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Senator James Welch, Co-Chairman of the Joint Committee on Financial Services
Room 413
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Representative James Murphy, Co-Chairman of the Joint Committee on Financial Services
Room 254
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Dear Senator Welch and Representative Murphy:

On behalf of my colleagues I am forwarding an updated supplemental analysis for the 2015 CHIA report regarding An Act Relative to Insurance Coverage for PANDAS/PANS (H.920/S.613). This was requested by Adam Horgan and Lisa Pellegrini, legislative research staff members for the Joint Committee on Financial Services at the time of our discussions on PANDAS Awareness Day, October 10, 2019.

Thank you for your continued consideration.

Sincerely,

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MEDICAL EFFICACY UPDATE:
AN ACT RELATIVE TO INSURANCE COVERAGE FOR PANDAS/PANS (H.920/S.613)

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HISTORY OF THE BILL

An Act Relative to Insurance Coverage for PANDAS/PANS (H.920/S.613) provides for insurance coverage of physician-recommended therapies, including intravenous immunoglobulin therapy (IVIG), for children suffering from Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS) and Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS).[1] Passage of this bill would give physicians access to higher level treatment options for their most ill PANDAS and PANS patients. According to an actuarial assessment conducted by the Center for Health Information Analysis (CHIA) of the Commonwealth of Massachusetts in 2015, coverage would have a minimal impact on premiums.[2,3] Recent literature, published in the four years following the initial CHIA report, recommends IVIG to treat a subset of patients with PANDAS and PANS and supports its efficacy. The present update summarizes these peer-reviewed studies, reviews, and international treatment guidelines.

PANDAS AND PANS

- PANS is a collection of disorders characterized by the abrupt onset of obsessive-compulsive disorder (OCD) or eating restrictions and a variety of cognitive, behavioral, neurological and somatic symptoms and signs. In most cases, neuroinflammation has been found to be the cause of the neuropsychiatric symptomatology.

- When PANS is triggered by prolonged infection with, or exposure to group A streptococcal (GAS) bacteria, it is known as PANDAS, or in severe cases, autoimmune encephalitis of the basal ganglia. Antigens on the strep bacteria's cell wall provoke the production of antibodies which cross-react with brain tissue, leading to neuroinflammation and the complex symptoms of PANDAS.

- PANDAS and PANS are most commonly recognized by the unusually abrupt and dramatic symptom onset. In addition to the primary symptoms of OCD or eating restrictions, children may experience personality changes, extreme emotional lability, severe separation anxiety, and neurologic signs, such as sensory abnormalities, motor and/or vocal tics, and cognitive changes. Other common symptoms include behavioral (developmental) regression, physical aggression, sleep difficulties, urinary frequency/enuresis, and a number of additional debilitating symptoms such as hallucinations and delusions.

- The diagnosis of PANDAS can be challenging because inciting streptococcal infections may be subclinical ("silent") and suspected only after the abrupt onset of this dramatic clinical picture. Although elevated GAS antibody tests are classically observed with immunologic complications of streptococcal infection, it is now recognized that these tests may be falsely negative rather frequently.[4] Thus, the recognition of the clinical features of PANDAS and the response to appropriate therapy are important in the clinical management of these children despite limitations in laboratory assessment.

SUMMARY OF TREATMENT

In 2017, the PANS Research Consortium (PRC) published a guideline series in four parts with contributing experts from more than two dozen academic institutions across the United States.[5,6,7,8] Researchers and clinicians from the National Institute of Mental Health (NIMH), Harvard, Yale, Georgetown, Columbia, Stanford and other academic institutions pooled their data and clinical experience with more than 1000

PANDAS and PANS patients to develop best practice recommendations. These can be summarized as: Treat the SYMPTOMS, remove the SOURCE, and modulate the IMMUNE SYSTEM to reduce neuroinflammation.[5]

Mild to moderate cases of PANDAS and PANS are often managed successfully with antibiotic and nonsteroidal anti-inflammatory therapy. More severely afflicted children frequently require prednisone and psychotropic medications. A small but significant subset, estimated to be 10-15% of referred children, fail to improve with these conventional measures and require immunomodulatory therapy with intravenous immunoglobulin (IVIG), therapeutic plasmapheresis (also known as plasma-exchange), or other modalities.[7]

CURRENT COVERAGE

Despite support of IVIG for severe cases of PANDAS and PANS in published studies and reviews and the PANS Research Consortium consensus, few insurers have recognized these recommendations and incorporated them into their policies. Tufts added coverage of IVIG for treatment of PANDAS for their Health Freedom plans on September 10, 2019.[9] Families have also accessed treatment through MassHealth, which includes IVIG therapy in their covered services for PANDAS and PANS. In their documentation, MassHealth has designated IVIG as medically necessary when approving it for these children.

Between 2017 and 2019, Illinois, Minnesota, Arkansas, Delaware, and New Hampshire adopted legislation requiring insurance coverage for immunomodulating therapies, including IVIG, for PANDAS and PANS. Ten additional states have legislation pending or in development.

COST OF IMPLEMENTING THE BILL

Given the narrow subset of patients requiring IVIG calculated by the 2015 CHIA report, coverage of treatment would result in a slight increase in premiums for insurance holders in the Commonwealth of Massachusetts. According to the report, "requiring coverage for this benefit by fully-insured health plans would result in an average annual increase, over five years, to the typical member's monthly health insurance premiums of between $0.003 (0.001%) and $0.039 (0.008%) per year."[2] We ask for this cost to be considered in contrast to the enormous financial burden on patients with PANDAS and PANS, their families, communities, and insurers when effective treatment is delayed or unavailable.

MEDICAL EFFICACY: AN UPDATE BASED ON EMPIRICAL EVIDENCE

In the four years following the 2015 CHIA Report, consensus guidelines, systematic reviews, and IVIG treatment studies were published representing experts in psychiatry, infectious disease, general pediatrics, immunology, rheumatology, neurology, neuroimmunology, and basic science. The PANS Research Consortium immunomodulatory task force included recommendations for IVIG therapy for patients with PANDAS and PANS in their 2017 consensus guidelines.[7]

> The authors believe that PANS patients presenting with severe symptoms and a chronic-static or chronic-progressive course require consideration of more intensive immunomodulatory approaches like those used for neuropsychiatric systemic lupus erythematosus (NPSLE), central nervous system (CNS) vasculitis, autoimmune encephalitis (AE), chronic-progressive MS, chronic-progressive Behçet's disease, and other persistent neuroinflammatory disorders.[7]

These guidelines further described the rationale for immunomodulatory therapy, including IVIG, in the treatment of PANDAS and PANS, in the context of its well-described predecessor and model, Sydenham chorea (SC).[7]

> Accumulating evidence supports conceptualizing PANS as an immune-mediated brain disease, akin to SC and PANDAS, involving the caudate, putamen, and other basal ganglia structures. Data supporting this model come from epidemiological, clinical, paraclinical, translational, and basic science investigations of PANDAS and SC.[7]

Several literature reviews categorized PANDAS and PANS with pediatric neurological, neurodevelop-mental, and neurodegenerative disorders in their determination of the medical efficacy of treatment with IVIG. A 2016 review acknowledged the wide use of IVIG despite the challenges and limitations of research in children with neurological and neurodevelopmental conditions such as PANDAS, SC, autoimmune encephalitis (AE), Myasthenia gravis, and Guillain-Barré syndrome (GBS). Their systematic analysis of data from previous studies supported IVIG treatment for many disorders, including PANDAS.[10]

> We conclude that it is likely that IVIG improves recovery in selected patients with paediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (level 2). We recommend that IVIG should be considered in selected patients with a diagnosis of paediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (grade B).[10]

Another systematic literature review determined that IVIG was effective in PANDAS and other neurodegenerative conditions including SC, Tourette's syndrome (TS), Multiple Sclerosis (MS), and acute disseminated encephalomyelitis (ADEM).[11] "In the studies we analyzed, IVIG was (sic) found to be efficient in the treatment of post-streptococcal neurodegenerative disorders, even if in PANDAS, plasma-exchange (PE) showed a higher efficiency."[11]

In the American Academy of Allergy, Asthma, and Immunology's 2017 Work Group Report, the authors conducted a rigorous review of literature prior to June, 2015, that referenced immunoglobulin therapy for an exhaustive list of conditions, including primary and secondary immunodeficiency as well as autoimmune, atopic, infection-related, and neurologic diseases.[12] According to the report, IVIG is a treatment recognized for anti-inflammatory, immunomodulatory, and infection-fighting capabilities. Taking into account factors such as benefit versus risk, finite supply, and often limited research, their analysis determined whether IVIG would provide benefit for each diagnosis. Regarding PANDAS, the authors concluded, "immune-based therapies should be used only in cases in which it is clear that the neuropsychiatric symptoms are related to an autoimmune response, as supported by laboratory evidence and in conjunction with neuropsychiatric professionals."[12] Consistent with this stipulation of the appropriateness of IVIG for these disorders, the PANS Research Consortium recommends evaluating children for immunodeficiency because inflammatory and/or autoimmune diseases such as PANDAS and PANS are "more common in patients with immunodeficiency."[6] In other words, children who have immunodeficiency are more at risk for PANDAS and PANS and such testing can confirm an inflammatory or autoimmune process underlying their illness.

The American Society for Apheresis (ASFA) included PANDAS in its guidelines published in the Journal of Clinical Apheresis (JCA) in its last two editions.[13,14] Their 2019 issue provided apheresis indications, including plasmapheresis and IVIG when appropriate, for 84 different diseases based on an extensive review of the literature.[14] When PANDAS was first included in the 2013 issue, the "strong recommendation" for treatment with therapeutic plasma exchange (TPE) by the ASFA set a new precedent given the rigorous, evidence-based methodology of this body.[13] "In severely symptomatic patients with PANDAS or SC, immunomodulatory

therapies, such as IVIG…or TPE, have been shown to be effective in reducing symptom severity or shorten the course." The strong endorsement of immunomodulatory therapies for PANDAS continues in the present edition.

A 2018 review published in the official journal of the European Paediatric Neurology Society evaluated treatment for immune-mediated movement disorders and classified IVIG as a first line therapy in the treatment of PANDAS and anti-NMDA encephalitis.[15.] The authors state that, although the pathophysiological processes differ in these conditions, "there are general themes that broadly apply including: early diagnosis and treatment is better, minimise the severity of disease, escalate treatment if the patient is not responding to initial treatments, and minimise relapse."

Two papers, a treatment study and a literature review were published in quick succession in the Journal of Neurology and Neurosurgery in 2016.[16,17] The treatment study found that up to 84% of pediatric patients exhibited benefit after IVIG and maintained this response even at 12 months.[16] Children with low IgA, IgG, or IgG subclass at baseline were more likely to achieve and maintain 100% improvement at one year. The review expanded on an understanding of inflammatory autoimmune disease: "From immunodeficiency to autoimmunity, the dynamic immunologic basis of PANDAS highlights the broad potential of high-dose IVIG therapy."[17] Their literature search yielded a total of eight studies comprised of 145 children who met PANDAS criteria and received IVIG. On the basis of their systematic analysis, IVIG was deemed to be a "safe and useful adjunctive therapy in the treatment of refractory neuropsychiatric symptoms due to PANDAS and its variants."

Additional recent treatment studies have documented that the response to IVIG is beneficial and appropriate for a subset of children with PANDAS. In an Italian study, 85% of "serious-severe" children with PANDAS showed a reduction or disappearance of the symptomatology following IVIG (1-2 cycles)."[18] For all pediatric participants in a blinded randomized control trial, a 62% mean decrease on the Children's Yale–Brown Obsessive Compulsive Scale (CY-BOCS), maintained at 6 month follow-up, was indicative of improvement.[19]

A 2019 case study published in the British Medical Journal illustrates the profoundly debilitating symptoms of PANDAS in the acute phase and the significant and swift recovery possible when appropriate therapy is administered expeditiously. [20]

> A 6-year-old Indian boy residing in Bahrain was referred to us by his general practitioner (GP) after experiencing 4 days of irritability in the form of increased proneness to anger, which got worse with time, sleep disturbances, severe eating restrictions, parental separation anxiety, emotional lability, personality changes, loss of speech and intermittent eye blinking. The onset of these symptoms was abrupt.…His mother further reported that he refused to eat on the day prior to his admission and was force fed.…Additionally, he injured two of his teeth as a result of his agitated behaviour.[20]

The patient's medical workup revealed an antistreptolysin O (ASO) titer approximately five times above the normal upper limit. Following a diagnosis of PANDAS, the patient received one dose of immunoglobulin (12 hour infusion) and IV ampicillin over the course of his hospital stay. Within 48 hours, all of his symptoms resolved to normal, including behavior, activity level, appetite, and speech. Upon discharge, he was prescribed prophylactic penicillin. The authors concluded, "PANDAS can be rapidly cured with appropriate antibiotics and immunoglobulin administration."[20]

STANDARDS OF MEDICAL PRACTICE AND MEDICAL NECESSITY

Evidenced-based best practices drive the decision-making of physicians as to the efficacy of IVIG to meet the needs of a given patient, and should be the basis of insurers' decision-making when determining authorization. In formulating and adopting medical policies with respect to covered services, it is understood that insurers shall rely on "generally accepted standards of medical practice" or "standards that are based on credible scientific evidence published in peer-reviewed medical literature generally recognized by the relevant medical community or otherwise consistent with the standards set forth in policy issues involving clinical judgment."[21,22] This includes the recommendations of physician specialty societies and practicing specialists.

