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Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS)

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Foreword

The inclusion of a chapter on pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (or PANDAS) is essential to provide a history of the disease and provide current information about its association with *Streptococcus pyogenes* (group A streptococci), tics, obsessive compulsive disorder (OCD) and its relationship to Sydenham chorea (SC), which is the neurologic manifestation of acute rheumatic fever. PANDAS has been misunderstood and confusing to doctors since its discovery, but the original group of the first 50 cases as described by Dr Susan Swedo (Swedo, et al., 1998) has a similarity to Sydenham chorea that distinguishes this initial group from tic and OCD cases. As this chapter will examine, the acute onset is an important feature of these disorders, as are their piano-playing choreiform movements, enuresis, night-time fears, separation anxiety, learning regression, and handwriting disabilities.

The most current literature, which has been recently published in the *Journal of Child and Adolescent Psychopharmacology* (Murphy, et al., 2015b; Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a; Toufexis, et al., 2015; Gerardi, Casadonte, Patel, & Murphy, 2015; Chang, et al., 2015), provides new insight into the clinical phenotype of PANDAS; namely, a subgroup of pediatric acute-onset neuropsychiatric syndrome (PANS), which has been proposed to have multiple etiologies, including those that are genetic and immunologic, and that present either with or without preceding infections, such as with *Streptococcus pyogenes* (Toufexis, et al., 2015). PANS is a subtype of obsessive compulsive disorder (OCD) that presents with an abrupt onset or exacerbation of neuropsychiatric symptoms (Murphy, et al., 2015b), including moderate or severe OCD. Elevated anti-streptococcal antibody titers tended to have higher OCD severity and the

symptoms tended to lead to sudden and severe impairment, due to comorbidities, such as anxiety, behavioral regression, depression, and suicidality. Comorbid tics in PANS were associated with decline in school performance, visuomotor impairment, eating disorders, deterioration of handwriting skills, and lower quality of life, as compared to children without tics (Murphy, et al., 2015b). In addition, clinical evaluation of youth with PANS and PANDAS and recommendations for diagnosis were reported from the 2013 PANS conference held at Stanford University where a group of clinicians and researchers who were academicians with clinical and research interest in PANDAS and PANS (Chang, et al., 2015). PANDAS is clearly a subtype of PANS (Murphy, et al., 2015b; Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a; Chang, et al., 2015) and not all PANS cases have an underlying streptococcal infection—but all PANDAS cases are associated with streptococcal infections, at least temporally.

When these diseases appear, treatment with antibiotics can be successful, and a treatment trial of cefdinir by Murphy and colleagues indicated that therapy with cefdinir, a β lactam antibiotic, provided notable improvements in tic symptoms rated by the Yale Global Tic Severity Scale (YGTSS) and OCD symptoms rated by the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS). However, the differences within the groups as a whole were not significant. β-lactam antibiotics have been proposed to be neuroprotective above and beyond their antibiotic efficacy (Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a). Anti-neuronal autoantibodies against the brain in SC and PANDAS react with brain antigens including dopamine receptors (Cox, et al., 2013; Brimberg, et al., 2012), lysoganglioside (Kirvan, Swedo, Heuser, & Cunningham, 2003; Kirvan, Swedo, Snider, & Cunningham, 2006a), and tubulin (Kirvan, Cox, Swedo, & Cunningham, 2007), as well as the activation of the calcium calmodulin-dependent protein kinase II (CaM KII) in human neuronal cells (Kirvan, Swedo, Heuser, & Cunningham, 2003). Human anti-brain antibodies expressed in Tg mice targeted dopaminergic neurons and signaled the dopamine D2 receptor (D2R) (Cox, et al., 2013). Evidence strongly suggests that human anti-brain autoantibodies induced by *Streptococcus pyogenes* infections target the dopamine receptors (Cox, et al., 2013; Brimberg, et al., 2012) and that animal models immunized with the *S. pyogenes* antigen develop obsessive behaviors and movement problems, along with antibodies that react with the dopamine receptors and signal the CaMKII, similar to antibodies found in humans with SC and PANDAS (Brimberg, et al., 2012; Lotan, et al., 2014a).

Introduction and Background

In the last half of the 1990s, a group of clinical researchers, including Swedo et al. (Swedo, et al., 1998; Snider, et al., 2002) at the National Institutes of Mental Health (NIMH) described a subgroup of children who presented with obsessive-compulsive disorder (OCD) and/or tic disorders following an infectious illness, in particular after streptococcal infections, and proposed the term pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) for this subgroup (Swedo, et al., 1998).

The background information for this proposal stems from different sources: some anecdotal reports on the relationship between OCD or tic symptoms and infectious illness (Selling, 1929; von Economo, 1931; Kondo & Kabasawa, 1978; Kiessling, Marcotte, & Culpepper, 1993); the observation of OCD, as well as tic symptoms, in patients with Sydenham chorea (SC) (Langlois & Force, 1965; Kerbeshian, Burd, & Pettit, 1990; Swedo, et al., 1989); and the observation of a fluctuating, infectious-related course of OCD in some patients without choreoathetoid movements of SC (Allen, Leonard, & Swedo, 1995; Swedo, 1994).

The original working criteria established by the NIMH group for the diagnosis of PANDAS included: 1) the presence of OCD and/or a tic disorder; 2) a pediatric onset; 3) an episodic course of symptom severity; 4) an association with streptococcal infections; 5) an association with neurological abnormalities, including piano-playing choreiform movements

of the fingers and toes, which suggests that PANDAS may be similar to SC. Moreover, besides these core features, the first 50 cases described in the original series showed emotional lability (66%), deteriorated school performance (60%), personality changes (54%), separation anxiety (46%), nightmares (18%), bedtime rituals (50%), deterioration in handwriting (36%), oppositional behaviors (32%), and motoric hyperactivity (50%), as seen in Table 1.

During the following years, the concept of PANDAS has become very popular and at the same time has sparked a heated debate among researchers and clinicians. To date, a large number of studies on different aspects of PANDAS have been published, as well as some comprehensive and recent reviews (Murphy, 2013; Macerollo & Martino, 2013).

Several researchers have examined two main critical aspects of PANDAS: the difficulty in establishing a tight link between the inciting streptococcal infection/exposure and the onset/recrudescence of OCD or tic symptoms, and the lack of reliable biological markers. These difficulties have led to a recent revision of the diagnostic criteria and to the proposal of a new clinical entity, the pediatric acute-onset neuropsychiatric syndromes (PANS), in which the key clinical feature is ‘‘acute and dramatic symptom onset.’’ There are some changes in the presenting symptoms (with special relevance to OCD and anorexia, and loss of prominence of tics) without any reference to their relationship with streptococcal infections (Swedo, Leckman, & Rose, 2012), although PANDAS would be included under a broader PANS group (Figure 2).

In this chapter, we will review some clinical, microbiological, and immunological aspects of PANDAS. Because tics and OCD symptoms are the main clinical features of PANDAS, a short review on their prevalence, appearance, and natural history will be provided first.

A brief review of tic disorders

Tics are rapid, recurrent, non-rhythmic, and stereotyped movements or vocalizations that can be simple or complex; they are usually suggestible, may be preceded by premonitory urges, and may be suppressed voluntarily. The last edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) categorizes tic disorders in three main groups, based on the length of the disorder (a duration of more or less than 1 year) and on the signs of symptoms (motor or phonic): “provisional tic disorder,” with motor or phonic tics that last less than 1 year); “persistent tic disorder,” with motor or phonic tics that last more than 1 year; and “Tourette’s disorder” (or TS), with motor and phonic tics that last more than 1 year.

Tics are considered the most prevalent movement disorder in childhood, even if their exact prevalence in the general population is unknown. In fact, many cases of tics don’t come to clinical attention, probably because of mild symptoms, they cause little functional or psychosocial impairment, or that the tics don’t cause parental concern in parents. This is true for all the clinical forms of tics, including the chronic ones. Moreover, the epidemiological studies on the incidence and the prevalence of tic disorders are biased by different factors, such as sampling methods, sample sizes, rate of subject participation, assessment methods, and diagnostic thresholds used to define cases. With these cautions, the prevalence of transient tics in school-age children is estimated to be from 11 to 20% (Snider, et al., 2002; Cubo, et al., 2011; Kurlan, et al., 2001; Linazasoro, Van Blercom, & de Zárate, 2006), while the prevalence of TS in school-age children is likely to fall somewhere between 5 and 7 cases per 1,000. For all tic disorders, there is a clear male prevalence, with the male-to-female ratio ranging from 2 to 1 to as high as 3.5 to 1.

Tic disorders are mainly a childhood and adolescent disturbance. In persistent or chronic forms, the onset of tics occurs between 2 and 7 years; the worst period of tic expression usually peaks around pre-adolescence (9–12 years); then there is a phase of stabilization and attenuation of symptoms during adolescence and early adulthood. Some studies report that more than around 40% of the TS children have no more tics during adult life; another 40% have minimal or mild tics that cause no interference in their lives; and only 20% continue to show moderate or even severe symptoms.

From a symptomatic point of view, in the majority of cases, the first tics are motor tics, eye tics (Martino, Cavanna, Robertson, & Orth, 2012) or facial movements. However, in some cases, vocal tics (shouts or vocalizations) and other motor tics (arm jerking, trunk spasms or other more complex movements) can be the first sign of the disorder, and can often pose problems of differential diagnosis with other movement disorders, particularly if their appearance is abrupt.

Typically, the onset of symptoms is sub-acute: the tics tend to slowly increase in frequency and intensity during months or years, and parents often have difficulties in recalling a precise date when tics began. The tics rarely appear acutely (seemingly overnight), though when this occurs, their intensity and frequency are very high from the beginning. This can often cause a great deal of anxiety in parents, who turn to emergency departments for consultations.

The severity of tic symptoms generally varies over time, and the waxing and waning course of chronic tic disorders is an universally recognized feature. Beside the natural course of chronic tic disorders, as described before, this variation of severity occurs over a period of weeks to months, and can even occur over the same day. A relationship between tics and environmental contingencies or emotional factors has been proposed: in particular, psycho-social stress (Lin, et al., 2010) or abnormalities in the cortisol circadian rhythm (Corbett, Mendoza, Baym, Bunge, & Levine, 2008) have been reported to affect the modulation of tic severity, but for many tics, it is difficult to establish a link between these fluctuations and a specific situation or environmental cause.

However, in the absence of a general agreement on the cut-off that defines a true exacerbation from the “normal” fluctuation of symptoms, the notion of tic exacerbation is quite vague. In a few studies, including those on some PANDAS patients (Lin, et al., 2002; Luo, et al., 2004; Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008; Leckman, et al., 2011; Martino, et al., 2011), tic exacerbation thresholds that incorporated the change score from the previous month and the current symptom score were estimated by using state-of-the-art bootstrap methods. Such methods agreed with the judgement of clinical experts. A seven-points increase of the global score (without impairment score) at the Yale Global Tic Severity Scale (YGTSS) has been considered in some studies to be a reliable cut-off that defines an exacerbation. Unfortunately, in an attempt to provide such a crucial definition, little help has come from the psychopharmacological studies on anti-tic medications, in which the definition of responsiveness or refractoriness to a single drug is quite vague.

Brief Review of OCD

OCD is a disorder with a lifetime prevalence of 1–3% in the general population. The disorder is characterized by the presence of obsession (i.e., recurrent and persistent thoughts, urges, or images that are experienced as intrusive and unwanted) and/or compulsions (i.e., repetitive behaviors or mental acts that an individual feels driven to perform in response to an obsession or according to rules that must be rigidly applied).

