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# ANTI-BRAIN ANTIBODIES IN ADULT PATIENTS WITH OBSESSIVE–COMPULSIVE DISORDER

Giuseppe Maina, Umberto Albert, Filippo Bogetto, Cristina Borghese, Alberto Cat Berro, Roberto Mutani, Ferdinando Rossi, Maria Claudia Vigliani

## Abstract

Background

An autoimmune hypothesis has been suggested for a subtype of Obsessive–Compulsive Disorder (OCD) with childhood onset: obsessions, compulsions and/or tics would result from anti-streptococcal antibodies that cross-react with basal ganglia tissue based on molecular mimicry. Consistent with this hypothesis anti-brain antibodies were detected in sera of children with OCD and/or Tourette's syndrome. In the present study, we tested whether adults with OCD have anti-brain antibodies or other antibodies that serve as markers of autoimmunity.

Methods

Seventy-four DSM-IV OCD (YBOCS ≥ 16) subjects were recruited and compared to 44 controls with a current Major Depressive Episode for neurological symptoms, ALSO titres, anti-tissue and anti-thyroid antibodies. Anti-brain antibodies were tested by immunohistochemistry and Western blotting methods.

Results

The proportion of subjects with tic comorbidity or positive ASLO titre (> 200 IU/ml) was significantly greater in OCD than in MDE patients (21.6 vs. 2.3% and 16.3 vs. 2.3%, respectively). No other differences in antibody parameters were found. 4/74 OCD patients (5.4%) and none of the controls resulted positive for anti-brain antibodies, with a band around 50–60 kDa at the Western blot analysis.

Limitations

The methodology used to assess anti-brain antibodies.

Conclusions

The majority of adult OCD patients do not seem to have autoimmunity disturbances as compared to a control group. However, a greater percentage of subjects with positive ASLO titres were found among OCD patients. For a small proportion of OCD patients, moreover, autoimmune reactions towards neuronal structures are present although further investigations are needed to demonstrate its etiopathogenetic relevance.

Keywords: Obsessive–compulsive disorder; Major Depressive Episode; Anti-brain antibodies; PANDAS; Anti-streptolysin O antibodies; Streptococcal infections

## 1. INTRODUCTION

An autoimmune hypothesis has been suggested for a subtype of Obsessive–Compulsive Disorder (OCD) with childhood onset: the term Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS) has been proposed for a syndrome believed to result from anti-streptococcal antibodies that cross-react with basal ganglia tissue based on molecular mimicry generating neuropsychiatric symptoms such as obsessions and/or compulsions, tics and soft neurological signs (Swedo et al., 1998 and Cunningham, 2000). Consistent with this hypothesis, significantly elevated anti-brain or, specifically, anti-basal ganglia antibodies were found in children with PANDAS (Swedo et al., 1991, Kiessling et al., 1994, Murphy et al., 1997, Church et al., 2004, Singer et al., 2004,

Pavone et al., 2004, Dale et al., 2005 and Morer et al., 2008) or with Tourette's syndrome (Singer et al., 1998 and Church et al., 2003), as in patients with Sydenham's Chorea (which is a manifestation of rheumatic fever following infection by group A β-hemolytic streptococci) (Kiessling et al., 1993, Kiessling et al., 1994 and Church et al., 2002). In support to the immune-mediated disorder hypothesis is the evidence of induced movements in rats after infusion of IgG of sera from patients with PANDAS (Taylor et al., 2002), the fact that antibodies from a patient with Sydenham's Chorea reacted against neuronal antigens also found at the GABHS surface (Kirvan et al., 2003), and MRI results finding larger caudate, putamen and pallidus in PANDAS patients (Giedd et al., 2000). It has to be acknowledged, however, that a negative study also exists which does not support the hypothesis that PANDAS and Tourette's syndrome in children are secondary to antineuronal antibodies (Singer et al., 2005) and that a prospective longitudinal study of children with tic disorders and/or OCD did not find evidence of a clear relationship between new GABHS infections and symptom exacerbations (Luo et al., 2004).

If confirmed even in a small proportion of patients, the autoimmune etiopathogenesis of the disorder would imply that therapeutic approaches other than serotonin reuptake inhibitors or cognitive-behavioural techniques could be effective in treating this subgroup of patients; preliminary findings, in fact, suggest that immuno-modulating strategies are effective in children with PANDAS (Singer, 1999, Garvey et al., 1999, Perlmutter et al., 1999, Murphy and Pichichero, 2002, Snider et al., 2005 and Besiroglu et al., 2007).