PANDAS Physicians Networks (PPN) in the US and UK represent physician specialty groups for practicing physicians in their respective countries. Their recommendations support a full range of treatment options for children with PANDAS and PANS, including IVIG. In particular, they underscore the favorable risk-benefit ratio for moderately severe to life-threatening cases "because the children's symptoms are causing significant impairment in daily functioning." The guidelines published by the PANS Research Consortium set standards of medical practice to support treating physicians who conclude that IVIG is medically necessary for children who are not responding to other therapies. The findings summarized in this update meet the criteria for medical necessity highlighted in the book, Essential Health Benefits: Balancing Coverage and Cost:[21,22]

> "Medically Necessary" or "Medical Necessity" shall mean health care services that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury, disease or its symptoms, and that are: a) in accordance with generally accepted standards of medical practice; b) clinically appropriate, in terms of type, frequency, extent, site and duration, and considered effective for the patient's illness, injury or disease; and c) not primarily for the convenience of the patient, physician or other health care provider, and not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.[21]

PARITY

Although symptoms of these disorders often present as primarily psychiatric, they are a manifestation of a neurological condition. PANDAS and PANS rest at the intersection of mental and physical health, yet these branches of care are not fully aligned and therefore often do not work together in an efficient manner. The stakes are high for children with psychiatric symptoms regardless of their root cause. The cost and the stigma are often crippling for families.

> Children with serious emotional disturbance are among the most fragile members of our society. … Prompt coordinated services that support a child's continuation in the home can allow even the most disabled child a reasonable chance at a happy, fulfilling life. Without such services, a child may face a stunted existence, eked out in the shadows and devoid of almost everything that gives meaning to life.[23]
>
> U.S. District Court Judge Michael A. Ponsor, Rosie D. v. Romney, January 26, 2006

For families whose children have PANDAS and PANS, the risks are no less significant and the implications of lack of access to adequate treatment are no less serious than in any other mental or physical illness. For children with PANDAS and PANS, parity between mental health coverage and medical health benefits is critical as the most effective intervention is that which not only equates but coordinates behavioral health support with medical evaluation and treatment.

CONCLUSION

The efficacy of immunomodulatory treatment has been rigorously examined since 2015. Recent evidence overwhelmingly supports inclusion of IVIG in the levels of treatment available for children with PANDAS and PANS. Based on extensive systematic reviews from several specialty areas, treatment studies, and the consensus guidelines of the PRC and PPN, IVIG is indicated for the treatment of a small but significant subset of children who meet the criteria.

The current gap in health insurance coverage is causing disruption in physicians' medical practices, as well as an undue financial burden on patients, families, schools, insurers, and communities. When children are significantly impacted or in crisis, medical, mental health, and educational services are required to meet their needs. Parents miss work to care for their children and take them to appointments; insurers pay for ambulance transport, emergency room treatment, and psychiatric hospitalization; and schools place students in special classrooms, provide one-on-one classroom aides, and send tutors to homes when children are unable to attend school. Taking these factors into account, it is not only medically necessary, but cost-effective to provide appropriate treatment to these children without delay. Further, by effectively treating PANDAS, in which psychiatric symptoms stem from an organic illness, physicians can mitigate the lasting impact of infection, inflammation, and/or immune dysfunction on the developing brain, allowing children to recover and regain function in the home, school, and community.

IVIG for the treatment of children with PANDAS and PANS is almost universally denied by commercial Massachusetts insurers. As a result, physicians cannot exercise their best and full clinical judgement in order to provide sufficient care for the most severe patients whose symptoms are extremely impairing and often life-threatening. The appeals process is not structured to include a physician who is an expert in the management of these conditions, and clinicians are frustrated by their inability to objectively discuss IVIG approval with a knowledgeable expert. We ask the insurers' Physicians Advisory Committees and Medical Policy Review Boards to review recent evidence for the efficacy of IVIG in the treatment of PANDAS and PANS, and to support access to a full range of treatment.

Today, severely ill children wait indefinitely for appropriate medical intervention due to the gap in coverage for immune therapy in Massachusetts. Now is the time to change the clinical outcomes for children. Passage of An Act Relative to Insurance Coverage for PANDAS and PANS (H.920/S.613) will ensure that a full range of treatment options is widely available.

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February 22, 2021

Dear Chair Representative Prusak, Vice-Chairs, and Members of the Committee,

I am writing to express my strongest support for Oregon's House Bill 2390 to cover the cost of treatment for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) and pediatric acute-onset neuropsychiatric syndrome (PANS), including but not limited to intravenous immunoglobulin therapy and plasmapheresis. As a basic scientist, I know that passage of the bill will significantly improve the health and well-being of patients with PANDAS/PANS and ease the financial and emotional burdens of their families.

Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) is characterized by the abrupt and dramatic onset of obsessive-compulsive symptoms, restricted intake of food or fluids (sometimes to the point of starvation or dehydration), anxiety, depression and suicidality, emotional lability, personality changes, sensory hypersensitivity, cognitive deficits and physical symptoms, such as arthralgias, urinary dysfunction, and severe insomnia. As its name implies, PANS affect children, primarily those aged 4 - 9 years. When Group A streptococcal infections (such as strep throat) triggers symptoms, the disorder is known as Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS). Recently, a number of studies have proven that PANS/PANDAS is a form of autoimmune encephalopathy—or inflammation of the brain. Treatment of PANS/PANDAS involves a three-pronged approach that utilizes psychiatric medications to provide symptomatic relief, antibiotics to eliminate the source of neuroinflammation and immune-modulating therapies to treat disturbances of the immune system. When these therapies are instituted promptly, many children recover completely and return to full functioning. Delays in obtaining treatment not only prolong the child's suffering needlessly but also increase the risk that the PANS/PANDAS symptoms will become entrenched, leading to long-term psychiatric, neurologic, and cognitive dysfunction.

Below, I outline several recent basic and clinical studies that demonstrate a very strong association of GAS with PANDAS and treatment strategies for PANDAS and PANS.

a) Basic studies in animal models of PANDAS/PANS have demonstrated that both cellular (Th17 lymphocytes) and humoral (antibodies) adaptive immunity, generated in response to multiple GAS infections, target the brain and trigger neuroinflammation, blood-brain barrier damage, neuroinflammation and neuronal dysfunction (Brimberg et al., 2012; Dileepan et al., 2016; Hoffman et al., 2004; Platt et al., 2020; Yaddanapudi et al., 2010). Moreover, Th17 lymphocytes, that are critical for pathogenesis in multiple autoimmune diseases such as Multiple Sclerosis,

Lupus, and Psoriasis, are also necessary for disease pathogenesis in rodent models for the PANDAS (Platt et al., 2020), suggesting a critical requirement for the adaptive cellular immune response in PANDAS pathogenesis in addition to the role of the humoral immune response.

b) Studies in sera of Sydenham's chorea, PANDAS and PANS have identified anti-neuronal autoantibodies targeting the basal ganglia, including the D1 and D2 dopamine receptors and recently cholinergic interneurons (Cox et al., 2013; Dale et al., 2012; Kirvan et al., 2003; Kirvan et al., 2006; Sinmaz et al., 2015; Xu et al., 2020). These antibodies induce neuronal dysfunction *in vitro* (Kirvan et al., 2003; Xu et al., 2020) and elicit behavioral abnormalities in rodents after adoptive transfer [reviewed in (Platt et al., 2017)], suggesting a critical role for the humoral immune response in the pathogenesis of these diseases. Moreover, the titer of these pathological antibodies is reduced in the sera of Sydenham's chorea, PANDAS or PANS patients during the convalescence period that corresponds with improved symptomatology (neurological and psychiatric manifestations) (Chain et al., 2020; Xu et al., 2020).

c) Recently, two large epidemiological cohort studies of children in Europe (N=1,068,000) (Orlovska et al., 2017) and Asia (N=28,600) (Wang et al., 2016) reported that children hospitalized with GAS infections had a 96% higher risk of neuropsychiatric disorders (Taiwan) (Wang et al., 2016), 51% higher risk for obsessive-compulsive disorder (OCD) and a 35% higher risk for tic disorders (Denmark) (Orlovska et al., 2017). These recent epidemiological studies together with previous findings that more than 25% of pediatric cases presenting with obsessive-compulsive disorders (OCD) and tic disorders (e.g. Tourette syndrome) originate as PANDAS (Swedo et al., 1998) strongly argue for a critical role of recurrent GAS infections in the etiology of PANDAS or PANS and that these diseases are rare similar in incidence to Lupus.

d) A recent clinical study has shown that in 41 pediatric subjects, followed for over a 24-month period, 65% of new GAS infections caused no symptoms, yet these subjects developed antibodies against GAS suggesting that **the majority of GAS infections are not detected in clinic** (Hysmith et al., 2017). This could result in missed opportunities for primary prevention of rheumatic fever and rheumatic heart disease, Sydenham's chorea or PANDAS with appropriate antimicrobial therapy.

e) The NIMH PANS consortium formed by a large number of experts from the disciplines of pediatrics, infectious disease, neurology, immunology and psychiatry have published the guidelines for treatment of PANDAS/PANS which rely on antibiotic therapy, steroids, IVIG, and psychiatric treatments (Thienemann et al., 2017; Frankovich et al., 2017: Cooperstock et al., 2017). The PANS Research Consortium has based its diagnosis and treatment guidelines on their experience of managing more than 1,000 patients in the U.S. The majority of the children are under age 13 and those who are left untreated can suffer dire consequences into young adulthood, including suicide.

f) PANDAS and PANS cases are increasingly being classified as a form of Autoimmune Encephalitis. The Mayo Clinic conducted a study in 2018 warning that more than 90,000 Autoimmune Encephalitis cases are being missed on an annual basis worldwide (Dubey et al., 2018). We contend that many PANDAS and PANS cases fall within that category as recently

discussed in detail in studies published in the American Academy of Neurology (Cellucci et al., 2020) and Lancet Psychiatry (Pollak et al., 2020). Furthermore, PANDAS and PANS are now considered as a form of basal ganglia encephalitis demanding attention and urgent care, as argued in recent editorial by esteemed physicians in Immunology, Neurology & Psychiatry of PANDAS/PANS. (Dale et al., 2017).

g) A Stage 3 Clinical Trial of IVIG will be conducted in January 2021, "A Superiority Study to Compare Panzyga Versus Placebo in Patients with PANS," Clinical Trials.gov, NCT04508530 in both Europe and USA in approximately 200 children to examine the effectiveness of IVIG in PANDAS and PANS children in a larger cohort.

Unfortunately, there are currently several barriers that delay or prevent treatment of PANS/PANDAS. At the outset, families are confronted with a paucity of physicians available to treat PANS/PANDAS. Oregon's House Bill 2390 would address this concern through providing insurance coverage for those whose severity requires it. Without such measures, many families must travel long distances to access treatment at great emotional and monetary expense. For others, the inability to travel due to financial circumstances or the severity of a child's illness postpones or precludes therapeutic interventions entirely.

Lack of insurance coverage for PANS/PANDAS further delays or, in some cases, completely prevents access to treatment. Particular difficulties are experienced with obtaining reimbursement for intravenous immunoglobulin (IVIG) and other immunotherapies. Insurers routinely deny insurance coverage, and a lengthy cycle of repeated denials and appeals frustrates both healthcare providers and families. More importantly, the denials/appeals process prolongs the patients' suffering and family trauma and increases the risk of serious neurological and psychological harm, long-term disability or even loss of life. Faced with continual denial of care, many families attempt to self-pay for the treatments, forcing them to take on heavy credit card debt, deplete retirement/college funds or sell their homes to raise funds to pay for a treatment that should be covered by insurance.

While I acknowledge that the cost of immunotherapies (particularly IVIG) is substantial, it is small in comparison with the cost of emergency interventions, in-patient psychiatric treatment, and/or pediatric hospitalizations for the complications of severe PANS/PANDAS, such as starvation/dehydration, aggressive behaviors, and self-injury or suicidality. Delayed or denied care also carries a risk of long-term care for serious neurological, emotional, and behavioral disabilities. In addition to the increased expenditures for medical care, untreated PANS/PANDAS also increase education-related costs, as children often require specialized, individualized instruction and significant accommodations for cognitive, neuropsychological, and psychological dysfunction.

In closing, I ask that you alleviate the burdens placed on families, physicians, and other community members who strive to serve the critical needs of children with PANS/PANDAS. Please enable their medical providers to make appropriate medical decisions free from administrative and time constraints posed by insurance coverage denials. I urge you to join your fellow legislators in Arkansas, Delaware, Indiana, Illinois, Minnesota, New Hampshire, Maryland, Massachusetts and require insurance coverage for PANS/PANDAS treatment. Your leadership on this important issue will help ensure children with PANS/PANDAS receive appropriate treatment, enabling them to experience all of the joys of childhood and reach their full potential.

Thank you for your time and consideration. Please feel free to contact me should you have any questions about this application.

Sincerely yours,

Dritan Agalliu Ph.D.

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PANDAS Physicians Network
117 Eastbend Court
Mooresville, NC 28117
FEIN# 46-3067699

November 20, 2020

To Whom It May Concern,

PANDAS Physicians Network publishes consensus diagnostic guidelines and treatment protocols for PANS (Pediatric Acute-onset Neuropsychiatric Syndrome) and PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections). These guidelines are developed by the PPN Diagnostic and Therapeutics Committee, Scientific Advisory Board, and clinicians with vast clinical experience in diagnosing and treating PANS/PANDAS.