In children and adolescents, OCD shows a bimodal age of onset: the first one peaks around 8-12 years, which is the so-called “early onset OCD” that is characterized by a frequent comorbidity with tics/TS, a male prevalence (nearly 25% of males with the disorder have an onset before 10 years), a different content of obsessions/compulsions or the presence of compulsions without obsessions, a different and reduced response to the pharmacological treatment, and a poorer prognosis. The second one peaks after puberty and is characterized by a slight female prevalence, a content of obsessions/compulsions similar to those seen in adulthood, a good response to treatment, and a better prognosis.

The bimodal age of the onset of OCD suggests some different etiological factors. In particular, patients with an early onset are likely to have a stronger genetic or biological component than patients with a late onset. In particular, family studies revealed higher familial aggregation among relatives of early-onset subjects (Geller, 2006).

The abrupt overnight onset of initial OCD symptoms reported in cases of PANDAS or PANS is characteristic of these disorders. However, in typical OCD, the onset of symptoms is more gradual. OCD is described as a chronic disorder with a fluctuation of symptoms (waxing and waning), even if an episodic course is described in some cases. Notably, in some longitudinal studies when both tic and OCD symptoms were present, there was a significant degree of covariation (Lin, et al., 2010; Luo, et al., 2004; Leckman, et al., 2011).

Pathophysiology of tics, TS, and OCD

In the last twenty years, a growing number of studies have investigated the neural and pathophysiological underpinnings of tics, TS, and OCD. Given the known role of the basal ganglia in motor control and in other movement disorders, these structures have been the primary focus of many studies that have investigated the neurobiology of these disorders.

The basal ganglia comprise a set of subcortical nuclei that include the caudate nucleus, putamen, globus pallidus, subthalamic nucleus, and substantia nigra. Their functional connections to several cortical regions have led to the conceptualization of the corticostriatal-thalamo-cortical (CSTC) circuits; namely, multiple parallel, segregated feedback circuits with outputs from striatum that target primary motor areas, and specific pre-motor and prefrontal cortical areas. The primary function of the CSTC circuits is to control and select goal-directed motor, cognitive and motivational behavior. Further, CSTC circuits are involved in inhibitory control (Aron, Behrens, Smith, Frank, & Poldrack, 2007) and habit formation (Graybiel, 2008).

Even if a clear explanation for the occurrence of tics hasn’t yet emerged, the most compelling finding so far is increased supplementary motor area (SMA) activity just prior to tic onset (Hampson, Tokoglu, King, Constable, & Leckman, 2009), which suggests that the SMA plays a role in the sensory phenomena that precede the execution of tics (premonitory urges).

Structural MRI studies have revealed reduced caudate volumes in children and adults with TS (Peterson, et al., 1993), with a negative correlation between caudate volume in childhood and the severity of symptoms later in life (Bloch, Leckman, Zhu, & Peterson, 2005).

The sensorimotor cortices are intuitive candidate cortical areas for investigation in TS due to the motor nature of tics and the sensory disturbances that frequently accompany them. MRI studies that have measured cortical thickness and grey matter volume in sensorimotor cortices in TS are limited in number, but they have provided consistent results. In particular, Sowell et al. (2008) found cortical thinning in sensorimotor cortex, along with

other regions (ventral frontal cortex, dorsal parietal cortex), in children with pure TS (Sowell, et al., 2008).

To date, neuroimaging studies of TS (and especially functional MRI studies) are limited and many study results are inconsistent. These inconsistencies could be due to the large heterogeneities in the samples that have been studied.

With regard to OCD, in recent years, a growing number of studies have identified the CSTC circuits as centrally implicated in the pathophysiology of the disorder (Saxena & Rauch, 2000). Most especially, the limbic or orbitofrontal circuit (orbitofrontal cortex, anterior cingulate cortex, and caudate nucleus) has consistently been shown to be involved in OCD symptoms. Imaging research (which concerns structural, functional, and connectivity investigations) have shown a particularly high degree of concordance across the studies and have led to the conceptualization of the CSTC model of OCD. This model has received further support through neuropsychological and treatment studies (Menzies, et al., 2008).

PANDAS phenotype

Most of the studies published on PANDAS investigated a possible relationship between the onset or recrudescence of symptoms—mainly tics—and clinical or biological signs of *S. pyogenes* infections in different populations (such as tics or TS patients) observed in cross-sectional or longitudinal ways; furthermore, other research was conducted to search for possible markers of the proposed autoimmune process. In most of these studies, the definition of PANDAS cases was made after a retrospective review of clinical records. As a matter of fact, up until now, little attention has been paid to the clinical signs that could differentiate PANDAS from tics, TS, or OCD patients, besides the inclusion criteria. To date, four studies reported data that was useful for a comparison.

In 2008, Kurlan et al. compared 40 PANDAS patients with 40 OCD or chronic tic disorder matched subjects, followed for a period of 24 months. From a clinical point of view, the groups were comparable, with the exceptions that the PANDAS case subjects seemed to more often have a psychiatric diagnosis other than tic disorder or OCD (Leckman, et al., 2011).

In 2010, Bernstein et al. compared 21 PANDAS children with a control group of 19 children with non-PANDAS OCD, with respect to ancillary symptoms, types of obsessions and compulsions, symptom severity, and co-morbid DSM-IV diagnoses. Both groups were retrospectively defined by reviewing their medical records. PANDAS children were significantly more likely to present with separation anxiety, urinary urgency, hyperactivity, impulsivity, deterioration in handwriting, and decline in school performance during their initial episode of neuropsychiatric illness, as compared with children with non-PANDAS OCD. The total number of tics was higher and the vocal tics were more severe in PANDAS children. Separation anxiety disorder and social phobia were more prevalent in non-PANDAS OCD children, and children with non-PANDAS OCD were significantly more likely to include others in their rituals (Bernstein, Victor, Pipal, & Williams, 2010).

In 2011, Leckman et al. conducted a multi-centric longitudinal study that compared 31 children who met the criteria for PANDAS with 53 TS or OCD non-PANDAS subjects. Both groups showed a similar severity of symptoms and a similar rate of tic or OCD symptom exacerbations. Only a quarter of exacerbations identified in the PANDAS group were associated with a simultaneous sudden acute onset with increase in anxiety, depression, and/or attention deficit and hyperactivity disorder (ADHD) symptoms (Martino, et al., 2011).

Finally, in 2012, Murphy et al. examined 109 children showing tics, TS, or OCD; the assignment to the PANDAS (41 subjects) or the non-PANDAS (68 subjects) group was based on the presence of PANDAS operational criteria, as developed by Swedo et al. in 1998. The clinical assessment didn't show any clinical difference between groups. Children classified as PANDAS had a high rate of dramatic symptoms onset and clumsiness; however, it should be noted that these are two of five criteria for PANDAS (Murphy, Storch, Lewin, Edge, & Goodman, 2012). (A subset of the non-PANDAS group would have met the criteria for PANS, but did not have the temporal association normally found with streptococcal infections).

Although these studies did not provide overwhelming evidence for the existence of ''PANDAS-specific'' phenomenological features, PANDAS is similar to SC and shows the choreiform movements of fingers and toes that may not have been observed in earlier comparative studies. The presence of these movements may constitute a red flag that signals a PANDAS diagnosis. However, a lack of choreiform movements does not exclude PANDAS, and these signs have to be regarded with caution, as they are also present in typically developing children and in children with other childhood disorders, such as ADHD and developmental coordination disorders.

PANDAS vs. Sydenham chorea

Sydenham chorea (SC) has provided a model for the conceptualization of PANDAS, and Swedo and the NIH group have shown that PANDAS is similar to SC and is characterized by choreiform piano-playing movements of the fingers and toes (Snider & Swedo, 2004). Both PANDAS and SC have immunological similarities, as a later section will show. Human sera studies in immunoassays suggest that human dopamine D2 receptor (D2R) is the target of autoantibodies that are produced in both SC and PANDAS (Cox, et al., 2013). Tics or OCD symptoms are often present in the early prodromal phases of SC, with choreoathetoid movements following. Deterioration in handwriting and irritability, often seen in early phases of SC, are also accompanying symptoms of PANDAS. However, the clinical course of PANDAS vs. SC is different: SC often is a monophasic illness, even if recurrent or persistent cases have been described (Cardoso, Vargas, Oliveira, Guerra, & Amaral, 1999). In contrast, the recurrence of tics or OCD symptoms after streptococcal (or other) infections has been one of the basic criteria for a PANDAS diagnosis. Finally, echocardiographic abnormalities (valvular incompetencies) are present in nearly 80% of SC patients, and they constitute a feature that often leads to the right diagnosis in the anamnestic or clinical doubtful cases. Conversely, PANDAS patients don't generally show signs of cardiac involvement (Snider, Sachdev, MacKaronis, St Peter, & Swedo, 2004); however, minimal echocardiographic abnormalities have been described in some patients with *S. pyogenes* related tics disorders (Cardona, et al., 2007) and in some children with PANDAS (Segarra & Murphy, 2008).

Association of *S. pyogenes* infections with tics/OCD

To fully understand the different studies published on the relationship between *S. pyogenes* and some neuropsychiatric disorders (such as tics, TS, or OCD) is not an easy task and has often raised more questions than answers. Some of these studies strongly support this association (Lin, et al., 2010; Murphy, Storch, Lewin, Edge, & Goodman, 2012; Cardona & Orefici, 2001; Leslie, et al., 2008; Mell, Davis, & Owens, 2005; Murphy & Pichichero, 2002) while others firmly deny it (Macerollo & Martino, 2013; Luo, et al., 2004; Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008; Leckman, et al., 2011), but all agree on the necessity of more research for a definitive demonstration of the existence/absence of this relationship and of the basic cellular and immune mechanisms involved. Table 2 provides a list of these studies.

The difficulty of having definitive results partially reflects the complexity and the possibly multifactorial nature of neuropsychiatric disorders. Animal models used (generally mice and rats) are not completely satisfactory and should be interpreted with caution; since humans are the only natural reservoir for *S. pyogenes* , these animal models may not exactly reproduce a human disorder. However, animal models in mice and rats have been very instructive in PANDAS and SC, with several studies indicating that immunization of rats and mice leads to behavioral alterations similar to SC and PANDAS (Brimberg, et al., 2012; Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004). In addition, passive transfer of anti-streptococcal antibodies to naïve rats or mice led to behavioral changes (Lotan, et al., 2014a; Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004).

After reviewing human studies, it is clear that many are looking at heterogeneous human populations; as a result, it may not be appropriate to analyze these data, which can be misleading when considered together. In some cases, the population studied is not in prepubertal age (Bencivenga, Johnson, & Kaplan, 2009; Schrag, et al., 2009; Morshed, et al., 2001); in others, there is a larger than usual presence of females; other studies are enhanced with more tic spectrum patients than acute onset OCD patients (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008; Leckman, et al., 2011), which indicates some bias in the selection of patients; in others the original population with tics/OCD described by Swedo for PANDAS (Garvey, Giedd, & Swedo, 1998) is enlarged by the inclusion of ADHD cases (Swedo, et al., 1998). In some studies, the involvement of *S. pyogenes* is investigated only through the detection of antibodies against one or two *S. pyogenes* antigens, without looking for the presence of the bacterium. It is important to note these differences when considering the results.