The vast majority of OCD patients, however, is diagnosed and treated for the first time while adults: a study, for example, found that the mean time from initial symptom manifestation to professional care seeking is approximately 10 years (Hollander et al., 1998). It remains to be demonstrated whether a subset of adult OCD patients have autoimmune abnormalities and could be potentially treated with immunomodulatory therapies.

Only two studies evaluated antineuronal antibodies or other markers of autoimmunity in samples of adult OCD patients; Black et al. (1998) tested the serum from 13 adult and chronic OCD patients for panels of autoantibodies that serve as markers of autoimmunity in the practice of neurology and internal medicine, including neuron-specific, other organ-specific and non-organ-specific autoantibodies. They found no humoral evidence of autoimmunity; limitations of that study include the fact that the sample was small and heterogeneous, severity of symptoms was not assessed at the time that blood was drawn, and an age- and gender-matched control group was not utilized. The second study (Morer et al., 2006) was performed in a sample of 40 adult OCD patients who were divided according to time of disease onset in a group with child onset (onset before the age of 11) and another with adult onset (after age 11). This study comprised a control group of 14 psychiatric patients. Child onset OCD was associated with higher mean ASLO titres, higher frequencies of history of tic disorders and tonsillitis in childhood, while no differences were found in D8/17 antibody titres or in other autoimmune parameters such as antinuclear antibodies, antimitochondrial antibodies, antithyroidal microsomal antibodies and serum antithyroglobulin antibodies (Morer et al., 2006). This study suggests that OCD in adults is a heterogeneous disorder and that only child onset OCD may be related to an autoimmune etiology. However, although controlled, this study had the limitation of the small sample size of both patients and controls.

It is possible that OCD in adults is not associated with autoantibodies while in children autoimmunity might play a role in the etiopathogenesis at least of some forms of OCD or Tourette's syndrome. It is also possible that antibodies responsible for OCD may be produced transiently at the onset of the illness only. Further research is needed in order to better characterize autoimmune parameters in adult OCD samples.

The aim of the present study was to test whether adults with OCD have autoimmune abnormalities, as evidenced by the presence of antibodies directed towards neuronal structures or antibodies that serve as markers of autoimmunity in the practice of neurology and internal medicine.

## 2. METHODS

### 2.1. Sample

Subjects for this study were recruited from all patients with a principal diagnosis of OCD consecutively referred to the Mood and Anxiety Disorders Unit, Department of Neuroscience, University of Turin (Italy). Inclusion criteria were:

principal diagnosis of OCD according to DSM-IV criteria, a minimum total score of 16 at the Yale–Brown Obsessive Compulsive Scale (Y-BOCS) (Goodman et al., 1989a and Goodman et al., 1989b). Furthermore, patients had to be at least 18 years of age, and be willing to voluntarily participate to the study. Informed consent from patients was obtained after the procedure had been fully explained. Exclusion criteria were considered as current or previous diagnosis of organic mental disorder, schizophrenia, schizophreniform or other psychotic disorder, or having an uncontrolled or serious medical condition.

A systematic face-to-face interview that consisted of structured and semistructured components was used to collect data. Diagnostic evaluation and Axis I comorbidities were recorded by means of the Structured Clinical Interview for the DSM-IV Axis I Disorders (First et al., 1997). All socio-demographic and illness characteristics were obtained through the administration of a semistructured interview, developed and used in previous studies (Bogetto et al., 1999, Albert et al., 2002, Albert et al., 2004 and Maina et al., 2004). Obsessive–compulsive symptomatology was assessed using the Y-BOCS Symptom Check List. The interview and all the ratings were completed by psychiatrists with at least 4 years' experience in anxiety and mood disorders. High reliability and diagnostic concordance have been documented in previous reports (Albert et al., 2004 and Maina et al., 2005).

In addition, all patients underwent a neurological examination aimed at identifying neurological abnormalities: the neurological examination was performed by a neurologist (MCV).

Seventy-four OCD patients were included; their mean age was 35.3 ± 12.7 years, the mean age at onset of the first obsessive–compulsive symptoms was 16.6 ± 8.8 years, the mean age at onset of the disorder was 21.8 ± 9.2 years, the mean educational level was 12.4 ± 3.4 years; the sample was composed of 43 (58.1%) males and 31 (41.9%) females; 40 (54.1%) subjects were single, 29 (39.2%) married, 4 (5.4%) separated and 1 (1.4%) widowed; 26 patients (35.1%) had an abrupt onset and 48 (64.9%) insidious; 14 subjects (18.9%) had an episodic course of the disorder while 60 (81.1%) chronic; the mean Y-BOCS total score was 24.8 ± 6.7, the mean obsession subscore was 13.0 ± 3.3 and the mean compulsion subscore was 12.1 ± 3.4.

