Association between genetic variants of serotonergic and glutamatergic pathways and the concentration of neurometabolites of the anterior cingulate cortex in paediatric patients with obsessive–compulsive disorder

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ABSTRACT

Objectives: The present study aimed to assess the relationship between variability in genes related to the pathophysiology of obsessive–compulsive disorder (OCD) and the concentration of different neurometabolites in the anterior cingulate cortex (ACC).

Methods: We concomitantly assessed neurometabolite concentrations using 3-T 1H-MRS and 262 single nucleotide polymorphism (SNPs) in 35 genes in 41 paediatric OCD patients.

Results: There were significant associations, after Bonferroni correction, between the concentration of inositol, glutamate and glutamine, and total choline and five polymorphisms located in genes related to serotonin and glutamate (i.e., the vesicular monoamine transporter 1 gene, SLC18A1 [rs6586896]; the serotonin receptor 1B gene, HTR1B [rs6296 and rs6298]; and the glutamate receptor, ionotropic, AMPA1 gene, GRIA1 [rs707176 and rs2963944]).

Conclusions: The association observed between these polymorphisms and the neurometabolite concentrations could indicate the presence of a biological interaction between the serotonin and the glutamate pathways that could be involved in the pathophysiology of OCD. More studies with this methodology could increase our understanding of the aetiology and pathophysiology of OCD in children.

Abbreviations: Cho: glycerophosphocholine plus phosphocholine; Cr: creatine plus phosphocreatine; Glx: glutamate plus glutamine; NAA: N-acetyl-aspartate plus N-acetyl aspartyl glutamate

Introduction

Obsessive–compulsive disorder (OCD) is a psychiatric disorder characterised by distressing, intrusive, repetitive, and often uncontrollable thoughts (obsessions) and the urge to engage in repetitive time-consuming behaviours (compulsions) that are enacted to reduce, neutralise, or prevent distress, dreaded experiences, or events (American Psychiatric Association 2013). OCD is estimated to affect 1–3% of the population (Heyman et al. 2001), with 30–50% of patients developing the disease in childhood (Stewart et al. 2004). It has been proposed that childhood onset of OCD may represent a developmental subtype of the disorder (Rosario-Campos et al. 2001), although not necessarily with a worse prognosis (Bloch et al. 2009, Miceli et al. 2010). Thus, it is essential that we clarify the neurobiology of OCD, characterise its subtypes, and improve the available treatment strategies in paediatric populations.

Although the exact aetiology of OCD is unknown, there is evidence to suggest that the disorder arises from a complex combination of genetic and environmental factors (Taylor et al. 2010). More than 80 genetic association studies of candidate OCD genes have been
published over the last decade. The associated single nucleotide polymorphism (SNP) may partially influence the pathophysiology and pharmacology of OCD. However, despite this strong genetic background and high familiarity (van Grootheest et al. 2005; Pauls 2008; Walitza et al. 2010), the identification of a single causal gene variant has remained elusive (Taylor 2013; Grünblatt et al. 2014).

The use of a variety of imaging techniques has led researchers to suggest that cortico-striatal-thalamocortical (CSTC) circuit dysfunction is a core pathophysiological feature of OCD (Saxena and Rauch 2000). In OCD patients, the key components of this circuit include the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC) and striatum (Brennan et al. 2013). The ACC has been suggested to be involved in OCD because of its role in error detection and monitoring and role in the processing of conflicting information (Gehring et al. 2000; van Veen and Carter 2002).

