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# Association of regulatory *TPH2* polymorphisms with higher reduction in depressive symptoms in children and adolescents treated with fluoxetine

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ABSTRACT

Genetic variability related to the brain serotonergic system has a significant impact on both the susceptibility to psychiatric disorders, such as major depressive disorder (MDD), and the response to antidepressant drugs, such as fluoxetine. *TPH2* is one of the most important serotonergic candidate genes in selective serotonin reuptake inhibitors (SSRIs) pharmacogenetic studies. The aim of the present study was to evaluate the influence of regulatory polymorphisms that are specifically located in human *TPH2* transcription factor binding sites (TFBSs), and therefore could be functional by altering gene expression, on clinical improvement in children and adolescents treated with fluoxetine. The selection of SNPs was also based on their linkage disequilibrium with *TPH2* rs4570625, a genetic variant with questionable functionality, which was previously associated with clinical response in our pediatric population. A total of 83 children and adolescents were clinically evaluated 12 weeks after initiating antidepressant treatment with fluoxetine for the first time. Clinical improvement was assessed by reductions in depressive symptoms measured using the Children's Depression Inventory (CDI) scale. The polymorphisms rs11179002, rs60032326 and rs34517220 were, for the first time in the literature, significantly associated with higher clinical improvement. The strongest association was found for rs34517220. In particular, minor allele homozygotes showed higher score reductions on the CDI scale compared with the major allele carriers. Interestingly, this polymorphism is located in a human *TPH2* TFBS for two relevant transcription factors in the serotoninergic neurons, Foxa1 and Foxa2, which together with the high level of significance found for this SNP, could indicate that rs34517220 is in fact the crucial functional genetic variant related to the fluoxetine response. These results provide new evidence for the role of regulatory genetic variants that could modulate human *TPH2* expression in the SSRI antidepressant response.

## 1. Introduction

In accordance with the serotonin dysregulation hypothesis, selective serotonin reuptake inhibitors (SSRI), such as fluoxetine, are among the first-line medications for different psychiatric diseases including major depressive disorder (MDD), obsessive–compulsive disorder (OCD) and Generalized Anxiety Disorder (GAD) (Helton and Lohoff, 2015). Moreover, both the effectiveness and safety of fluoxetine have been demonstrated in children and adolescents (Keeton et al., 2009; Usala et al., 2008).

Genetic variability in genes associated with the brain serotonergic system has a significant impact on both the susceptibility to these psychiatric disorders (Gao et al., 2012; Mas et al., 2014; Taylor, 2013; Xia and Yao, 2015) and the response to antidepressant drugs (Brandl

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et al., 2012; Gassó et al., 2017; Helton and Lohoff, 2015; Mas et al., 2016; Porcelli et al., 2011). *TPH2* is one of the most important serotonergic candidate genes in MDD (Gao et al., 2012) and the pharmacogenetics of the antidepressant response (Porcelli et al., 2011). This gene codifies for tryptophan hydroxylase (TPH), the enzyme that catalyzes the rate-limiting step in serotonin biosynthesis. There are two isoforms, TPH1 and TPH2, but the latter seems to be more selectively expressed in the brain system (Zill et al., 2007). Long-term treatment with fluoxetine was found to be associated with up-regulation of *TPH2* mRNA expression, which seems to occur simultaneously with the antidepressant effect (Shishkina et al., 2007). In fact, there are numerous antidepressant therapies that influence *TPH2* gene expression. By increasing the understanding of *TPH2* regulation, this gene could be used as a target to develop new or optimize current therapies to improve the prevention and treatment of a wide variety of stress disorders (Chen and Miller, 2013).

Despite the lack of replicability of many results, as shown in the vast majority of pharmacogenetic studies, several *TPH2* single nucleotide polymorphisms (SNPs) have been associated with the antidepressant response to SSRI treatment. They include promoter and nonsynonymous coding SNPs, based on their presumable effect on gene expression or protein function, and also intronic variants for which there is still no evidence of their functionality (Peters et al., 2004; Rotberg et al., 2013; Tsai et al., 2009; Tzvetkov et al., 2008; Su et al., 2016; Xu et al., 2016; Zhang et al., 2005). In a previous study by our group, in which 47 candidate genes were assessed, *TPH2* rs4570625 was associated with clinical improvement after fluoxetine treatment (Mas et al., 2016). In detail, we analyzed 10 SNPs in *TPH2,* and the strategy for their selection was based on previously reported genetic associations and the tagging analysis to capture the most common variants throughout the gene. The associated polymorphism (rs4570625, also known as -703G > T) is located in the *TPH2* promoter region and is one of the most frequently assessed variants in this gene. It has been associated with major depression in a meta-analysis (Gao et al., 2012), but also with other psychiatric disorders including OCD (Mössner et al., 2006) and anxiety (Chi et al., 2013). Regarding antidepressant treatment, it has been related to citalopram and escitalopram response in independent populations (Rotberg et al., 2013; Su et al., 2016). However, it should be noted that no effect on antidepressant response was found for this particular *TPH2* polymorphism in a recently published study (Xu et al., 2016). Although it has been shown that a haplotype containing the minor allele rs4570625 was able to reduce gene expression in cell culture (Chen et al., 2008), the lack of influence of this polymorphism on *TPH2* transcription has also been reported (Scheuch et al., 2007). This raises the question whether this is the true functional variant or whether it could be other polymorphisms in linkage disequilibrium (LD) that have a real effect on human *TPH2* expression.