2.2. Controls

A control group of 44 patients with a current Major Depressive Episode (within a diagnosis of Major Depressive Disorder: N = 26, Bipolar Disorder type I: N = 1 or Bipolar Disorder type II: N = 17) consecutively referred to the Mood and Anxiety Disorders Unit, Department of Neuroscience, University of Turin (Italy) during the same period was recruited. Their mean age was 56.0 ± 16.8 years, the mean age at onset of the Mood Disorder was 37.5 ± 15.7 years, the mean age at onset of the current MDE was 55.7 ± 16.8 years, the mean educational level was 11.7 ± 4.5 years; the sample was composed of 18 (40.9%) males and 26 (59.1%) females; 14 (31.8%) subjects were single, 19 (43.2%) married, 4 (9.1%) separated and 7 (15.9%) widowed.

2.3. Procedures

Sera were obtained from all patients and controls and stored at − 80 °C prior to serological investigation. We tested the serum of patients for panels of autoantibodies that serve as markers of autoimmunity in the practice of neurology and internal medicine:

a) ASLO

Anti-streptolysin O titres (ASLO) were determined using the standard haemagglutination procedure in all subjects. ASLO titres above 200 IU/ml were defined as positive, according to World Health Organization guidelines (Spaun et al., 1961). Streptococcal serological measurements took place in a different laboratory from that where the anti-brain antibodies were searched for. Investigators performing streptococcal serological measurements were masked to anti-brain antibody results.

b) Anti-tissue antibodies

Anti-nuclear antibodies (ANA), anti-double stranded DNA (anti-dsDNA) antibodies, antibodies to soluble extractable nuclear antigen (anti-ENA), anti-cardiolipin IgM and IgG antibodies, antineutrophil cytoplasmic antibodies (anti-PR-3

antibodies — c-ANCA and anti-myeloperoxidase (MPO) antibodies — p-ANCA), anti-thyroid peroxidase antibodies (TPOAb), anti-thyroglobulin (TgAb) antibodies, and anti-thyrotropin receptor (TRAb) antibodies were assessed.

c) Anti-brain antibodies

Anti-brain antibodies were tested by immunohistochemistry and Western blotting methods. All experiments on animals were carried out in strict accordance with the Italian Ministry of Health guidelines for the care and use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering.

2.3.1. Immunohistochemistry

The presence of total IgG class antineuronal antibodies was determined using indirect immunoperoxidase. Two adult (3-month old; Charles River, Milan, Italy) Wistar rats maintained in a controlled environment (14 h light/10 h dark) with food and water ad libitum were used. Animals were anesthetized (Pentothal sodium; Gellini, Italy; 60 mg/100 g i.p.) and perfused intracardially with a heparinized saline solution (25 IU/ml in 0.9% NaCl, during 2–3 min) followed by a freshly prepared solution of 4% paraformaldehyde, in 0.1 M sodium phosphate buffer, pH 7.4. Brains were dissected and post-fixed overnight (other animal tissues were post-fixed for 2–4 h), then cryoprotected, frozen in liquid nitrogen-cooled isopentane at − 80 °C, and cryostat sectioned in series. Immunohistochemistry was carried out on cryostat sections (30 μm thick) incubated with the serum of each patient (OCD patients and controls, dilution 1:100) overnight at 4 °C and revealed by using double indirect peroxidase techniques with biotinylated secondary antibodies (Dako System), detected with 3,3-diaminobenzidine (DAB). All sera were diluted in 0.01 M PBS containing 0.5% Triton X-100.

2.3.2. Western blotting

Bovine brain and rat brain were collected after death without delay; dissected portions of the basal ganglia and of the cortex of cow brain and entire rat brain were immediately placed in liquid nitrogen. Proteic samples were prepared crashing in liquid N2 small amounts of frozen basal ganglia or cortex of bovine brain and of full brain of rat tissue. Samples were then diluted in lysis buffer (62.5 mM Tris, pH 6.8, SDS 10%) at 100 °C for 3 min. After centrifugation (10,000 rpm for 20 min) protein concentration of supernatant was determined by Lowry method. Equal amounts of proteins were diluted in SDS 10%/Bromophemol/βmercaptoetanol/glycerol and Tris/HCl (pH 6.8), loaded onto 12% Acrylamide/Bis Acrylamide gel (Acrylamide/Bis Acrylamide 37.5:1 BioRad) and then blotted to pure nitrocellulose membranes (0.46 μm Trans-blot Transfer Medium, BioRad). Membranes, after blocking with non-fat dried milk for 60 min, were incubated with patient's sera (1:1000, 1 h at room temperature), and then, after washing with TBS-T, were exposed to anti-human IgG AP-conjugated secondary antibodies (1:1000, Dako System). Immunoreactivity was detected using BCIP/NBT substrate.

