| Fur-mark; 2008 | Medication-free patients (9/16; 35.6±10.7 yrs) diagnosed with SAD (18 generalized, 7 nongeneralized) using SCID. 8 patients had a comorbid anxiety disorder: specific phobia (n=5), generalized anxiety disorder (n=2), panic disorder (n=1) and mild posttraumatic stress disorder (n=1). Exclusion criteria: SAD treatment of social anxiety disorder in 6 months preceding the study, current serious or dominant psychiatric disorder other than SAD, chronic use of prescribed medication, alcohol or drug abuse, pregnancy, menopause, left handedness, previous | | PET scan during stressful speaking task<br><br>Responders vs non-responders<br><br>Post- vs pretreatment | **rs4570625** | T allele carriers showed a smaller rCBF decrease from pre- to posttreatment (8 weeks) with placebo in the right and left ($p_{uncorr} = 0.009$) amygdala.<br><br>Pre- to posttreatment differences in amygdala rCBF were only significantly reduced in homozygotic G allele patients<br><br>The rs4570625 SNP was a predictor for clinical placebo response on day 56.<br><br>Homozygosity for the G allele was associated with better outcome. |

| | | | | | |
|---|---|---|---|---|---|
| | PET examination and any somatic or neurologic disorder that could be expected to influence the outcome of the study. | | | | |
| Gut-knecht; 2007 | German patients diagnosed with cluster B (133/183; 35.3±13.0 yrs) or cluster C (39/65; 38.5±12.7 yrs) personality disorder using SCID-II. A subgroup of 166 pts also had anxiety-related comorbidity comprising neurotic, stress-related or somatoform disorders. Inclusion criteria: diagnosis and age=18-60. Exclusion criteria: medical conditions that have significantly changed the previous level of functioning, lifetime diagnosis of psychotic disorders. 73% of the pts also had a DSM-IV axis I diagnosis.<br><br>German controls (89/247; 22.4±5.3 yrs). | 420<br><br>336 | Pairwise linkage disequilibrium analysis<br><br>Allele frequency analysis in pts vs HC<br><br>Personality measures | **rs4570625**<br><br>**rs11178997** | T allele frequency was higher in pts with anxiety-related disorders (n=166) relative controls.<br><br>T allele frequency was higher in pts with adjustment disorders (n=99) relative controls.<br><br>No sig. differences in allele frequency in pts. relative controls |

| | | | | | |
|---|---|---|---|---|---|
| | Personality measures were taken with German versions of the NEO-PI-R and the TPQ. | | | | |

Note. a: yrs = years , b: LD = linkage disequilibrium

**Table 2-2:** Summary of studies investigating the link between panic disorder and $TPH_2$ polymorphisms.

| First author; year | Participants (male/female; mean age) | n | Measurements | SNP | Association |
|---|---|---|---|---|---|
| Campos; 2010 | Caucasian bipolar pts (82/203; 41.9±12.3 yrs[a]) diagnosed with MINI-PLUS. A subgroup of 45 pts had lifetime PD comorbidity (6/39; yrs). Exclusion criteria: history of alcohol and drug abuse and dependence. | 274<br><br><br><br><br><br><br><br><br><br>241 | Bipolar pts with PD comorbidity vs controls<br><br>Bipolar patients with PD comorbidity vs Bipolar patients without PD comorbidity | **rs4448731**<br><br><br><br><br><br>**rs4565946**<br><br><br><br><br><br>**rs11179000** | Higher frequency of the T allele and T/T genotype in BPD pts with PD relative controls and BPD pts with relative without PD<br>Higher frequency of the C allele and CC genotype ($p$ = 0.058; PMT) in BPD pts with PD relative controls and BPD pts with relative without PD<br>Higher frequency of the T allele in BPD pts with relative without PD<br>Higher frequency of the C allele and CC genotype in BPD pts with |

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|---|---|---|---|---|---|
| | Healthy controls (52.6±18.9 yrs) | | | **rs4760820**<br><br>**rs1487275**<br><br>**rs10879357**<br><br>**rs7955501**<br>**rs10506645** | PD relative controls and BPD pts with relative without PD<br>Higher frequency of the C allele in BPD pts with PD relative controls and BPD pts with relative without PD<br>Higher frequency of the A allele (PMT) and AG genotype ($p$ = 0.063; PMT) in BPD pts with PD relative controls.<br>Higher frequency of the A allele and AG or AA genotype (PMT) in BPD pts with relative without PD<br>No sig differences in allele frequency or genotype in all analyses<br><br>Higher frequency of the rs4448731-rs4565946 T-C haplotype in BPD pts with relative without PD<br>Lower frequency of the rs4448731-rs4565946 C-T haplotype and the rs10506645-rs4760820 C-C haplotype in BPD patients with relative without PD |
| Kim; 2009 | Unrelated Korean patients (50/58; 41.8±10.1 yrs) diagnosed with | 108 | Pts vs controls<br><br>Pts vs | **rs4570625** | Higher frequency of the G allele in:<br>- Pts relative controls<br>- Female patients relative female |

| | | | | | |
|---|---|---|---|---|---|
| | SCID Exclusion criteria: comorbid mood disorders, psychotic disorders or other anxiety disorders. Severity of the patients' symptoms was assessed with the STAI, PDSS, ASI, API and HAMD.<br><br>Unrelated matched Korean healthy controls (122/125; 39.4±9.0 yrs)<br>Exclusion criteria: History of personal or family psychiatric disorders or psychotropic medication. | 247 | controls in gender subgroups<br><br>Male vs female pts/controls<br><br>Symptom severity | | controls<br>- Male relative female controls<br><br>No sig. difference in allele frequency in:<br>- Male patients relative male controls<br>- Male relative female patients<br><br>No sig. difference in genotype in pts relative controls<br><br>Lower frequency of the TT genotype in pts relative controls ($p$ = 0.055) |
| Maron; 2007 | Estonian patients (49/164; 37.9±12.6 yrs) diagnosed with the MINI. Patients were included with comorbid agoraphobia (58%), major depression (51%), bipolar | 213 | Pts vs controls<br><br>Pts vs controls in gender subgroups<br><br>Pts vs | **rs1386494**<br>**rs1386483** | No sig. differences in allele frequency and genotype in pts. relative controls in whole sample<br><br>Sig. higher frequency of the rs1386494 A allele and the A/G genotype in female PD patients without comorbidity (n=52) relative female controls. (In the |

| | | | | | |
|---|---|---|---|---|---|
| | disorder (14%) and other anxiety disorders (4%). Exclusion criteria: Other psychiatric comorbidity.<br><br>Matched Estonian healthy controls (91/212; 39.3±13.3 yrs). Exclusion criteria: Personal or family history of psychiatric disorders. | 303 | controls in a subgroup without comorbidity | | whole sample PD patients without comorbidity relative controls $p$ = 0.07 for genotype and $p$= 0.06 for allele analysis)<br><br>The rs1386494-rs1386483 C-G haplotype showed a lower frequency ($p$ = 0.07) and the T-A haplotype showed a higher frequency ($p$ = 0.05) in female patients without comorbidity relative female controls. |
| Mossner; 2006 | Unrelated German patients (49/85; 45.7±11.4 yrs) diagnosed with SADS-LA, CIDI and IDCL. Comorbidity with other anxiety disorders or depressive disorder was allowed as long as panic disorder was predominant.<br><br>Unrelated German controls (45.2±10.2 | 134<br><br>134 | Pts vs controls | **rs4570625**<br><br>**rs4565946** | No sig. difference in allele frequency and genotype in pts relative controls<br><br>No sig. difference in allele frequency and genotype in agoraphobic pts relative control |

| | | | | | |
|---|---|---|---|---|---|
| | yrs) matched for gender and age. Controls were not screened for personal or family history of psychiatric disorders | | | | |

