

# Antineuronal antibodies in a group of children with obsessive–compulsive disorder and Tourette syndrome

Astrid Morer <sup>a,\*</sup>, Luisa Lázaro <sup>a</sup>, Lidia Sabater <sup>b</sup>, Joan Massana <sup>c</sup>,  
Josefina Castro <sup>a,1</sup>, Francesc Graus <sup>b,1</sup>

<sup>a</sup> Department of Child and Adolescent Psychiatry and Psychology, Institut Clinic de Neurociències, Hospital Clínic Universitari, Carrer Villarroel 170, Barcelona 08036, Spain

<sup>b</sup> Service of Neurology and Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Institut Clinic de Neurociències, Hospital Clínic Universitari, Carrer Villarroel 170, Barcelona 08036, Spain

<sup>c</sup> Department of Psychiatry, Institut Clinic de Neurociències, Hospital Clínic Universitari, Carrer Villarroel 170, Barcelona 08036, Spain

Received 8 March 2006; received in revised form 22 September 2006; accepted 25 September 2006

## Abstract

An autoimmune hypothesis has been suggested for early onset obsessive–compulsive disorder and Tourette syndrome. The term: *Paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection* (PANDAS) has been proposed as an aetiological subtype of OCD and TS, related to a Group A beta haemolytic streptococcal (GABHS) infection that triggers an autoimmune response. Antineuronal antibodies have been studied and found in the sera of some patients with these disorders, and they are thought to cross-react with streptococcal and basal ganglia antigens. The present study included 32 prepubertal-onset OCD patients, 21 with TS diagnosis (some of them meeting criteria for PANDAS) and 19 normal children, all aged between 9 and 17 years. Antibodies were assayed by immunohistochemistry and immunoblot. Special attention was paid to the methodology and a high serum dilution was used to minimize non-specific binding. No anti-basal ganglia antibodies were detected by immunohistochemistry in any of the samples. Two proteins, with approximate molecular weights of 86 kDa and 55 kDa, were found in sera from 7 patients. Though the study supports the hypothesis of an autoimmune process underlying OCD or TS in some patients, further research is needed.

© 2006 Elsevier Ltd. All rights reserved.

**Keywords:** Antineuronal antibodies; PANDAS; Autoimmunity; Obsessive–compulsive disorder; Tourette syndrome

## 1. Introduction

Early onset obsessive–compulsive disorder (OCD) is often described as a subtype of the condition with special epidemiological and clinical characteristics (Eichstedt and Arnold, 2001; Geller et al., 1998; Rapoport et al., 1992). Childhood-onset OCD is also associated with comorbid Tourette syndrome (TS). A familial relationship between TS and OCD has been demonstrated, and an autoimmune hypothesis has been suggested for both syndromes (Morero

et al., 2005b). The controversial concept *paediatric autoimmune neuropsychiatric disorders associated with streptococcal infection* (PANDAS) was proposed to describe an aetiological subtype of OCD and TS, related to a Group A beta haemolytic streptococcal (GABHS) infection that triggers an autoimmune response based on molecular mimicry (Cunningham, 2000). Interestingly, recent research indicates a possible relation between streptococcal infection and the risk of obsessive–compulsive disorder (Mell et al., 2005). However, many issues remain to be resolved before the autoimmune hypothesis can be accepted, especially questions concerning the detection of antineuronal antibodies (Church et al., 2002; Husby et al., 1976; Kotby et al., 1998; Morshed et al., 2001; Swedo et al., 1993) and specific markers (Morero et al., 2005a).

\* Corresponding author. Tel.: +34 932275400x3474/9970; fax: +34 932275662.

E-mail address: [amorero@clinic.ub.es](mailto:amorero@clinic.ub.es) (A. Morero).

<sup>1</sup> Both authors contributed equally to this work.