Streptococcus pyogenes is known to be a complex organism with a vast repertoire of virulence factors produced for bacterial adhesion and invasion, for evasion of phagocytosis, or for modulating host defenses (Sjöholm, Karlsson, Linder, & Malmström, 2014). It is able to change over time by the acquisition of new mechanisms and structures to avoid host defenses (Bryant, et al., 2014; Hertzén E. , et al., 2012) or to have long intracellular persistence (Wang, Li, Southern, & Cleary, 2006; Hertzén E. , et al., 2010; Kaplan, Gastanaduy, & Huwe, 1981; Kaplan, Chhatwal, & Rohde, 2006). The same strain may cause suppurative diseases, non-suppurative sequelae, toxic shock, or may colonize carriers without provoking an infection.

Many human studies have focused more on clinical findings or on host immune responses to specific antigens than on the bacterium itself, and as a result, only the presence or absence of *S. pyogenes* is reported. Microbiologists often act as simple blinded operators, and no information is reported on the methods used for taking specimens or isolating the strain. This result is evident from differences in the percentage of *S. pyogenes* positive samples found in different studies, or in multicenter studies, by the different microbiology results between participating centers.

Unfortunately, while a *S. pyogenes* -positive sample demonstrates the real presence of the bacterium in the infected or carrier host throat, a negative swab is not really informative; in particular, a swab that contains just a few colonies may be not detected as positive by routine laboratory methods. Since a true infection may be accompanied by very few colonies in the throat swab (Johnson, Kurlan, Leckman, & Kaplan, 2010), the percentage of positive samples found largely depends on the methods used to detect them. In our experience, at the time of their first visit for tics, children rarely present clinical signs of pharyngitis, and a percentage of them often carry even fewer than 10 colonies/plate in their throat swabs. That requires great care in taking the swab, to avoid *S. pyogenes* being covered by a too large amount of saprophytic flora present in the sample, and the use of selective media and careful

methods in processing the swab, even different from those routinely in use for pharyngitis. If samples of children with tics but without clinical signs of pharyngitis are processed in the same way as those of children with sore throats, there are strong possibilities that the result will be below the threshold of detection and that the culture will be considered negative. *S. pyogenes* colonizing tonsillar criptae have been found in greater than 30% of children undergoing tonsillectomy for recurrent *S. pyogenes* tonsillopharyngitis and in children with no history of previous frequent infections who underwent surgery for different reasons, who were selected as healthy controls (Roberts, et al., 2012). These percentages are higher than all those found in PANDAS studies or in carriers, which indicates that even with accurate methods, many positive subjects are not detected; but, in the studies on the association between *S. pyogenes* infection/exposure and neuropsychiatric symptoms, false negative results can result in the true differences in positivity between cases and controls being entirely undetectable.

A second important point to consider is that *S. pyogenes* is not only an extracellular pathogen, but can survive to phagocytosis inside the cells. M-protein–expressing *S. pyogenes* strains can survive after phagocytosis by human neutrophils (Staali, Mörgelin, Björck, & Tapper, 2003) and the surface M-anchored protein has been identified as the pivotal factor that affects the phagosomal maturation in macrophages (Hertzén, et al., 2012; Hertzén, et al., 2010). During the intracellular phase, the expression of many genes—namely, the majority of those involved in cell wall synthesis and energy production—is significantly altered; after a replicative phase, *S. pyogenes* egress after having destroyed the host cells, and are fit to infect new cells and may persist in the throat for a long time, releasing any type of streptococcal antigens and causing the permanence of high-antibody titers even in the absence of overt disease. This may also account for the intermittent presence of the same serotype in the throat of tic patients and for the high percentage of carriers seen in some studies after treatment (Pichichero, et al., 1999). Host cells are a useful niche to escape many antibiotic drugs used against *S. pyogenes* (such as penicillin, for instance), and microorganisms during carriage or infection are selected on the basis of their capacity to enter and survive the treatment (Kaplan, Gastanaduy, & Huwe, 1981; Sela, Neeman, Keller, & Barzilai, 2000; Park, Francis, Yu, & Cleary, 2003).

The last point to consider concerns the level of antibodies (Anti Streptolysin O, or ASLO, and Anti DnaseB, or ADB) used to indicate the infection. In several cases (Johnson, Kurlan, Leckman, & Kaplan, 2010), a true infection causes a moderate increase in this level, though it remains below the threshold considered the upper limit of normal titers (ULN) and, in the absence of an accurate monitoring of the subject, it is disregarded. On the other hand, choosing a too low level of antibodies as the ULN may result in undetectable differences between cases and controls.

All these considerations show the difficulties in studying the association between *S. pyogenes* infection/exposure and neuropsychiatric symptoms and in explaining why, despite the large number of studies published, the demonstration of PANDAS following the original definition given by Swedo et al. (Garvey, Giedd, & Swedo, 1998) is debated. Initially, studies of the association of SC with streptococcal infection determined that chorea can occur anywhere from several weeks to nine months following a streptococcal infection (Cardoso, Vargas, Oliveira, Guerra, & Amaral, 1999).

Epidemiologic evidence for some *S. pyogenes* involvement in tic disorders comes from administrative data from a health maintenance organization in the Seattle area where 144 new cases of TS and OCD/tics were matched with 609 controls (Mell, Davis, & Owens, 2005). A significant association was found (13-fold more for TS) with prior *S. pyogenes* infections diagnosed either 3 months or 1 year before the onset of disturbance. The presence

of multiple *S. pyogenes* infections in the previous 12 months significantly increased the risk of TS, which indicates a sort of threshold of anti-streptococcal antibodies to be reached before the onset of the manifestation.

A strong association with a prior *S. pyogenes* infection was also found in an USA national health insurance study where 479 cases of OCD, tics, and TS were matched with 3647 controls, but the results of the study did not include a rigorous ascertainment of tic or OCD from consistent diagnostic criteria (Leslie, et al., 2008).

In a retrospective study, 80 consecutive children (15–17 years) were investigated through a structured clinical interview to establish if infection and an abrupt onset of symptoms could be identified; 53% of the patients reported such an abrupt onset and 21% of this subset had it within 6 weeks of infection (Singer, Giuliano, Zimmerman, & Walkup, 2000). It was suggested that in some cases, the abrupt onset might have been exaggerated by a biased memory of parents.

In the UK, Schrag et al. (Schrag, et al., 2009), on the contrary, were unable to support an association with *S. pyogenes* infection through a database analysis. However, in this case, both the mean age of their patients (16 years instead of prepubertal age) and the lack of established analysis parameters to determine the time between streptococcal infection and onset of tics/OCD (up to 3 years) could have accounted for the temporal association and thus the different results.

The age of the population studied is very important: in a large study that included 3006 school children, the percentage with motor or vocal tics was 22.3% for preschool children, 7.8% for elementary school, and 3.4% for secondary school, with the male/female ratio of 3.8/1 in the elementary school group and 6.1/1 for the secondary school group (Gadow, Nolan, Sprafkin, & Schwartz, 2002). Therefore, results using patients not in prepubertal age or with a too low rate of male/female subjects need to be carefully evaluated, since the population may be different from that of other studies.

Many of the studies performed on tic disorders are cross-sectional: clinical, serological, and microbiological data are collected at the time of the neuropsychiatric manifestation, onset, or increase of tics /OCD, but are not monitored over the time. This type of study may give useful insights (and they often do) to demonstrate that patients with tics differ from healthy normal people for a higher exposure to *S. pyogenes* antigens, but these studies are inadequate to demonstrate the overall PANDAS concept (*S. pyogenes* clinical infection with the subsequent or antecedent rise of antibodies associated with onset /recrudescence of symptoms in a pediatric population), which can be only assessed through sequential observations.

In a case-control study performed between March 1996 and November 1998, 150 children were examined for sudden onset, recrudescence, or protracted duration of their tic disorders (Cardona & Orefici, 2001). The controls were 150 healthy children without tics. In this study, 38% of the cases (in comparison with 2% of the controls had ASLO titers higher than 500 IU with a mean ASLO titer of 434 IU, in comparison with 155 IU in controls ($p < 0.01$). Moreover, 58% had a family history of tics and frequent upper respiratory tract infections and 17% had a throat swab positive for *S. pyogenes* . At the time of the visit, none of the patients had clinical evidence of pharyngitis and, if analyzed by standard methods, the rate of *S. pyogenes* positive specimens was very low, with only a few colonies per plate; as a result, an old pour plate method was used (Taranta & Moody, 1971) that gave better results than the one routinely used for pharyngitis (Johnson, et al., 1997), which made it easier to detect and isolate every single colony (see Figures 1A and B).

In several studies, the increase of the immune response to those streptococcal antigens (ASLO and ADB) generally used as indicators of *S. pyogenes* infection is considered to be evidence of infection. It is interesting that when studying more or less the same matter (i.e., the possible involvement of *S. pyogenes* in tic disorders), the results and the conclusions produced by different groups were different. Loiselle et al. (Loiselle, Wendlandt, Rohde, & Singer, 2003) were unable to confirm differences in ASLO, anti-DNase B, and anti-basal ganglia (ABGA) titers in 41 children with TS and ADHD and 38 controls, even if ASLO titers were significantly higher in children with ADHD, as compared to the non-ADHD group.

In another study, sera from 30 children with PANDAS, 30 with TS, and 30 controls were examined for ABGA. Though more antibody positivity and a higher immunofluorescence against human striatum samples were found in samples from children with PANDAS and TS in comparison with controls, no statistically significant association was found between immunofluorescent reactivity and the diagnosis (Morris, Pardo-Villamizar, Gause, & Singer, 2009).

In a cross-sectional study on 100 British patients with TS (50% children), Church et al. (Church, Dale, Lees, Giovannoni, & Robertson, 2003) found that 64% of children and 68% of adults had a significantly higher ASLO titer, as compared to 15% of children with recent uncomplicated streptococcal pharyngitis, but no attempts to study the presence of *S. pyogenes* was reported.

A higher ASLO titer in comparison with controls was also found in an American study on 81 patients with TS (Morshed, et al., 2001); antistreptococcal higher titers (ASLO, ADB, anti-M12 and anti-M19) were also found in a sample of German patients (Corbett, Mendoza, Baym, Bunge, & Levine, 2008).

In a cross-sectional case-control study on 69 Italian TS children, Martino and Rizzo et al. found that 59% of the patients had significantly high ASLO titers (> 400 IU), in comparison with 19% of the controls (Martino, et al., 2011); a high percentage of children with high ABGA was also found. Finally in another cross-sectional study (Geller, 2006), the mean ASLO titers (246 IU vs 125 IU, $p>0.01$), the number of positive *S. pyogenes* throat cultures (8%, compared with 2%; $p=0.009$) and the positivity of anti-basal ganglia antibodies (ABGA) (23% in comparison with 8%; $p >0.001$) were significantly higher in TS patients than in controls, but no difference in ASLO titers was detected between ABGA-positive and ABGA-negative children

These are just some examples that show the elusiveness of the matter and the difficulty in reaching firm conclusions from published studies. Retrospective studies from health maintenance data may be a good source of information, even if they are not wholly collected with an ad hoc questionnaire.

Longitudinal studies to demonstrate the relationship between new infections and recrudescences of symptoms: the problem of carriers

The main difficulty in demonstrating the concept of PANDAS is showing the association of a new *S. pyogenes* infection with a sudden exacerbation of tics or OCD. In fact, this association can't be demonstrated by cross-sectional studies and requires monitoring the microbiological and serological parameters of patients and controls for a long time, both to detect infections and to establish that these infections are new and are caused by *S. pyogenes* types that were not previously present. Longitudinal studies (in particular, those that follow patients from the initial tic manifestation) are informative, but are much more difficult to perform, and only a limited number of patients can be followed.