**Table 2-3:** Summary of studies investigating the link between OCD and $TPH_2$ polymorphisms.

| First author; year | Participants (male/female; mean age) | n | Measurements | SNP | Association |
|---|---|---|---|---|---|
| 2011; Rocha | Brazilian-Caucasian pts (18-65 yrs[a]) diagnosed using the MINI-PLUS. Exclusion criteria: major depression, bipolar disorder, substance-related disorder, psychotic disorder, lifetime history of traumatic / vascular brain injury<br><br>Brazilian-Caucasian | 107<br><br>214 | Pts vs controls | **rs4448731**<br>**rs4565946**<br>**rs11179000**<br>**rs7955501**<br>**rs10506645**<br>**rs4760820**<br>**rs1487275**<br>**rs10879357** | No sig. difference in allele frequency or genotype between pts and controls.<br><br>Higher frequency of the rs448731-rs4565946-rs10506645 T-C-T and the rs4565946-rs7955501-rs10506645 C-A-T haplotypes in pts relative controls |

| | | | | | |
|---|---|---|---|---|---|
| | matched controls screened using the MINI-PLUS. Free of psychiatric illness and no family history of Axis I psychiatric disorder. | | | | |
| 2005; Mössner | 71 German families of children (41/30; 13.29±2.6 yrs) diagnosed using the C-YBOCS[c]. Comorbid disorders were assessed using DIPS[d]. All pts had received in-patient treatment at the University of Aachen. Exclusion criteria: lifetime history of psychotic disorder, Tourette's syndrome, autistic disorder, alcohol dependence, IQ<70 and if obsessive compulsive symptoms occurred only as part of another disorder. | 71 | TDT[b] | **rs4570625**<br><br>**rs4565946** | No preferential allele transmission<br><br>Preferential transmission of the C allele in pts ($p$ = 0.059) Higher transmission frequency of the CC compared to the TT genotype<br><br>Preferential transmission of the G-C haplotype in pts. The G-T haplotype was transmitted to pts less frequently. |

Note. a: yrs = years, b: TDT = transmission disequilibrium test, c: C-YBOCS = Yale–Brown Obsessive Compulsive Scale

d: DIPS = Diagnostisches Interview bei psychischen Sto¨rungen im Kindes- und

Furthermore polymorphisms in the promoter region of the $TPH_2$ gene have been reported to be associated with personality traits of harm avoidance and disorders related to emotional dysregulation in healthy samples (Gutknecht et al., 2007; Reuter, Ott, Vaitl, & Hennig, 2007).

**3. Anxiety, Serotonin and Gender**

As indicated above, epidemiological findings reveal a gender difference in anxiety: females are predisposed to experience anxiety more likely than males (American Psychiatric & American Psychiatric Association. Task Force on, 2000; Yonkers, Bruce, Dyck, & Keller, 2003). Given the role of 5-HT in anxiety, one of the explanations accounting for this difference in prevalence may be related to gender differences in the regulation of 5-HT. For instance, an in vivo Positron Emission Tomography (PET) study has shown that the rate of 5-HT synthesis in healthy males is 52% higher than in healthy females (Nishizawa et al., 1997). Moreover; females show a greater behavioral response to acute tryptophan depletion procedures (Booij, van der Does, Haffmans, Spinhoven, & McNally, 2005; Booij et al., 2002). These results pointed to the importance of studying gender differences in the occurrence of anxiety disorders, as a function of 5-HT genotypes. This however has to our knowledge not been investigated yet.

## 4. Summary

Dysregulation of the serotonergic system has been shown to be an important factor in the pathophysiology of several psychiatric diseases. In the previous sections we have focused on several researches showing an association between 5-HT polymorphisms and anxiety disorders. However, as indicated in the tables above, research is not always consistent. With regard to $HTR_{2A}$ two animal studies demonstrated a relation between $HTR_{2A}$ and anxiety-related behavior or anxiety disorders. In the human studies, whereas two studies demonstrated a relation between $HTR_{2A}$ and anxiety disorders in the clinical samples (Inada et al., 2003; Maron et al., 2005) two other studies found no association (Fehr et al., 2001; Rothe et al., 2004). Regarding the $TPH_2$ gene, conflicting results were found in human studies. While two studies did not show significant associations between $TPH_2$ and anxiety disorders (Mossner et al., 2006; Staals et al., 2011) other three studies demonstrated a relation between $TPH_2$ and anxiety disorders association(Lin, Ko, Chang, Yeh, & Sun, 2009; Maron et al., 2007). A limitation of these studies however is that the majority of them measure anxiety at a single point in time, are cross-sectional and most of the studies are in adults. Thus, the role of 5-HT genes in the longitudinal developmental course of anxiety is not known. Compared to cross-sectional studies, longitudinal studies have clear advantages, especially when they are based on representative community sample followed over a long period of time (from childhood to adulthood) with multiple measurements over a long period of time. Hence, a strength of the current study was its longitudinal design, allowing having multiple measures of anxiety and

careful investigation of the developmental course in behaviors, over a long period of time.

**Objectives**

The aim of the present study was to investigate the relationships between developmental patterns of anxiety in childhood/adolescence, 5-HT genes and the development of psychopathology in adulthood, using a longitudinal design with a focus on the 5-$HT_{2A}$ gene polymorphisms as well as $TPH_2$. Specific aims were to:

A) Examine the association between specific 5-$HT_{2A}$ and $TPH_2$ polymorphisms and trajectories of anxiety in childhood and adolescence. Since most of the specific polymorphisms available in the investigated sample have not been tested yet in other studies, no specific hypotheses in terms of direction of effect can be defined.

B) Examine whether a trajectory of anxiety in childhood-adolescence predicted clinical diagnosis in adulthood.

C) Examine the association between 5-$HT_{2A}$ and $TPH_2$ polymorphisms and clinical diagnosis in adulthood. It was examined whether polymorphisms of the 5-$HT_{2A}$ and $TPH_2$ gene could predict anxiety disorders in adulthood.

D) Gender differences were tested.