Complementary approaches examining regional neurochemistry now hold the promise of additional insights into the neurobiology of OCD. In particular, proton magnetic resonance spectroscopy (1H-MRS) permits in vivo quantification of specific neurochemicals in various brain regions. The technique records a series of peaks or resonances, each representing the local concentration of a different neurometabolite or small family of chemically related neurometabolites. Compounds that can be measured at present, include myo-inositol (Ins), glutamatergic compounds (Glx), total choline (Cho), N-acetyl-aspartate (NAA) and creatine (Cr). Ins can be regarded as a glial cell marker that is intimately connected to the osmoregulation of astrocytes, so its increase probably reflects glial activation; in addition, Ins is considered a degradation product of myelin (Starck et al. 2008). Glx is a useful measure of glutamatergic excitatory neurotransmission (Starck et al. 2008), while Cho levels reflect choline-containing compounds such as phosphocholine and acetylcholine. Decreased Cho concentrations have been observed in regions of acute demyelinisation in patients with multiple sclerosis and are believed to reflect abnormalities of myelination (Smith et al. 2003). NAA is a marker for neuronal viability and declines in neural tissue before neuronal loss is detected by structural magnetic resonance imaging (MRI). Decreased NAA concentrations are therefore associated with impaired neuronal function (Birken and Oldendorf 1989). Altered brain creatine-phosphocreatine concentrations might also reflect changes in brain energy use (Mirza et al. 2006). Despite the many literature reports of alterations in these neurometabolites in different areas of the brain OCD, the results of 1H-MRS studies are heterogeneous. This is likely due to the reliance on small and heterogeneous study samples (age, illness duration, illness severity, comorbidity and concomitant medications), together with the widely varying imaging methodologies used (Brennan et al. 2013).

Although no differences between patients and controls have been reported in some studies (Ebert et al. 1997; Whiteside et al. 2006; Starck et al. 2008), several studies have observed abnormal changes in the concentrations of neural metabolites in the ACC of patients with OCD (Rosenberg et al. 2004; Yücel et al. 2008; Tükel et al. 2014). An emerging body of evidence from animal models, genetics, neuroimaging and clinical trials supports the hypothesis that dysregulation of glutamate neurotransmission may contribute to the pathophysiology of OCD (Ting and Feng 2008). Following this line of research, some studies have examined glutamatergic changes after treatment; however, most of them did not find any differences between OCD patients and controls in the ACC as well as other regions (O’Neill et al. 2012; Whiteside et al. 2012; Zurowski et al. 2012). Regarding Ins, several studies in OCD patients have assessed concentrations of this neurometabolite versus healthy controls; one of them (Yücel et al. 2008) found significantly increased levels in right rostral and dorsal ACC in adult OCD, whereas another (Whiteside et al. 2006) found significantly decreased levels of Ins/Cr in the caudate. In relation to the concentrations of Cho, some studies have found increased Cho in OCD versus healthy individuals in the thalamus (Rosenberg et al. 2001; Smith et al. 2003; Mohamed et al. 2007) and hippocampus (Atmaca et al. 2009), although one found decreased Cho in the left striatal area (Lázaro et al. 2012).

Another way to clarify the genetic basis of OCD would be to identify the endophenotypes associated with OCD, including the identification of neuroimaging endophenotypes. This approach could delineate pathways linking risk genes to disorders (Meyer-Lindenberg and Weinberger 2006), thereby increasing our understanding of the mechanisms of psychiatric diseases and identifying potential therapeutic targets. To our knowledge, only one previous genetic study of paediatric OCD used neuroimaging phenotypes using spectroscopic techniques (Arnold et al. 2009). That study reported an association between a polymorphism of the glutamate receptor and glutamate concentrations in the ACC. No other studies have been published in recent years that evaluate genetic and neurochemical variables concurrently.

This preliminary study applied an exploratory approach to assess the relationship between the genetic variability associated with pathophysiology of OCD and the concentration of different neurometabolites in the ACC.
Therefore, we assessed neurometabolite concentrations using 3-T $^1$H-MRS against 262 SNPs in 35 genes in 41 paediatric OCD patients. We hypothesised that altered neurometabolite concentrations in the ACC may be at least partly genetically determined.

**Materials and methods**

**Subjects**

We recruited 87 patients meeting the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) diagnostic criteria for OCD from the Department of Child and Adolescent Psychiatry and Psychology at the Hospital Clinic in Barcelona (American Psychiatric Association 2000) following referral to a specialist OCD unit at the Hospital Clinic of Barcelona. OCD was the main diagnosis in all cases.

The Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime Version (K-SADS-PL) interview (Kaufman et al. 1997) validated in Spanish (Ulloa et al. 2006) was used to confirm the main diagnosis and any additional diagnoses. This interview was administered with one or both parents and the child as informants. Diagnostic assessment was first conducted by two experienced psychiatrists. Then, two researchers with experience in the use of semi-structured diagnostic instruments administered the K-SADS to assess past and current comorbidity.

