ANALYSIS OF POLYMORPHISMS IN CANDIDATE GENES IN EARLY ONSET OBSESSIVE-COMPULSIVE DISORDER. RELATIONSHIP WITH CEREBRAL ABNORMALITIES AND SYMPTOM DIMENSIONS

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1. Summary

The main aim was to study genetic polymorphisms in OCD-related candidate genes and their relationships with neuroanatomical abnormalities and symptom dimensions.

Methodology: One hundred patients with a diagnosis of OCD according to DSM-IV (American Psychiatric Association, 1994) criteria and their first-degree relatives were included. Single-locus and haplotype analyses were performed using the family-based association test and the transmission disequilibrium test. A complete psychopathological assessment was carried out with patients and relatives. Patients were assessed via single-voxel proton magnetic resonance spectroscopy (1H-MRS) and diffusion tensor magnetic resonance imaging (DTI). A control group (N=47) was assessed with the same neuroimaging protocol to establish whether there are differences in glutamate expression in brain and in white matter (WM) architecture between OCD and healthy subjects.

Results: Twenty SNP were overtransmitted from parents to probands with early-onset OCD, with values of P<0.05. Three of these polymorphisms yielded P<2x10^-4 after Bonferroni correction: rs8190748 and rs992990 located in GAD2 and rs2000292 located in HTR1B. Regarding neuroimaging assessments, there were no significant differences in glutamate concentration adjusted for cerebrospinal fluid (CSF) between OCD patients and healthy controls. However, lower glutamate levels were found in OCD patients with duration of disorder of more than 24 months compared to less than 24 months (P=0.024). Furthermore, patients with OCD exhibited a lower volume in the WM in the cingulate area and the left dorsolateral regions. In addition, OCD patients compared with healthy controls showed a lower fractional anisotropy (FA) in the anterior corpus callosum and a higher mean diffusivity (MD) in the anterior corpus callosum extending to WM of the anterior cingulate cortex and the right medial frontal and bilateral superior frontal gyri; the anterior and posterior lobe of the cerebellum and the pons; the left inferior frontal gyrus and the left lenticular nucleus; and
lingual gyrus of the occipital lobe. The results regarding the relationship between brain abnormalities and risk genes for OCD are still preliminary.

2. Results

Clinical characteristics of obsessive-compulsive patients
The mean age of the OCD group was 15.40 years (SD=2.19) and mean age at onset was 12.91 years (SD=2.63). The mean duration of disease until assessment was 28.29 months (SD=24.16), with a range of 3-112 months. There were 53 boys (53%) and 47 girls (47%) in the case group. The principal dimension of the OCD categories was harm avoidance and checking in 59 (59%), cleanliness and washing in 24 (24%), and symmetry and ordering in 17 cases (17 %). No patient presented the hoarding dimension. The mean symptom severity score, as assessed by the CY-BOCS (Children Yale-Brown Obsessive-Compulsive Scale) at the time of the MRI examination, was 18.03±8.23. The mean symptom severity score at the worst point of the illness was 26.8±7.18. Nineteen per cent of the sample was characterized by a very early onset (<10 years old) and 82% had an early onset (≥10 years old).

Transmission disequilibrium study
Eighty-six children and adolescents diagnosed with OCD according to DSM-IV (American Psychiatric Association, 1994) and their parents (N=158) were initially included for genetic study. Finally 75 complete trios were included in the analysis. The genes and pathways selected for this study are shown in table 1.
Table 1

<table>
<thead>
<tr>
<th>Pathways</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>DRD2, DRD3, DRD4, SLC6A3, COMT, MAOA</td>
</tr>
<tr>
<td>Serotonin</td>
<td>HTR1B, HTR2A, HTR2C, SLC6A4, SLC18A1, TPH2</td>
</tr>
<tr>
<td>Glutamate</td>
<td>GRIK2, GRIN2B, GRIA1, GRIA3, SLC1A1, DLGAP3</td>
</tr>
<tr>
<td>GABA</td>
<td>GAD1, GAD2</td>
</tr>
<tr>
<td>BDNF</td>
<td>NTRK2, NTRK3, BDNF, AKT1, GSK3B</td>
</tr>
<tr>
<td>Neuroregulin</td>
<td>NGFR, ERBB4, NRG1, OLIG1, OLIG2</td>
</tr>
<tr>
<td>Others</td>
<td>LMX1A, BDKRB2, CDH9, KCNN3, EFNA5</td>
</tr>
</tbody>
</table>