A prospective microbiology study analyzed the data of 160 children with tics enrolled in two clinical studies with identical protocols (Johnson, Kurlan, Leckman, & Kaplan, 2010). The goal of the study was to provide a description of the long-term kinetics of the immune response in patients with tics, either with *S. pyogenes* infections or who were carriers. The presence of *S. pyogenes* in the throat samples, M types, and the variation of antibody responses to ASLO and ADB were reported without specific attempts to correlate *S. pyogenes* infection with the concurrent recrudescences of tics. Pharyngeal swabs were taken every month for 120 weeks, and blood was examined approximately every three months. The study was very accurate, and shows that a true rise in anti-streptococcal antibodies may occur at levels below the ULN used in many studies. It stresses the need to use at least two antibodies (ASLO and ADB) for the diagnosis of new infection (since sometimes only a single antibody titer increases) and clarifies that a true infection with a significant antibody response may be associated with cultures with <10 colonies per plate, which demonstrates that many infections would be unidentified without a longitudinal observation. Obviously, only a limited number of example cases were reported; therefore, not much detailed information is available on the total population studied (number of *S. pyogenes* positive children, total number of infections seen during the entire study, characteristics of all *S. pyogenes* isolated) to enable comparisons to other large studies.

The conclusions of this research are convincing. Unfortunately, the same accuracy is difficult to reach in clinical practice, where the clinician is not necessarily informed on the basal level of anti-streptococcal antibody titers before the onset of the clinical manifestation or on the previous microbiology of *S. pyogenes* in the throat.

S. pyogenes throat infections and carriage are rather common in school-age children: several previous studies (Kaplan E. L., 1980) and in a recent meta-analysis (Shaikh, Leonard, & Martin, 2010) found 37% *S. pyogenes* positivity in children with sore throat and a 12% positivity in healthy children. In the Johnson study (Johnson, Kurlan, Leckman, & Kaplan, 2010) and in other studies, patients bearing the same *emm* type for a long time without changes in antibody levels were considered “carriers,” since the presence of *S. pyogenes* in these cases could not be considered a new infection. Nevertheless, the status of a “carrier” is difficult to define: historically, *carriage* was defined as the prolonged permanence of *S. pyogenes* in the pharynx without evidence of immune or inflammatory response (Kaplan, 1980; Tanz & Shulman, 2007). Carriage is a very complex phenomenon, in which the *S. pyogenes* strain, the host immune response, and environmental factors all play roles, as demonstrated by the fact that the same strain can provoke pharyngitis, invasive disease, or simply be carried by a healthy population. We agree with Kaplan (Kaplan, 1980) in that “antibody titers remain elevated as long as the organism is present in the upper respiratory tract,” and that “in many so-called prolonged carriers, there was a continual anti-streptococcal immune response.”

In Johnson’s study and other examples, there are some cases where the same strain was repeatedly isolated and the antibody titers remained high for a long time (therefore, correctly defined as “no new infection”), which made it difficult to differentiate a true carriage from a persistent infection. In these cases, it would be interesting to see if these “carriers” are more frequent in the tic population, and to characterize these strains to evaluate if this long presence of *S. pyogenes* (either simply as carriage or as a persistent infection), together with other environmental factors, may be associated with a higher frequency of tic recrudescences. The model of colonization followed by a long “carriage” with a rise in antibody titers without evidence of clinical infection has been described by Ashbaugh in baboons (Ashbaugh, et al., 2000), and had already been described by Kaplan in humans (Kaplan, 1980). In any case, patients with tics and long-lasting antibody titers should be carefully studied, even if the patients have negative swabs. If that does not change anything

for the clinical treatment of a single patient, it may nevertheless give new insights into the role played by *S. pyogenes* in these movement, tic, and neuropsychiatric disorders. In 2007, Murphy et al., a school study showed that those with repeated *S. pyogenes* infections had higher rates of behavioral and movement findings. Although the strains were not characterized, the findings suggest that a carrier state could contribute to neuropsychiatric symptoms (Murphy, et al., 2007).

In other longitudinal studies, patients were followed for a long time for clinical and immunological findings to verify if recrudescences may be temporally associated with a *S. pyogenes* infection, as described in the PANDAS definition. In a 3-year prospective study, 12 children with new-onset PANDAS were followed (Murphy & Pichichero, 2002). All tested throat-positive for *S. pyogenes* , and had neuropsychiatric symptoms along with signs of tonsillo-pharyngitis with rises in antibody titers. Antibiotics were effective in both eradicating *S. pyogenes* and suppressing tics at the first episode and at recurrences.

In another prospective longitudinal study on 47 patients and 19 controls, *S. pyogenes* infection rate in children with TS and/or OCD was 0.42 per year, as compared with 0.28 per year in non-tic patients**,** but the association of symptom exacerbations and new *S. pyogenes* infections was not higher than those on the basis of chance. Therefore, the study suggested no clear relationship between exacerbations and new *S. pyogenes* infections (Luo, et al., 2004). Since school-aged children may have a higher rate of *S. pyogenes* positivity or carriage due to classroom exposures, it may be difficult to demonstrate new infection and its association with exacerbation in some studies.

Murphy et al. (Murphy, et al., 2004) investigated the relationship between *S. pyogenes* infections and symptom fluctuations in 25 children followed for 9–22 months with visits every 6.2 weeks on average for each subject. Authors reported that a part of their patients named ESC (episodic/sawtooth course) closely approximated the criteria described for PANDAS. In this study, beside ASLO and ADB titers, antibodies against the streptococcal capsular polysaccharide (ACHO) were measured. The typical pattern reported for one of these patients showed that, unlike ASLO and ADB, ACHO antibodies that target the group A streptococcus carbohydrate after a rapid increase remain elevated for a long time; moreover, a positive correlation between YGTSS and ACHO (p=0.063) or CYBOCS and ACHO (p=0.013) was found. This observation is interesting since in tic, TS, OCD or PANDAS studies, only the variation of antibody titers against protein antigens (ASLO and ADB) is usually considered, while ACHO is known from studies in SC to be a key antigen in rheumatic disease (Martins, et al., 2008; Cunningham, 2012). Moreover, it has been observed that for many patients with PANDAS, symptoms appeared only after repeated *S. pyogenes* infections.

Mell also reported this higher risk of tic development in children with frequent infections (Mell, Davis, & Owens, 2005), who noticed that for some PANDAS patients, symptoms emerged only after repeated *S. pyogenes* infections and that increases in behavioral and motor symptoms were found in children with repeated *S. pyogenes* infections (Murphy, et al., 2007); these findings suggest that a threshold of antibodies is needed to trigger symptoms. It is still unclear and unproven whether a true *S. pyogenes* clinical infection is really needed to develop symptoms, or whether repeated exposure to *S. pyogenes* antigens (maybe together with other external agents such as stress or a concomitant viral infection) may stimulate recrudescences on its own.

An accurate analysis was used to validate the PANDAS entity in a longitudinal study (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008) which followed a group of 40 PANDAS cases, matched to 40 controls with TS without any documented

association of recrudescences with *S. pyogenes* infection, for two years. Results showed that even if not statistically significant, the group of PANDAS had more exacerbations than controls (65 clinical exacerbations in total: 40 in PANDAS, 25 in the control group). Moreover, among the 43 definite or probable *S. pyogenes* infections, 31 were in 22 PANDAS cases and 12 were in 9 subjects of the control group. The number of exacerbations associated with *S. pyogenes* infections (defined as hits) was outside the 95% confidence limit for the mean number of hits, which suggests that PANDAS exacerbations are significantly associated with an antecedent *S. pyogenes* infection. On the other hand, 75% of exacerbations had no observed temporal relationship with *S. pyogenes* infection. After noting that the number of recrudescences was lower and milder than expected, the authors concluded that the vast majority of PANDAS clinical recrudescences could not be linked to *S. pyogenes* infections, and that children with PANDAS represented a subgroup of patients with TS or OCD who are susceptible to *S. pyogenes* infections as part of their initial symptoms. An interesting note is that 22.5% of the PANDAS cases (compared with 5.3% of the controls) have a family history of rheumatic fever, which might indicate a special genetic predisposition in PANDAS cases that perhaps makes them more prone to develop *S. pyogenes* infections. Similar data were also reported by other authors (Cardona & Orefici, 2001).

Following the same study design and the same protocol, Leckman et al. (Leckman, et al., 2011) did not find an increase in exacerbations in the group of PANDAS, but on the contrary, a higher number was detected in the non-PANDAS group. As in the Kurlan study (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008), the total number of recrudescences and *S. pyogenes* infections reported was lower than previously estimated, which raises suspicions that the study was underpowered; in contrast to what had been described in the definition of PANDAS, only a small number of recrudescences was associated with a sudden increase of tic /OCD severity. Again, 20 out of 31 children in the PANDAS group (in comparison to 8 of 53 in the non-PANDAS group) had a family history positive for rheumatic fever, which suggests some genetic predisposition and susceptibility to *S. pyogenes* sequelae. It should be noted that in both these latter studies, patients could continue to have their usual tic medications and that based on laboratory results, physicians were free to prescribe antibiotics. In particular, 28% of the controls (in comparison with 60% of the PANDAS group) were treated with antibiotics. This might partially account for the results achieved, and could be an unintentional indirect support for antibiotic treatment to suppress recrudescences.

In another multicenter longitudinal study on TS patients (Martino, et al., 2011), *S. pyogenes* infections ASLO titers, ADB, and anti-basal ganglia antibodies (ABGA) antibodies were compared among 168 children with TS, and 177 matched controls with epileptic or sleep disorders without tics. Seven definite (2%) and 32 possible (10%) infections were reported with a rise in ASLO titers in 26 (18%) of the subjects and in 11 (8%) of the ADB titers; 14% of patients had ABGA test persistently positive over at least 2 consecutive visits, and 20% became ABGA positive throughout the study. Nevertheless, it was not possible to correlate *S. pyogenes* infections with exacerbations, and the occurrence of a new identification of ABGA did not predict the occurrence of an exacerbation. Authors concluded that children and adolescents with TS show an increased exposure to and immune response against *S. pyogenes* and increased expression of antineuronal antibodies. This supports the view that patients with TS, independently of the PANDAS definition, may be more prone to *S. pyogenes* infections and may develop stronger immune responses against streptococcus, possibly as a result of immune dysregulation.

Other infections

S. pyogenes is not the only pathogen thought to be associated with the onset or recrudescence of tics. Other pathogens, such as intracellular microorganisms with the capacity of living and replicating inside host cells (Riedel, Straube, Schwatz, Wilske, & Müller, 1998; Müller, et al., 2004; Krause, et al., 2010), have been described as possibly being involved in these movement disorders, and particularly associated with recrudescences, but no strict observance of common parameters have been reported. Hoekstra (Hoekstra, Manson, Steenhuis, Kallenberg, & Minderaa, 2005) found a recrudescence association with the common cold, but it is difficult to evaluate this report because of the vague assessment of "common cold" and of the low isolation of *S. pyogenes* from patients.