# Method

**Study sample**

Hypotheses were tested in participants of a longitudinal study on Kindergarten Children in Quebec (ELEMQ). The community sample was representative from all Quebec regions, including both rural and urban areas. This cohort consists of 3185 boys and girls (1669 boys and 1516 girls) selected in 1986 and 1987 from Kindergartens in Quebec's (Canada) French speaking schools. Participants had been followed extensively from age 6 to age 14, and at regular intervals until adulthood. Most of participants (82.9%) were living with both biological parents and the remaining 17% were living within other family structures. The mothers of the participants have completed an average of 11.79 years of schooling, and the mother`s mean age at birth of their first child was 24.54 (SD = 3.83). 5-HT genotyping data are available for 1068 cohort members, of who were 436 boys and 632 girls. The study was approved by research ethics boards of the CHU Saint-Justine and the University of Montreal. Written informed consent was obtained from all participants.

**Developmental trajectories of anxiety**

Items of the Social Behavior Questionnaire (SBQ) (Tremblay et al., 1991) were used to assess children's anxiety. The SBQ is a 38 item questionnaire which has been divided into four components: a) disruptiveness b) anxiety c) inattention and d) prosocial (Tremblay et al., 1991).

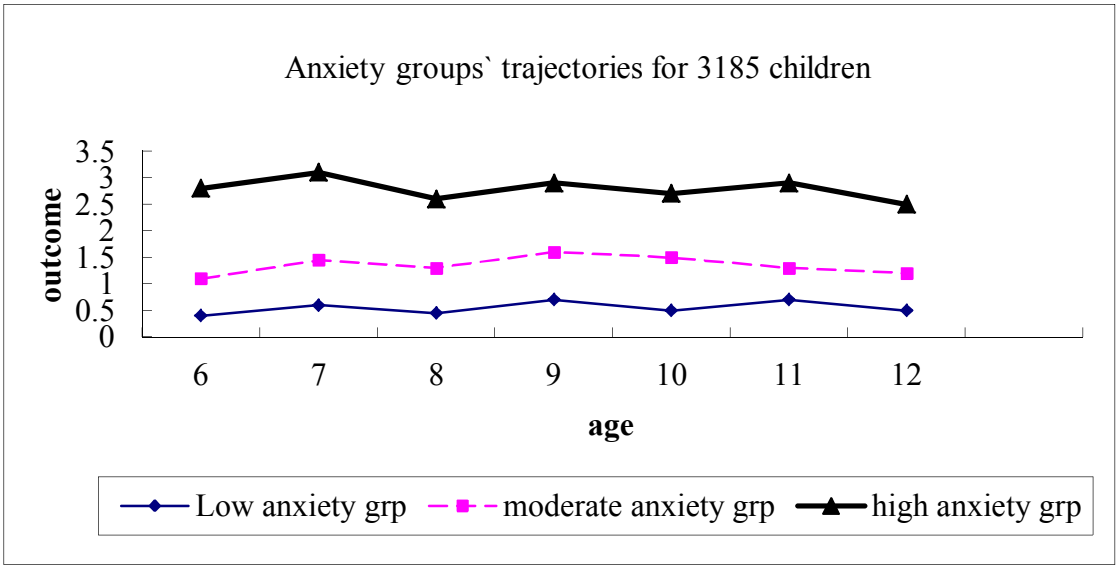
Children's anxiety was rated by means of the SBQ by their teachers when they were in kindergarten and continued over the next seven years while they attended elementary school.

For anxiety, the following items were scored by their teachers: worries (worries about many things); fearfulness tendency (fearfulness or afraid of new things or new situation); cries easily.

Seven yearly assessments of anxiety symptoms between ages 6 and 14 were used to estimate the behavioral trajectories.

The trajectory of anxiety was modeled on a group-based semi parametric mixture method. This semi parametric mixture method is suited to assess population heterogeneity over time considering the behavior (Jones, Nagin, & Roeder, 2001; Nagin, 1999). The model describes the shape of trajectory for each group and estimates the probability of belonging to a group for each individual. Using this model, the following developmental trajectories were identified for anxiety (Figure 4): a) Low level of anxiety (29.6 %, 942 boys/girls) b) Moderate level of anxiety (61.5 %, 1958 boys/girls) c) High level of anxiety (8.9%, 285 boys/girls). Figure 4 below shows the development trajectory of anxiety in these participants based on age and semi-parametric model outcome. The low anxiety trajectory consisted of 480 boys and 462 girls of cohort members, 1039 boys and 919 girls of members were in moderate level anxiety trajectory and the high anxiety trajectory consisted of 150 boys and 135 girls.

**Figure 4:** Development trajectories of anxiety, between age of 6 and 12 years.




* group = grp

**Diagnostic Interview Schedule for Adults (DIS)**

In early adulthood (age 21), participants were diagnosed in accordance to the Diagnostic and Statistical Manual of Mental Health Disorders, Third Edition(American Psychiatric. A., 1987). Mood, substance abuse disorders and anxiety (generalized anxiety, panic and phobias) were assessed through an interview by psychology students who had experience using the DIS. A total of 1085 cohort members were interviewed in which 239 of them were diagnosed having anxiety disorders (general anxiety, panic and phobias). Only anxiety disorders (having either GAD, PD or phobia) were analyzed in the present thesis.

**Genotyping**

In the current study we analyzed a total of 37 single nucleotide polymorphism (SNPs) within the 5-hydroytryptamine receptor 2A ($HTR_{2A}$) and

tryptophan hydroxylase-2 ($TPH_2$). Specific polymorphisms were determined based on a literature review (Brezo et al., 2010). Using HapMap data for the Utah residents with Northern and Western European ancestry and Tagger`s multimarker-tagging procedure (r 2> 0.8), common tag SNPs (minor allele frequency (maf) > 5%) were chosen for each gene. SNPs located up to 5 kbp upstream of the transcription site (maf > 5%)(Brezo et al., 2010). For more specific literature for the $HTR_{2a}$ and $TPH_2$ gene, see page 31-43.

**Statistics**

In order to examine the influence of $TPH_2$ and $HTR_{2A}$ on the trajectory of anxiety in detail, two approaches were applied based on two key outputs of the model: a) the estimated proportion (probability) of children belonging to each trajectory (continuous approach), and b) the similarities in the shape of their trajectory (categorical approach).

***Continuous approach***

Prior to analyses, statistical assumptions were tested using the Shapiro-Walk test and by visual inspection using histograms (normality assumption) and Levin's test (homogeneity of variance assumption). Data transformation (logit) was applied on the probability data since these data were highly-skewed. However, untransformed values are displayed in the text and graphs to facilitate interpretation. One-way analyses of variance (ANOVAs) were used to test for differences in probability to be in the high anxiety trajectory across genotypes, using the probability to be in the high anxiety trajectory as dependent variable and genotype as between-subjects factor. For all analyses, post-hoc tests for multiple

comparisons were conducted in case of three group analyses. Values are expressed as mean (± SD).