2.4. Statistical analysis

Treatment group comparisons were done using a chi-square test for categorical variables and the Student's t test for continuous ones. Statistical significance was defined as a 2-sided p value $\leq 0.05$. A pair wise deletion of missing data was used for statistical analyses.

## 3. RESULTS

Table 1 presents the results of the antibody screening. In the group of patients with OCD, the frequency of current or lifetime tic disorders and that of positive ASLO titre (> 200 IU/ml) subjects were significantly higher than in the control group. No other statistically significant differences in antibody parameters were found.

*Table 1. Comparison between OCD patients and controls.*

| | **OCD** | **Controls** | ***t* or $\chi^2$** | **df** | ***p*** |
|---|---|---|---|---|---|
| Tic comorbidity, *N* (%) | 16 (21.6) | 1 (2.3) | 8.377 | 1 | 0.004 |
| Tourette's syndrome comorbidity, *N* (%) | 2 (2.7) | 0 (0) | 1.210 | 1 | 0.271 |

| | OCD | Controls | $t$ or $\chi^2$ | df | $p$ |
|---|---|---|---|---|---|
| Neurological soft signs, *N* (%) | 31 (41.9) | 20 (45.5) | 0.143 | 1 | 0.706 |
| ASLO titre (IU/ml), mean (± SD) | 98.21 ± 92.49 | 70.86 ± 63.42 | 1.612 | 85 | 0.111 |
| ASLO positive (> 200 IU/ml), *N* (%) | 7 (16.3) | 1 (2.3) | 5.109 | 1 | 0.024 |
| ANA positive (> 1:80), *N* (%) | 10 (25.0) | 5 (11.4) | 2.656 | 1 | 0.103 |
| Anti-dsDNA (IU/ml), mean (± SD) | 3.36 ± 2.93 | 4.91 ± 12.33 | − 0.773 | 82 | 0.442 |
| Anti-dsDNA positive (> 50 IU/ml), *N* (%) | 0 (0) | 1 (2.3) | 0.920 | 1 | 0.337 |
| Anti-ENA positive, *N* (%) | 0 (0) | 0 (0) | – | – | – |
| Anti-cardiolipin IgM antibodies (MPL/ml), mean (± SD) | 2.85 ± 1.95 | 2.91 ± 2.04 | − 0.143 | 81 | 0.887 |
| Anti-cardiolipin IgM antibodies positive (> 10 MPL/ml), *N* (%) | 1 (2.6) | 1 (2.3) | 0.007 | 1 | 0.931 |
| Anti-cardiolipin IgG antibodies (GPL/ml), mean (± SD) | 3.39 ± 2.77 | 4.52 ± 2.85 | − 1.841 | 81 | 0.069 |
| Anti-cardiolipin IgG antibodies positive (> 10 GPL/ml), *N* (%) | 2 (5.1) | 2 (4.5) | 0.015 | 1 | 0.902 |
| c-ANCA positive (> 2 U/ml), *N* (%) | 0 (0) | 0 (0) | – | – | – |
| p-ANCA positive (> 6 U/ml), *N* (%) | 0 (0) | 0 (0) | – | – | – |
| TPOAb (IU/ml), mean (± SD) | 26.79 ± 84.69 | 67.98 ± 191.93 | − 1.250 | 82 | 0.215 |
| TPOAb positive (> 35 IU/ml), *N* (%) | 3 (7.5) | 6 (13.6) | 0.825 | 1 | 0.364 |
| TgAb (IU/ml), mean (± SD) | 36.54 ± 89.44 | 66.74 ± 187.48 | − 0.937 | 83 | 0.352 |
| TgAb positive (> 40 IU/ml), *N* (%) | 4 (9.8) | 6 (13.6) | 0.308 | 1 | 0.579 |
| TRAb (U/l), mean (± SD) | 4.52 ± 3.56 | 5.14 ± 4.74 | − 0.660 | 81 | 0.511 |
| TRAb positive (> 14 U/l), *N* (%) | 1 (2.6) | 1 (2.3) | 0.007 | 1 | 0.931 |
| Anti-brain antibodies, positive, *n* (%) | 4 (5.4) | 0 (0) | 2.462 | 1 | 0.117 |

Legend:

ASLO = Anti-streptolysin O.
ANA = Anti-nuclear antibodies.
Anti-dsDNA = anti-double stranded DNA antibodies.
Anti-ENA = antibodies to soluble extractable nuclear antigen.
c-ANCA = antineutrophil cytoplasmic antibodies − anti-PR-3 antibodies.
p-ANCA = antineutrophil cytoplasmic antibodies − anti-myeloperoxidase (MPO) antibodies.
TPOAb = anti-thyroid peroxidase antibodies.
TgAb = anti-thyroglobulin antibodies.
TRAb = anti-thyrotropin receptor antibodies.