Since the involvement of *S. pyogenes* in PANDAS has never been completely demonstrated, and there is no complete evidence of which *S. pyogenes* antigen(s) (if any) could be associated with tics, it is also impossible to define the way in which these other microorganisms are related to tics: could they be the causes, or could they collaborate with *S. pyogenes* in triggering symptoms? Are some of their antigenic determinants common with *S. pyogenes* ? Or are none of them really associated with tics? In any case, even if some reports have been published, they could support the hypothesis that the genetic background of these patients (including any immunodeficiencies) may generally make them more susceptible to certain infectious organisms and more prone to develop antibodies against microbial and brain antigens.

Plurality of antigens involved

This last hypothesis stated above seems to be supported by the results of a study (Bombaci, et al., 2009) that used a protein array to test the antibody responses of children with tics to a panel of more than 100 recombinant *S. pyogenes* antigens. These patients had chronic tic disorders, but no overt pharyngitis and no previous rheumatic diseases; their results were compared with those of healthy control children without tics and with children with microbiologically demonstrated *S. pyogenes* pharyngitis. The results showed that a group of 25 antigens were recognized by sera of all three groups; 21 antigens reacted with sera of tic and pharyngitis patients, but poorly with control sera; and 5 antigens were preferentially recognized by sera from children with chronic tics. Moreover, the overall response to the tested antigens appeared to be stronger in tic patients than in pharyngitis cases. What is most interesting is that this strong response to streptococcal antigens in the absence of clinical evidence of pharyngitis was independent of ASLO titers or an *S. pyogenes* positive throat culture. The results of the study indicated that a subgroup of tic patients show a typical profile of subjects who mount a broad, specific immune response to *S. pyogenes* antigens in the absence of clinical pharyngitis, and suggests that in genetically predisposed patients, a strong anti- *S. pyogenes* response due to a lengthy exposure to streptococcal antigens (like in long-term carriers or children with frequent pharyngitis) may produce a cumulative threshold of antibodies that are needed to produce recrudescences. The effect of other environmental factors (stress or other concomitant infections) may help in triggering the disorder in the absence of overt infection. The results show how ASLO, ADB, and the positive *S. pyogenes* throat swab may be sufficient to predict children who are at risk of developing neuropsychiatric movement or tic-like symptoms.

The high number of proteins tested in the study, and the strong preferential response by the tic sera to five of them, does not necessarily mean that these antigens are involved in tics, since only protein antigens have been examined, and because of the broad plasticity demonstrated by *S. pyogenes* in switching on or off the genes (Hertzén, et al., 2012) on the basis of the intracellular or extracellular environment. However, the stronger response in the

children with tics suggests that they responded more strongly to streptococcal antigens than did children with pharyngitis or children in the control group. This could be due to repeated streptococcal infections, as is believed to occur with rheumatic fever.

Characterization of the strains

No attempts to characterize the strains from tic patients for their possible specific virulence factors or antibiotic resistance have been made, nor have studies been attempted of M proteins (the fundamental antigens of *S. pyogenes*), which are involved in pathogenicity and used in characterization of the strains. Could M proteins play any role in the development of tics, or are specific M types more frequently found in these movement disorders? In one study (Müller, et al., 2001), Mueller detected antibodies against M1 and M 23, and in the Johnson study (Johnson, Kurlan, Leckman, & Kaplan, 2010) some M types of the strains isolated from swabs are reported, but not many studies that specifically examine the M types of the *S. pyogenes* isolated in these patients have been published.

Creti et al. (Creti, et al., 2004) examined 100 strains collected from 368 children with tics during years 1996-2001. Strains were typed by M protein agglutination and *emm* molecular typing. Sixty-seven children (18%), 53 males and 14 females had one or more throat swab test positive for *S. pyogenes* . Notably, while no problems were found with the molecular typing, 35% of the isolates resulted in being non-typable by anti M-protein sera even after repeating the typing in 3 centers (Rome, Prague, and Minneapolis), which indicates a very scarce presence of M protein on the surface.

No specific *emm* types associated with these patients were found, but 5 types, namely M12 (11.40%), M22 (11.40%), M5 (8.86%), M3 (6.32%), and M89 (6.32%) accounted for 44.3% of the strains, while M4, M2, and M1 accounted for 5.06% each. A large number of different types was found, and in some cases, a type was represented by only one or two isolates. M3 and M5 were generally associated with ASLO titers higher than 407 IU, but the numbers were too small to make a comparison to other M types. It is interesting that, even if the rank order appeared different from that of strains from pharyngitis (Dicuonzo, et al., 2001) or invasive disease (Creti, et al., 2007) isolated from the same area in the same period, the same M types presented the same “virulence and antibiotic string” (*spe* A, *spe* C, *mef* A, *erm* A, *erm* B), independent from the source of isolation (tic, pharyngitis or invasive disease). In Italy, M12 is the M type most frequently isolated from carriers, and M4 is frequently associated with scarlet fever epidemics; the M types typical for rheumatic disease, like M1, M3, M5, and M18 (Shulman, Stollerman, Beall, Dale, & Tanz, 2006), when found, were never related to previous histories of rheumatic fever.

Even when taken with caution (due to the small amount of data available), the M types found represent the serotypes present in a “normal” population with no specific M types or particular virulence factors. In this sense, the observation of the scarce typability by anti-M protein sera, the fact that different *emm* types were isolated in strains in the following years (2002–2007)—as happens in the natural selection of the strains—and that strongly mucoid strains, which are traditionally “rheumatogenic,” were never found, all support the hypothesis that the strains found were those that circulate in the normal population. On the other hand, changes in the rank of frequency can be expected when a limited number of strains are studied; this possibly reflects the normal epidemiological relationship between circulating types and the population immunological condition and dynamics (when antibodies have been raised against one serotype among the population, this type decreases its frequency). Nevertheless, this finding could be important, along with immunologic and genetic study of these patients, to determine why individuals are more prone to streptococcal infections, to improve the knowledge about these isolated strains, and to detect if there is

any specific reason that facilitates their colonization or their long-term residence in the throat.

The effect of a genetic predisposition for rheumatic disease has already been described by Bryant et al. (Bryant, et al., 2014), who investigated whether any difference in immune response detectable by gene expression can be found between individuals susceptible to ARF and those who are not. The authors found that 34 genes were significantly and differentially expressed between ARF-susceptible and ARF-resistant subjects, 7 of which were involved in immune response genes, chemotaxis, and apoptosis.

Kotb et al. (Kotb, et al., 2008), used *S. pyogenes* as a model to demonstrate in cell cultures and in animal models (including transgenic mice) how the host's genetically determined response may be modulated by environmental factors and how the response to some streptococcal superantigens may account for the different severity of the invasive disease determined. The same type of study might be useful for PANDAS and for tics in general.

The role of stress

Studies on TS/OCD report the relevance of psychosocial stress, which suggests that these disorders are sensitive to stress and show a high stress response (Corbett, Mendoza, Baym, Bunge, & Levine, 2008; Chappell, et al., 1994; Buse, Kirschbaum, Leckman, Münchau, & Roessner, 2014); these findings offer further evidence that many different factors contribute to these movement disorders. A cohort of 86 children diagnosed with TS/OCD and 41 matched controls were followed in a longitudinal study to verify if TS/OCD patients showed higher levels of psychosocial stress, as compared to the healthy population (Lin, et al., 2007). Notably, while levels of psychosocial stress were modest but were significant predictors of future tic symptom severity, current tic severity was not a significant predictor of psychosocial stress.

The same group in a longitudinal study monitored 45 cases (with 11 defined as PANDAS) and 41 healthy controls for 2 years with thrice-yearly visits and monthly telephone conversations to examine the impact of new *S. pyogenes* infections and psychosocial stress on future fluctuations of tic/OCD and the severity of depressive symptoms. PANDAS cases had higher (even if not significant) number of *S. pyogenes* infections compared to normal controls or non-PANDAS cases. Psychosocial stress and newly defined (or possible diagnosed) *S. pyogenes* infections were significant predictors of future symptom severity: newly diagnosed *S. pyogenes* infections increased by a factor of more than three, which indicates the power of psychosocial stress to predict future symptom severity. The study suggests that a minority of children with tics or OCD are sensitive to antecedent *S. pyogenes* infections, and that psychosocial stress is a potent factor associated with future worsening of tics (Lin, et al., 2010).

Clinical trial in PANDAS

Another source of information on the relationship between *S. pyogenes* and neuropsychiatric symptoms comes from clinical trials. Based on experience with acute rheumatic fever (ARF), in which secondary prophylaxis with penicillin reduced recurrences of ARF or SC by preventing *S. pyogenes* infections, some studies were conducted on patients with PANDAS.

The first one was an 8 month, double-blind, balanced cross-over study (Garvey, et al., 1999). Thirty-seven children who met the five classical criteria for PANDAS, were randomized to receive either 4 months of the active compound (twice daily oral 250 mg penicillin V) followed by 4 months of a placebo, or a placebo followed by penicillin V. Subjects were evaluated monthly for eight consecutive visits in order to assess the clinical

features (ratings of tics, obsessive compulsive symptomatology, anxiety, and depression) as well as undergo laboratory evaluation (including serum titers of antistreptolysin-O (ASLO), anti-deoxyribonuclease B (anti-DNaseB) and throat cultures). These results showed no significant difference between the two phases in the number of both streptococcal infections and symptom exacerbations. The authors reported a lack of compliance by 26 of the children, which they attributed to an overall failure to achieve the aims of the study.

Some years later, the same group conducted another double-blind, randomized controlled trial (Snider, Lougee, Slattery, Grant, & Swedo, 2005); in this study, 23 subjects with PANDAS received an antibiotic prophylaxis with penicillin or azithromycin for 12 months. In particular, subjects were randomized in a double-blind fashion to receive either penicillin V-K 250 mg two times a day, or azithromycin 250 mg capsules two times a day, on one day of the week and placebo capsules taken two times a day on the other six days. The rate of streptococcal infections and symptom exacerbations were assessed during the study year by monthly visits and laboratory evaluation (ASLO and Anti-DNase B titers) and were then compared with those of a baseline year (for which subjects/parents were asked to retroactively recall the number of clinical exacerbations and streptococcal infections; medical records were also reviewed). Results showed a significant reduction (96%) of the rate of streptococcal infections, as well as of neuropsychiatric symptoms (64%) in both groups.

The authors concluded that both penicillin and azithromycin are effective in preventing *S. pyogenes* infections, and that both penicillin and azithromycin may be effective in preventing *S. pyogenes* -triggered neuropsychiatric exacerbations in children in the PANDAS subgroup. However, they also suggest great caution when interpreting the data, due to the small number of patients and the lack of a placebo. It should be mentioned that this study received several criticisms, including from the Tourette's Syndrome Study group (Budman, et al., 2005), about many of the study's methodological aspects. Most recently, a report published by Murphy et al. suggested that a reduction of symptoms using the β-lactam antibiotic cefdinir was observed in PANS (Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a), which supports the potential usefulness of antibiotics in these diseases.

To our knowledge, apart from the above-mentioned studies and a case series report (Murphy & Pichichero, 2002), no other data have been published to establish a treatment protocol for antibiotic therapy or prophylaxis in PANDAS subjects. Caution is advised against the over-use of antibiotic treatments of PANDAS or PANS patients, as well as of subjects that show only a single exacerbation of neuropsychiatric (tics or OCD) symptoms, which has been reported in the US (Gabbay, et al., 2008) and in other countries.