Regulatory SNPs can modulate gene expression through multiple mechanisms, transcription factor binding being one of the most important. These SNPs can be located in functional elements including promoters, enhancers and silencers, and can destroy, create, alter or modify sequences for transcription factors (TFs), leading to changes in the location, timing and level of gene expression, and ultimately being responsible for complex traits and diseases (Huang, 2015).

Based on all this background, we decided to study the genetic variability that could affect *TPH2* expression and, therefore, response to antidepressants. To this end, we evaluated the influence of regulatory polymorphisms in high LD with rs4570625 that are specifically located in human *TPH2* transcription factor binding sites (TFBSs) on clinical improvement in children and adolescents treated with fluoxetine.

**Table 1**
Demographic, clinical, and genotype data of the study population.

| | Patients (N = 83) |
|---|---|
| Male gender, N (%) | 26 (31.3) |
| Age (years, mean ± SD) | 14.7 ± 1.7 |
| Diagnosis, N (%) | |
| MDD | 57 (68.7) |
| OCD | 16 (19.3) |
| GAD | 10 (12) |
| Co-medication, N (%) | |
| Antipsychotics | 9 (10.8) |
| Benzodiazepines | 7 (8.4) |
| Antipsychotics + Benzodiazepines | 2 (2.4) |
| Antipsychotics + Mood stabilizers | 2 (2.4) |
| Genotype frequencies, N (%) | |
| rs58344694 | |
| CC | 59 (71.1) |
| CT | 21 (25.3) |
| TT | 9 (10.8) |
| rs11178997 | |
| TT | 67 (80.7) |
| TA | 16 (19.3) |
| AA | 0 (0.0) |
| rs11179002 | |
| CC | 38 (46.3) |
| CT | 35 (42.7) |
| TT | 9 (10.9) |
| rs17110489 | |
| TT | 44 (53.0) |
| TC | 36 (43.4) |
| CC | 3 (3.6) |
| rs60032326* | |
| GG | 49 (59.0) |
| GA | 31 (37.3) |
| AA | 3 (3.6) |
| rs34517220 | |
| AA | 35 (42.2) |
| AG | 40 (48.2) |
| GG | 8 (9.6) |

Abbreviations: CDI, Children's Depression Inventory; GAD, Generalized Anxiety Disorder; MDD, Major Depression Disorder; OCD, Obsessive Compulsive Disorder.

* This SNP was in complete linkage disequilibrium (LD) with rs57706328 for which the same percentages for the three genotypes (CC, CG and GG) were found.

## 2. Subjects and methods

### 2.1. Patients and clinical improvement assessment

A complete description of the population included in the study is given elsewhere (Gassó et al., 2014; Mas et al., 2016). Briefly, 83 children and adolescents receiving fluoxetine treatment for the first time were recruited. Patients were diagnosed with MDD, OCD or GAD according to the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) (American Psychiatric Association, 1994). The study was conducted at the Child and Adolescent Psychiatry and Psychology Service of the Institute of Neuroscience at the Hospital Clinic in Barcelona. Exclusion criteria were mental retardation, somatic or neurological disease, autism, psychotic disorders, and non-Caucasian ethnicity. All subjects began fluoxetine treatment in the initial phase of the study, this being the first time that they had received antidepressant treatment. Some patients were temporarily co-medicated with antipsychotics, benzodiazepines or mood stabilizers (Table 1).

Information on illness severity was obtained in the initial phase of the study through the assessment of different clinical scales, which were re-administered after a 12-week fluoxetine treatment in order to evaluate the pharmacological response (Gassó et al., 2014; Mas et al., 2016). Clinical improvement assessed using the Children's Depression Inventory (CDI) scale was previously associated with a *TPH2* genetic marker in our pediatric population (Mas et al., 2016). CDI evaluates the presence and severity of specific depressive symptoms in youth. It contains 27 items, each scored from 0 to 2. Depressed children were