Antineural antibodies have been found and studied in the sera of some patients with Sydenham's Chorea (SC), Tourette syndrome or tic disorder, and in obsessive–compulsive disorder, and they are thought to cross-react with streptococcal and basal ganglia antigens (Kiessling et al., 1993, 1994; Morshed et al., 2001; Singer et al., 1998). In SC, antineuronal antibodies have been detected and quantified by several methods. In a recent study, anti-basal ganglia antibodies were found in 95% of acute and 56% of persistent SC patients (Church et al., 2002). Western blot analyses detected anti-60 kDa antibodies which occurred more frequently in tic disorders or TS patients (Hoekstra et al., 2003; Singer et al., 1998; Trifiletti and Packard, 1999). Recently, positive anti-basal ganglia antibodies were found in 64% of PANDAS patients but in only 9% of controls with a documented streptococcal infection but no neuropsychiatric symptoms (Pavone et al., 2004). Immunoblotting has also identified multiple bands against the caudate supernatant fraction in PANDAS with primary tics, which differed from the control group (Church et al., 2004). Few studies have specifically looked for antineuronal antibodies in OCD. Recently a consolidated group reported the presence of antibrain antibodies in 42% of an OCD group of 50 children compared with rates between 2% and 10% in three different control groups (paediatric autoimmune, neurological and streptococcal). Again, the most frequent molecular weights were 40, 45 and 60 kDa (Dale et al., 2005).

A pathogenic role for anti-basal ganglia antibodies has been suggested by two studies describing induced movements in rats after infusion of IgG of sera from patients with PANDAS (Taylor et al., 2002), but a recent multicentre study failed to show behavioural abnormalities when sera of patients with high titre antineuronal antibodies were microinfused into rat striatum (Singer et al., 2005b). Recently, it has also been reported that antibodies from an SC patient reacted against lysoganglioside and *N*-acetyl-beta-D-glucosamine, a neuronal antigen that is also found at the GABHS surface (Kirvan et al., 2003), and Dale et al. have identified antibodies against neuronal glycolytic enzymes autoantigens in 20 unselected post-streptococcal CNS patients compared to 20 controls (Dale et al., 2006). Volumetric resonance imaging (MRI) studies also support these features, as does the immune-mediated aetiological hypothesis of a larger caudate, putamen and pallidus in PANDAS patients in comparison with healthy children (Giedd et al., 2000).

As far as the immune-mediated disorders hypothesis is concerned, a single study has demonstrated an improvement of obsessive–compulsive symptoms after plasmapheresis or intravenous immunoglobulin treatment (Perlmutter et al., 1999).

In these disorders, antineural antibodies have been studied by several methods: immunofluorescent antibody staining in human basal ganglia, indirect immunofluorescence in rat striatum, enzyme–immunosorbent assays on human post-mortem basal ganglia tissue, and Western blotting techniques. Western blot allows the presence of specific

antibodies against the brain fraction to be investigated where serum is exposed, and it is the best procedure for determining their molecular weight. The reproducibility of the studies described is unclear, and certain discrepancies in their methods and data make them unreliable: for example, differences in brain regions studied, differences in tissue conditions, different and always low serum dilutions (some of them 1:10 or 1:25, and never higher than 1:300) and differences in the analysis of the results (comparison of specific bands between cases and controls or discriminant analyses that show different antibody patterns in subjects and controls).

The aim of the present study using immunohistochemistry and immunoblotting techniques was to detect the presence of specific antineural antibodies in children with OCD or TS, some of whom met criteria for PANDAS, compared with healthy children. Special attention was paid to the methodology and a high serum dilution was used to minimize non-specific binding.

## 2. Material and methods

The study sample included 32 prepubertal-onset OCD children and adolescents (15 male, 17 female), 21 TS children and adolescents (18 male, 3 female) and 19 normal children (10 male, 9 female), all of them aged between 9 and 17 years. The diagnosis of OCD and TS was made according to DSM-IV criteria (First et al., 1997), and the diagnosis of PANDAS was made in those who met the criteria proposed by Swedo et al. (1998). All patients were seen at the Child Psychiatry Department of the Hospital Clinic in Barcelona, while control subjects were recruited from the community and had no personal history of PANDAS, SC, tics or OCD. The sample was recruited during the period 2001–2003. The procedures were approved by the institution's Ethics Committee and written informed consent was obtained from all parents of subjects under study.