Anti-Neuronal Autoantibodies in Sydenham Chorea and Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococci (PANDAS)

Sydenham chorea (SC) is well established as the neurologic manifestation of acute rheumatic fever (Taranta & Stollerman, 1956), and is characterized by antibodies found in the cytoplasm of neurons in the caudate and putamen regions of the human brain (Husby, van de Rijn, Zabriskie, Abdin, & Williams, Jr., 1976). Little was known about the antibodies and how they affected the brain until human mAbs were derived from SC (Kirvan, Swedo, Heuser, & Cunningham, 2003) and were found to react with the group A streptococcal carbohydrate epitope N-acetyl-beta-D-glucosamine and brain antigens lysoganglioside (Kirvan, Swedo, Heuser, & Cunningham, 2003) and tubulin (Kirvan, Cox, Swedo, & Cunningham, 2007). Evidence from studies of human chorea-derived mAbs strongly suggests that autoantibody crossreactivity between streptococci and brain is an important feature in Sydenham chorea (Kirvan, Swedo, Heuser, & Cunningham, 2003; Kirvan, Cox, Swedo, & Cunningham, 2007; Kirvan, Swedo, Kurahara, & Cunningham, 2006b). Human

mAbs and antibodies in sera or cerebrospinal fluid from the SC-activated calcium calmodulin dependent protein kinase II (CaMKII) in human neuronal cells (Kirvan, Swedo, Heuser, & Cunningham, 2003) and led to an increase in dopamine release from the human neuronal cell line using tritiated dopamine assays (Kirvan, Swedo, Kurahara, & Cunningham, 2006b). Further study indicated that chorea-derived mAb (24.3.1) induced tyrosine hydroxylase activity in dopaminergic neurons after the intrathecal transfer of purified human mAb 24.3.1 (Kirvan, Swedo, Heuser, & Cunningham, 2003) into a Lewis rat brain (Kirvan, Swedo, Kurahara, & Cunningham, 2006b). The removal of IgG from serum caused a loss of neuronal cell-signaling activity (Brimberg, et al., 2012; Kirvan, Swedo, Heuser, & Cunningham, 2003), and plasmaphoresis was found to improve chorea symptoms (Perlmutter, et al., 1999; Garvey, Snider, Leitman, Werden, & Swedo, 2005). Therefore, antibody-mediated neuronal cell signaling was induced by IgG antibodies in serum or cerebrospinal fluid from SC, and the presence of these signaling autoantibodies were associated with symptoms (Kirvan, Swedo, Heuser, & Cunningham, 2003; Ben-Pazi, Stoner, & Cunningham, 2013). Antibody-mediated neuronal cell signaling in SC is a novel pathogenic mechanism which is important in the movement and neuropsychiatric disorder of acute rheumatic fever (Kirvan, Swedo, Heuser, & Cunningham, 2003). SC may be a model for other movement and neuropsychiatric disorders associated with infections, such as PANDAS (Swedo, et al., 1998).

To further the studies of the antibodies in SC and related diseases, a novel transgenic mouse model expressing an SC-derived brain autoantibody was developed to gain insight into *in vivo* functional antibody targets that may be involved in the mechanisms of SC, and to test the hypothesis that autoantibodies from movement and behavioral disorders target neurons and possibly the dopamine D2 receptor (D2R) in the brain (Cox, et al., 2013). Transgenic mice expressed chorea-derived, human mAb 24.3.1, heavy and light chain variable region (V_H and V_L) genes as part of a chimeric (human V gene/mouse constant region) $IgG1^a$ antibody construct (Figure 3). Mice transgenic for mAb 24.3.1 V genes were validated by characteristic cross-reactive anti-neuronal antibody specificities in serum, and of mAbs produced from lymphocytes from spleens of transgenic mice. In our SC transgenic mouse model, chimeric 24.3.1 antibody expressed in mouse B cells and serum penetrated the brain and dopaminergic neurons in the basal ganglia of transgenic mice. Expression of the V genes of SC mAb 24.3.1 (Cox, et al., 2013) in transgenic mice demonstrated that the SC antibody V gene expression in the serum of transgenic mice targeted dopaminergic tyrosine hydroxylase positive neurons in the basal ganglia of the transgenic mice (Cox, et al., 2013), as shown in Figure 2. These results were consistent with evidence seen in human SC (Husby, van de Rijn, Zabriskie, Abdin, & Williams, Jr., 1976). In addition, human mAb 24.3.1 from SC was shown to react with and signal the human dopamine D2 receptor expressed in transfected cell lines (Cox, et al., 2013). Evidence using a flag-tagged D2 receptor, as well as signaling of the human D2 receptor in transfected cell lines, demonstrated that human mAb, as well as human SC sera IgG, targeted the dopamine D2 receptor (Cox, et al., 2013). In addition, antibodies (IgG) were also present in serum against the human D1 receptor, and further studies suggested that the ratio of the anti-D1R/D2R antibodies correlated with symptoms (Ben-Pazi, Stoner, & Cunningham, 2013). The studies also showed that anti-D1 receptor and anti-D2 receptor antibodies (IgG) were significantly elevated in serum from SC, as well as PANDAS, as described by Cox et al. (Cox, et al., 2013).

PANDAS shares similar antibodies against the dopamine receptors, as does SC (Cox, et al., 2013). The symptoms of PANDAS, as originally reported, appear as small choreiform piano-playing movements of the fingers and toes which were reported in the first 50 cases by Swedo et al. (Swedo, et al., 1998). PANDAS is characterized by tics and OCD; which, in addition to the fine choreiform movements, are not as obvious as those movements seen in

SC (Garvey, Snider, Leitman, Werden, & Swedo, 2005; Garvey & Swedo, 1997). The fine choreiform movements of lower amplitude than chorea may go unnoticed in PANDAS and can lead to poor handwriting associated with learning and behavioral regression, enuresis, separation anxiety and night-time fears, and anorexia in approximately 17 percent of cases (Swedo, et al., 1998). The appearance of PANDAS is very striking because the onset is very sudden, such as overnight behavioral changes.

For years the focus of research on SC was primarily on the chorea and involuntary movements, with little attention given to the neuropsychiatric obsessive-compulsive symptoms which predate the chorea and characterize the neurological manifestations of acute rheumatic fever (Ben-Pazi, Stoner, & Cunningham, 2013). These manifestations may be seen in other types of infections, and in these cases, is termed pediatric acute onset neuropsychiatric syndrome or PANS (Swedo, Leckman, & Rose, 2012). There have been many questions about PANDAS/PANS, which current research is attempting to answer. Clearly, the original PANDAS group has many similarities to SC, including a previous *S. pyogenes* infection; however, unlike SC, PANDAS has a male predominance (Swedo, et al., 1998; Murphy, Parker-Athill, Lewin, Storch, & Mutch, 2015a; Swedo, et al., 1989; Swedo, 1994; Swedo, Leckman, & Rose, 2012; Snider & Swedo, 2004). PANS and more chronic types of tics and OCD are not always associated with *S. pyogenes* infections. More chronic tics and OCD may not display the small choreiform piano-playing movements of the fingers and toes, and are not similar to SC in their anti-neuronal antibody patterns of antibodies against the dopamine D2 receptor (Cox, et al., 2015; Singer, et al., 2015). More chronic forms of tics and OCD do not have the IgG antibodies against the D2 receptor (Cox, et al., 2015; Singer, et al., 2015; Morris-Berry, et al., 2013). PANDAS with small choreiform piano-playing movements of the fingers and toes (Swedo, et al., 1998) share the antibodies against both D1 and D2 receptors with SC (Cox, et al., 2013; Brimberg, et al., 2012; Ben-Pazi, Stoner, & Cunningham, 2013) and also have elevated antibodies against tubulin and lysoganglioside (Cox, et al., 2013; Brimberg, et al., 2012; Kirvan, Cox, Swedo, & Cunningham, 2007; Ben-Pazi, Stoner, & Cunningham, 2013). Both tics and OCD, including the original PANDAS (Swedo, et al., 1998) and the more chronic tics and OCD are both temporally associated with *S. pyogenes* infection and have a significantly elevated abnormal CaMKII (Kirvan, Swedo, Snider, & Cunningham, 2006a; Cox, et al., 2015; Singer, et al., 2015). More studies of PANS are required to study children who have OCD and tics that are not associated with *S. pyogenes* infection.

Animal models of movement and obsessive compulsive symptoms have been studied in a mouse model and Lewis rat model, where both models show positive evidence that symptoms are associated with anti-streptococcal antibodies. Immunization of a mouse model (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004) with streptococcal components in Freund's complete adjuvant led to behavioral alterations and compulsions, and a subset of mice with antibody deposits in several brain regions, including deep cerebellar nuclei (DCN), globus pallidus, and the thalamus (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004). Group A streptococcal immunized mice with increased deposits of IgG in the deep cerebellar nuclei exhibited increased rearing behavior, as compared to controls. These data suggested that immune responses against *S. pyogenes* were associated with motoric and behavioral disturbances, and suggested anti- *S. pyogenes* antibodies that cross-react with brain components may lead to symptomatology (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004). Passive transfer of anti-streptococcal antibodies from the immunized mice into naïve mice led to autoantibody deposits in the brain, as well as behavior changes (Yaddanapudi, et al., 2010).

Another animal model of SC, and potentially PANDAS, was created in the Lewis rat (Brimberg, et al., 2012), which demonstrated that exposure to group A streptococcal

antigens during immunization led to behaviors characteristic of SC and PANDAS. After at least two immunizations, rats were not able to hold a food pellet as well as control rats, and also could not traverse a narrow beam as well as control rats (Brimberg, et al., 2012). In addition, the rats demonstrated a compulsive grooming behavior. Antibody IgG deposits were observed in the Lewis rat striatum, thalamus, and frontal cortex, and concomitant alterations in dopamine and glutamate levels in the cortex and basal ganglia were observed, which were consistent with SC and its related neuropsychiatric disorder. In the rat model, serum from group A streptococcal immunized rats activated CaMKII in SKNSH neuronal cells (Brimberg, et al., 2012) like that observed for sera from acute SC (Kirvan, Swedo, Heuser, & Cunningham, 2003). The expression of SC mAb V genes in transgenic mice demonstrated that antibody in SC most likely targets the dopamine receptors on dopaminergic neurons, since the antibody was observed in the cytoplasm of dopaminergic neurons in the basal ganglia (Cox, et al., 2013) and was found to signal the dopamine D2 receptor, as well as associate with the flag-tagged D2 receptor on transfected cells (Cox, et al., 2013). The reactivity of chorea-derived mAb 24.3.1 or SC IgG with D2R was also confirmed by the blocking of Ab reactivity by an extracellular D2R peptide (Cox, et al., 2013).

To summarize, the anti-neuronal antibodies present in SC and PANDAS with fine choreiform piano-playing movements include anti-lysoganglioside (Kirvan, Swedo, Snider, & Cunningham, 2006a), anti-tubulin (Kirvan, Cox, Swedo, & Cunningham, 2007), anti-dopamine D2 receptor (D2R) (Cox, et al., 2013; Brimberg, et al., 2012; Ben-Pazi, Stoner, & Cunningham, 2013), and anti-dopamine D1 receptor (D1R) (Ben-Pazi, Stoner, & Cunningham, 2013) antibodies. In SC, the ratio of the anti-dopamine D2 receptor / anti-dopamine D1 receptor antibodies correlated with the UFMG-Sydenham's-Chorea-Rating-Scale (USCRS) clinical rating scale of neuropsychiatric symptoms (Ben-Pazi, Stoner, & Cunningham, 2013). Most importantly, these antibodies in both SC and PANDAS signaled the SKNSH human neuronal cell line and activated calcium calmodulin-dependent protein kinase II (CaMKII) (Kirvan, Swedo, Heuser, & Cunningham, 2003; Kirvan, Swedo, Snider, & Cunningham, 2006a), which may have led to excess dopamine release (Kirvan, Swedo, Kurahara, & Cunningham, 2006b). Figure 4 shows a model diagram from a recent review (Cunningham, 2012).