PANS and PANDAS are treatable. Early intervention and treatment are keys to expediting a full recovery. Lack of treatment may lead to psychological distress for the child, school disruption, family disruption, suicidal ideation, psychiatric hospitalization, death in severe case of restrictive eating, and evolution of the disease into a chronic state.

According to PPN's therapeutic guidelines, treatment options are tied to the severity of symptoms. Treatments range from antibiotics to treat infections to anti-inflammatories and immunomodulatory therapies. Moderate and severe cases may require Intravenous immunoglobulin (IVIG) or plasmapheresis. For all PANS/PANDAS patients, Cognitive-Behavioral Therapy (CBT) with Exposure and Response Prevention (ERP) should be started as soon as the child can tolerate it. If the child is not able to engage in CBT/ERP due to the severity of symptoms, learning parent management techniques may be beneficial for the family. CBT/ERP may also help with residual OCD symptoms and managing flare-ups. A referral with a psychiatrist to help with symptom management may also be considered.

Diagnosing and treating immune-mediated brain diseases can drastically alter the course of a child's life. It is imperative to provide physicians and medical professionals with the tools and resources they need to help their patients recover. Thank you.

Sincerely,

PANDAS Physicians Network

Quick references:
PANDAS Physicians Network website: www.pandasppn.org
Seeing Your First Child with PANDAS/PANS: www.pandasppn.org/seeingyourfirstchild
PANS Research Consortium guidelines: www.pandasppn.org/jcap

Enrolled
Senate Bill 628

Sponsored by Senator LIEBER, Representative GRAYBER; Senators BONHAM, CAMPOS, DEMBROW, FREDERICK, GELSER BLOUIN, GORSEK, HANSELL, HAYDEN, JAMA, KNOPP, LINTHICUM, MANNING JR, PATTERSON, PROZANSKI, SMITH DB, SOLLMAN, THATCHER, WAGNER, WEBER, Representatives BOICE, BOWMAN, CATE, CHAICHI, CONRAD, DIEHL, EVANS, HELM, HUDSON, JAVADI, KROPF, LEVY B, MARSH, MORGAN, NGUYEN D, NOSSE, OWENS, PHAM H, RUIZ, SCHARF, SMITH G, STOUT, TRAN (Presession filed.)

CHAPTER .....

AN ACT

Relating to pediatric mental health disorders; creating new provisions; and amending ORS 750.055.

Be It Enacted by the People of the State of Oregon:

SECTION 1. Sections 2 and 3 of this 2023 Act are added to and made a part of the Insurance Code.

SECTION 2. (1) A health benefit plan, as defined in ORS 743B.005, must cover the cost of up to three monthly immunomodulatory courses of intravenous immunoglobulin therapy for the treatment of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections and pediatric acute-onset neuropsychiatric syndrome, when the following conditions have been met:

(a) Clinically appropriate trials, which may be done concurrently, of two or more less-intensive treatments were:

(A) Not effective;

(B) Not tolerated; or

(C) Did not result in sustained improvement in symptoms, as measured by a lack of clinically meaningful improvement on a validated instrument directed at the patient's primary symptom complex; and

(b) A pediatric subspecialist was consulted and the pediatric subspecialist and the patient's primary care provider recommend the treatment. For an adolescent patient, the consultation may be with an adult subspecialist.

(2) The health benefit plan may require that the patient be clinically reevaluated at three-month intervals.

(3) This section is exempt from ORS 743A.001.

SECTION 3. For billing and diagnostic purposes, the coverage described in section 2 of this 2023 Act may be coded as autoimmune encephalitis until the American Medical Association and the Centers for Medicare and Medicaid Services create and assign a specific billing and diagnostic code for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections and pediatric acute-onset neuropsychiatric syndrome.

SECTION 4. ORS 750.055, as amended by section 11, chapter 37, Oregon Laws 2022, is amended to read:

750.055. (1) The following provisions apply to health care service contractors to the extent not inconsistent with the express provisions of ORS 750.005 to 750.095:

(a) ORS 705.137, 705.138 and 705.139.

(b) ORS 731.004 to 731.150, 731.162, 731.216 to 731.362, 731.382, 731.385, 731.386, 731.390, 731.398 to 731.430, 731.428, 731.450, 731.454, 731.485, as provided in subsection (2) of this section, ORS 731.488, 731.504, 731.508, 731.509, 731.510, 731.511, 731.512, 731.574 to 731.620, 731.640 to 731.652, 731.730, 731.731, 731.735, 731.737, 731.750, 731.752, 731.804, 731.808 and 731.844 to 731.992.

(c) ORS 732.215, 732.220, 732.230, 732.245, 732.250, 732.320, 732.325 and 732.517 to 732.596, not including ORS 732.582.

(d) ORS 733.010 to 733.050, 733.080, 733.140 to 733.170, 733.210, 733.510 to 733.680 and 733.695 to 733.780.

(e) ORS 734.014 to 734.440.

(f) ORS 742.001 to 742.009, 742.013, 742.016, 742.061, 742.065, 742.150 to 742.162 and 742.518 to 742.542.

(g) ORS 743.004, 743.005, 743.007, 743.008, 743.010, 743.018, 743.020, 743.022, 743.023, **743.025,** 743.028, 743.029, 743.038, 743.040, 743.044, 743.050, 743.100 to 743.109, 743.402, 743.405, 743.406, 743.417, 743.472, 743.492, 743.495, 743.498, 743.522, 743.523, 743.524, 743.526, 743.535, 743.550, 743.650 to 743.656, 743.680 to 743.689, 743.788 and 743.790 and section 8, chapter 37, Oregon Laws 2022.

(h) ORS 743A.010, 743A.012, 743A.014, 743A.020, 743A.034, 743A.036, 743A.040, 743A.044, 743A.048, 743A.051, 743A.052, 743A.058, 743A.060, 743A.062, 743A.063, 743A.064, 743A.065, 743A.066, 743A.068, 743A.070, 743A.080, 743A.082, 743A.084, 743A.088, 743A.090, 743A.100, 743A.104, 743A.105, 743A.108, 743A.110, 743A.124, 743A.140, 743A.141, 743A.148, 743A.150, 743A.160, 743A.168, 743A.170, 743A.175, 743A.185, 743A.188, 743A.190, 743A.192, 743A.250, 743A.252 and 743A.260 and section 2, chapter 771, Oregon Laws 2013, and sections 6 and 7, chapter 37, Oregon Laws 2022**, and section 2 of this 2023 Act**.

(i) ORS [*743.025,*] 743B.001, 743B.003 to 743B.127, 743B.128, 743B.130, 743B.195, 743B.197, 743B.200, 743B.202, 743B.204, 743B.220, 743B.222, 743B.225, 743B.227, 743B.250, 743B.252, 743B.253, 743B.254, 743B.255, 743B.256, 743B.257, 743B.258, 743B.280 to 743B.285, 743B.287, 743B.300, 743B.310, 743B.320, 743B.323, 743B.330, 743B.340, 743B.341, 743B.342, 743B.343 to 743B.347, 743B.400, 743B.403, 743B.407, 743B.420, 743B.423, 743B.450, 743B.451, 743B.452, 743B.453, 743B.470, 743B.475, 743B.505, 743B.550, 743B.555, 743B.601, 743B.602 and 743B.800.

(j) The following provisions of ORS chapter 744:

(A) ORS 744.052 to 744.089, 744.091 and 744.093, relating to the regulation of insurance producers;

(B) ORS 744.602 to 744.665, relating to the regulation of insurance consultants; and

(C) ORS 744.700 to 744.740, relating to the regulation of third party administrators.

(k) ORS 746.005 to 746.140, 746.160, 746.220 to 746.370, 746.600, 746.605, 746.607, 746.608, 746.610, 746.615, 746.625, 746.635, 746.650, 746.655, 746.660, 746.668, 746.670, 746.675, 746.680 and 746.690.

(2) The following provisions of the Insurance Code apply to health care service contractors except in the case of group practice health maintenance organizations that are federally qualified pursuant to Title XIII of the Public Health Service Act:

(a) ORS 731.485, if the group practice health maintenance organization wholly owns and operates an in-house drug outlet.

(b) ORS 743A.024, unless the patient is referred by a physician, physician assistant or nurse practitioner associated with a group practice health maintenance organization.

(3) For the purposes of this section, health care service contractors are insurers.

(4) Any for-profit health care service contractor organized under the laws of any other state that is not governed by the insurance laws of the other state is subject to all requirements of ORS chapter 732.

(5)(a) A health care service contractor is a domestic insurance company for the purpose of determining whether the health care service contractor is a debtor, as defined in 11 U.S.C. 109.

(b) A health care service contractor's classification as a domestic insurance company under paragraph (a) of this subsection does not subject the health care service contractor to ORS 734.510 to 734.710.

(6) The Director of the Department of Consumer and Business Services may, after notice and hearing, adopt reasonable rules not inconsistent with this section and ORS 750.003, 750.005, 750.025 and 750.045 that are necessary for the proper administration of these provisions.

SECTION 5. ORS 750.055, as amended by section 21, chapter 771, Oregon Laws 2013, section 7, chapter 25, Oregon Laws 2014, section 82, chapter 45, Oregon Laws 2014, section 9, chapter 59, Oregon Laws 2015, section 7, chapter 100, Oregon Laws 2015, section 7, chapter 224, Oregon Laws 2015, section 11, chapter 362, Oregon Laws 2015, section 10, chapter 470, Oregon Laws 2015, section 30, chapter 515, Oregon Laws 2015, section 10, chapter 206, Oregon Laws 2017, section 6, chapter 417, Oregon Laws 2017, section 22, chapter 479, Oregon Laws 2017, section 10, chapter 7, Oregon Laws 2018, section 69, chapter 13, Oregon Laws 2019, section 38, chapter 151, Oregon Laws 2019, section 5, chapter 441, Oregon Laws 2019, section 85, chapter 97, Oregon Laws 2021, and section 12, chapter 37, Oregon Laws 2022, is amended to read:

750.055. (1) The following provisions apply to health care service contractors to the extent not inconsistent with the express provisions of ORS 750.005 to 750.095:

(a) ORS 705.137, 705.138 and 705.139.

(b) ORS 731.004 to 731.150, 731.162, 731.216 to 731.362, 731.382, 731.385, 731.386, 731.390, 731.398 to 731.430, 731.428, 731.450, 731.454, 731.485, as provided in subsection (2) of this section, ORS 731.488, 731.504, 731.508, 731.509, 731.510, 731.511, 731.512, 731.574 to 731.620, 731.640 to 731.652, 731.730, 731.731, 731.735, 731.737, 731.750, 731.752, 731.804, 731.808 and 731.844 to 731.992.

(c) ORS 732.215, 732.220, 732.230, 732.245, 732.250, 732.320, 732.325 and 732.517 to 732.596, not including ORS 732.582.

(d) ORS 733.010 to 733.050, 733.080, 733.140 to 733.170, 733.210, 733.510 to 733.680 and 733.695 to 733.780.

(e) ORS 734.014 to 734.440.

(f) ORS 742.001 to 742.009, 742.013, 742.016, 742.061, 742.065, 742.150 to 742.162 and 742.518 to 742.542.

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(h) ORS 743A.010, 743A.012, 743A.014, 743A.020, 743A.034, 743A.036, 743A.040, 743A.044, 743A.048, 743A.051, 743A.052, 743A.058, 743A.060, 743A.062, 743A.063, 743A.064, 743A.065, 743A.066, 743A.068, 743A.070, 743A.080, 743A.082, 743A.084, 743A.088, 743A.090, 743A.100, 743A.104, 743A.105, 743A.108, 743A.110, 743A.124, 743A.140, 743A.141, 743A.148, 743A.150, 743A.160, 743A.168, 743A.170, 743A.175, 743A.185, 743A.188, 743A.190, 743A.192, 743A.250, 743A.252 and 743A.260 and sections 6 and 7, chapter 37, Oregon Laws 2022**, and section 2 of this 2023 Act**.

(i) ORS [*743.025,*] 743B.001, 743B.003 to 743B.127, 743B.128, 743B.130, 743B.195, 743B.197, 743B.200, 743B.202, 743B.204, 743B.220, 743B.222, 743B.225, 743B.227, 743B.250, 743B.252, 743B.253, 743B.254, 743B.255, 743B.256, 743B.257, 743B.258, 743B.280 to 743B.285, 743B.287, 743B.300, 743B.310, 743B.320, 743B.323, 743B.330, 743B.340, 743B.341, 743B.342, 743B.343 to 743B.347, 743B.400, 743B.403, 743B.407, 743B.420, 743B.423, 743B.450, 743B.451, 743B.452, 743B.453, 743B.470, 743B.475, 743B.505, 743B.550, 743B.555, 743B.601, 743B.602 and 743B.800.

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(A) ORS 744.052 to 744.089, 744.091 and 744.093, relating to the regulation of insurance producers;

(B) ORS 744.602 to 744.665, relating to the regulation of insurance consultants; and

(C) ORS 744.700 to 744.740, relating to the regulation of third party administrators.

(k) ORS 746.005 to 746.140, 746.160, 746.220 to 746.370, 746.600, 746.605, 746.607, 746.608, 746.610, 746.615, 746.625, 746.635, 746.650, 746.655, 746.660, 746.668, 746.670, 746.675, 746.680 and 746.690.

(2) The following provisions of the Insurance Code apply to health care service contractors except in the case of group practice health maintenance organizations that are federally qualified pursuant to Title XIII of the Public Health Service Act:

(a) ORS 731.485, if the group practice health maintenance organization wholly owns and operates an in-house drug outlet.