Of the 266 SNPs analyzed, 20 were over-transmitted from parents to OCD probands and presented nominal point-wise p<0.05 values (Figure 1). These SNPs mapped in 10 different genes. Among the genes with more than one significant SNP, GAD2 and HTR1B each had five, and SLC18A1 and GAD1 showed two significant SNPs in each gene. Three of these polymorphisms achieved P<2x10^{-4}, the significant p-value after Bonferroni corrections: rs8190748 and rs992990 localized in GAD2 and rs2000292 in HTR1B. No gene of the glutamatergic or the neuroregulin pathways was overexpressed.

Figure 1: Association results of 266 validated SNPs using parent TDT analysis in 75 early-onset OCD trios. The X-axis indicates various SNPs ordered by gene and chromosome position. The horizontal lines at -log(P) 1.3 and 3.6 correspond to nominal P =0.05 and P =0.0002 respectively.
When we stratified our sample according to gender, no SNP achieved a significant p-value after Bonferroni correction. However, different trends were observed between males and females. In males SNP rs2000292 (*HTR1B*) showed the lowest p-value (p=0.0006) whereas the SNPs in *GAD2*, such as rs8190748 and rs992990, were only marginally significant (p=0.01). In contrast, in females *HTR1B* polymorphisms were not significant whereas rs8190748 (*GAD2*) showed the lowest p value (p =0.0006).

These results agree with several lines of evidence that indicate a role for the of serotonin and γ-aminobutyric acid pathways in the risk of early-onset OCD, and in gender differences already mentioned in the pathophysiology of OCD.

**Glutamate function in early onset OCD**

The purpose of this study was to measure in vivo $^1$H-MRS neurometabolite concentrations in the ACC of children and adolescents with OCD in order to identify metabolite abnormalities compared to healthy controls and their relationship with clinical variables. 3T proton-magnetic resonance spectroscopy was used to probe ACC biochemistry in 47 pediatric and adolescent OCD patients (11-18 years old) and 31 healthy subjects of similar age, sex and estimated intellectual quotient were compared. There were no significant differences in the concentration of glutamate adjusted for CSF between OCD patients and healthy controls ($F_{1,74}=0.00; p=0.943$) but there were significant differences in the concentration of glutamate adjusted for CSF in child and adolescent OCD patients according to time of evolution (less than or more than 24 months) [$F_{2,73}=3.95; p=0.024$]. In addition, we found significantly lower levels of myo-inositol adjusted for CSF in the ACC ($F_{1,74}=5.686; p=0.02$). Later, $^1$H-MRS glutamate concentrations and genetic risk variables were combined. Ten patients presented GLX concentration levels adjusted for CSF higher than 12, 6 of whom were carriers of one or two risk genotypes: rs2498794-Akt1 outside the path of BDNF, rs4921691-gen and rs6586896-gen SLC18A1 (serotonergic gene pathway), 3 of these 10 patients are double heterozygotes. These results
are still preliminary and we are preparing a final analysis to get more conclusive results.

**Structural and microstructural white matter abnormalities in OCD patients**

Sixty-two child and adolescent OCD patients (11-18 years old) and 46 healthy subjects of the same gender and similar age and estimated intellectual quotient were assessed by magnetic resonance imaging (MRI). Axial three-dimensional T1-weighted images were obtained in a 3T scanner and analyzed using optimized voxel-based morphometry (VBM). Compared with healthy controls, OCD patients showed a smaller WM volume in the left dorsolateral and cingulate regions involving the WM of superior and middle frontal gyri and anterior cingulate gyrus \( (p_{\text{FWE-corr}}<.05: \text{coordinates MNI } -13 48 18, n^0 \text{ of voxels } 2231, \text{peak t score } 4.47, p_{\text{FWE-corr}}=.046) \) (Figure 2).