Non-Caucasian patients were excluded. Ethnicity was determined by self-reported ancestries, and we excluded participants with non-European grandparents. Genetic and $^1$H-MRS data were obtained from 75 and 66 patients, respectively. In three cases, the examination was terminated due to claustrophobia before the $^1$H-MRS measurement was performed. We excluded 16 participants who presented low quality images (the criterion of “low quality image” will be explained in the next section on the methodology $^1$H-MRS). Finally, 41 patients of the 47 for whom both genetic and $^1$H-MRS data were available were included in the analysis. Genetic data were not available from six patients because the methodology in the previous study was based on trios. OCD severity was measured with the Children’s Yale-Brown Obsessive-Compulsive Scale (CY-BOCS; Scahill et al. 1997).

The procedures were approved by our hospital’s Ethics Committee. Written informed consent was obtained from all parents, and verbal informed consent was given by all participants following explanation of the procedures involved. The researchers undertook to preserve the anonymity of patients at all times and to use the information collected solely for the purposes indicated. All participants received reimbursement as compensation for their time.

**$^1$H-MRS study**

The methods used for the $^1$H-MRS study have been previously reported (Ortiz et al. 2015). Briefly, $^1$H-MRS was acquired in a 3-T TIM TRIO scanner (Siemens, Erlangen, Germany) using a 32-head channel coil. A volume of interest (VOI) of 3 cm$^3$ (15 × 20 × 10 mm$^3$) was determined in the ACC and the voxel was placed using two planes from the high-resolution T1-weighted image obtained previously. First, the voxel was placed in the sagittal plane, above the genu of the corpus callosum and centred on the ACC. Confirmation that the ACC was in the voxel was then made in the axial plane. $^1$H-MRS data were only acquired from the ACC. Although there are other important brain regions, we selected the ACC because it is one of the regions with the most evidence of involvement in the neurobiology of OCD. Figure 1 shows the representative voxel placement. This procedure was applied in the same manner in all participants, and care was taken to ensure standard placement. Spectra were acquired with the use of a double-spin echo point-resolved spectroscopy sequence with a repetition time of 200 ms and an echo time of 35 ms.

Metabolite concentrations were quantified by a user-independent frequency domain-fitting programme (LCModel, version 6.1-4A) (Provencher 2001), applying the eddy current correction and using an internal water signal reference to calculate absolute metabolite concentrations. We only considered the absolute metabolite values with a Cramer-Rao lower bound below 20% and a signal-to-noise ratio greater than 10, so that these metabolites could be reliably estimated (Provencher 2001). An additional structural image (3D T1-weighted MPRAGE sequence with isometric voxel of 1 × 1 × 1 mm$^3$) was recorded in the same scanning session. The structural image was segmented using customised tissue probability maps (TPMs) and following the new segmentation model provided in SPM8 (Ashburner 2009). To generate customised TPMs, white matter (WM) and grey matter (GM) images obtained from standard segmentation were normalised to MNI using DARTEL (Ashburner 2007) at a resolution of 2 × 2 × 2 mm$^3$, and averaged and smoothed with a Gaussian kernel of 8 mm. The metabolite concentrations were corrected for differences in the cerebrospinal fluid (CSF) content of the VOI using homemade software. The residual percentage of GM in the VOI, after removing the CSF component, was used as a confounding variable in the statistical analyses (Guerrini et al. 2009).
A neuroradiologist confirmed that all MRI scans were free of gross structural abnormalities.

**Sample preparation**

Blood samples were collected from the individuals in ethylenediamine tetraacetic acid (EDTA) (K2EDTA BD Vacutainer EDTA tubes; Becton Dickinson, Franklin Lakes, NJ, USA) and genomic DNA was extracted with the MagNA Pure LC DNA isolation Kit III and the LC MagNA Pure system (Roche Diagnostics GmbH, Mannheim, Germany). The DNA concentration was determined by absorbance (ND1000, NanoDrop, Wilmington, DE, USA).