(b) ORS 743A.024, unless the patient is referred by a physician, physician assistant or nurse practitioner associated with a group practice health maintenance organization.

(3) For the purposes of this section, health care service contractors are insurers.

(4) Any for-profit health care service contractor organized under the laws of any other state that is not governed by the insurance laws of the other state is subject to all requirements of ORS chapter 732.

(5)(a) A health care service contractor is a domestic insurance company for the purpose of determining whether the health care service contractor is a debtor, as defined in 11 U.S.C. 109.

(b) A health care service contractor's classification as a domestic insurance company under paragraph (a) of this subsection does not subject the health care service contractor to ORS 734.510 to 734.710.

(6) The Director of the Department of Consumer and Business Services may, after notice and hearing, adopt reasonable rules not inconsistent with this section and ORS 750.003, 750.005, 750.025 and 750.045 that are necessary for the proper administration of these provisions.

SECTION 6. Section 2 of this 2023 Act and the amendments to ORS 750.055 by sections 4 and 5 of this 2023 Act apply to health benefit plans and health care service contracts issued, renewed or extended on or after the effective date of this 2023 Act.

SECTION 7. Section 3 of this 2023 Act is repealed on January 2, 2028.

Passed by Senate March 14, 2023

.....
Lori L. Brocker, Secretary of Senate

.....
Rob Wagner, President of Senate

Passed by House May 9, 2023

.....
Dan Rayfield, Speaker of House

Received by Governor:

.....M.,....., 2023

Approved:

.....M.,....., 2023

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Tina Kotek, Governor

Filed in Office of Secretary of State:

.....M.,....., 2023

.....
Secretary of State

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Evaluation of Intravenous Immunoglobulin in Pediatric Acute-Onset Neuropsychiatric Syndrome

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Abstract

Objectives: Pediatric acute-onset neuropsychiatric syndrome (PANS) is a clinical diagnosis in children who have an acute manifestation of varied neuropsychiatric symptoms, including obsessive compulsive disorder, eating disorders, tics, anxiety, irritability, and problems with attention/concentration. PANS may develop as a result of a postinfectious syndrome and may represent a new form of postinfectious autoimmunity. To test the hypothesis that multiple, consecutive infusions of intravenous immunoglobulin (IVIG) for PANS can be efficacious, a multisite, open-label study was designed.

Methods: The primary endpoint was evaluation of the efficacy of IVIG [Octagam 5%] in PANS over a period of 6 months (six infusions) based on mean changes in psychological evaluation scores using 6 different assessments, including the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS), Clinical Global Impression of Severity, and the Parent-Rated Pediatric Acute Neuropsychiatric Symptom Scale (PANS Scale).

Results: The final cohort consisted of 21 subjects (7 per site) with moderate to severe PANS. The mean age was 10.86 years (range: 4–16 years). Results demonstrated statistically significant reductions in symptoms from baseline to end of treatment in all six assessments measured. CY-BOCS results demonstrated statistically significant reductions in obsessive compulsive symptoms ($p < 0.0001$), resulting in >50% improvement sustained for at least 8 weeks after the final infusion and up to 46 weeks in a subset of subjects.

Conclusions: In PANS, which may be associated with an underlying immune dysregulation, sequential infusions of IVIG [Octagam 5%] successfully ameliorated psychological symptoms and dysfunction, with sustained benefits for at least 8 weeks, and up to 46 weeks in a subset of subjects. In addition, baseline immune and autoimmune profiles demonstrated significant elevations in a majority of subjects, which requires further evaluation, characterization, and study to clarify the potential immune dysfunction by which PANS manifests and progresses.

Keywords: IVIG, PANS, PANDAS, Octagam

Introduction

Rapid onset of obsessive compulsive disorder (OCD) and/or tic disorder in children following streptococcal infections was initially explored at the National Institutes of Mental Health (NIMH) in the late 1990s. The researchers who initially reported this syndrome used the terminology, ''pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections,'' or PANDAS, to describe the disorder (Swedo et al. 1998). The criteria established by the NIMH for the diagnosis of PANDAS included ''(1) the presence of OCD and/or a tic disorder; (2) 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 Sydenham's chorea (SC)'' (Swedo et al. 1998).

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As a result of the inherent difficulties in defining the inciting incident/infection associated with PANDAS in a pediatric population, as well as a lack of precise testing modalities and biological markers, the diagnostic criteria were revised. A broader definition of this clinical entity was proposed. The preferred terminology is now, ''pediatric acute-onset neuropsychiatric syndrome,'' or PANS, in which the key clinical features include, ''acute and dramatic symptom onset of OCD and/or severely restrictive food intake with at least two coinciding abrupt onset, equally debilitating symptoms (anxiety; dysregulation; irritability, aggression, oppositionality; behavioral regression; cognitive deterioration; sensory or motor abnormalities; somatic symptoms)'' (Swedo et al. 2012). Based on these new criteria, PANDAS is now considered a subgroup of PANS.

The first PANS Consensus Conference was assembled at Stanford University in 2013 with a group of clinicians and researchers from several different geographic areas and specialties, including general and developmental pediatrics, infectious diseases, immunology, rheumatology, neurology, and child psychiatry. Because the diagnostic boundaries of PANS were ambiguous and somewhat debatable, the goal of the meeting was to develop key clinical and behavioral criteria. The result of this important conference was a Consensus Statement proposing recommendations for the diagnostic evaluation of youth presenting with PANS (Chang et al. 2015).

Guidelines for treating PANS/PANDAS were published as a three-part series of articles published in 2017 (Cooperstock et al. 2017; Frankovich et al. 2017; Thienemann et al. 2017) by the PANS Research Consortium (PRC). Current treatment modalities for PANS include psychiatric and behavioral interventions as well as the use of nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotic therapy, corticosteroids, plasmapheresis, and intravenous immunoglobulin (IVIG). As per the guidelines, for moderate to severe PANS, oral or intravenous corticosteroids may be sufficient, however, IVIG is often the preferred treatment for these patients by most PRC members (Frankovich et al. 2017).

An increasing body of clinical, preclinical, and basic science research data support conceptualizing PANS and PANDAS as immune-mediated neurological disorders, similar to SC, and suggest that immune dysfunction may contribute to disease manifestation and progression (Hornig 2013; Hornig and Lipkin 2013; Frankovich et al. 2015; Murphy et al. 2015; Cutforth et al. 2016). The hypothesis is that PANS may represent a new form of post-infectious autoimmunity, through molecular mimicry, suggesting a potential mechanism by which the disorder evolves. Therefore, a multisite study was proposed to explore the efficacy of multiple, consecutive infusions of IVIG for PANS treatment.

Methods

Participants and study design

This open label study was conducted at three clinical/research sites in the United States: IMMUNOe Research Center (Centennial, CO); Midlands Pediatrics (Papillion, NE); and Allergy, Asthma & Immunology Relief Research Institute (Charlotte, NC). A central Institutional Review Board approved the study (IntegReview). Participants were recruited from direct referrals from clinicians as well as ClinicalTrials.gov (NCT03348618). The parents of participants provided informed consent, and study participants provided assent, when appropriate.

To be eligible for the study, participants between 4 and 16 years of age were required to have a diagnosis of moderate to severe PANS based on accepted criteria (Swedo et al. 2012) as validated by the Pediatric Acute Neuropsychiatric Symptom Scale (PANS Scale), Parent Version conducted during a prescreening phone call (for additional information, see Behavioral Assessments section) (PANS Scale 2012) (Supplementary Appendix S1). It is also important to note that all patients presented with symptoms that were not controlled using standard PANS therapy (e.g., antibiotics, selective serotonin reuptake inhibitors [SSRIs], corticosteroids, medications for attention-deficit/hyperactivity disorder [ADHD] such as methylphenidate, and so on). Therefore, according to published treatment recommendations, they required more aggressive immunomodulatory interventions (e.g., IVIG) (Frankovich et al. 2017). Antecedent therapies following date of onset of PANS symptoms (before enrollment), as well as PANS triggers, were reported during initial intake.

Participants who were using prophylactic antibiotics were required to be on a stable dose for ≥3 months. In addition, potential participants were excluded if they had a history of rheumatic fever, including SC (with neurologic manifestations), previous IVIG therapy within 6 months before screening, and/or use of corticosteroids within 6 weeks before screening. If potential participants had been prescribed antibiotics for an acute infection, a washout period of 7 days following completion of dose was required.

Behavioral assessments

For the primary outcome measures, licensed independent (from the clinician's study center) psychologists administered validated psychometric scales, including the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS), Clinical Global Impression of Severity (CGI-S), Yale Global Tic Severity Scale (YGTSS), and the Anxiety Disorders Interview Schedule for DSM-IV, Child/Parent versions (ADIS). In addition to these assessments, two parent-rated questionnaires were utilized during the study. The PANS Scale, Parent Version (PANS Scale 2012) (Supplementary Appendix S1) was administered as a prescreening measure for validation of the PANS diagnosis and to provide a baseline measurement of disease severity. Subsequent evaluations of the PANS Scale, following IVIG treatment, were also utilized to assess efficacy.

In addition to the PANS Scale, the Parent-Rated PANS Questionnaire (PRPQ) was developed specifically for this study and completed by parents at every treatment visit (Supplementary Appendix S2). This questionnaire takes 10–20 minutes to complete and contains 58 items selected as key symptoms of interest for data analysis per the most important PANS characteristics reported in the literature (Bernstein et al. 2010; Swedo et al. 2012).

Exploratory assessments

Exploratory outcome measures included evaluation of key neuroimmune panels (Cunningham Panel [Moleculera Labs, Oklahoma City, OK], Neural Zoomer [Vibrant Wellness, San Carlos, CA]), as well as immune, infectious, and atopic laboratory panels. The Cunningham Panel includes five assays, including immunoglobulin G (IgG) levels by enzyme-linked immunosorbent assays directed against (1) dopamine D1 receptor (D1R), (2) dopamine D2L receptor (D2LR), (3) lysoganglioside-GM1, and (4) tubulin. A fifth assay is a cell stimulation assay which measures the ability of a patient's serum IgG to stimulate calcium/calmodulin-dependent protein kinase II (CaMKII) activity in human neuronal cells (Shimasaki et al. 2020). The Neural Zoomer panel evaluates 16 neurological autoantibodies, including, among others, antitubulin IgM/IgG+IgA, antimyelin basic protein IgM/IgG+IgA, antineuron-specific enolase IgM/IgG+IgA, and anti-GM1/GM2 IgM/IgG+IgA (Vibrant Wellness 2020).

Based on the work by Swedo et al. (2012), motor abnormalities occurring in PANS include a variety of signs and symptoms. Dysgraphia and fine motor skills may abruptly deteriorate following onset of symptoms. Therefore, obtaining a drawing sample during the acute phase, and during an asymptomatic period, is a relatively simple way to document motor changes. For these reasons, optional drawing/writing samples were collected from participants as an additional measure of assessment both before and following treatment.

Safety assessments

All subjects were given a patient diary and asked to catalog all adverse events (AEs). In addition, a follow-up phone call 72 hours postinfusion by a research coordinator was also implemented to gather AEs. The parents were instructed to record the following data in the diary: any suspected AEs, temperature (using same method for every time), infections (serious acute bacterial infections had to be validated), physician/emergency room visits, hospitalizations (overnight stays), school/work days missed because of infections or illness, and concomitant medications, especially antibiotics. The diary was reviewed, and AEs were monitored, at every treatment visit following the first IVIG infusion.

Visit schedule and procedures

The study consisted of a prescreening phone call, followed by 10 visits. During the prescreening phone call, the PANS Scale (2012) (Supplementary Appendix S1) was administered to assess disease severity. If the potential participant met the criteria of moderate to severe PANS, a subsequent on-site screening/baseline visit (Visit 0) was scheduled and included both the potential participant and parent(s). At Visit 0, medical history and concomitant/antecedent medications were assessed, baseline psychometric evaluations were conducted (CY-BOCS, CGI-S, YGTSS, ADIS), and blood was drawn for initial panel, biomarker, and safety assessments. In addition, optional pretreatment writing and/or drawing samples were gathered from participants and parents. Four weeks later, eligible participants received IVIG infusions every 21 days (± 3 days) for a total of 6 infusions over a period of 18 weeks (Visits 1–6). In addition to IVIG infusions, AEs (including review of diaries) and concomitant medications were assessed, and parents completed the PRPQ, at each treatment visit.

Follow-up included a visit ∼1 week after the final infusion (Visit 7) and a visit 7 weeks after the final infusion (Visit 8), the latter of which was considered the end of study (EOS) visit. At Visits 7 and 8, all psychometric evaluations (CY-BOCS, CGI-S, YGTSS, and ADIS) and the PANS Scale were administered. In addition, blood was drawn for posttreatment evaluation of all panel, biomarker, and safety assessments.

A late study visit (up to 46 weeks following the final infusion) was added to the study design to gather additional psychometric evaluations (CY-BOCS, CGI-S, YGTSS, and ADIS) in a subset of available participants (Visit 9) to assess durability of response.

Study drug and dosage/administration

IVIG has been used to treat primary and secondary immunodeficiencies at replacement doses of 0.2–0.6 g/kg body weight every 3–4 weeks and enhances immune homeostasis by modulating expression and function of Fc receptors, interfering with activation of complement and production of cytokines, providing anti-idiotypic antibodies, and affecting the activation and effector functions of T and B cells (Cunningham-Rundles et al. 1984; Perez et al. 2017; Melamed et al. 2019). In higher doses of 1–2 g/kg body weight, IVIG has been shown to induce immune modulation and suppress systemic inflammation, and has long been used in the treatment of autoimmune and inflammatory conditions (Dwyer 1992; Nimmerjahn and Ravetch 2007; Ballow 2014; Joao et al. 2018).