In our most recent studies of tics and OCD, anti-neuronal autoantibodies were investigated as well as antibody-mediated neuronal cell signaling activity as previously reported for SC and PANDAS to determine immunological profiles for a large cohort (n=742) of children with tics and/or OCD (Cox, et al., 2015). The goal of this study was to expand upon these earlier observations and to investigate whether sera from patients with OCD, tics, or both resulted in higher CaMKII induction, as compared to healthy controls, and also to see if sera from patients with OCD, tics, or both showed elevated reactivity to previously tested neuronal antigens, tubulin and lysoganglioside, and to dopamine D1 and D2 receptors, which appeared to be targets of autoantibodies in an animal model as well as human sera IgG from PANDAS sera (Brimberg, et al., 2012). In addition, the link between streptococcal infection and OCD, tics, or both was investigated. The study focused on 311 of the 742 participants who had a history of neuropsychiatric illness with streptococcal infections or not, and tics, OCD, or both. Not all 311 subjects fell into every category studied, which resulted in only 261 of the 311 participants with confirmed tics, OCD, or both. Of the 311 individuals, 222 (71%) had evidence of a confirmed group A streptococcal infection, which was associated with tics and/or OCD status (p=0.0087) (Cox, et al., 2015). In our study, the presence of OCD and/or tics was associated with positive streptococcal infection status (p = 0.0087). It was also found that subjects who were positive for streptococcal infection were more likely to have both OCD and tics (51%), as opposed to those who were negative for

streptococcal infection (30%), while there was no significant association when tics or OCD were considered alone, relative to streptococcal infection.

Individuals with tics and/or OCD (n=261) had evidence of elevated serum IgG antibodies against human D1R ($p<0.0001$) and lysoganglioside ($p=0.0001$), and higher activation of CaMKII activity ($p<0.0001$) in a human neuronal cell line, as compared to healthy controls (n=16). Furthermore, children with tics and/or OCD had significantly increased activation of CaMKII activity, as compared to children with only tics or only OCD ($p<0.033$ for each) (Cox, et al., 2015).

Our new study also revealed two important correlations that involved CaM kinase II activation: one, the presence of OCD and/or tics was positively associated with CaM kinase II activation ($n = 261$, $p = 0.0008$); and two, CaM kinase II activation was elevated for children with OCD and/or tics (n=261), with the median percentile of CaMKII increased values ranging from 149 to 162 percentile units above the baseline enzyme activity, while CaMKII activation remained unaffected in healthy controls, with a median of 94 (equivalent to baseline CaMKII activity at approximately 100) ($n = 16$, $p < 0.0001$) (Cox, et al., 2015). The difference in the median value for CaMK II activation between patient samples and healthy controls is similar to what was found for PANDAS sera and non-PANDAS sera in previous studies (Kirvan, Swedo, Snider, & Cunningham, 2006a).

Our study showed that sera IgG from cases with OCD, tics, or both reacted more significantly with human D1 receptor antigen, as compared to healthy controls in direct ELISA ($p \leq 0.0001$) (Cox, et al., 2015). Clearly, serum IgG from our tics and OCD cohort did not react significantly above normal values with the human D2 receptor and were determined to be more chronic, since the symptoms were present for >1 year or longer in our cohort. Reactivity of the original acute onset PANDAS and SC sera IgG as tested in direct ELISA reacted more significantly with the dopamine D2 receptor antigen, as compared to healthy controls, while and PANDAS sera reacted more significantly with both the D1 and D2 receptor antigens when compared with healthy controls. Additionally, when the sera of 261 patients diagnosed with OCD, tics, or both was found to react in a direct ELISA with lysoganglioside as the antigen, sera IgG had statistically significant higher titers than healthy controls ($p = 0.0001$) (Cox, et al., 2015). The direct ELISA with tubulin as the antigen did not show a statistically significant difference between sera from tics, OCD, or both, versus healthy controls.

To summarize this study, the presence of OCD and/or tics was associated with positive streptococcal infection status ($p = 0.0087$). It was also found that subjects who tested positive for streptococcal infection were more likely to have both OCD and tics (51%) versus those who tested negative for streptococcal infection (30%), while there was no significant association when tics or OCD were considered alone, relative to streptococcal infection. As a result, it is possible that subjects that present with both OCD and tics are more likely to have had streptococcal infections. Presentations of OCD and tics alone are potentially manifestations of disorders not associated with *S. pyogenes* . The study also suggested a significant correlation of streptococcal associated tics and OCD with elevated anti-D1R and anti-lysoganglioside anti-neuronal antibodies concomitant with the higher activation of CaMKII in human neuronal cells. The statistically significant correlation between a history of chronic tics/OCD with anti-neuronal antibodies against the D1R and lysoganglioside and functional activation of CaMKII suggests that at least some pediatric neuropsychiatric disorders may be associated with autoimmunity against the brain. The functional activity of the autoantibodies which signal CaMKII in human neuronal cells suggests that antibodies could target receptors in the brain and alter dopamine neurotransmission, which could lead to neuropsychiatric symptoms.

The mechanisms and effects of anti-neuronal antibodies on the brain include alterations in dopamine transmission, including the release of excess dopamine from neuronal cells. Excess dopamine was released from the SKNSH cell line when treated with a human mAb from SC (Kirvan, Swedo, Kurahara, & Cunningham, 2006b) and human mAb from PANDAS was found to cause alterations in the sensitivity of the receptors to dopamine (Zuccolo, 2015). Evidence in animal models and humans strongly suggest that antibodies mediate inflammatory consequences in SC, PANDAS, and PANS (Brimberg, et al., 2012; Lotan, et al., 2014a; Perlmutter, et al., 1999; Lotan, Cunningham, & Joel, 2014b). There may be other brain antigens targeted by autoantibodies in PANDAS/PANS and related autoimmune diseases that may affect memory and behavior (Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004; Yaddanapudi, et al., 2010; Huerta, Kowal, DeGiorgio, Volpe, & Diamond, 2006; Kowal, et al., 2004; DeGiorgio, et al., 2001).

Finally, molecular mimicry between *S. pyogenes* and the brain is supported by evidence from studies of human mAbs and serum IgG antibodies from rheumatic fever (Kirvan, Swedo, Heuser, & Cunningham, 2003; Galvin, Hemric, Ward, & Cunningham, 2000). The investigation of human mAbs from SC has supported the hypothesis that antibodies against the *S. pyogenes* carbohydrate epitope GlcNAc (Kirvan, Swedo, Heuser, & Cunningham, 2003) recognize crossreactive structures on neuronal cells in the brain, which may lead to the onset of SC. In the brain, antibody-mediated neuronal cell signaling may be a mechanism of antibody pathogenesis in SC. The emerging theme in mimicry suggests that crossreactive autoantibodies target intracellular antigens—but for disease pathogenesis, the antibodies must target the surface of neuronal cells by affecting the signaling pathways in neurons. These mechanisms of molecular mimicry lead to the effects seen in acute rheumatic fever and related autoimmune sequelae associated with *S. pyogenes* infections.

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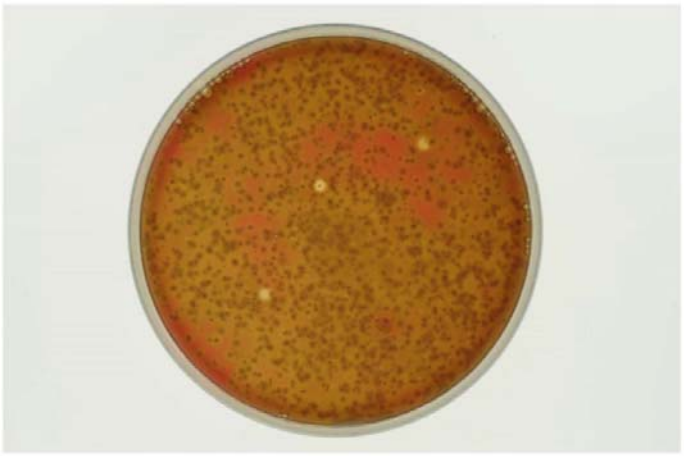
Streptococcus pyogenes

Streptococcus pyogenes

A.



B.

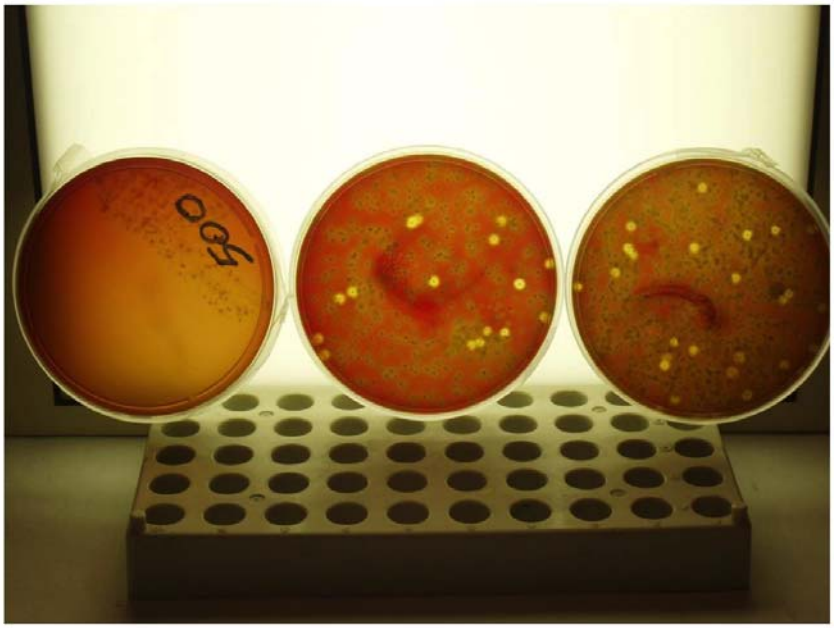


Figure 1.

A. 300 *S. pyogenes* colony-forming units (CFUs) were suspended in 5 mL Todd Hewitt broth in an 0.1mL plated (pour plate) in blood Columbia agar. The recovery was about 50%. **B.** 500 *S. pyogenes* CFUs put in 5 mL Todd Hewitt Broth. 0.1mL and 0.2 ml were pour plated in blood Columbia agar and compared to 0.1 mL directly streaked on the surface.