Fisher exact test: $p < 0.05$.

Out of 74 OCD patients, 4 (5.4%) resulted positive for anti-brain antibodies, as revealed by immunohistochemical staining of rat brain sections with patient's sera. In contrast, none of the depressed patients showed any anti-brain positivity. In OCD patients, immunolabelling was not restricted to the basal ganglia and consistently localised in cell nuclei, though the pattern of staining of each case showed some distinctive features that were paralleled by different results of the Western blot (Fig. 1A–E). Patient 1 showed a positivity of nuclei and of some long axons both in the cortex and in the basal ganglia; both bovine and rat WB were negative. Patients 2 and 3 showed a positivity of nuclei and (rarely) of some axons both in the cortex and basal ganglia. Bovine WB showed a positivity around 60 kDa with bovine extract of basal ganglia in both patients, but only patient 2 showed the same positivity with rat entire brain extract. Patient 4 showed a positivity limited to the nuclei of the cortex and basal ganglia and bovine and rat WB were negative. Two patients (A and D) resulted positive for ANA antibodies (Table 2).

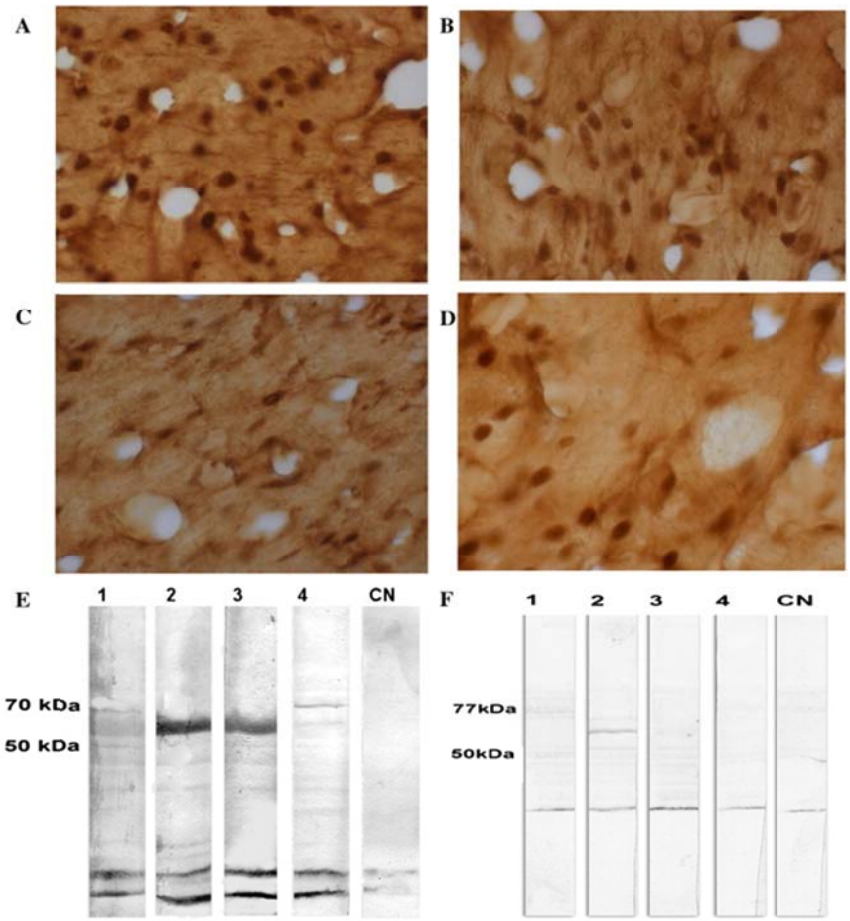


*Fig 1 Immunohistochemistry (A–D) and Western blotting analyses (E bovine extract of basal ganglia, F entire brain rat extract) of the four patients positive for anti-brain antibodies.*