The design of the study included on-site administration of IVIG [Octagam 5%] at a dosage of 1 g/kg of body weight every 21 days (±3 days) for a total of six infusions (cycles) over a period of 18 weeks. While a dose of 2 g/kg of IVIG has been used for immunomodulation in adults, we have found that it is a very large dose for pediatric patients and requires administration over 2–4 days. A dose of 1 g/kg can be administered in 1–2 days in the majority of pediatric patients, which is much more manageable in this population (Melamed et al. 2018). In our clinical experience before initiation of this study, we also found a dose of 1 g/kg to be effective in reducing/eliminating symptoms in PANS patients. In a previous study in pediatric patients with autism spectrum disorder (ASD), a dose of 1 g/kg of a 5% IVIG was well tolerated and significant improvements in behavioral and cognitive assessments were demonstrated (Melamed et al. 2018). A dose of 1 g/kg has also been shown to be effective in pediatric patients with immune thrombocytopenic purpura (Warrier et al. 1997).

The IVIG study drug [Octagam 5%] was specifically chosen based on our positive clinical experiences of tolerability in pediatric patients (Melamed et al. 2018). Although higher percentage concentrations are available, we prefer 5% (vs. 10%), again, due to our perception of improved clinical tolerability in this population. The study drug was provided in bottles from the manufacturer [Octapharma], and was labeled and stored appropriately for investigational use. The study drug was administered intravenously directly from the bottle by a health care provider according to the labeled infusion rates (which should not exceed 3.33 mg/kg/min [200 mg/(kg·h)]. Vital signs were monitored throughout each infusion.

It is important to note that the number of sequential IVIG infusion cycles (×6) evaluated in this study is a unique treatment model that, to the best of our knowledge, has not been utilized in any previously reported assessment of IVIG treatment efficacy in the PANS population.

Statistical analysis

Unadjusted descriptive statistics were conducted to summarize the endpoints for eligible participants to detect the mean, standard deviation for continuous variables, and percentages for categorical variables. In adjusted descriptive statistics, outliers present in data sets will often be removed to determine the adjusted mean because they can have a large impact on the calculated means of small populations. To maintain the integrity of the data, we did not adjust the statistics in this manner to correct statistical averages to compensate for data imbalances and variances. Differences between subjects were tested using Student's t test for continuous variables and Fisher's exact tests were used for categorical variables. Analyses were conducted using SAS 9.4 software (SAS Institute, Cary, NC). A two-sided p-value <0.05 was considered statistically significant.

Results

Study population

A total of 26 patients were screened and 21 patients met the criteria for participation in the study (7 subjects at each site) (Table 1). The five screened patients who were unable to participate

Table 1. Sociodemographic and Baseline Clinical Characteristics

Characteristic	n *(%)*	*Mean ± SD*
Age	21 (100)	10.86 ± 2.88
Sex		
Male	13 (62)	
Female	8 (38)	
Race		
White	19 (90)	
Asian	1 (5.0)	
Asian/White	1 (5.0)	
Weight (kg)	20 (95)	43.83 ± 21.18
PANS with streptococcal relationship	14 (67)	
Years of PANS symptoms (before enrollment)		4.3 ± 2.2
PANS Scale, OCD Symptom Score (0–25)	19 (90)	21.32 ± 5.22
CY-BOCS total (0–40)	21 (100)	22.10 ± 7.82
CGI-S		4.67 ± 0.84
Moderate (4)	10 (48)	
Marked (5)	6 (28)	
Severe (6)	5 (24)	
CaMKII		
Serum	21 (100)	130.85 ± 25.01
Elevated (>130)	7 (33)	
Antitubulin antibodies		
Serum	21 (100)	1880.95 ± 1252.66
Elevated (≥1000)	20 (95)	

CaMKII, calcium/calmodulin-dependent protein kinase II; CGI-S, Clinical Global Impressions of Severity; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; OCD, obsessive compulsive disorder; PANS Scale, Pediatric Acute Neuropsychiatric Symptoms Scale; SD, standard deviation.

had scheduling conflicts related to IVIG infusion dates, decided that they did not want to participate, or did not meet inclusion criteria for severity. The enrolled subjects included 13 males (62%) and 8 females (38%). The majority of subjects were white with a mean age of 10.86 ± 2.88 and weight of 43.83 kg ±21.88. The onset of PANS symptoms was obtained for all subjects enrolled in the study. The mean number of years of PANS symptoms before enrollment was 4.3 ± 2.2 (range of 3–9 years). There were 14 subjects (67%) who had a streptococcal infection associated with their initial onset of PANS symptoms. In the remaining subjects, PANS was associated with sinusitis and/or respiratory infection ($n = 3$ [14%]) or the etiology was unknown ($n = 4$ [19%]). Antecedent medications for PANS were also recorded at enrollment. All subjects ($n = 21$ [100%]) had received antibiotics. There were three subjects (14%) who had received corticosteroids, three subjects (14%) who had received SSRIs, and three subjects (14%) who had received ADHD medications (methylphenidate, guanfacine). In addition, one subject had previously received two infusions of IVIG ~6 years before study start. Several subjects ($n = 12$ [57%]) had also received NSAIDs and/or antihistamines as needed for symptom management. However, because these medications are available over-the counter, it is difficult to accurately quantify use before enrollment.

As expected, the mean PANS Scale OCD Symptom Score at baseline was high at 21.32 ± 5.22 (scoring system of 0–25). As per the CGI-S, 10 (48%) of participants presented with moderate PANS symptoms, 6 (28%) with marked symptoms, and 5 (24%) were considered severe. Again, it should be noted that mean baseline serum measurements of CaMKII and antitubulin antibodies were both elevated.

Primary efficacy endpoints

The primary efficacy endpoints were validated psychometric assessments (CY-BOCS, CGI-S, YGTSS, and ADIS) and parent observations (PANS Scale, PRPQ). Statistically significant improvements were demonstrated in all psychometric assessments and parent questionnaires from baseline to end of treatment and in early/late follow-up visits (Figs. 1–4 and Tables 2 and 3). In a subset of subjects ($n = 12$) who participated in a late follow-up visit (29–46 weeks following the final infusion), results indicate that tics returned, although they were still below baseline levels (Fig. 3 and Table 2). One of the most important assessments was the PRPQ, in that it demonstrates the efficacy of IVIG following each infusion (Fig. 4 and Table 3). As the other primary efficacy assessments were only performed at baseline, the final infusion (Visit 7), and early/late follow-up visits (Visits 8 and 9), the PRPQ is the only assessment that provides interim efficacy data. Statistically significant reductions in symptoms were noted by the third IVIG infusion per the PRPQ assessment data (Fig. 4 and Table 3).

Exploratory endpoints

Biomarker evaluations. Several baseline immune, atopic, and infectious laboratory variables as well as neuroimmune panels (Cunningham Panel, Neural Zoomer) were explored as possible predictors or moderators of response. Of these, only CaMKII elevation ($n = 7$) (Table 1) was found to be potentially related to response based on CY-BOCS total scores at EOS. While there was a minor difference in mean CY-BOCS total score between the two groups (elevated CaMKII CY-BOCS score: 10.5 ± 10.7; normal CaMKII CY-BOCS score: 7.4 ± 8.4), the difference did not reach statistical significance.

Drawing/writing samples. A dramatic example of the potent effects of IVIG in this patient population is demonstrated in drawing samples of the PANS subjects before and after the administration of IVIG (Figs. 5 and 6). As can be seen in the examples, drawing skills/perspective and mood/outlook may abruptly deteriorate following onset of symptoms with resolution following immunomodulatory treatment.

Adverse events

AEs that were considered related to the IVIG infusions included three severe headaches. One patient required sumatriptan, and no medication was required for resolution in the other two subjects. Following resolution, there were no further complications.

The remaining related AEs were rated as mild or moderate and included headache, ($n = 10$ [48%]), nausea/vomiting ($n = 3$ [14%]), and rash ($n = 3$[14%]). All mild and moderate AEs resolved without further complication. No serious AEs occurred during the study.

Discussion

To the best of our knowledge, this is the first study to assess a total of six infusions for the treatment of PANS. The results of this prospective, open-label, proof-of-concept study substantiate earlier randomized, controlled clinical trials of the benefits of IVIG in controlling PANS symptoms (Perlmutter et al. 1999; Williams et al.

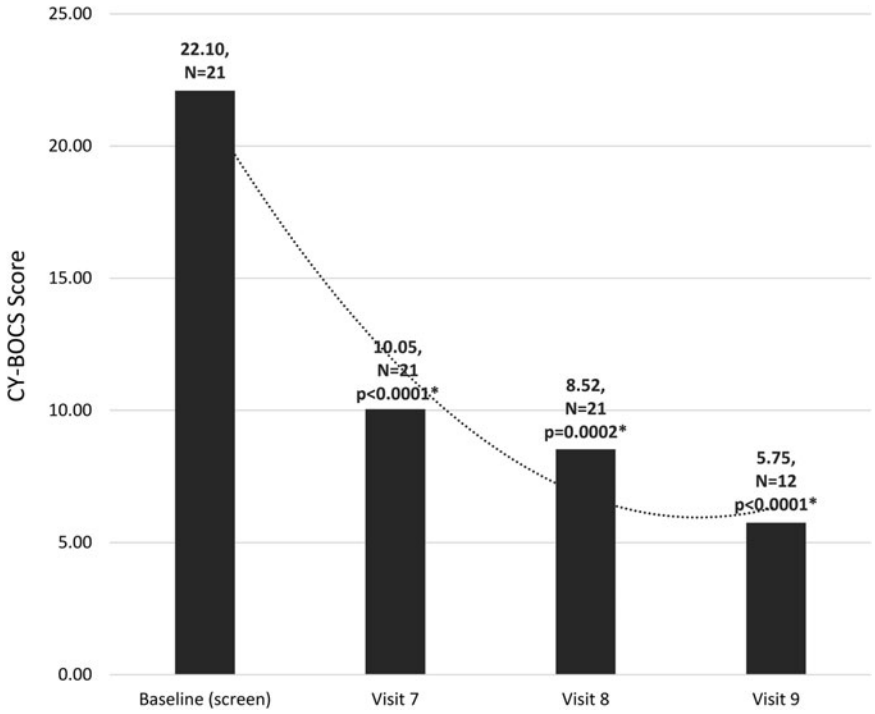

FIG. 1. Unadjusted mean CY-BOCS total scores (*$p < 0.05$ was considered statistically significant). Note that in a subset of subjects ($n = 12$) who participated in a late follow-up visit (29–46 weeks following the final infusion), results continued to improve compared to baseline. The timing of evaluations is as follows: Visit 7 (19 weeks after baseline), Visit 8 (26 weeks after baseline), Visit 9 (29–46 weeks after Visit 8/final infusion and 55–72 weeks after baseline). CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale.

2016), however, the extended dosing strategy in this study demonstrated durability of effects up to 46 weeks following the final infusion. It is notable that per the interim measurements provided by the PRPQ, statistically significant drops in symptom scores did not occur until third infusion (Fig. 4 and Table 3). The dosing strategy in earlier randomized, controlled studies was 1 g/kg administered over 2 consecutive days (2 g/kg total) (Perlmutter et al. 1999; Williams et al. 2016). In this study, we utilized a total dose of 1 g/kg every 3 weeks for a total of six infusions. While a dose of 2 g/kg of IVIG is routinely used for immunomodulation in adults, it is a very large dose in the pediatric population and must be administered over 2–4 days. A dose of 1 g/kg can be administered in 1–2 days in the majority of pediatric patients, which is much more manageable in this population (Melamed et al. 2018). In a previous

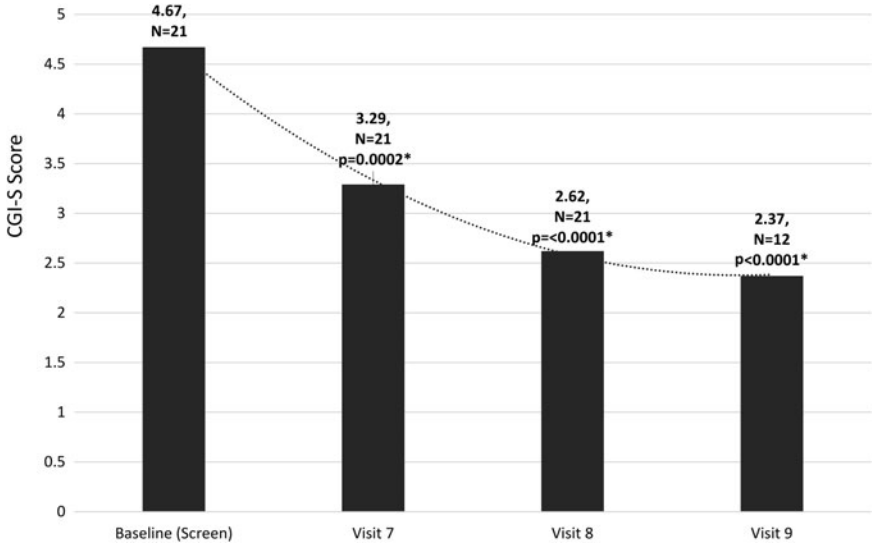

FIG. 2. Unadjusted mean CGI-S scores (*$p < 0.05$ was considered statistically significant). Note that in a subset of subjects ($n = 12$) who participated in a late follow-up visit (29–46 weeks following the final infusion), results continued to improve compared to baseline. The timing of evaluations is as follows: Visit 7 (19 weeks after baseline), Visit 8 (26 weeks after baseline), Visit 9 (29–46 weeks after Visit 8/final infusion and 55–72 weeks after baseline). CGI-S, Clinical Global Impression of Severity.