***Categorical approach***

Data were analyzed by chi-square tests in order to assess the link between genotypes and the anxiety trajectories. Next, two sets of logistic regression analyses were performed. First we analyzed the association dichotomously. Specifically, the extreme group(s) of the trajectory was compared with the other group(s) (dichotomous dependent variable) across the genotypes (independent variable) using binary logistic regression. Also the moderate anxiety group was compared with the two extreme groups. Thus, the following three associations were analyzed: a high anxiety group contrasted with a combined low/moderate group; a low anxiety group contrasted with a combined high/moderate anxiety group; and a combined low/high anxiety group contrasted against a group of only moderate anxiety cohort members.

Secondly, using multinomial logistic regression (allowing more than two categories as dependent variable) we analyzed the association between membership in three trajectory groups (dependent variable) and genotypes (independent variable).

Finally, we used a binomial logistic regression model to predict later diagnoses of anxiety disorders. We used the diagnosis of anxiety disorders (whether or not participant had been diagnosed with GAD or PD or phobias) as dependent variable and analyzed the association within polymorphisms (genes) as independent variable. By using an additive interaction model in logistic regression

we also evaluated whether the magnitude of the association between genes-anxiety differs across gender.

# Results

**Relationship between 5-HT genes and anxiety trajectories**

As described in the previous section, the relationship between 5-HT genes, specifically $HTR_{2A}$ and $TPH_2$, and anxiety trajectories were analyzed using both a *continuous approach* and *categorical approach.* First we applied a continuous approach in order to find the effects of genotype on the *probability* to be in the high anxiety trajectory. Then the categorical approach was used to a) investigate differences in genotype distribution between the different anxiety *trajectories,* b) investigate if membership in trajectory groups in childhood could predict clinical diagnoses of anxiety in adulthood c) examine the association between genotype and clinical diagnosis in adulthood and d) examine the gender differences.

***Continuous approach***

**Effects of genotype on probability of high anxiety**

Overall ANOVAs analyses for $HTR_{2A}$, demonstrated a significant effect of one SNP (rs1328684) on the probability of being in the high anxiety trajectory ($F(2, 1067) = 3.225$, $p = .039$). Post-hoc analyses with a Tukey correction showed that homozygous carriers of genotype C/C (M = .179, SD = .029) were more likely to be in the high anxiety trajectory than carriers of T/C (M =.079, SD = .008). There were no differences between C/C and T/T and T/C and T/T.

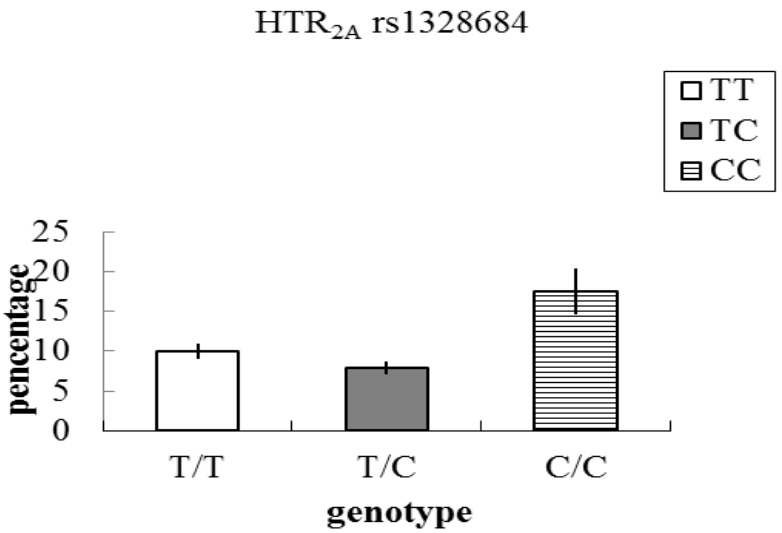
Allelic similar analysis of SNPs rs1328684 (T/C+C/C vs. C/C; $F(1, 1066) = 6.443$, $p = .011$) and rs95534511 (C/C+T/C vs T/T; $F(1, 1066) = 4.105$, $p = .043$) also showed significant associations with the probability of high anxiety. Specifically, for rs1328684, children carrying at least one C allele (C/T, C/C: M =.176, SD = .029) had a greater probability to be in the high anxiety trajectory

than children homozygous for T allele (T/T: M = .090, SD = .006). For rs95534511, children homozygous for T allele (M = .112, SD = .015) had a greater probability to be in the high anxiety trajectory than children with two C alleles (M =.095, SD = .007). There were no other group differences on any of the investigated 5-$HT_{2A}$ genotypes (Figure 5-1).

When similar analyses were repeated for $TPH_2$, the tag SNPs rs11179050 (F (2, 1065) = 2.747; d*f* = 2; *p* =.070) and rs11179052 (F (2, 1066) = 2.664; p =.077) approached significance associations on high anxiety probability. Specifically homozygosity for the C allele (M = .130, SD =.019) in rs11179050 was associated with a greater probability to be in high anxiety group than homozygosity for the T allele (M =.083, SD = .009, *p* = .043). By contrast, for rs11179052, locus T/T carriers (M =.134, SD =.093, *p* = .047) had a greater probability to be in high anxiety group than C/C carriers (M =.086, SD =.008) (Figure 5-2). No other differences for any of the other $TPH_2$ polymorphisms were found.

**Figure 5-1:** Probability to be in the high anxiety trajectory, as a function of 5-$HT_{2A}$ genotype rs1328684 (a) and rs95534511 (b). Probabilities are expressed in percentages.

a)




b)