**SNP selection, genotyping, and quality control**

A total of 304 SNPs were selected in 35 candidate genes involved in different pathways including the dopaminergic, serotonergic, glutamatergic, GABAergic, BDNF and neuroregulin-related pathway (Table I). The method of SNP selection has been previously reported (Mas et al. 2014). Briefly, SNPs were selected following one of three strategies: (1) tagging analysis (as implemented in Haploview 4.2) at an \( \rho^2 \) threshold of 0.8 to capture 98% of the most common HapMap phase II variants based on the CEU panel (minor allele frequency \( \geq 0.1 \)) (range 91–100% for individual genes); (2) suspected SNP functionality according to data published in Ensembl (http://www.ensembl.org), dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and the PupaSuite 3 (http://pupasuite.bioinfo.cipf.es/) databases, with a validated minor allele frequency \( > 0.1 \) in the Caucasian population; and (3) previous associations reported in the literature, either with OCD or other psychiatric diseases. SNPs were genotyped by the MassArray assay with the Sequenom genotyping system (San Diego, CA, USA) at the Santiago de Compostela Node of the Spanish National Genotyping Centre (CeGen). For quality control, 13 samples were genotyped in duplicate for all the SNPs analysed. There was a 100% concordance rate.

We excluded some SNPs from the analysis because they were incompatible with the genotyping design (\( n = 7 \)) or were monomorphic (\( n = 1 \)), because they showed Mendelian errors (\( n = 5 \)), inconsistent clustering, or low genotyping rates (fewer than 90% of samples), or because they were out of Hardy–Weinberg equilibrium (\( n = 28 \)). Finally, 262 validated SNPs in 35 candidate gene regions (covering target loci and upstream and downstream regions) were used for the genetic analysis.
association analysis (Supplemental Table S1, available online).

### Statistics

Data were analysed using IBM SPSS statistics 20 (IBM Corp., Chicago, IL, USA). Means and standard deviations were computed for continuous variables. To identify variables associated with metabolite concentrations, the Spearman correlation, Student t-test, analysis of variance (ANOVA), Mann–Whitney U-test, or Kruskal–Wallis test were used according to the distribution and scales of the variables. Hardy–Weinberg equilibrium for each SNP was analysed. To estimate the independent contribution of each SNP to determine metabolite concentration measurements, multivariate methods based on logistic regression analysis were performed under codominant, dominant, overdominant, recessive, and additive models. The analysis was adjusted for the variables identified in the univariate analysis. The best model was selected using Akaike information criteria. For those purposes, we used the SNPAssoc R package (González et al. 2007). To avoid false positive results we applied Bonferroni correction for multiple testing (significant P value <1.9 × 10^{-4} after Bonferroni correction).

For each associated polymorphism, we analysed the linear regression model, including the adjusted covariates, using IBM SPSS statistics 20 (IBM Corp.). Standardised regression coefficients (β) and coefficients of determination (R^2) were calculated to estimate the proportionate amount of variation in the brain concentration of each neurometabolite explained by the associated polymorphisms.

### Results

The mean age of the OCD group was 15.5 years (SD = 2.9) and the mean age at onset of OCD was 12.1 years (SD = 3.3). There were 21 boys (51.2%) and 20 girls (48.8%). The mean disease duration before evaluation was 25.2 months (SD = 20.9), with a range of 3–137 months. Another axis I diagnosis was found in 58.5% of the patients: generalised anxiety disorder (22%, n = 9), attention-deficit/hyperactivity disorder (12.2%, n = 5), anorexia nervosa (9.8%, n = 4), hypomania (4.9%, n = 2), panic disorder (2.4%, n = 1), phobia simple (2.4%, n = 1), bulimia nervosa (2.4%, n = 1) and oppositional defiant disorder (2.4%, n = 1). The second and third co-morbid diagnoses were not in an acute phase of disease. Patients with an eating disorder were not overweight and patients with a mood disorder were psychopathologically stable. There were no significant differences between neurometabolite concentrations and the presence or absence of comorbid diagnosis. With regard to pharmacological therapy, 17.1% (n = 7) of the OCD patients were not receiving any medication at the time of the evaluation, 58.5% (n = 24) were receiving an antidepressant, and 24.4% (n = 10) were under antidepressant and antipsychotic treatment. Although certain pharmacological effects on brain metabolite concentration have been reported, previous results of our group showed no differences in neurometabolite concentrations between OCD patients who received pharmacological treatment and those who did not; nor did the type of psychopharmacological treatment have an effect (Ortiz et al. 2015). Similar results in adult OCD samples have been reported by Yücel et al. (2008).