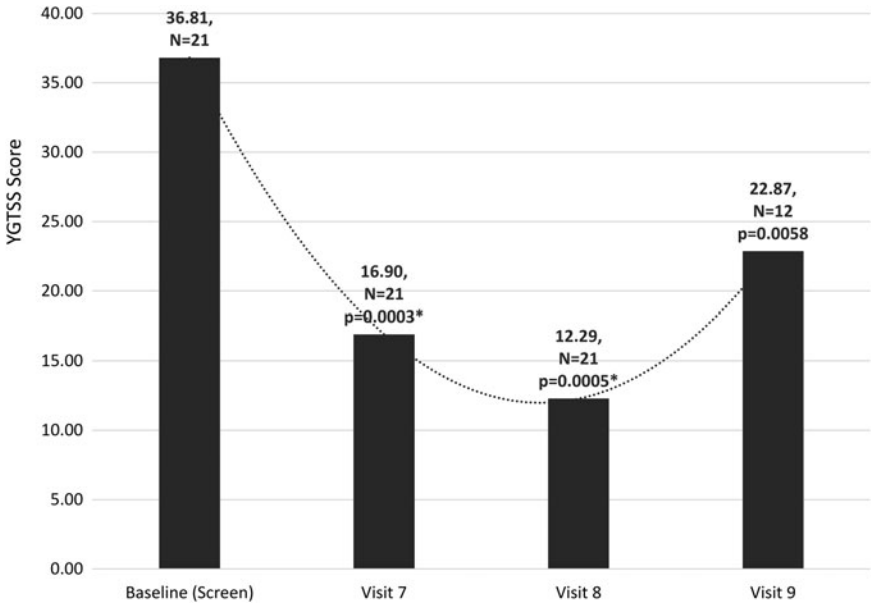

FIG. 3. Unadjusted mean YGTSS scores (*$p < 0.05$ was considered statistically significant). Note that in a subset of subjects ($n = 12$) who participated in a late follow-up visit (29–46 weeks following the final infusion), results indicate that tics returned, although they were still below baseline levels. The timing of evaluations is as follows: Visit 7 (19 weeks after baseline), Visit 8 (26 weeks after baseline), and Visit 9 (29–46 weeks after Visit 8/final infusion and 55–72 weeks after baseline). YGTSS, Yale Global Tic Severity Scale.

study in pediatric patients with ASD conducted by the lead author, a dose of 1 g/kg of a 5% IVIG was well tolerated. In addition, significant improvements in behavioral and cognitive assessments were demonstrated (Melamed et al. 2018). A total dose of 1 g/kg of IVIG has also been shown to be effective in pediatric patients with immune thrombocytopenic purpura (Warrier et al. 1997). The study drug [Octagam 5%] was specifically chosen based on our positive clinical experiences of tolerability in pediatric patients, which was also demonstrated in this study and the previous study in ASD (Melamed et al. 2018).

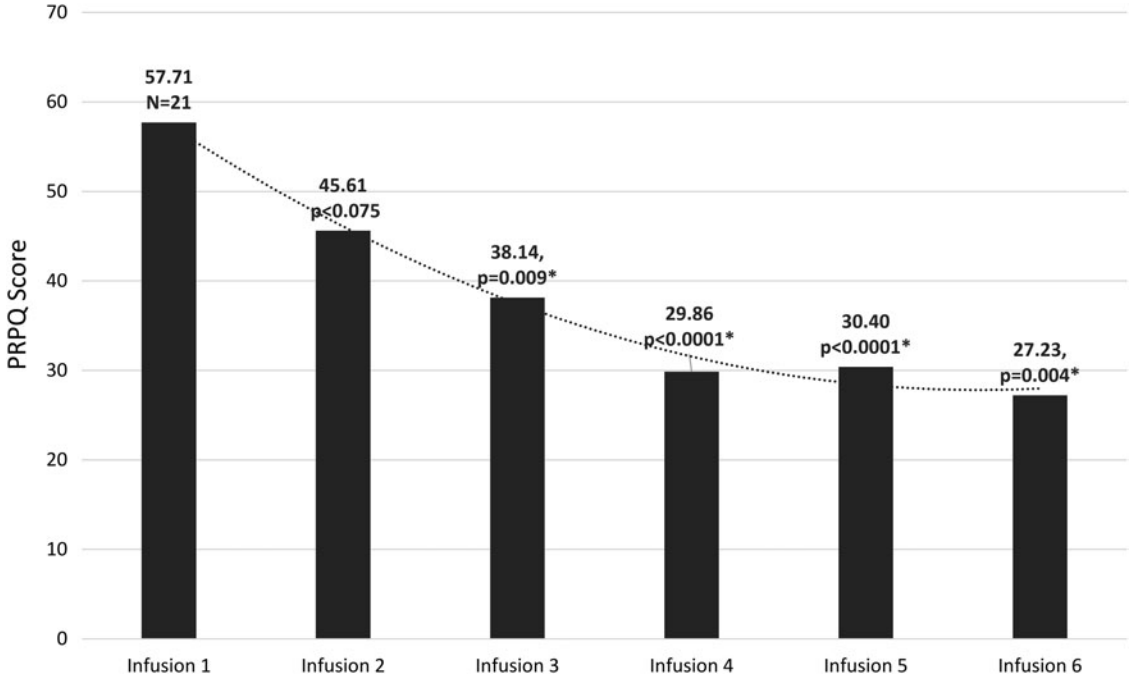

FIG. 4. Unadjusted mean scores from infusion 1 to infusion 6 (infusions occurred every 3 weeks) of the PRPQ (Supplementary Appendix S2) (*$p < 0.05$ was considered statistically significant). This questionnaire takes 10–20 minutes to complete and contains 58 items selected as key symptoms of interest for data analysis per the most important PANS characteristics reported in the literature. The importance of this assessment, compared to the others conducted in this study, is that it demonstrates the efficacy of IVIG following each infusion. Statistically significant reductions in symptoms were noted by the third IVIG infusion. IVIG, intravenous immunoglobulin; PANS, pediatric acute-onset neuropsychiatric syndrome; PRPQ, Parent-Rated Pediatric Acute-Onset Neuropsychiatric Syndrome Questionnaire.

Table 2. Behavioral Assessment Outcomes

Assessment	*Baseline (screen)*	*Visit 7*	*Visit 8*	*Visit 9*[a]
CY-BOCS mean total scores ± SD	22.10 ± 8.02	10.05 ± 8.53	8.52 ± 9.70	5.75 ± 8.53
Percentage change[b]		–54.52%	–61.45%	–71.01%
Mean change[b]		–12.05	–13.58	–14.08
CY-BOCS *p* values[c]		<0.0001	0.0002	<0.0001
CGI-S mean scores ± SD	4.67 ± 0.86	3.29 ± 1.35	2.62 ± 1.13	2.37 ± 1.30
Percentage change[b]		–29.55%	–43.90%	–46.23%
Mean change[b]		–1.38	–2.05	–2.04
CGI-S *p* values[c]		0.0002	<0.0001	<0.0001
YGTSS mean scores ± SD	36.81 ± 26.67	16.90 ± 19.16	12.29 ± 14.27	22.87 ± 26.46
Percentage change[b]		–54.09%	–66.61%	–44.70%
Mean change[b]		–19.91	–24.52	–18.50
YGTSS *p* values[c]		0.0003	0.0005	0.0058

[a]Subset of patients ($n = 12$) that participated in a late follow-up visit 29–46 weeks after Visit 8/final infusion and 55–72 weeks after baseline (screen). Percentage change and mean change were only calculated for the subset of participating patients.
[b]Percentage and mean change calculated from baseline (screen).
[c]*p*-Values <0.05 were considered statistically significant.
CGI, Clinical Global Impressions of Severity; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; SD, standard deviation; YGTSS, Yale Global Tic Severity Scale.

In the first double-blind, placebo-controlled investigation conducted by Perlmutter et al. (1999), therapeutic plasma exchange (TPE; five single-volume exchanges over 2 weeks), IVIG (1 g/kg daily on 2 consecutive days), or placebo (saline solution given in the same manner as IVIG) were compared. Results demonstrated that IVIG and TPE were both effective in reducing OCD symptoms in PANDAS patients (by 45% and 58%, respectively), whereas a placebo infusion had no discernable effect (Perlmutter et al. 1999). In contrast, non-PANDAS OCD (Nicolson et al. 2000) and tic disorders (Hoekstra et al. 2004) do not demonstrate benefits in TPE and IVIG, respectively.

Although the use of IVIG in the treatment of PANS has been utilized clinically, no additional placebo-controlled trials were conducted until 2016 (Williams et al. 2016). The study consisted of four visits: baseline, week 6 (end of the blinded phase), week 12 (end of the open-label phase), and week 24 (follow-up). At baseline, participants received either IVIG (2 g/kg per day administered at 1 g/kg over 2 days; $n = 17$) or placebo ($n = 18$). Six weeks following baseline, participants were evaluated, and a ''responder'' was defined as a decrease in CY-BOCS score of ≥30%, and ''Much'' or ''Very Much'' improved rating on CGI-I. Nonresponders to the blinded infusion were offered an open-label IVIG infusion.

At 6 weeks, the mean decrease in OCD severity was greater in the IVIG cohort than in placebo, but this difference did not reach statistical significance. It was determined that the study's power to detect between-group differences was tempered by the high variability in individual improvement after double-blind administration of IVIG. It was also known to the participants and their parents that those who did not meet the criteria for a ''responder'' in the 6-week portion of the study would receive an open-label IVIG infusion. The OCD severity scores for those receiving open-label IVIG (regardless of whether they had received a placebo or blinded IVIG infusion) decreased roughly 50% in 6 weeks. Because these improvements were only demonstrated during the open-label phase of the trial, it was not possible to definitively determine the efficacy of IVIG. In particular, participants may have overreported symptom severity in the double-blind portion of the study to increase the possibility of getting open-label IVIG at 6 weeks.

The limitations of this study include the small sample size, lack of a control group, and a heterogeneous patient population with differing durations of illness and antecedent treatments, as well as diverse PANS triggers (although the majority did have a relationship to a streptococcal infection). Although neuroimmune, atopic, and biomarker panels from baseline to end of treatment were explored, interpretation of results was complicated by the interference of the immunologic components of IVIG following infusion. The clinical laboratory testing problems associated with IVIG have been reported (Branch 2019), including interference with accurate antibody detection and antiglobulin testing. In future studies, it may be useful to include late neuroimmune, atopic, and biomarker panels in the design (e.g., following a washout period of 6–12 months) for comparison to baseline assessments. In addition, it is important to note the inherent difficulties in measuring systemic serum biomarkers for a localized brain disease such as PANS. It may be that the immunologic ''action'' is localized within brain tissue and central nervous system, and blood measurements are too remote, diffuse, and insensitive. In animal models that include cerebrospinal fluid measurements,

Table 3. Parent-Rated Pediatric Acute-Onset Neuropsychiatric Syndrome Questionnaire Outcomes

PRPQ	*Infusion 1*	*Infusion 2*	*Infusion 3*	*Infusion 4*	*Infusion 5*	*Infusion 6*
Mean scores ± SD	57.71 ± 36.40	45.61 ± 31.21	38.14 ± 24.77	29.86 ± 22.17	30.40 ± 26.25	27.23 ± 33.23
Percentage change[a]		20.97%	34.22%	48.26%	47.32%	52.82%
Mean change[a]		–12.10	–19.75	–27.85	–27.31	–30.48
PRPQ *p* values[b]		0.075	0.009	<0.0001	<0.0001	0.004

[a]Percentage and mean change calculated from infusion 1.
[b]*p*-Values <0.05 were considered statistically significant.
PRPQ, Parent-Rated PANS Questionnaire; SD, standard deviation.