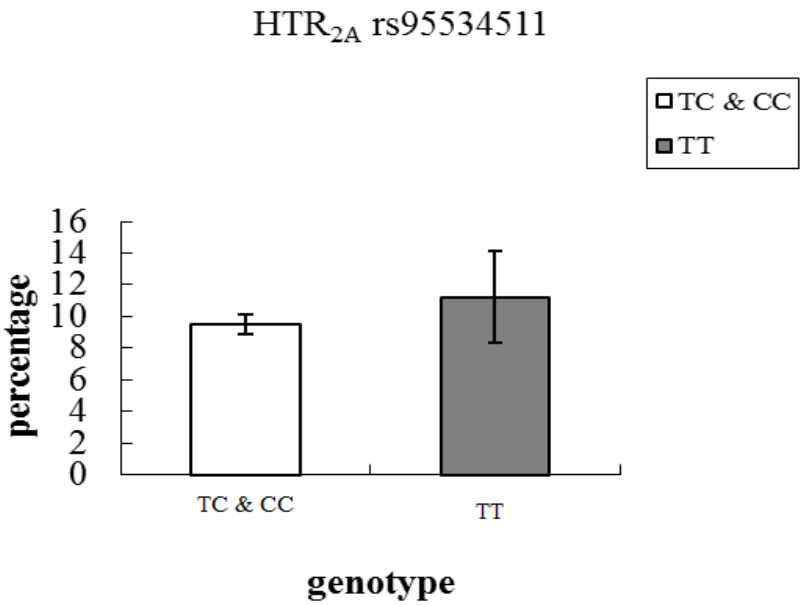
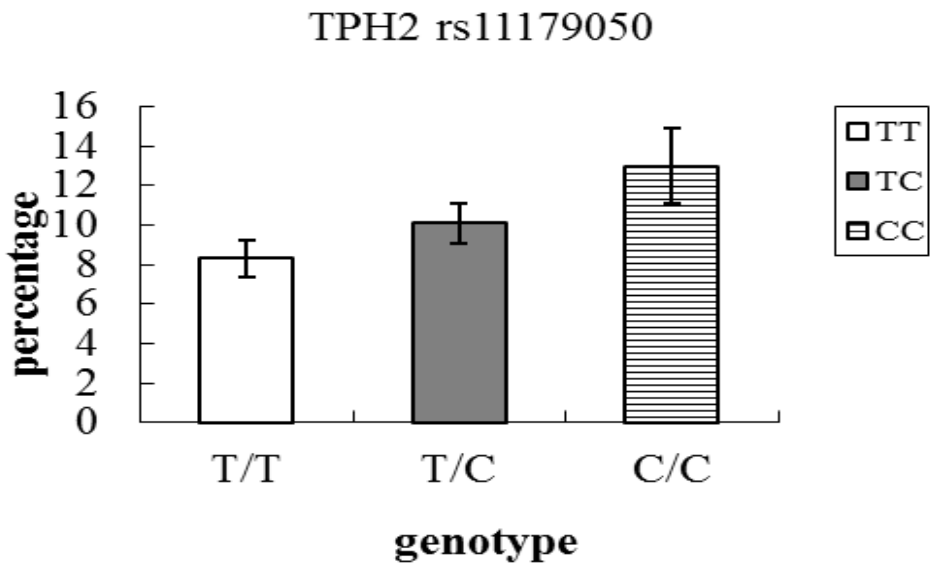
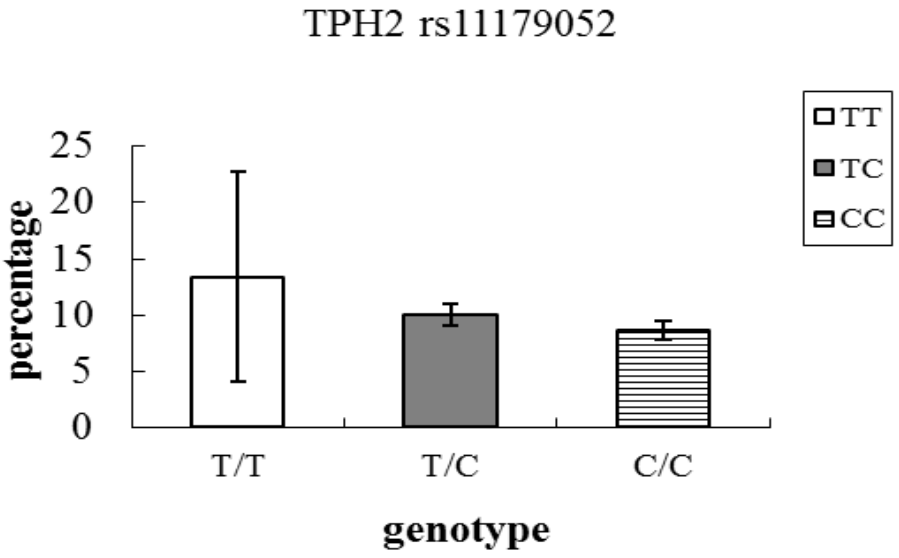


**Figure 5-2:** Probability to be in the high anxiety trajectory, as a function of $TPH_2$ genotype rs11179050 (a) and rs11179052 (b). Probabilities are expressed in percentages.

a)




b)




***Categorical approach***

**Effect of genotype on anxiety trajectory classification**

Chi-square statistics showed significant differences in the genotype frequencies (across anxiety trajectories) for three SNPs of $HTR_{2A}$; rs7330636, rs1745837 and rs1328684. For both $HTR_{2A}$ rs7330636 and rs1745837 variants, individuals in the high anxiety trajectory group were more often carriers of the A/G genotype than the G/G and A/A genotypes. For $HTR_{2A}$ SNP rs1328684, individuals in the high anxiety trajectory group had more often the T/T genotype than genotype T/C and C/C (See Table3). Among the investigated $TPH_2$ SNPs, only rs1386498 showed statistically significant associations with the anxiety trajectories. Specifically, individuals in the high anxiety trajectory group had more often the T/C genotype than genotypes C/C and T/T (see Table 3).

**Table 3:** Distribution of genotype frequencies of $HTR_{2A}$ and $TPH_2$ polymorphisms as a function of anxiety trajectory (percentage of genotype frequency).

| | **SNPs** | **Genotype frequency distribution within low, moderate and high anxiety trajectory (%)** | | | | $\chi^2$ | ***P*** |
|---|---|---|---|---|---|---|---|
| $HTR_{2A}$ | rs7330636 | Genotype low/moderate/high | A/G (51/44/55) | A/A (13/19/12) | G/G (35/37/34) | 10.140 | .038 |
| $HTR_{2A}$ | rs1745837 | Genotype low/moderate/high | A/G (41/39/46) | A/A (53/52/40) | G/G (6/10/14) | 9.783 | .044 |
| $HTR_{2A}$ | rs1328684 | Genotype low/moderate/high | T/C (45/48/32) | C/C (8.5/9/23) | T/T (47/43/45) | 18.688 | .001 |
| $TPH_2$ | rs1386498 | Genotype low/moderate/high | T/C (48/42/56) | C/C (34/41/31) | T/T (18/17/13) | 9.583 | .048 |

Next, a series of logistic regression analyses were conducted to test further the association between genotypes and trajectories. Contrasts between the trajectories groups were distinct in the way that a combination of two groups could be compared to a third, reference group. Thus the combined low and medium trajectory group was compared with the high anxiety trajectory group. *Analysis of $HTR_{2A}$*- The analysis showed significant associations between $HTR_{2A}$ rs1745837, rs1328684, rs7984966 and membership in the high anxiety trajectory versus low+ medium group. Specifically, there was a significant association between rs1745837 and membership in the high trajectory relative to the low-medium group. Individuals who were homozygous for the A allele were more often in the high anxiety trajectory group compared to those that were G

homozygotes (A/A vs. G/G, β = .824, SE = .357, Wald = 5.313, *p* = .021). Also for rs1328684, genotype C/C predicted membership in the high anxiety group compared to genotype T/T or T/C (C/C vs T/T, β = .903, SE = .304, Wald = 8.818, *p* = .003; C/C vs T/C, β = 1.282, SE = .321, Wald = 15.953, *p* = .001)

Another significant association was found between $HTR_{2A}$ variant rs 7984966 and the high anxiety trajectory. Specifically the analysis demonstrated that carriers of homozygous C/C were more likely to be in the high anxiety group than carriers of T/T (C/C vs T/T, β = .790, SE = .367, Wald = 4.633, *p* = .031).