*Table 2. Characteristics of patients positive for anti-brain antibodies.*

| | Patient A | Patient B | Patient C | Patient D |
|---|---|---|---|---|
| Actual age | 26 | 26 | 45 | 41 |
| Gender | Female | Male | Male | Male |
| Age at onset symptoms (years) | 10 | 10 | 7 | 30 |
| Age at onset disorder (years) | 24 | 16 | 18 | 30 |
| Type of onset[a] | Insidious | Insidious | Insidious | Abrupt |
| Course[b] | Chronic, waxing and waning | Chronic, waxing and waning | Chronic, waxing and waning | Chronic, waxing and waning |
| YBOCS total score | 23 | 21 | 21 | 33 |
| YBOCS obsessions subscore | 12 | 10 | 9 | 17 |
| YBOCS compulsions subscore | 11 | 11 | 12 | 16 |
| Type of obsessions (YBOCS Check-List) | Aggressive | Symmetry | Aggressive | Hoarding |
| | Somatic | | Contamination | Contamination |
| | | | Hoarding | |
| Type of compulsions (YBOCS Check-List) | Checking | Checking/repeating | Checking | Hoarding |
| | | | Cleaning | Cleaning |
| | | | Hoarding | Checking |
| Family history | No | No | OCD | No |
| Current comorbid disorder | No | No | No | No |

| | Patient A | Patient B | Patient C | Patient D |
|---|---|---|---|---|
| Lifetime comorbid disorder | Panic disorder | Major depression<br>Specific phobia | Major depression | Bipolar disorder |
| History of or current tics | No | Yes | Yes | No |
| Neurological soft signs (tics, enuresis, micturition urgency) | No | Yes | Yes | Yes |
| ASLO titres (IU/ml) | 89 | 55 | 78 | 95 |
| ASLO positive (> 200 IU/ml) | No | No | No | No |
| ANA positive (> 1:80) | Yes | No | No | Yes |
| Anti-dsDNA positive (> 50 IU/ml) | No | No | No | No |
| Anti-ENA positive | No | No | No | No |
| Anti-cardiolipin IgM antibodies positive (> 10 MPL/ml) | No | No | No | No |
| Anti-cardiolipin IgG antibodies positive (> 10 GPL/ml) | No | No | No | No |
| c-ANCA positive (> 2 U/ml) | No | No | No | No |
| p-ANCA positive (>6 U/ml) | No | No | No | No |
| TPOAb positive (> 35 IU/ml) | No | No | No | No |
| TgAb positive (> 40 IU/ml) | No | No | No | No |
| TRAb positive (> 14 U/l) | No | No | No | No |

Legend:
ASLO = Anti-streptolysin O.
ANA = Anti-nuclear antibodies.
Anti-dsDNA = anti-double stranded DNA antibodies.
Anti-ENA = antibodies to soluble extractable nuclear antigen.
c-ANCA = antineutrophil cytoplasmic antibodies − anti-PR-3 antibodies.
p-ANCA = antineutrophil cytoplasmic antibodies − anti-myeloperoxidase (MPO) antibodies.
TPOAb = anti-thyroid peroxidase antibodies.
TgAb = anti-thyroglobulin antibodies.
TRAb = anti-thyrotropin receptor antibodies.

a
The onset was considered abrupt when the symptoms reached clinically significant intensity within 1 week of onset. All other types of onset were considered insidious.
b
The course of the disorder was considered episodic when at least one circumscribed symptom-free interval (6 months) was present; all other types of course were considered chronic.

3.1. Relation to OCD clinical phenotype

Table 2 shows characteristics of the four OCD patients positive for anti-brain antibodies. Three of them had an earlier (pre-pubertal) onset of symptoms, two had also tic disorder comorbidity (although none of them had Tourette's syndrome), and three of them had neurological soft signs (enuresis and micturition urgency together with tics). Two patients also showed a low titre positivity for ANA. None of them was positive for ASLO or anti-dsDNA.

The two patients with a similar pattern of immunohistochemistry positivity had a similar clinical profile: early (paediatric) and insidious onset of the disorder with soft neurological signs (enuresis, micturition). The vast majority of patients with early onset or soft neurological signs, however, did not result positive for any anti-brain antibodies.

## 4. DISCUSSION

The aim of the present study was to test whether adults with OCD have autoimmune abnormalities, as evidenced by the presence of antibodies directed towards neuronal structures (through immunohistochemistry and Western blotting) or antibodies that serve as markers of autoimmunity in the practice of neurology and internal medicine. We also examined ASLO titres as possible signs of previous or present streptococcal infections and neurological abnormalities (such as tics, choreiform movements, enuresis or micturition) as those described in PANDAS subjects. In fact, we sought to examine the hypothesis of an autoimmune etiopathogenesis for at least some forms of OCD in adults as proposed for PANDAS in children (Swedo et al., 1998). We included as a control group a sample of subjects with a current Major Depressive Episode.