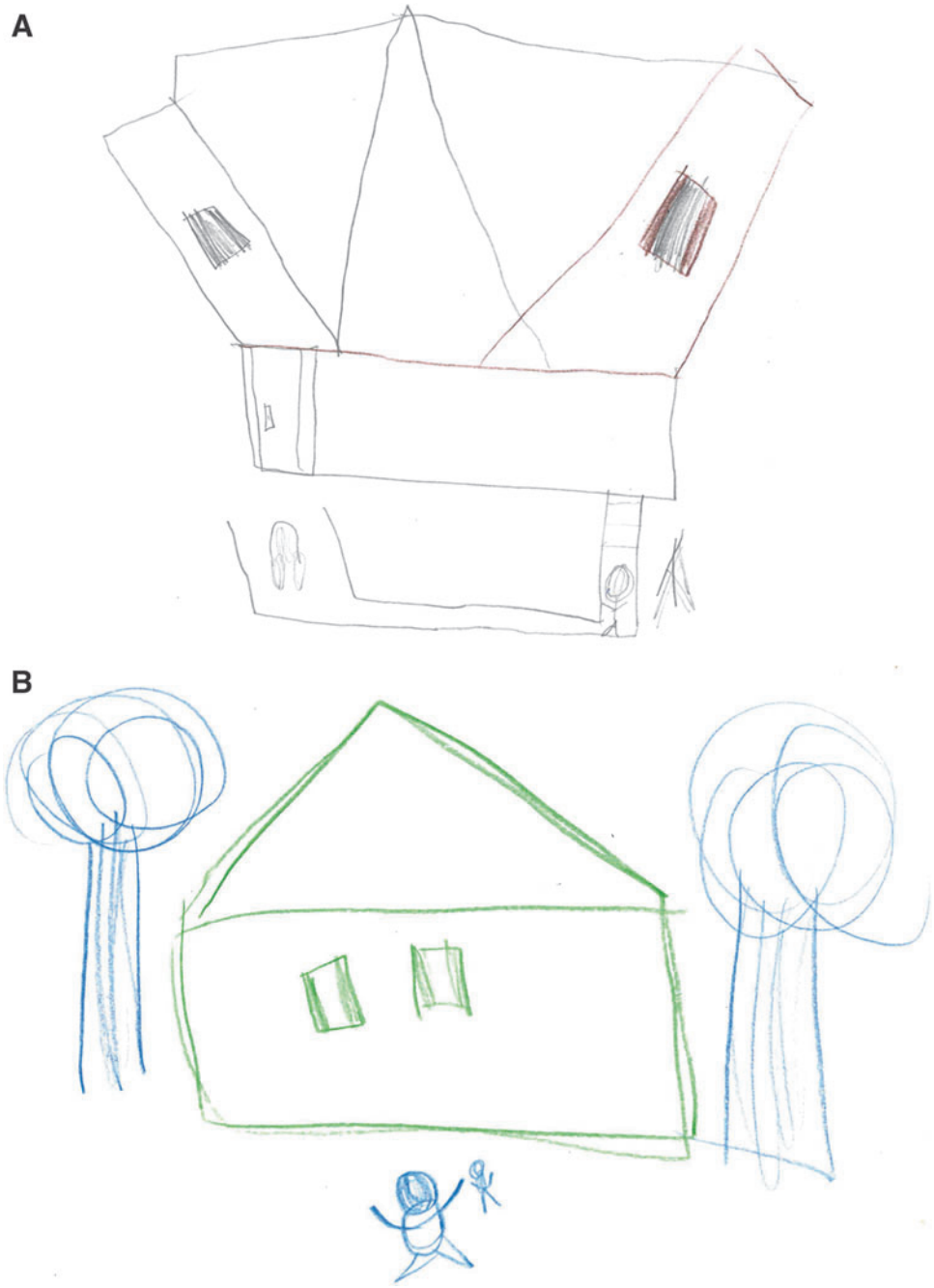

FIG. 5. The subject was asked to draw a house. **(A)** Subject's drawing before treatment. **(B)** Subject's drawing following IVIG treatment. IVIG, intravenous immunoglobulin.

brain tissue biopsies, and so on, results are impressive and convincing. Obviously, such studies are difficult, if not impossible, to conduct in children for a variety of reasons.

The positive results from this study contribute to the gathering evidence in support of conceptualizing PANS as an immune-mediated brain disease, similar to SC, involving the caudate, putamen, and other basal ganglia structures. Published data support the premise that PANS is an autoimmune disorder in susceptible children resulting in immune dysregulation involving autoantibodies, autoreactive T cells, disruption in T-regulatory cell function, microglial cell dysregulation, inappropriate release of or response to inflammatory cytokines, and autoreactive B cells, which result in an inflammatory disorder of the basal ganglia (Hornig 2013; Hornig and Lipkin 2013; Williams and Swedo 2015; Cutforth et al. 2016; Frick and Pittenger 2016; Frankovitch et al. 2017). Therefore, the use of a broad-spectrum immunomodulatory agent, such as IVIG, should result in changes in behavior brought on by abnormal inflammation (Ballow 2014; Spinello et al. 2016; Frankovitch et al. 2017; Joao et al. 2018). In other words, if PANS were not an autoimmune, autoinflammatory disease, then an immunomodulatory intervention, such as IVIG, should not have any impact on psychometric and clinical measurements. As the results

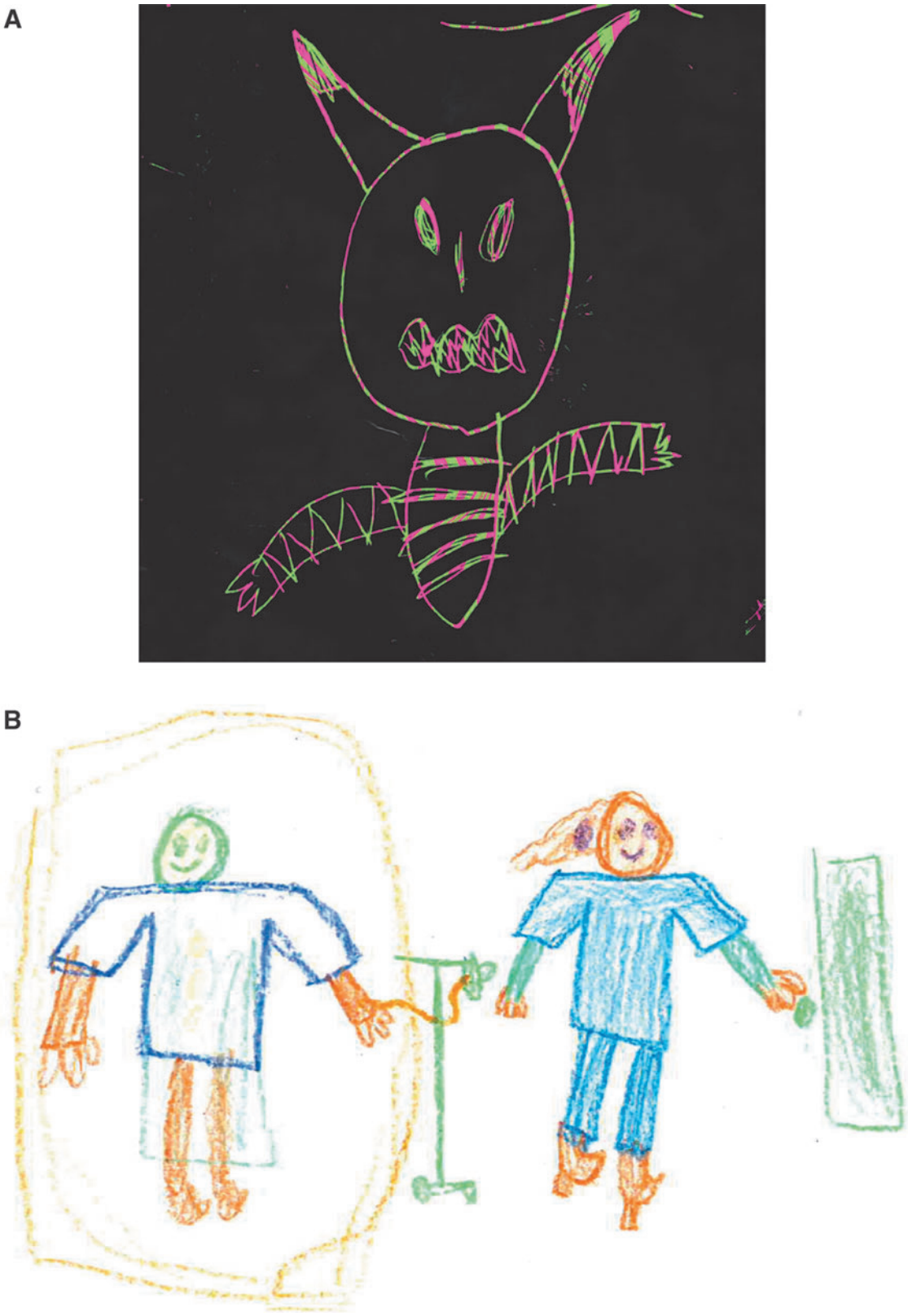

FIG. 6. The subject was asked to draw, ''self and others.'' **(A)** Subject's drawing before treatment. **(B)** Subject's drawing following IVIG treatment. IVIG, intravenous immunoglobulin.

of our study demonstrate, sequential infusions of IVIG had a significant, positive impact on PANS patients, supporting the characterization of PANS as an autoimmune disorder.

Conclusions

The results of this study demonstrated that in PANS, which may be associated with an underlying immune dysregulation, sequential infusions of IVIG [Octagam 5%] successfully ameliorated psychological symptoms and dysfunction, with sustained benefits for at least 8 weeks, and up to 46 weeks in a subset of subjects, following the final infusion. In addition, baseline immune and autoimmune profiles demonstrated significant elevations in a majority of subjects, which requires further evaluation, characterization, and study to clarify the potential immune dysfunction by which PANS manifests and progresses.

Clinical Significance

The limitations of this open-label pilot study include the small sample size and lack of a control group. However, in this population of PANS subjects, all psychometric endpoints studied exhibited statistically significant decreases following six infusions of IVIG. These positive results warrant a randomized, placebo-controlled trial to definitively evaluate the impact of multiple, sequential IVIG infusions on PANS symptoms. The durability of response is also noteworthy. Although the majority of PANS symptoms were still under control at the late follow-up visit (up to 46 weeks), it is of interest that tics returned in a subset of subjects following washout of IVIG. For these patients, additional infusions may be required to ameliorate recurrent symptoms.

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Supplementary Material

Supplementary Appendix S1
Supplementary Appendix S2

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Differential binding of antibodies in PANDAS patients to cholinergic interneurons in the striatum

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Abstract

Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus, or PANDAS, is a syndrome of acute childhood onset of obsessive-compulsive disorder and other neuropsychiatric symptoms in the aftermath of an infection with Group A beta-hemolytic *Streptococcus* (GABHS). Its pathophysiology remains unclear. PANDAS has been proposed to result from cross-reactivity of antibodies raised against GABHS with brain antigens, but the targets of these antibodies are unclear and may be heterogeneous. We developed an *in vivo* assay in mice to characterize the cellular targets of antibodies in serum from individuals with PANDAS. We focus on striatal interneurons, which have been implicated in the pathogenesis of tic disorders. Sera from children with well-characterized PANDAS (n = 5) from a previously described clinical trial (NCT01281969), and matched controls, were infused into the striatum of mice; antibody binding to interneurons was characterized using immunofluorescence and confocal microscopy. Antibodies from children with PANDAS bound to ~80% of cholinergic interneurons, significantly higher than the <50% binding seen with matched healthy controls. There was no elevated binding to two different populations of GABAergic interneurons (PV and nNOS-positive), confirming the specificity of this phenomenon. Elevated binding to cholinergic interneurons resolved in parallel with symptom improvement after treatment with intravenous immunoglobulin. Antibody-mediated dysregulation of striatal cholinergic interneurons may be a locus of pathology in PANDAS. Future clarification of the functional consequences of this specific binding may identify new opportunities for intervention in children with this condition.

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Disclosure

Dr. Pittenger has served as a consultant in the past year for Biohaven Pharmaceutical Holding Company, Ltd., and is working under a contract with Blackthorn Therapeutics, Ltd., on unrelated projects. All other authors report no competing interests.

INTRODUCTION

Obsessive-compulsive disorder (OCD) and tic disorders often first appear in childhood [1–3]. In a minority of pediatric OCD cases, onset is unusually abrupt and is accompanied by a range of comparably severe associated neuropsychiatric symptoms. This syndrome has been named Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) [4, 5]. In some instances, this abrupt onset is seen with or after resolution of an infectious illness, suggesting an immune-mediated pathogenesis [6]. Temporal association with infection by group A beta-hemolytic *Streptococcus* infection (GABHS, or *Streptococcus pyogenes*) has been noted with particular frequency; this association has been termed Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus, or PANDAS [7–9].

By analogy with the pathophysiology of Sydenham's chorea, a neuropsychiatric disorder that also occurs following GABHS infection, it was proposed that infection in susceptible children triggers an autoimmune reaction through molecular mimicry, a process in which host antibodies directed against *Streptococcus pyogenes* cross-react with human proteins [5, 8, 10–12]. In Sydenham's chorea (SC), for example, antibodies from patients have been found to cross-react both against neuronal lysoganglioside and streptococcal N-acetyl-beta-D-glucosamine [12]. Other antibody targets have been described in SC, including the dopamine D2 receptor [13, 14]. Numerous studies have sought to better characterize the PANDAS clinical subgroup, clarify the associated pathophysiology, and identify the brain targets of the autoantibodies [8]. Studies in animals have confirmed the ability of anti-Streptococcal antibodies to produce neural and behavioral abnormalities, further justifying the pursuit of antibody targets that may explain pathogenesis [15–19]. Despite this progress, the PANDAS diagnosis remains somewhat controversial, and its pathophysiology remains to be clearly elucidated [9].

Based on the hypothesized autoimmune etiology, a variety of immunomodulatory therapies have been investigated in children with PANDAS [8]. An early controlled study indicated efficacy of both plasmapheresis and intravenous immunoglobulin (IVIG), compared to placebo [20]. Subsequent clinical experience has continued to suggest benefit from these approaches in some cases [21]. A recent two-site study, performed at Yale and the National Institute of Mental Health, identified children with PANDAS by particularly stringent criteria and treated them with IVIG or placebo (NCT01281969). While IVIG did not separate from placebo during the blinded phase, response rates were robust after the administration of open-label IVIG, which all participants were offered, if their symptoms remained severe after completion of the double-blind phase [22].

Functional and structural abnormalities of the cortico-basal ganglia circuitry have been described in both OCD and tic disorders and are central to most current thinking about their pathophysiology [23–26]. Pathological abnormalities in the striatum have been also reported in PANDAS. Giedd and colleagues [27, 28] found enlarged striatal volume in patients with PANDAS, similar to that seen in those with acute Sydenham's chorea. Striatal abnormalities have been reported to resolve in conjunction with symptoms, either after plasmapheresis [28] or spontaneously [29]. More recently, inflammation of the striatum has been reported in

PANDAS and Tourette syndrome patients, as measured by positron emission tomography using a marker of microglial activation [30].