*Analysis of $TPH_2$*- For $TPH_2$, significant associations between rs10748185, rs11179050, rs11179052, rs 11179056 and membership in the high anxiety trajectory relative to the low-medium anxiety group were found.

For SNP rs10748185, genotype A/G predicted membership in the high anxiety group compared to the genotype A/A (A/G vs A/A, β = . 800 , SE = .319, Wald = 6.292, *p* = .012). Also, for rs11179050, children who were homozygous for the T allele were more often in the high anxiety trajectory than those who were homozygous for the C allele (C/C vs T/T, β = .672, SE = .334, Wald = 4.036, *p* = .045). In contrast, for rs11179052, individuals who were homozygous for C are more likely to be in the high anxiety group than children were homozygous for the T allele (T/T vs C/C, β = .649, SE = .324, Wald = 3.890, *p* = .049). Finally, for rs11179056, carriers of the G/G and A/G genotype were more often in the high anxiety trajectory than carriers who were homozygous for the A allele (A/A vs G/G, β = 2.084, SE = .921, Wald = 5.118, *p* = .024, A/G vs A/A, β = 2.030, SE = .961, Wald = 4.448, *p* = .035).

**Anxiety trajectories and clinical diagnosis in adulthood**

We analyzed whether membership in trajectory groups in childhood were associated with anxiety disorders at age 21. Chi-square analyses showed that there was no association between trajectories of anxiety in childhood / adolescence and anxiety diagnosis in adulthood ($\chi^2 = 1.852$, $p = .396$).

5-HT genes and clinical diagnosis

Binomial logistic regression models showed that the genotype distributions were significantly different between those with and without an anxiety diagnosis for $HTR_{2A}$, rs3125, rs9567739, rs1885884, rs2224721 and $TPH_2$, rs4760820. Specifically, for SNP rs3125, genotype G/G or G/C predicted a diagnosis of anxiety compared to genotype C/C (G/G vs G/C, $p$ =.015; G/C vs C/C, $p$ = .026) (for complete statistics see Table 4).

Also for rs9567739 and rs1885884 carriers of C/G were more likely to be diagnosed with an anxiety disorder compared to carriers of homozygous C allele and G allele consecutively (C/G vs C/C, $p$ = .031; C/G vs G/G, $p$ = .036). Finally for $HTR_{2A}$ rs2224721, anxiety disorders were associated with the A/C genotype compared to the A/A genotype (AC vs AA, $p$ = .015). For $TPH_2$ polymorphism rs4760820, analysis showed that the carriers of two C alleles and were less likely to be diagnosed with an anxiety disorder than carriers of the G/G genotype (C/C vs. G/G, $p$ = .048) (see Table 4).

**Table 4:** Logistic regression analyses, predicting anxiety diagnosis in adulthood from $HTR_{2A}$ and $TPH_2$ genotypes.

| | **SNPs genotype** | **β** | **SE** | **Wald** | ***p*** |
|---|---|---|---|---|---|
| $HTR_{2A}$ | **rs3125** | | | | |
| | C/C vs G/G | 1.19 | .49 | 5.87 | .015 |
| | C/C vs G/C | 1.14 | .51 | 4.98 | .026 |
| $HTR_{2A}$ | **rs9567739** | | | | |
| | C/G vs C/C | .45 | .18 | 4.78 | .031 |
| $HTR_{2A}$ | **rs1885884** | | | | |
| | G/C vs G/G | .35 | .19 | 4.80 | .036 |
| $HTR_{2A}$ | **rs2224721** | | | | |
| | A/C vs A/A | .47 | .17 | 5.36 | .015 |
| $TPH_2$ | **4760820** | | | | |
| | C/C vs G/G | - .47 | .23 | 3.90 | .048 |

Note: There were no associations between anxiety diagnosis and other $TPH_2$ and 5-$HTR_{2A}$ polymorphisms.

**Gender differences**

**Effect of genotype on anxiety trajectory classification**

The association between genotype distributions and anxiety trajectories were significant when looking separately in boys and girls for three $HTR_{2A}$ SNPs and one $TPH_2$ polymorphism (see Table 5 and Table 6).

For boys, the association between anxiety trajectory and genotype distribution of three $HTR_{2A}$ rs1928042, rs9316235, rs1328684 ($\chi^2$ = 2.976, $p$ = .020*), (*$\chi^2$ =10.998, $p$ = .027*)*, ($\chi^2$ = 9.732, $p$ = .045) variants and one $TPH_2$ polymorphism rs11179000 ($\chi^2$ = 10.47, $p$ = .047) were significant. Specifically, in children characterized by a high anxiety trajectory, the prevalence of rs1928042 genotype A/A was 9-fold higher than the genotype C/C and ~ 3-fold higher than genotype A/C. For variant rs9316235, carriers of homozygous G were more likely to be in the high anxiety trajectory than carriers of the A/G and A/A genotype. Also for SNP rs1328684, boys who were homozygous for T allele were more often in the high anxiety trajectory than boys with at least one C allele. For $TPH_2$ rs11179000, boys with T/T genotype were more often in the high anxiety trajectory than boys with the T/A genotype.

In contrast, in girls, three $THP_2$ SNPs rs11179050, rs1487275, rs11179052 ($p$ = .046, $\chi^2$ = 9.583), ($p$ = .035, $\chi^2$ = 10.374), ($p$ = 0.025, $\chi^2$ =11.55) and only one $HTR_{2A}$ variant rs1328684 showed significant associations between genotype and anxiety (see Table 6). Specifically the analyses for the $TPH_2$ gene indicated that for rs11179050, girls with T/C genotype were more frequent in the high anxiety group than girls who were homozygous for the T or C allele. For SNP

rs1487275, girls homozygous for C/C were more likely to be in the high anxiety trajectory than girls with the A/C and A/A genotype. In addition, for $TPH_2$ variant rs11179052, girls with the T/C genotype were for often in the high anxiety trajectory than girls who were homozygous for the C or for the T allele. A similar effect was seen for girls for rs1328684, with a greater prevalence of the T/C genotype in girls with a high anxiety trajectory compared to the T/Tor C/C genotype.