Axial three-dimensional T1-weighted images were also obtained in a 3T scanner and analyzed using diffusion tensor imaging (DTI) in 63 child and adolescent OCD patients (11-18 years old) and 46 healthy subjects of the same gender and similar age and estimated intellectual quotient. Voxel-based analysis revealed a significant decrease in mean fractional anisotropy (FA) in the anterior region of the corpus callosum (genu and the anterior portion of the body) in the ODC group \( (p_{\text{FDR-corr}}=0.049) \). A map of reduced FA is shown in Figure 3. Mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) values were significantly increased in ODC patients
when compared with controls, in the same regions involving the anterior region of the corpus callosum extending into the WM of the anterior cingulate and medial frontal gyri bilaterally and of the right superior frontal gyrus; the cerebellum involving the anterior and posterior lobes and the pons; the left inferior frontal gyrus and the left lentiform nucleus; and the lingual gyrus of the occipital lobe. The analysis comparing patients with harm and checking symptoms versus controls found decreased FA in corpus callosum and right and left cingulate anterior and frontal lobe subgyral ($p_{\text{FDR-corr}}=0.010$). Contamination and washing OCD patients showed significant increases in MD (AD and RD) compared to controls in both anterior lobe of cerebellum and pons ($p_{\text{FDR-corr}}=0.001$). No significant differences were found between symmetry/ordering and control subjects.

**Genetic variants and abnormalities in the white matter microstructure**

In order to study whether there is any association between the genetic variants analyzed in the study of risk of OCD and alterations in cerebral white matter, measured by DTI, a first statistical analysis was performed. Fifty-four patients for whom we had genetic data and evaluation with DTI participated in this analysis. We analyzed a total of 250 SNPs in 35 candidate genes related to fractional anisotropy values (FA) and mean diffusivity (MD) measured in brain regions which previously described significant differences between patients with OCD and healthy controls were found. To estimate the contribution of each SNP in the determination of these measurements the genotypes observed were compared with the five possible models (dominant, codominant, recessive, additive and superdominant) by logistic regression using the statistical package SNPassoc R. Only SNPs that passed the Bonferroni correction ($p<0.0002$) were considered significant. While no association was found between SNPs analyzed and fraction anisotropy, several polymorphisms seem to affect the mean diffusivity. Specifically, two variants located in the DRD3 gene (rs3773679) and SLC1A1 (rs3087879) overcame the Bonferroni corrections and were associated with mean diffusivity measured in cerebellum and pons respectively ($p=0.00015$, $p=0.00004$). Carriers of the less common allele (A) polymorphism of DRD3 showed higher mean diffusivity values whereas
in the SLC1A1 gene polymorphism the homozygotes for the most frequent allele (A) were those that showed the highest values of this variable in different regions of the brain (corpus callosum, \( p=0.0003 \); cerebellum and pons, \( p=0.00004 \) and lingual gyrus and occipital cortex: \( p=0.0005 \)). Although these results are preliminary, it appears that genetic variants in the dopamine D3 receptor and glutamate transporter, involved in two of the key neurotransmitter systems in OCD, could determine alterations in white matter in different brain regions.

### 3. Relevance and possible implications

The results of this study support the evidence that OCD remains a model of neuropsychiatric disease with an underlying neurobiological abnormality. Its varied clinical presentation makes it a heterogeneous disorder. This heterogeneity had previously obscured the results of clinical studies and treatment response. This study has recruited more homogeneous groups and improved the study of the genetic variants involved in the pathophysiology of the disease. The early onset of the disease has certainly influenced the results of the genetic analysis. This study contributes to a better understanding of the genetic features underlying the onset of OCD. Moreover, brain imaging can help us to investigate the neural correlates of psychiatric disorders and thus better understand the alterations underlying these disorders. The whole project was intended to validate the previous results of the group and to improve knowledge of the etiology of OCD children in a large sample of patients.

### 4. Literature generated

Ortiz AE, Ortiz AG, Falcon C, Morer A, Plana MT, Bargalló N, Lázaro L. 1H-MRS of the anterior cingulate cortex in childhood and adolescent Obsessive-Compulsive Disorder: a case-control study (submitted)