According to the autoimmune hypothesis, anti-streptococcal antibodies produced during GABHS infection are involved in a cross reaction in the nervous system and through an autoimmune pathway affect the basal ganglia. The NIMH diagnostic criteria for PANDAS are: 1) presence of OCD and/or tic disorder, 2) paediatric onset of symptoms (age 3 years to puberty), 3) episodic course of symptom severity, 4) temporal association of symptom onset or exacerbation with GABHS infection, and 5) association with neurological abnormalities (motoric hyperactivity, choreiform movements). Even though PANDAS is by definition a paediatric disorder, patients with adult onset OCD or tic disorder related to streptococcal infections have been described (Bodner et al., 2001, Mathew, 2001, Church and Dale, 2002 and Martinelli et al., 2002). These cases support the hypothesis that post-streptococcal disease may result in adult-onset OCD in some patients. Moreover, it may also be that GABHS infection just serves as a trigger in childhood, and that, subsequently, autoimmune antibodies directed against neuronal structures maintain obsessive–compulsive symptoms without new infections; in that case adult OCD with childhood onset may show anti-brain antibodies without elevated ALSO titres or other signs of recent streptococcal infections.

In our sample of adults with OCD we did not observe neurological abnormalities more frequently than in the control group of depressed subjects (42% vs. 45%) except for tics that were significantly more frequent in OCD than in controls (21.6% vs. 2.3% $p = 0.004$). This is consistent with literature data indicating a preferential association between OCD and tic disorders and does not allow drawing any conclusions concerning similarities between adult OCD and PANDAS patients.

One of the proposed criteria for PANDAS is the temporal association of symptom onset or exacerbation with GABHS infection as evidenced by positive ASLO titres. In our sample of adults with OCD we found a proportion of subjects with positive ASLO titres (> 200 IU/ml) significantly greater than in the control group composed of depressed patients (16.3% vs. 2.3% $p = 0.024$). We have however to consider that this result may partially be a consequence of the younger mean age of our OCD cohort as compared to that of the controls; it has been reported in the general population, in fact, that ASLO titres are higher in childhood, adolescence and early adulthood (Blyth and Robertson, 2006). Concerning ASLO titres, we found a difference in the percentage of patients positive when considering a cut-off of 200 IU/ml; however a study by Church and Dale (2002), although concluding that streptococcal serology might be a useful diagnostic tool for assessing the etiology of new cases of neuropsychiatric diseases in adults, proposed an ASLO titres of 270 IU/ml as the upper limit of normal for adults. According to this cut-off, only four OCD subjects could be classified as positive, without significant differences with respect to the control group. Our data, then, do not allow us to draw definitive conclusions concerning a possible role of streptococcal infections in the pathogenesis of OCD in adults.

We did not observe differences in anti-tissue markers between OCD and control patients. These results in adult OCD patients are in agreement with those of the only two studies which assessed autoimmune markers in adults: Black et al. (1998) found no evidence of humoral autoimmunity in the sera of 13 adult OCD patients while Morer et al. (2006) found that only adult OCD patients with child onset had higher ASLO titres while no other immune parameters were found to be different. Both studies, however, were limited by the small sample size, while our study has the strength of having included 74 OCD patients. We can then conclude that adults with OCD do not show an immunological profile suggesting a more diffuse systemic disorder of autoimmunity.

Although the statistical analysis did not find a significant difference in the proportion of subjects positive for anti-brain antibodies, we found 4 adult OCD patients with autoantibodies directed towards neuronal structures. Being true that the results are not statistically significative, the observation corroborates previous studies that have shown that the presence of antineuronal antibodies are only present in a small group of patients. In this study the use of bovine brain instead of

human putamen or caudate could be responsible of the even lower fraction of patients found positive for antineuronal antibodies. The observed positivity is around 60 kDa at the Western blot analysis, corresponding to the molecular weight observed in PANDAS, in some patients with Tourette's syndrome and in some paediatric OCD patients (Morshed et al., 2001, Church et al., 2002, Hoekstra et al., 2003, Dale et al., 2005 and Morer et al., 2008). Interestingly, three out of these four subjects had a prepubertal onset of obsessive–compulsive symptoms (≤ 10 years), and the two patients with a similar pattern both at immunohistochemistry and WB analysis had also some neurological symptoms (enuresis and micturition urgency) other than tics. These clinical characteristics fulfil three out of the five NIMH criteria for a diagnosis of PANDAS. Concerning the course of the disorder, none of the patients positive for anti-brain antibodies had an episodic course (at least one circumscribed symptom-free interval); however, they all had a chronic waxing and waning course of symptoms, although, because of the retrospective examination, we were not able to assess the temporal relationship between symptoms exacerbations and eventual GABHS infections. It has to be acknowledged, moreover, that some of the adult PANDAS cases described in the literature to date (Bodner et al., 2001 and Hornig et al., 1999) had also a chronic course of the disorder. Our patients with anti-brain antibodies did not show a high ASLO titre; this negativity does not exclude that a previous streptococcal infection, for instance occurred in paediatric age, played a role in the onset of the autoimmune reaction as hypothesized for PANDAS patients. It is possible, then, that autoimmunity might play a role in the etiopathogenesis of a very small subgroup of patients with OCD, and that OCD subjects whose onset is in childhood still show anti-brain antibodies years after the onset of the disorder without any other signs of streptococcal infection. Because onset of symptoms occurred during the pre-adolescent period, moreover, evidence of streptococcal infections acting as triggers (such as frequent throat infections) was not reliable, and this limits our ability to draw definitive conclusions.