The mean age ( $\pm$ SD) of the three groups was as follows: OCD group,  $13 \pm 2.9$  years; TS group,  $12.1 \pm 1.9$  years; and healthy patients group,  $12.5 \pm 2.9$  years. These differences were not significant ( $p = 0.07$ ). Eight patients met criteria for PANDAS, three of them from the OCD group (2 males, 1 female) and five from the TS group (5 males). Tic severity was assessed using the Yale Global Tic Severity Scale (YGTSS). This instrument has separate scales for motor and vocal tics (Leckman et al., 1989). For obsessive–compulsive symptoms the Children's Yale-Brown obsessive–compulsive scale (CY-BOCS) (Goodman et al., 1989) was used. The CY-BOCS mean  $\pm$  SD score for OCD patients was  $27.5 \pm 5.6$  (range 14–36), while the mean YGTSS score in TS patients was  $41.05 \pm 17$  (range 20–70). Antistreptolysin-O titres were determined using the standard haemagglutination procedure in all subjects. ASLO titres above 200 U/ml. were defined as positive.

Anti-basal ganglia antibodies were tested by immunohistochemistry on frozen sections of putamen from a normal subject using an avidin–biotin immunoperoxidase

Table 1  
Characterization of subjects showing positive antibody reaction

Patient	1	2	3	4	5	6	7
Sex	M	M	M	M	F	M	F
Age	8	12	11	16	13	11	14
Diagnosis	OCD	OCD	TS-PANDAS	TS	OCD	TS-PANDAS	OCD
ASLO	0	200	200	400	0	200	0
AN AB	55, 86	55	55	55	55	86	86

ASLO: antistreptolysine O titers. AN AB: antineuronal antibodies band.

technique, as previously described [10]. Antibodies were also detected by the standard immunoblot technique of human putamen homogenates, as described elsewhere (Graus et al., 1997). Serum was used at dilutions of 1:500 for immunohistochemistry and 1:1000 for immunoblot studies. Normal putamen was procured from the Brain Bank of the University of Barcelona. Molecular weight was determined by comparison with commercially available standards.

All statistics were calculated using SPSS. Fisher's exact test was used to compare proportions of antibody reaction between patients and controls.

### 3. Results

No anti-basal ganglia antibodies were detected by immunohistochemistry in any of the samples. Immunoblotting screen showed antibody positive reactions to neural proteins of putamen in some patients. Two proteins with approximate molecular weights of 86 kDa and 55 kDa were found reacting with the sera of seven patients. The 86 kDa protein was found in 4 patients (3 with OCD and 1 with a diagnosis of TS that met PANDAS criteria), and the 55 kDa in 4 patients (2 with an OCD diagnosis and 2 with a diagnosis of TS, one of whom met PANDAS criteria). Clinical characteristics of the children in whom anti-basal ganglia antibodies were detected are described in Table 1. Immunoblots of two positive patients are shown in Fig. 1. Serum from control subjects did not show any positive reaction. Although the antigen–antibody reactions described were only found in patients, comparison of proportions of positive subjects between patients and controls was not significant ( $p = 0.178$ ). No significant differences were found in the mean ASLO titres in the three groups (OCD mean ASLO = 138, ST mean ASLO = 291 and control group mean ASLO 314). Five of the seven patients (71.4%) with positive bands also had an increased ASLO titre, compared with figures of 45% in OCD group and 62% in TS group. Between-group differences did not turn out to be significant ( $p = 0.33$ ), probably due to the small sample size resulting in low statistical power.

### 4. Discussion

We report the detection in seven patients of two different bands, corresponding to antigens with approximate molecular weights of 55 and 86 kDa. These findings may be