Most of the neurometabolite values obtained by 1H-MRS correlated with age. No other clinical variables were associated with the neurometabolites values. Therefore, the genetic analysis of neurometabolites was adjusted by age and the percentage of GM in the ACC (for conservativeness) as covariates.

The genetic analysis of the 262 validated polymorphisms showed that the concentration of Ins adjusted for CSF was associated with two polymorphisms (rs6296 and rs6298) located in the serotonin receptor 1B (HTR1B)

### Table II. Genetic associations (P < 0.00019, Bonferroni corrected P value) obtained between polymorphisms and concentrations of neurometabolites in the ACC.

| Gene concentration and genotype | N | Mean (SD) | P value | β | R^2 |
|-------------------------------|---|-----------|---------|---|-----|
| **Ins concentration** | 39 | 5.90 (0.10) | 0.0001383 | 0.567 | 0.418 |
| HTR1B rs6296/rs6298 A/A-A/C | | | | | |
| C/C | 2 | 3.69 (0.21) | | | |
| Glx concentration | 35 | 10.79 (0.32) | 0.001167 | 0.536 | 0.289 |
| SLC18A1 rs6586896 A/A-A/C | | | | | |
| C/C | 5 | 13.03 (0.54) | | | |
| Cho concentration | 16 | 1.53 (0.06) | 0.000532 | 0.454 | 0.620 |
| GRIA1 rs707176/n9263944 A/A | | | | | |
| C/C | 22 | 1.56 (0.06) | | | |
| **Mean concentrations obtained in each genotype group are expressed as mean ± SD.** |
| **Ins, inositol; Glx, glutamate + glutamine; Cho, glycerophosphocholine + phosphocholine; β, standardised regression coefficient; R^2, squared regression coefficient.** |
| **Neurometabolite concentrations adjusted for CSF and covarying by age and GM.** |
gene. These SNPs showed significant $P$ values after Bonferroni correction (Figure 2). Particularly, the concentration of Ins adjusted for CSF was lower in the minor allele homozygous (CC) for the two polymorphisms of HTR1B. The polymorphisms significantly correlated with Ins concentration ($β = 0.567$, $P < 0.001$) in the regression model, which accounted for 41.8% of its variance (Table II).

The concentration of Glx adjusted for CSF measured in our paediatric OCD population was associated with just one polymorphism in the vesicular monoamine transporter 1 (SLC18A1) gene (rs6586896). This showed significant $P$ values after Bonferroni correction (Figure 2). The concentration of Glx adjusted for CSF was proportionally higher with the number of minor frequency alleles (C) of SLC18A1 rs6586896 ($P = 0.0001167$). This polymorphism significantly correlated with Glx concentration ($β = 0.536$, $P < 0.001$) in the regression model, which accounted for 28.9% of its variance (Table II).

The concentration of Cho adjusted for CSF was associated with two polymorphisms in the glutamate receptor, ionotropic, AMPA1 (GRIA1) gene (rs707176 and rs2963944). These also showed significant $P$ values after Bonferroni correction (Figure 2). The concentration of Cho adjusted for CSF was proportionally higher with the number of minor frequency alleles (C) of both GRIA1 polymorphisms. The polymorphisms were significantly correlated with Cho concentration ($β = 0.454$, $P < 0.001$) in the regression model, which accounted for 62.0% of its variance (Table 2). In this model, age also showed a high correlation with Cho concentration ($β = 0.800$, $P < 0.001$).

No other significant genetic associations were found after Bonferroni correction, specifically for the concentrations of NAA and Cr neurometabolites.