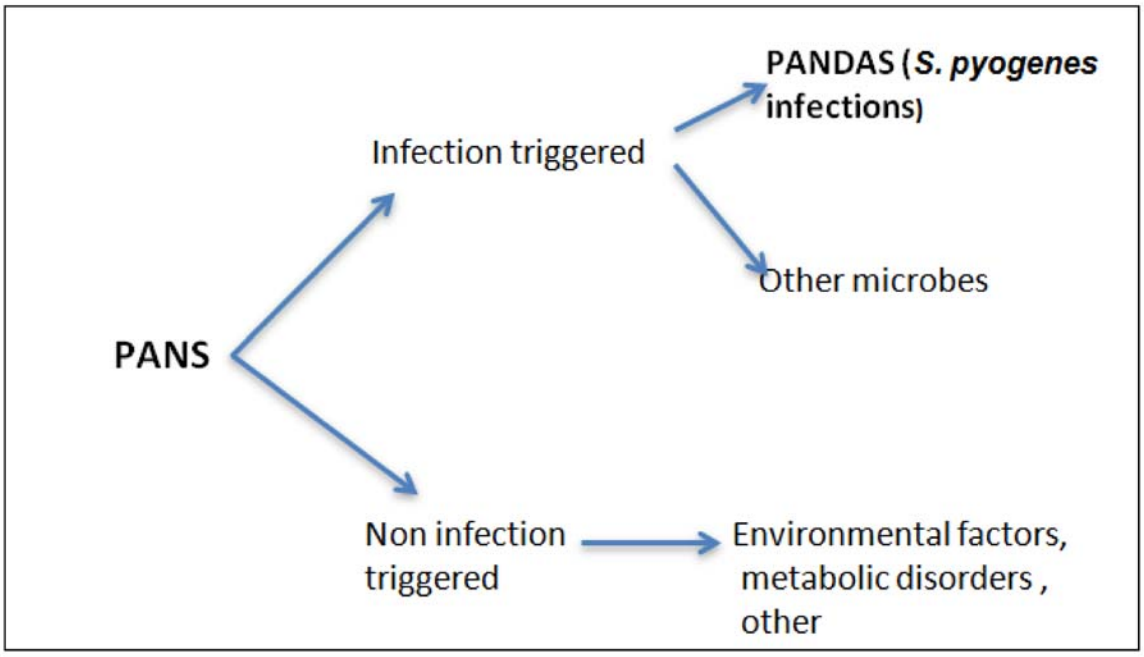


Figure 2.
Evolution criteria of PANDAS and PANS (modified from (Swedo, Leckman, & Rose, 2012)

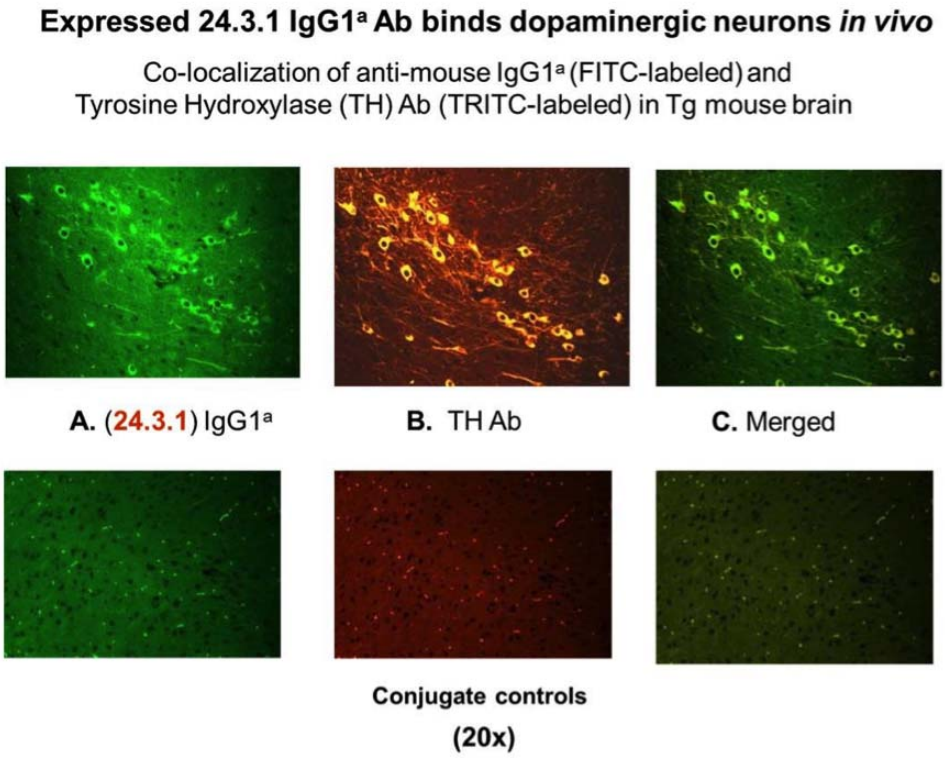


Figure 3.
Human Sydenham chorea 24.3.1 V gene expressed as a human V gene-mouse IgG1a constant region in Transgenic(Tg) mice targets dopaminergic neurons in the basal ganglia (most likely substantia nigra, based on location). Chimeric Tg24.3.1 VH

IgG1a Ab expressed in Tg mouse sera penetrated dopaminergic neurons in Tg mouse brain in vivo. Colocalization of Tg 24.3.1 IgG1a (anti-IgG1a Ab, green Left Panel) and Tyrosine Hydroxylase Antibody (anti-TH Ab, yellow Middle Panel). TH is a marker for dopaminergic neurons. Left panel shows IgG1a (FITC labeled), center panel shows TH Ab (TRITC labeled), and right panel is merged image (FITC-TRITC). Brain sections (basal ganglia) of VH24.3.1 Tg mouse (original magnification 320), showing FITC labeled anti-mouse IgG1a (A), TRITC-labeled anti-TH Ab(B), and merged image (C). Controls treated with secondary antibody are negative. Figure 3 is similar to the figure shown in Cox et al. (Cox, et al., 2013).

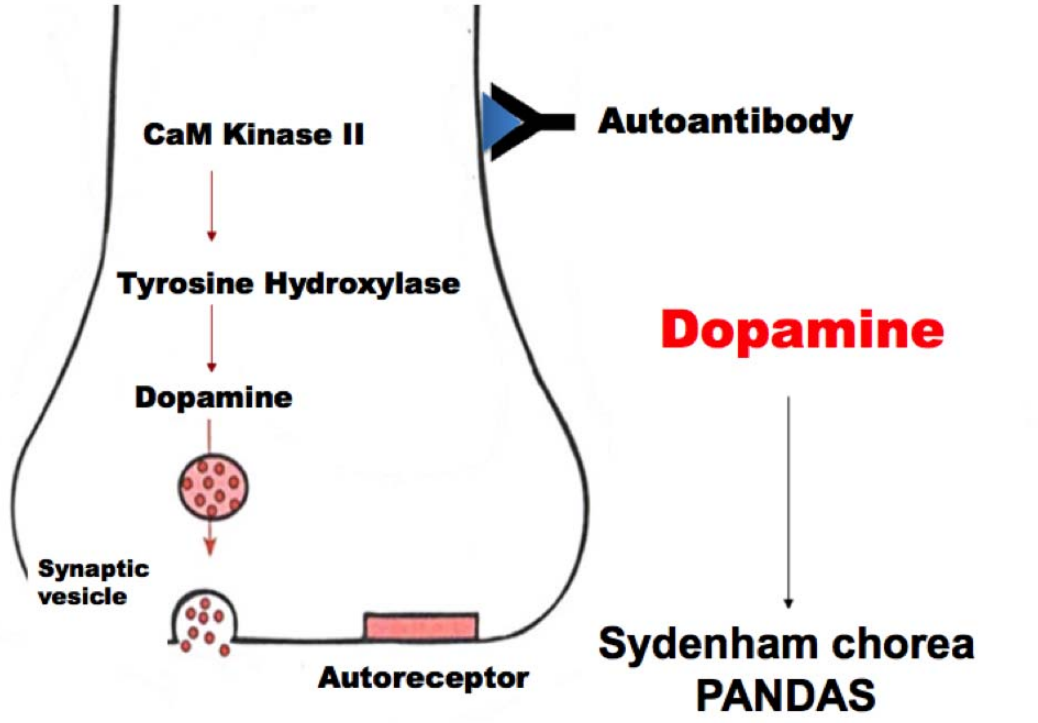


Figure 4.

Simplified illustration of a potential pathogenic mechanism of antibody mediated neuronal cell signaling in Sydenham chorea and PANDAS. Antineuronal antibody (IgG) may bind to receptors (blue triangle) on the surface of neuronal cells and trigger the signaling cascade of CaMKII, tyrosine hydroxylase, and dopamine release, which may potentially lead to excess dopamine and the manifestations of Sydenham chorea. Similar to figure shown in Cunningham 2012 review (Cunningham, 2012).

Table 1:

Historical PANDAS diagnostic criteria (from (Swedo, et al., 1998))

All five criteria must be met	
	Presence of obsessive-compulsive disorder (OCD) and/or a tic disorder
	Prepubertal symptom onset
	Acute symptom onset and episodic (relapsing remitting) course
	Temporal association between S. pyogenes infection and symptom onset /exacerbation
	Associated with neurologic abnormalities (choreiform movements, motoric hyperactivity)

Table 2:

Studies to establish the association of *S. pyogenes* and other infections or stress situation with some neuropsychiatric disorder (tics/OCD, TS)

	Pros/Conclusive	**Cons/Inconclusive**
Anti-*S. pyogenes* antibodies and neuropsychiatric behaviors in animal models of group A streptococcal immunization and passive antibody transfer	(Hoffman, Hornig, Yaddanapudi, Jabado, & Lipkin, 2004) (Yaddanapudi, et al., 2010) (Brimberg, et al., 2012) (Lotan, et al., 2014a) (Lotan, Cunningham, & Joel, 2014b)	
Retrospective studies associate *S. pyogenes* with tics and OCD	(Mell, Davis, & Owens, 2005) (Singer, Giuliano, Zimmerman, & Walkup, 2000) (Cox, et al., 2015)	(Schrag, et al., 2009)
Cross sectional studies associate *S. pyogenes* with tics and OCD	(Swedo, et al., 1998) (Cardona & Orefici, Group A streptococcal infections and tic disorders in an Italian pediatric population, 2001) (Cardona, et al., 2007) (Garvey, Giedd, & Swedo, 1998) (Macerollo & Martino, 2013)	
Longitudinal studies associate *S. pyogenes* with tics and OCD	(Murphy & Pichichero, 2002) (Murphy, et al., 2004) (Martino, et al., 2011) (Murphy, et al., 2007) (Singer, et al., 2015)	(Luo, et al., 2004) (Kurlan, Johnson, Kaplan, & Tourette Syndrome Study Group, 2008) (Leckman, et al., 2011) (Morris-Berry, et al., 2013)
Antibodies against streptococcal antigens in tics and OCD	(Morshed, et al., 2001) (Müller, et al., 2001) (Church, Dale, Lees, Giovannoni, & Robertson, 2003) (Lin, et al., 2010) (Martino, et al., 2011) (Bombaci, et al., 2009)	(Loiselle, Wendlandt, Rohde, & Singer, 2003)
Antibodies against anti-basal ganglia in *S. pyogenes* sequelae, tics and OCD	(Dale, et al., 2001) (Kirvan, Swedo, Heuser, & Cunningham, 2003) (Kirvan, Swedo, Snider, & Cunningham, 2006a) (Martino, et al., 2011) (Dale, et al., 2012) (Cox, et al., 2013)	(Loiselle, Wendlandt, Rohde, & Singer, 2003) (Morris, Pardo-Villamizar, Gause, & Singer, 2009)
Other pathogens associated with tics and OCD	(Riedel, Straube, Schwatz, Wilske, & Müller, 1998) (Müller, et al., 2004) (Hoekstra, Manson, Steenhuis, Kallenberg, & Minderaa, 2005) (Krause, et al., 2010)	
Involvement of psychosocial stress in tics and OCD	(Chappell, et al., 1994) (Lin, et al., 2007) (Corbett, Mendoza, Baym, Bunge, & Levine, 2008) (Buse, Kirschbaum, Leckman, Münchau, & Roessner, 2014)	