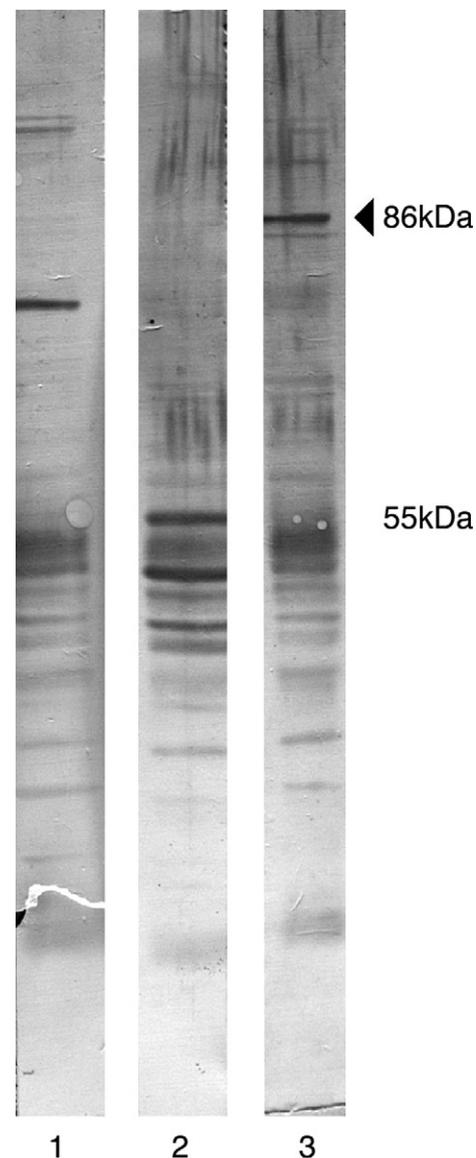


Fig. 1. Immunoblots of two patients where 55 kDa and 86 kDa bands were found. First strip corresponds to normal human sera. Second and third strips are from patients.

related to previous reports in SC, tic disorders or TS and PANDAS (Church et al., 2004; Dale et al., 2005; Hoekstra et al., 2003; Pavone et al., 2004). Since the determination of molecular methods has a certain error range, depending on the electrophoresis condition of gel (over and under); it

may be that the 55 kDa and the 86 kDa bands correspond to the previously described bands of 60 kDa (Church et al., 2002; Dale et al., 2005; Hoekstra et al., 2003) and 83 kDa (Pavone et al., 2004). Five of these seven patients had positive ASLO titres, which forms a higher proportion than in the patient groups in general and the healthy controls. Between-group differences did not reach significance, however, probably due to the low number of patients involved. In the patient group, where an antigen–antibody reaction was found, this feature may suggest an immunological basis for disease pathogenesis, and may be responsible for a range of symptoms, tics, extra-pyramidal movements and obsessions or compulsions. As regards the pathogenic role of these antibodies, recent reports found specific binding with molecules from the GABHS surface, such as lyso-ganglioside or glucosamine, and more neuronal glycolytic enzymes as piruvate kinase, aldolase or enolase (Dale et al., 2006; Kirvan et al., 2003). However, these antibodies may also be produced as a consequence of local damage or by alternative immune mechanisms.

Our study failed to identify significant differences in specific antibodies between OCD/TS patients and normal controls, though, in agreement with previous reports (Singer et al., 2004) the percentage of patients with some anti-basal ganglia antibodies in serum was higher than that found in controls. Failure to confirm an association between OCD/TS and specific antineural antibodies has recently been reported by a consolidated group. In that study Western blot analyses showed complex staining patterns with no differences in any tissue region based on the number of bands or reactivity peaks at molecular weights 98, 60, 45, and 40 kDa in patients with TS (Singer et al., 2005a). Those authors found no autoantibody binding against brain autoantigens (neuronal glycolytic enzymes) previously described. As noted by Dale et al. (2006) this lack of positive confirmation of the autoantibody binding in the Singer group study may be due to the use of rabbit neural antigens with low homology with the human isoforms instead of human antigens.

The differences with respect to previous studies (which found greater positivity of anti-basal ganglia antibodies in both patient samples and controls) may partially be due to the higher dilutions used in our study in order to reduce non-specific immunity as much as possible. Whereas the serum dilution used in previous studies was 1:25–1:250, we used at least 1:500. When we tried lower dilutions, the background was unacceptable. High staining background is the non-specific binding, that is also found in controls, that cannot be attributed to specific antigen–antibody interaction; this optimal dilution was obtained in previous experiments by Graus et al. for the identification and immunological characterization of a neuronal antibody (anti-Tr) (Graus et al., 1997). In addition, our results may be affected by the use of the putamen, a different tissue from other studies based on caudate or globus pallidus; the condition of the tissue is also relevant here. In the current study immunohistochemistry failed to

identify antiputamen antibodies, which is not an uncommon observation when human sera are evaluated by immunohistochemistry and immunoblot (Saiz et al., 1999).