**Discussion**

We conducted an exploratory analysis of the association between putative genetic polymorphisms related to OCD and the concentration of different neurometabolites in the ACC by 1H-MRS in children with OCD. Our results revealed significant associations, after Bonferroni correction, between the concentrations of Ins, Glx and Cho and five polymorphisms in HTR1B (rs6296 and rs6298), SLC18A1 (rs6586896) and GRIA1 (rs707176 and rs2963944).

Differences in the concentration of Ins between controls and paediatric OCD patients may not only reflect dysfunctional neurotransmission in these patients but also glial abnormalities. In fact, this neurometabolite has been closely connected with the osmoregulation of astrocytes (Govindaraju et al. 2000), although its exact significance remains unclear. About two decades ago, several studies reported inconsistent findings in the role of Ins as a synergistic compound in the treatment of OCD (Fux et al. 1996, 1999). Several studies have assessed the concentrations of Ins in OCD patients versus healthy controls and have shown differences, including significantly increased concentrations in the right rostral and dorsal ACC (Yücel et al. 2008) and significantly decreased concentrations in the caudate (Whiteside et al. 2006). Interestingly, decreased concentrations of Ins in the ACC were previously found (Ortiz et al. 2015) in the same paediatric patients included in the present study when compared with healthy controls of the same age and sex. Now, we have associated the concentrations of this neurometabolite with two polymorphisms in the HTR1B gene. Although they give rise to a synonymous change in the protein, these SNPs do seem to have some functional effect, being associated with altered response to antidepressant treatment (Villafuerte et al. 2009; Xu et al. 2012). Moreover, studies have reported associations between other HTR1B SNPs and OCD (Mundo et al. 2002; Kim et al. 2009) or other endophenotypes, such as the OFC volume (Atmaca et al. 2009) and severity of obsessions measured by the Y-BOCS score (Camarena et al. 2004). In fact, in a recent meta-analysis Taylor (2013) identified a trend for the involvement of HTR1B in OCD. It is also noteworthy that this same gene, HTR1B, was also identified by our group in a transmission disequilibrium study of early-onset OCD, in which we included many of the same patients as in the present study (Mas et al. 2014).

The serotonin pathway became a leading target for investigation of the neurobiology of OCD largely because of the remarkable therapeutic effects of serotonin re-uptake inhibitors on obsessions and compulsions (Greist et al. 1995). Our study identified a polymorphism in another serotonin-related gene, specifically, a polymorphism located in the vesicular monoamine transporter 1 gene, the SLC18A1 (rs6586896), was associated with Glx concentrations in the ACC of paediatric OCD patients. The gene encoding SLC18A1 is located on chromosome 8p21, a region implicated in linkage studies of schizophrenia, bipolar disorder and anxiety-related phenotypes (Lohoff 2010). In fact, several genetic case-control studies have documented an association between common missense variations in the SLC18A1 gene and susceptibility to bipolar disorder and schizophrenia (Bly 2005; Lohoff et al. 2006; Chen et al. 2007). Interestingly, this gene was also associated with anorexia nervosa and OCD in paediatric patients, some of whom were included in the present study (Mas et al. 2013).
Figure 2. Association results for single nucleotide polymorphisms in candidate genes for the different models of inheritance. Genetic association with the concentration of myo-inositol (Ins) (A), glutamatergic compounds (Glx) (B) and total choline (Cho) (C). The y-axis indicates the $-\log$ of the likelihood ratio tests computed for 262 validated SNPs adjusted for age of onset or gender as appropriate. The x-axis indicates specific SNPs ordered by gene. The horizontal lines at $-\log(p)$ 1.3 and 3.7 correspond to nominal $P$ values of 0.05 and 0.00019, respectively.
There is growing evidence that disrupted glutamate neurotransmission within CSTC circuits is important in the pathogenesis of OCD (Pittenger et al. 2006; Wu et al. 2012). Previous results by our group have also shown significant differences in Glx concentrations in the ACC in the same pediatric OCD patients (Ortiz et al. 2015). The significance of Glx concentrations in $^1$H-MRS is currently being debated. Although abnormal Glutamate concentrations in the ACC have been identified in children and adults with OCD (Rosenberg et al. 2004; Yücel et al. 2007), these findings must be interpreted cautiously, because other studies have failed to find these differences (Starck et al. 2008; O'Neill et al. 2012; Whiteside et al. 2012). Only one other study has assessed the associations between Glx concentrations in the ACC and genetic variants in pediatric patients with OCD, and this showed a significant association for a polymorphism of the glutamate receptor GRIN2B gene. Interestingly, two polymorphisms (rs707176 and rs2963944) of GRIA1, a gene encoding another glutamate receptor, were associated with the concentrations of Cho. Although they are synonymous and intronic SNPs, respectively, they could have some functional effect given their previous association with schizophrenia (Magri et al. 2006). It has to be noted that a third gene encoding yet another glutamate receptor, GRI3, was also associated with anorexia nervosa and OCD in children (Mas et al. 2013).