**Table 5:** Distribution of genotypes frequencies of $HTR_{2A}$ and $TPH_2$ polymorphisms within low, moderate and high anxiety trajectories in boys (percentage of genotype frequency), $p < .05$

| | **SNPs** | **Genotype frequency distribution within low, moderate and high anxiety trajectory for boys (%)** | | | | **Chi-sq.** | ***p*** |
|---|---|---|---|---|---|---|---|
| $HTR_{2A}$ | rs1928042 | Genotype | A/C | A/A | C/C | 2.976 | .020 |
| | | low/moderate/high | 33/44.5/25 | 64/51/67.5 | 2.5/4/7.5 | | |
| $HTR_{2A}$ | rs9316235 | Genotype | G/G | A/G | A/A | 10.998 | .027 |
| | | low/moderate/high | 57.5/59/62.5 | 36/38/22.5 | 6/3/15 | | |
| $HTR_{2A}$ | rs1328684 | Genotype | T/T | T/C | C/C | 9.732 | .045 |
| | | low/moderate/high | 47/38/57.5 | 45/51/27.5 | 8/11/15 | | |
| $TPH_2$ | rs11179000 | Genotype | T/T | T/A | A/A | 10.47 | .047 |
| | | low/moderate/high | 58/60/72.5 | 35/38/27.5 | 7/2/0 | | |

**Table 6:** Distribution of genotypes frequencies of $HTR_{2A}$ and $TPH_2$ within low, moderate and high anxiety trajectories in girls (percentage of genotype frequency), $p < .05$

| | **SNPs** | **Genotype frequency distribution within low, moderate and high anxiety trajectory for girls (%)** | | | | **Chi- Sq.** | ***P Girl*** |
|---|---|---|---|---|---|---|---|
| $HTR_{2A}$ | rs1328684 | Genotype low/moderate/high | T/T (47/47/34) | T/C (44/45/36) | C/C (9/8/29.5) | 19.721 | .001 |
| $TPH_{2A}$ | rs11179050 | Genotype low/moderate/high | T/T (40/37/27) | T/C (50/47/48) | C/C (10/17/25) | 9.583 | .046 |
| $TPH_{2A}$ | rs1487275 | Genotype low/moderate/high | C/C (53/51/45.5) | A/C (43.5/42/39) | A/A (3.5/7/16) | 10.347 | .035 |
| $TPH_2$ | rs11179052 | Genotype low/moderate/high | C/C (43/42/36.5) | T/C (48.5/42.5/39) | T/T (9/15.5/25) | 11.155 | .025 |

**Additive effect of gender on genotype and anxiety trajectories**

Finally, we estimated the interaction on an additive scale between gender differences and genotype on membership in high anxiety group by a logistic regression model. There was "negative" interaction for $HTR_{2A}$ rs1328684 on additive scale ($\beta$ = -1.414, SE = .640, Wald = 4.881 $p$ = .027). Using the Formula of RERI (see Knol, van der Tweel, Grobbee, Numans, & Geerlings, 2007 for description of RERI), the amount of additive interaction was equal to – 0.24. This means that the relative risk of being in high anxiety trajectory group versus low-medium group was 0.24 less for boys than for girls (for rs1328684). There were no other additive interactions with gender.

# Discussion

The goal of the present study was to investigate the influence of 5-HT gene variation on the development of anxiety during the elementary school years (from kindergarten) until adolescence in a large longitudinal community sample of children. The first aim was to examine the relationships between thirty-seven SNPs within $HTR_{2A}$ and $TPH_2$ genes and anxiety developmental trajectories. The second aim was to investigate the association between the 5- $HTR_{2A}$ and $TPH_2$ genes, anxiety trajectories and clinical diagnosis in adulthood. The third aim was to explore gender differences. Trajectories of anxiety were based on teacher ratings conducted between the age of 6 and 12 years using the Social Behavior Questionnaire. These trajectories were defined as low anxiety trajectory, moderate anxiety trajectory and high anxiety trajectory, as described and calculated previously (Cote, Tremblay, Nagin, Zoccolillo, & Vitaro, 2002). Out of 37 investigated polymorphisms, several 5-$HTR_{2A}$ (rs1328684, rs95534511, rs1745837, rs7984966, 7330636) and $TPH_2$ (rs11179050, rs11179052, rs1386498) polymorphisms were associated with a high anxiety trajectory.

With regard to the $HTR_{2A}$ gene, the results are remarkably consistent with two different animal studies. As described in the introduction of this thesis, one study found that global disruption of the function of the $HTR_{2A}$ receptor (knockout model) led to an increase in risk behavior in a number of conflict anxiety paradigms in mice, while depression-related behaviors remained unaffected (Weisstaub et al., 2006). Another study found lower 5-$HT_{2A}$ receptor binding index in bilateral frontocortical regions of the brain of sixteen dogs

diagnosed with anxiety disorders, relative to 22 normal-behaving dogs (Vermeire et al., 2009).

In humans, polymorphic variation of the $HTR_{2A}$ gene has been assessed in relation to anxiety disorders in clinical samples, though with conflicting results (Fehr et al., 2001; Inada et al., 2003; Maron et al., 2005; Rothe et al., 2004). For instance, two studies found no association between $HTR_{2A}$ (T102C) and anxiety disorders (PD, GAD) (Fehr et al., 2001; Rothe et al., 2004). In contrast, two other studies (one conducted in Japan and another one in Estonia) demonstrated a positive association between $HTR_{2A}$ gene and PD (Inada et al., 2003; Maron et al., 2005). No previous study has investigated the link between the 5-$HTR_{2A}$ gene and anxiety in children, nor in relation to non-clinical levels of anxiety. The present study is the first study showing that polymorphic variation in the $HTR_{2A}$ gene is associated with variation in anxiety in a non-clinical community sample of children. Meta-analytic studies are needed to carefully compare the strength of effects across studies.

In addition, we also found significant associations between $TPH_2$ (rs11179050, rs11179052, rs1386498) and anxiety trajectories. The relevance of the $TPH_2$ gene for brain 5-HT synthesis has previously been demonstrated in animals (Zhang et al., 2004) and in humans (Booij et al., 2011). However, researches investigating associations between the $TPH_2$ gene and anxiety are inconsistent. Whereas some studies in Caucasians did not demonstrate significant associations between anxiety disorders (e.g.; PD, SAD) and $TPH_2$ polymorphisms (Mossner et al., 2006; Staals et al., 2011) another study in a Han Chinese

population in Taiwan, showed a positive association (Lin et al., 2009). Interestingly, in another study (Estonian population), the association between PD and $TPH_2$ polymorphisms was limited to female patients (Maron et al., 2007). The present study is the first to show the link between $TPH_2$ variation and developmental patterns of anxiety in a young sample. Again, future meta-analytic studies are needed to compare effect sizes across studies.

Though trajectories of anxiety in childhood and adolescence did not predict any diagnosis at age 21, relationships were found between $HTR_{2A}$ and $TPH_2$ SNPs and anxiety diagnosis at age 21. Interestingly, the SNPs for which we found associations with clinical anxiety diagnosis ($HTR_{2A}$: rs3125, rs9567739, rs1885884, rs222472, $TPH_2$: rs4760820) were not the same as the ones for which we found associations with developmental trajectories of anxiety assessed in childhood. This suggests that the genetic predisposition to develop anxiety in childhood may not be the same as the genetic predisposition to develop anxiety disorder in adulthood. No other studies were found that made such comparison directly. However, the findings of the present study are consistent with the observation that the core diagnostic criteria of anxiety might be different across children and adults. The present observations highlight to use special assessment and treatment strategies for this age group (Beesdo et al., 2009).