The limitations of the present study include the small number of patients that could be diagnosed as PANDAS; indeed, the study sample comprises a rather heterogeneous group of patients. Negative results in some patients could be due to the point in the disease history at which blood was drawn, which did not always correspond to the most symptomatic period. Clinical categorization, according to the disease course (OCD/TS or PANDAS), is thus needed to obtain more homogeneous samples.

Although the hypothesis of an autoimmune process underlying OCD or TS in some patients is supported by the present study, further studies are needed to reach a more definitive conclusion. It would be of great interest to characterize the autoantigens involved in this autoreactivity in terms of immunoreactivity against neural tissue, and to use a molecular cloning approach to identify target molecules, as has been done in other neural pathologies (Bataller et al., 2004).

#### Acknowledgement

We are grateful to Mercé Bonastre, Laura Rubio and Maria Romera for their expert technical assistance. The study was supported by FIS:01/1529.

#### References

- Bataller L, Sabater L, Saiz A, Serra C, Claramonte B, Graus F. Carbonic anhydrase-related protein VIII: autoantigen in paraneoplastic cerebellar degeneration. *Annals of Neurology* 2004;56:575–9.
- Church AJ, Cardoso F, Dale RC, Lees AJ, Thompson EJ, Giovannoni G. Anti-basal ganglia antibodies in acute and persistent Sydenham's chorea. *Neurology* 2002;59:227–31.
- Church AJ, Dale RC, Giovannoni G. Anti-basal ganglia antibodies: a possible diagnostic utility in idiopathic movement disorders? *Archives of Disease in Childhood* 2004;89:611–4.
- Cunningham MW. Pathogenesis of group A streptococcal infections. *Clinical Microbiology Reviews* 2000;13:470–511.
- Dale RC, Candler PM, Church AJ, Wait R, Pocock JM, Giovannoni G. Neuronal surface glycolytic enzymes are autoantigen targets in post-streptococcal autoimmune CNS disease. *Journal of Neuroimmunology* 2006;172:187–97.
- Dale RC, Church AJ, Candler PM, Chapman M, Martino D, Giovannoni G. Serum autoantibodies do not differentiate PANDAS and Tourette syndrome from controls. *Neurology* 2006;66:1612.
- Dale RC, Heyman I, Giovannoni G, Church AW. Incidence of anti-brain antibodies in children with obsessive–compulsive disorder. *British Journal of Psychiatry* 2005;187:314–9.
- Eichstedt JA, Arnold SL. Childhood-onset obsessive–compulsive disorder: a tic-related subtype of OCD? *Clinical Psychology Review* 2001;21:137–57.
- First MB, Spitzer RL, Gibbon M, Williams JBW. Structured clinical interview for DSM-IV axis I disorders, research version, patient edition (SCID-I/P). New York: New York State Psychiatric Institute; 1997.
- Geller DA, Biederman J, Jones J, Shapiro S, Schwartz S, Park KS. Obsessive–compulsive disorder in children and adolescents: a review. *Harvard Review of Psychiatry* 1998;5:260–73.
- Giedd JN, Rapoport JL, Garvey MA, Perlmutter S, Swedo SE. MRI assessment of children with obsessive–compulsive disorder or tics