Choline-containing compounds are components of cell membranes and increased Cho concentrations have been identified in several neurodegenerative disorders (Jenkins et al. 1993; Meyerhoff et al. 1994), perhaps reflecting membrane breakdown associated with neuronal loss. Thus, the occasional findings of increased Cho in OCD (Kitamura et al. 2006; Mohamed et al. 2007; Atmaca et al. 2009) might indicate myelin breakdown. This interpretation is strengthened by findings of WM abnormalities in OCD patients (Szczeglo et al. 2005; Stewart et al. 2007; Lázaro et al. 2014a, 2014b) and the potential association between OCD and the genes involved in myelination (Stewart et al. 2007). Conversely, Cho concentrations seem normal in other CSTC circuits in OCD (Ebert et al. 1997; Barth et al. 1998; Rosenberg et al. 2000), which weighs against the demyelization hypothesis (Brennan et al. 2013). The concentrations of Cho in OCD have been well studied, as reported by Brennan et al. (2013): some studies have found increased Cho in OCD versus healthy individuals in the thalamus (Rosenberg et al. 2001; Smith et al. 2003; Mohamed et al. 2007), parietal WM (Kitamura et al. 2006), and hippocampus (Atmaca et al. 2009), although one found decreased Cho in the left striatal area (Lázaro et al. 2012).

The association observed between the polymorphisms in HTR1B, SCL18A1 and GRIA1 and the neurometabolite concentrations in the ACC indicate a role for the biological interaction between the serotonin and glutamatergic pathways in the pathophysiology of OCD. Several direct and indirect relationships have been described between these systems, especially in the fronto-striatal circuits (Marsh et al. 2009; Drago et al. 2011). In addition, molecular interactions suggest that there is a subcellular cross-talk between the two systems (Ciranna 2006; López-Gil et al. 2010). Considerable attention has been paid to the symptomatic heterogeneity of OCD in recent years in the attempt to identify biological markers, genetic transmission mechanisms or ways of predicting treatment response (Mataix-Cols et al. 2005).

The present exploratory study has several limitations. Firstly, although our results are promising, the lack of a control group with healthy individuals makes it impossible to directly relate the associations observed between genetic variants and neurometabolite concentrations to OCD pathophysiology. Secondly, $^1$H-MRS data were only acquired from the ACC, not from other brain regions that are similarly important in the neurobiology of OCD. Thirdly, the sample size was modest, which limits the statistical power of the study and makes it difficult to detect small or modest effects associated with common variants. Nevertheless, this is the largest study to be based on $^1$H-MRS data in patients with OCD. Further $^1$H-MRS studies using special sequences that allow the separation of glutamate from glutamine signals may gauge glutamatergic neurotransmission better because they reflect Glu release and the reciprocity of Glu and Gln (Brennan et al. 2013).

To our knowledge, this study is the first to examine the relationship between differences in the concentrations of several neurometabolites and known genetic variants in children with OCD. Only one previous genetic study of pediatric OCD specifically examined glutamatergic concentrations in the brain (Arnold et al. 2009). More studies with this methodology could hold the promise of increasing our knowledge of the pathophysiology of OCD. Such investigations can offer insights into brain alterations that may clarify how genetic changes affect brain structure, chemistry and function. Consequently, there is a clear need for further research with these innovative approaches to confirm our preliminary findings.

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Statement of interest
None to declare.

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