A possible limitation of the present study is the different composition of the two samples (OCD and MDE patients) with respect to mean age and gender distribution: the mean age of the OCD sample was 35 years vs. 56 years of the MDE group, and approximately 40% of OCD patients were females as compared to 60% of the MDE subjects. These differences are however consistent with literature reports of clinical characteristics of OCD and MDE samples. Moreover, female gender and increasing age have been found, in affective disorders, to be related to higher autoantibody disturbances (increasing levels of ANA and anticardiolipin antibodies) (Hornig et al., 1999); consistent with this report we would have expected to find higher anti-tissue antibodies disturbances in MDE subjects, which we actually did not. Another limitation of our study is that not all patients underwent all the autoimmune screenings, and this might have limited the statistical power to detect differences between the two groups. However, this is, to our knowledge, the largest study to date which examined autoimmunity in OCD patients. A third limitation can be represented by the methods, immunohistochemistry and Western blot, used in the present study to assess anti-brain antibodies. In fact the risk of background reactivity can attain 8–12% of the healthy population; anyway, we tried to overcome such a problem using a quite numerous control group represented by 44 depressed patients and none of them was positive. Moreover, it cannot be excluded that the positivity in the nuclei of neuronal cells observed in our patients with the immunohistochemistry method was due to antinuclear antibodies, which were detected in two out of the four positive patients. However it must be stressed that ANA positivity was in all cases a low-level positivity (1:40), while sera was tested at 1:100 in our immunostaining setting. Moreover, in order to rule out this hypothesis, we repeated the immunohistochemistry analysis looking for autoantibodies on rat liver sections and we could not find any positivity (data not shown). We did not perform a systematic study of all the brain limiting our analysis to some slides of cortex and basal ganglia; as a consequence we cannot add more about the topographical distribution of immunohistochemistry positivity.

In addition, Western blot analysis is considered the best procedure to determine the molecular weight of a possible protein target of the immunoreaction. Using this technique and the homogenate of bovine basal ganglia, we found in two patients a band around 60 kDa, while only one patient showed the same positivity when the homogenate of the full rat brain was used. The discrepancy can be due to the use of homogenate of the full-brain in the case of rat and of homogenate of a more restricted area, basal ganglia,/cortex, in the case of cow, with a higher sensitivity of this last method. Anyway in both the species the same band can be observed in at least one patient; this is in accordance with results of other authors in disorders such as PANDAS, Tourette's syndrome or paediatric OCD (Morshed et al., 2001, Church et al., 2002, Hoekstra et al., 2003, Dale et al., 2005 and Morer et al., 2008). We cannot exclude that the use of human material could further increase the sensitivity of the method with a higher percentage of positive patients. Nonetheless, even if we found evidence that a small proportion of adults with OCD have anti-brain antibodies, similar to those found in other disorders where autoimmunity might play a role in the etiopathogenesis, in our opinion it is too

early to consider these antibodies as etiopathogenetically relevant for OCD. In fact, they could just represent an epiphenomenon in a chronic disease of the nervous system.

Despite all these limitations, we can conclude that the vast majority of adult OCD patients do not seem to have autoimmunity disturbances or elevations in ASLO titres as compared to a control group made of patients with another psychiatric disorder. However, a greater percentage of subjects with positive ASLO titres were found among OCD patients. For a small proportion of OCD patients, moreover, autoimmune reactions towards neuronal structures are present although further investigations are needed to demonstrate its etiopathogenetic relevance. It seems mandatory for Psychiatry to clarify this point as the possible autoimmune etiopathogenesis in some OCD patients could open new therapeutic scenarios in adults as already suggested in children (Singer, 1999, Garvey et al., 1999, Perlmutter et al., 1999, Murphy and Pichichero, 2002, Snider et al., 2005 and Besiroglu et al., 2007). In fact, given that a significant proportion of adult OCD patients do not respond to conventional treatment strategies, the search for alternative and hypothesis-driven treatments is highly needed.

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Conflict of interest

All the authors declare that they have no conflicts of interest.

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