- associated with streptococcal infection. *American Journal of Psychiatry* 1999;157:281–3.
- Goodman WK, Price LH, Rasmussen SA, Mazure C, Fleischmann RL, Hill CL, et al. The Yale-brown obsessive compulsive scale I. Development, use, and reliability. *Archives of General Psychiatry* 1989;46:1006–11.
- Graus F, Dalmau J, Valldeoriola F, Ferrer I, Rene R, Marin C, et al. Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *Journal of Neuroimmunology* 1997;74:55–61.
- Hoekstra PJ, Horst G, Limburg PC, Troost PW, van Lang N, de Bildt A, et al. Increased seroreactivity in tic disorder patients to a 60 kDa protein band from a neuronal cell line. *Journal of Neuroimmunology* 2003;141:118–24.
- Husby G, van de Rijn I, Zabriskie JB, Abdin ZH, Williams Jr RC. Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. *Journal of Experimental Medicine* 1976;144:1094–110.
- Kiessling LS, Marcotte AC, Culpepper L. Antineuronal antibodies in movement disorders. *Pediatrics* 1993;92:39–43.
- Kiessling LS, Marcotte AC, Culpepper L. Antineuronal antibodies: tics and obsessive-compulsive symptoms. *Journal of Developmental and Behavioral Pediatrics* 1994;15:421–5.
- Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. *Nature Medicine* 2003;9:914–20.
- Kotby AA, El Badawy N, El Sokyary S, Moawad H, El Shawarby M. Antineuronal antibodies in rheumatic chorea. *Clinical and Diagnostic Laboratory Immunology* 1998;5:836–9.
- Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *Journal of the American Academy of Child and Adolescent Psychiatry* 1989;28:566–73.
- Mell LK, Davis RL, Owens D. Association between streptococcal infection and obsessive-compulsive disorder, Tourette's syndrome, and tic disorder. *Pediatrics* 2005;116:56–60.
- Morer A, Vinas O, Lazaro L, Bosch J, Toro J, Castro J. D8/17 monoclonal antibody: an unclear neuropsychiatric marker. *Behavioural Neurology* 2005a;16:1–8.
- Morer A, Vinas O, Lazaro L, Calvo R, Andres S, Bosch J, et al. Subtyping obsessive-compulsive disorder: clinical and immunological findings in child and adult onset. *Journal of Psychiatry Research* 2005.
- Morshed SA, Parveen S, Leckman JF, Mercadante MT, Bittencourt Kiss MH, Miguel EC, et al. Antibodies against neural, nuclear, cytoskeletal, and streptococcal epitopes in children and adults with Tourette's syndrome, Sydenham's chorea, and autoimmune disorders. *Biological Psychiatry* 2001;50:566–77.
- Pavone P, Bianchini R, Parano E, Incorpora G, Rizzo R, Mazzone L, et al. Anti-brain antibodies in PANDAS versus uncomplicated streptococcal infection. *Pediatric Neurology* 2004;30:107–10.
- Perlmutter SJ, Leitman SF, Garvey MA, Hamburger S, Feldman E, Leonard HL, et al. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *Lancet* 1999;354:1153–8.
- Rapoport JL, Swedo SE, Leonard HL. Childhood obsessive compulsive disorder. *Journal of Clinical Psychiatry* 1992;53(Suppl.):11–6.
- Saiz A, Dalmau J, Butler MH, Chen Q, Delattre JY, De Camilli P, et al. Anti-amphiphysin I antibodies in patients with paraneoplastic neurological disorders associated with small cell lung carcinoma. *Journal of Neurology Neurosurgery and Psychiatry* 1999;66:214–7.
- Singer HS, Giuliano JD, Hansen BH, Hallett JJ, Laurino JP, Benson M, et al. Antibodies against human putamen in children with Tourette syndrome. *Neurology* 1998;50:1618–24.
- Singer HS, Hong JJ, Yoon DY, Williams PN. Serum autoantibodies do not differentiate PANDAS and Tourette syndrome from controls. *Neurology* 2005a;65:1701–7.
- Singer HS, Loisel CR, Lee O, Minzer K, Swedo S, Grus FH. Anti-basal ganglia antibodies in PANDAS. *Movement Disorders* 2004;19:406–15.
- Singer HS, Mink JW, Loisel CR, Burke KA, Ruchkina I, Morshed S, et al. Microinfusion of antineuronal antibodies into rodent striatum: failure to differentiate between elevated and low titers. *Journal of Neuroimmunology* 2005b;163:8–14.
- Swedo SE, Leonard HL, Schapiro MB, Casey BJ, Mannheim GB, Lenane MC, Rettew DC. Sydenham's chorea: physical and psychological symptoms of St Vitus dance. *Pediatrics* 1993;91:706–13.
- Swedo SE, Leonard HL, Garvey M, Mittleman B, Allen AJ, Perlmutter S, et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *American Journal of Psychiatry* 1998;155:264–71.
- Taylor JR, Morshed SA, Parveen S, Mercadante MT, Scahill L, Peterson BS, et al. An animal model of Tourette's syndrome. *American Journal of Psychiatry* 2002;159:657–60.
- Trifiletti RR, Packard AM. Immune mechanisms in pediatric neuropsychiatric disorders. Tourette's syndrome, OCD, and PANDAS. *Child and Adolescent Psychiatric Clinics of North America* 1999;8:767–75.