Interestingly, the association between $HTR_{2A}$ and $TPH_2$ genes and anxiety was different for boys and girls. Specifically, there was generally a stronger association between the high anxiety trajectory and $TPH_2$ genotype for girls than for boys. Whereas three $TPH_2$ SNPs (rs11179050, rs1487275, rs11179052) were

associated with the high anxiety trajectory in girls, just one $THP_2$ variant rs11179000 demonstrated this association in boys. These results are consistent with a study of Maron et al., showing an association between $TPH_2$ rs1386494 and PD in adult female patients whereas there was no such association for male patients (Maron et al., 2007). Interestingly, while for $TPH_2$ the effects were stronger for girls than for boys, the opposite was observed for $HTR_{2A}$ in our study. The evidence from different studies suggests that the impact of genetic factors on anxiety risk differ for men and women. Interestingly, gender also plays a role in responses to pharmacological treatment for anxiety disorders (Kinrys & Wygant, 2005). Furthermore, women are more responsive to serotonergic challenge procedures (Booij et al., 2002; Booij et al., 2005). The finding that the effects of the TPH2 gene on behavior as observed in the present study is greater in females than in males is in line with the observation that women have lower brain 5-HT synthesis than males (Nishizawa et al., 1997). Although speculative, genetic factors may play a more prominent role in the production of serotonin in females than in males, while other factors known to affect brain serotonin synthesis, such as the environment (Booij et al., 2012) may be more crucial for the brain serotonin production in men. A reverse mechanism may exist for the receptor gene. There are no studies however that compared the genetic and/or environmental contribution in serotonin neurotransmission between males and females, and future studies should investigate this further. Nevertheless, the results of the present study suggest that it is important to take gender into account when analyzing associations between serotonergic genes and behaviors.

Though the underlying mechanism remains unknown, these results demonstrated the importance of taking into account gender in the investigation of the link between 5-HT genes and anxiety.

The present study had a number of strengths. To our knowledge, this study was the first to investigate the association of $HTR_{2A}$ and $TPH_2$ genes with the developmental course of anxiety trajectories in a community sample. Moreover, most previous studies on the role of 5-HT genes in anxiety disorders were cross-sectional and were done in adult patients; therefore they revealed little information about the genetic underlying of the developmental pattern of anxiety. In addition, when using trajectories our analyses were based on multiple measurements of anxiety, rather than an anxiety measurement taken at a single point in time. This may have provided a better understanding of natural course of anxiety symptoms. Finally, this is one of the few studies taking gender differences into account.

The present study has the following limitations. First, we did not take into account the influence of environmental factors. Several studies show 5-HT gene X environmental interactions in the prediction of clinical diagnosis (Booij et al., 2011; Caspi et al., 2005). Exposures to environmental factors such as stressful life events, major health problems, childhood maltreatment and even socioeconomic factors have been associated with increased risk for anxiety and also interact with different genetic variants to predispose to aggression(Craig, 2007; Kim-Cohen, Moffitt, Taylor, Pawlby, & Caspi, 2005; Moffitt, 2005). However, in healthy samples, a number of studies have shown main effects of

genes on behavioral traits in nonclinical samples, while gene X environmental interaction effects were minimal or absent (Verhoeven, Booij, Kruijt, Cerit, & Van der Does, Manuscript in preparation). Nevertheless, even though adversity was expected to have a small effect on the outcome in this sample, it would be of interest to investigate these interactions further. Secondly, the diagnosed adult sample was covering PD, GAD and phobias together and because of sample size we did not have the statistical power to investigate associations between 5-HT genes and the specific anxiety diagnosis. Thus the relationships between 5-HT genes and PD, 5-HT and GAD, 5-HT and phobias specifically was not assessed. Third, although a candidate gene approach offer a powerful way of investigating possible genetic linkage directly, candidate gene studies, including the present study, typically involve a multitude of analyses, potentially causing many false positives. As we included a large number of polymorphisms in our study for exploratory purposes, as it is the case for many published candidate gene studies, power was insufficient to correct for multiple comparisons. Consequently, we cannot rule out the possibility of type I errors. Additional studies are needed to further replicate these findings.

Importantly, the functional relevance of most of the SNPs investigated in the present study should be further investigated. The potential functional role of some $TPH_2$ variants has been shown in several previous studies. Most of the evidence is related to the G1473A polymorphism, showing that homozygous carriers of the 1473G mutant had lower 5-HT synthesis in the frontal cortex and striatum (Zhang et al., 2004). However in humans this mutant is rare, if not absent

(Booij et al., 2011). In a recent study the possible role of some intronic $TPH_2$ variants for human brain 5-HT synthesis in the orbitofrontal cortex was investigated (Booij et al., 2011). The functional relevance of the $TPH_2$ variants tested in our study however is yet unknown.

Regarding the functional relevance of $HTR_{2A}$, studies in post-mortem brain samples demonstrated an association between genotype 1438A/G and 5-$HT_{2A}$ receptor expression (Myers, Airey, Manier, Shelton, & Sanders-Bush, 2007). Whereas some studies showed the relationship between T-allele of T102C SNP and higher 5-$HT_{2A}$ receptor binding (Khait et al., 2004; Turecki et al., 1999) others reported no association between 5-$HT_{2A}$ receptor density and genotype (Du et al., 1999; Kouzmenko et al., 1997; Kouzmenko et al., 1999). The functional relevance of the $HTR_{2A}$ SNPs used in the present study has not yet been investigated. Thus, more in vitro and in vivo studies are needed to understand the functional relevance of the $HTR_{2A}$ and $TPH_2$ variants in the current study.

In addition to genetic factors, behavioral, cognitive, interpersonal and contextual factors have to be considered for a better understanding of the origin of anxiety disorders (Essau & Petermann, 2002; Grahame-Smith, 1964; Vasey & Dadds, 2001). Thus prospective-longitudinal studies are necessary to investigate if adversity increases the risk for onset of an anxiety disorder in addition of the association between 5-$HTR_{2A}$/$TPH_2$ polymorphisms and development of anxiety in childhood. Furthermore, one of the problems of genetic studies is the heterogeneity and the types of genetic variation across a certain population (i.e. population effect) (Burmeister, McInnis, & Zöllner, 2008; Grahame-Smith, 1964).

As the large majority of the present sample was from Québec French speaking population, future studies should study whether the effects can be generalized to specific other ethnic groups. Finally taking into account that anxiety problems often co-occur with other psychiatric problems, future studies should take comorbidity into account in the analyses.

In conclusion, this is the first study reporting an association with $HTR_{2A}$, $TPH_2$ variants and trajectories of anxiety in children. Whereas diagnosis at adulthood was not predictive of trajectories of anxiety in childhood, the result of this study confirmed the link between some $HTR_{2A}$ and $TPH_2$ polymorphisms and diagnosis in adulthood. Moreover, this study also illustrates the importance of taking gender into account in genetic analyses. Future studies should replicate the findings in larger samples, in individuals with different ethnicities and further examine the interaction with early stress. Moreover, the functional relevance of the polymorphisms of investigation should be further investigated.

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