The hypothesis that PANDAS derives from molecular mimicry implies the presence of antibodies in PANDAS patients that cross-react with brain antigens, as has been documented in Sydenham's chorea [12]. Indeed, such reactivity has been documented *ex vivo* [31]. Reactivity of antibodies from individuals with PANDAS against several neuronal proteins, including tubulin, lysoganglioside, and dopamine receptors, has been reported, as has antibody-mediated activation of CaM kinase II; these findings have not been consistent across all studies, perhaps due to etiological heterogeneity [11, 32–35].

The current investigation draws upon recent evidence implicating striatal interneuronal abnormalities in the pathophysiology of tic disorders. *Post mortem* analyses have identified reduced density of specific populations of striatal interneurons in patients with Tourette syndrome; these include cholinergic interneurons (CINs) and GABAergic interneurons expressing the markers parvalbumin and nNOS [36–38]. Our laboratory has shown that recapitulation of these post-mortem findings in mice, using experimental depletion of cholinergic or parvalbumin-expressing interneurons in the dorsal striatum, produces tic-like phenomenology [39–41]. This suggests that striatal interneuronal dysfunction may play a causal role in tic pathophysiology. The contribution of interneuronal pathology to OCD is less clear.

To date, no studies have investigated reactivity of antibodies from individuals with PANDAS with epitopes present on interneurons. We investigated interneuron targets of PANDAS antibody reactivity using an *in vivo* model. We infused serum from children with PANDAS into the striatum of mice and characterized the cellular targets of serum antibodies using double immunofluorescence with a panel of cell-specific markers. Samples were drawn from the treatment trial described above, NCT01281969; cases matched particularly stringent clinical criteria for PANDAS (not just PANS), including acute onset of OCD symptoms, presence of characteristic associated symptoms, and documented GABHS infection [22]. Individuals who responded clinically to IVIG treatment were selected for analysis, as responders may be most likely to have antibody-mediated pathophysiology. We report the first evidence for interneuron-reactive antibodies in the pathogenesis of PANDAS.

METHODS AND MATERIALS

Samples from patients diagnosed with PANDAS

Sera from children with PANDAS were obtained from a recent IVIG trial, NCT01281969 [22]. This randomized, double-blind trial investigated the efficacy of IVIG on PANDAS symptoms. Subjects in the clinical trial were required to meet all diagnostic criteria for PANDAS, including a positive test for beta hemolytic *Streptococcus*, and to have moderate to severe OCD symptoms, as assessed by the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS [42]). Ratings were performed by trained clinicians, blinded to the treatment status [12].

For the pilot study (Supplementary Figure 2), three children with PANDAS were selected from this cohort, without applying further selection criteria. To enhance the probability of identifying subjects with antibody-mediated pathophysiology in the main experiment (Figures 1–3), we selected subjects who had a positive clinical response to IVIG, defined as a >35% decrease in the Y-BOCS score (see Figure 4A). One subject from the pilot experiment met these additional criteria and was included in the main experiment (subject B in the pilot, #3 in the main experiment). Two subjects in the main experiment received IVIG during the blinded treatment phase; two received placebo during the blinded phase and IVIG during the subsequent open-label phase, 6 weeks later. The fifth subject received open-label IVIG infusion and follow-up on the same schedule as the others after being excluded from the trial due to anxiety associated with a lumbar puncture. Serum samples used in this analysis were obtained at baseline and at 12 weeks (time points 1 and 3 in the clinical trial; see Figure 4A). The second time point was thus either 6 (2 subjects) or 12 weeks (3 subjects) after IVIG infusion. Of the five subjects in the main experiment, two had mild tics at baseline; three had no tics.

Control subjects for the pilot experiment were drawn from healthy control samples collected at Yale. For the main experiment, well-screened healthy subjects matched to the PANDAS sample for age and gender, also evaluated at the NIMH and stored under equivalent conditions and for equivalent time, were used as controls (see Table 1). None of these healthy controls had clinically significant OCD or tic symptoms.

Serum samples were stored at −80°C until use. These investigations were approved by the Human Investigations Committees of Yale University and the National Institute of Mental Health. All subjects provided assent and parental informed consent. Samples were fully anonymized at the clinical site at NIMH before being sent to the laboratory at Yale for analysis.

Mice

All experiments were conducted under the auspices of the Yale Institutional Animal Care and Use Committee, in accordance with NIH guidelines. Adult female and male C57Bl/6 wild-type or bitransgenic BAC transgenic mice [43], aged 2–4 months, were used for all experiments; there were no significant differences between results from male and female mice. (These BAC transgenic mice were used to identify binding to D1- and D2-expressing medium spiny neurons; this staining was uninformative and is not reported here.) 16 mice were infused in the pilot experiment (2–3 mice/serum sample); 20 mice were infused in the first experiment (2 mice with each of 10 sera), and 20 were used in the follow-up treatment experiment (5 patients; 2 mice with serum from before IVIG treatment and 2 mice with serum from after IVIG treatment).

Surgery and serum infusions

Custom bilateral cannulae were manufactured to target the striatum (3.25 mm long; 4.6 mm space between guides; Plastics One, Roanoke, VA). Cannulae were chronically implanted using standard stereotaxic technique, targeting +0.50 mm anteroposterior, +/−2.3 mm mediolateral, relative to bregma [44]. Animals were anesthetized during surgery with

ketamine:xylazine 100:10 mg/kg. Guide cannulae were affixed to the skull using C&B-Metabond® Quick! Cement System (Parkell Inc.). Guide cannula patency was ensured with customized dummy cannulae that projected 0.5 mm beyond the end of the guide. Animals recovered for at least 5 days prior to first serum infusion.

Undiluted serum was infused into the striatum simultaneously in the two hemispheres at a fixed rate of 0.1μl/min through customized injectors projecting 0.5 mm beyond the end of the guide cannulae, using a PHD Ultra Pump (Harvard Apparatus).

Pilot experiments determined optimal infusion parameters. Piloting was performed with pooled human serum (Sigma: H4522). Serum was infused unilaterally, varying the volume and number of infusions and the time between the last infusion and animal sacrifice; saline was infused on the contralateral side (Supplementary Figure 1). IgG deposition was identified using immunohistochemistry with rabbit anti-human IgG (1:200 Abcam, Cambridge, MA) and visualized using diaminobenzidine (ABC Kit; Vector Laboratories, Burlingame, CA, USA). Optical density was quantified using ImageJ (NIH, Bethesda, MD, USA), as previously described [45]. Optimal IgG deposition was achieved when animals were infused for 5 consecutive days with 0.5 μl serum and then sacrificed 5 days after the last infusion; these conditions were used for all subsequent experiments.

Immunofluorescence and confocal microscopy

Mice were euthanized without perfusion. Brains were quickly dissected, fixed in 4% paraformaldehyde for 48h at 4°C, and then transferred into a 30% sucrose solution and allowed to equilibrate at 4°. Brains were sectioned at 30 μm using a cryostat; sections were stored in cryoprotectant solution (30% glycerin + 30% ethylene glycol in PBS) at −20°C until use.

Free-floating sections were washed with PBS, treated with Bloxall Solution (Vector Laboratories), washed again in PBS, incubated in 5% normal donkey serum solution in PBS + 0.3% Triton X100 for 1 hour at room temperature, and then overnight at 4° C in the same solution with rabbit anti-human IgG 1:200 (Abcam), together with goat anti-choline acetyltransferase (ChAT; 1:1000, Sigma, St. Louis, MO, USA), mouse anti-neuronal nitric oxide synthase (nNOS; 1:100, Santa Cruz Biotechnology, Dallas, TX), or mouse anti-parvalbumin (PV; 1:500; Sigma). For the anti-nNOS and anti-PV antibodies, an additional blockade with Mouse-on-Mouse (MOM) reagent (Vector Laboratories) was included to prevent reactivity of the secondary antibody with endogenous mouse immunoglobulin, following the manufacturer's instructions. Slices were washed 3X with PBS and then incubated with fluorescent secondary antibodies (1:400, Invitrogen, Carlsbad, CA, USA) for one hour at room temperature. Secondary antibodies were: for ChAT immunostatining, Alexa 555-conjugated donkey anti-rabbit and Alexa 633-conjugated donkey anti-goat; for nNOS and PV immunostaining, Alexa 555-conjugated donkey anti-rabbit and Alexa 488-conjugated donkey anti-mouse. Samples were washed again and mounted in Vectashield HardSet Mounting Medium (Vector Labs).

Confocal imaging was performed by sequential scanning of slices on an Olympus Fluoview FV-1000 confocal microscope, using a Kalman filter with an acquisition speed of 4 pixel/sec. 15 μm z-stacks were generated by using a fixed step size of 0.5 μm.

Quantification of serum antibody binding to interneurons

Two images were obtained from each slice (one per side); several slices per mouse were used to provide a representative sample of the entire mouse striatum, for a total of 8–12 images per mouse. Single- and double-positive cells were counted throughout all images, blind to experimental condition. Cell counts were averaged to produce a single value for each animal. Two mice were infused with each serum; technical variation in infusion and cell counting was very low.

Statistical analysis

Data were analyzed using Prism 6.0 (GraphPad), except for linear mixed model analysis, which was performed using SPSS 24 (IBM). The unit of analysis was the patient/serum sample, not the slice or the animal. Each data point in the final analysis averages cell counts from ~20 slices (8–12 slices from each of 2 animals).

We have examined a small number of rigorously characterized samples, rather than a larger but potentially more heterogeneous clinical group. With an N of five samples per group in the main experiment, we therefore have 80% power to detect only very large effects ($d > 1.7$). We reasoned that examination of a small, homogeneous sample would lead to smaller variance and thus to greater power than examination of a larger but more heterogeneous group.

RESULTS

Pilot study

In a small pilot study, we infused serum from three individuals with PANDAS and three controls into the striatum of adult mice. Binding to ChAT-positive interneurons was elevated after infusion of PANDAS serum, relative to controls; binding to PV-positive interneurons was unchanged (Supplementary Figure 2). These pilot data motivated a larger experiment with more carefully selected PANDAS subjects and optimally matched controls.

Serum infusion produced microgliosis and astrocytosis in the affected striatum. However, this glial activation was qualitatively equivalent after infusion of control and PANDAS serum (Supplementary Figure 3).

Main experiment: Subjects

Five subjects with PANDAS were selected from the participants in the clinical trial [22] on the basis of their robust clinical response to IVIG; one of these overlapped with the smaller group used in the pilot experiment. These subjects had no history of acute rheumatic fever or Sydenham's chorea, autism, or schizophrenia, or a history of previous immunomodulatory therapy. They were matched for age and gender with 5 healthy controls (see Table 1).

PANDAS subjects had slightly but significantly higher IgG levels at baseline than controls ($p = 0.02$).

Elevated binding of IgG from PANDAS serum to cholinergic but not GABAergic interneurons

IgG deposition onto ChAT-positive cholinergic interneurons (CINs) was seen after infusion of both PANDAS and control serum. A larger number of ChAT-positive interneurons, expressed as a fraction of total $ChAT^+$ cells, was bound by antibodies in serum collected at baseline from PANDAS patients, compared to controls (Figure 1). This increased binding had a very large effect size of $d = 8.1$. The total number of ChAT-positive interneurons was not altered by PANDAS serum infusion, indicating that this difference does not derive from a non-specific toxic effect ($t[8] = 0.89$, $p > 0.4$).

We pooled these CIN binding data with data from the pilot experiment (Supplementary Figure 2) using a linear mixed model, coding serum number as a random variable to account for the fact that one sample was included in both experiments. This confirmed a highly significant effect of diagnosis ($F[1,23] = 75.8$; $p < 0.001$).

In contrast, binding of IgG to PV^+ and $nNOS^+$ interneurons did not differ between PANDAS and control samples (Figure 2, 3). The nominal trend was towards decreased binding in both cases.

Reversal of CIN antibody deposition after IVIG treatment

These five patients were selected in part because they all improved substantially following IVIG treatment (Figure 4A). In an independent experiment, we infused sera from the same patients, collected at baseline and 6–12 weeks after IVIG treatment. Infusion was again into 2 mice per serum sample. Binding of IgG to CINs was significantly reduced in convalescent serum (Figure 4B). Improvement in CY-BOCS after treatment correlated significantly with reduction of IgG binding to cholinergic interneurons (Figure 4C).

DISCUSSION

We have identified elevated binding of serum antibodies to cholinergic interneurons (CINs) in the striatum in children with stringently defined PANDAS that responded to IVIG treatment. This represents the first time that immunoreactivity against interneurons in the striatum has been examined in PANDAS and identifies CINs as a potentially important locus of pathology in the disorder.

Several lines of evidence implicate CINs in tic disorders. In postmortem brain from patients with Tourette syndrome, ChAT-positive CINs are reduced in number by approximately 50% in the caudate and the putamen [37, 38]. Moreover, selective virus-mediated depletion of CINs in the dorsolateral striatum of mice, which reproduces the ~50% loss seen post-mortem, leads to tic-like stereotypies after either acute stress or psychostimulant challenge [39]. Mutations in the Slit and Trk-like family member 1 (SLITRK1) gene have been associated with Tourette syndrome in rare cases [46, 47]; in the adult striatum, expression of this gene is restricted to CINs [48]. Finally, expression of a number of acetylcholine-related

genes in blood has been reported to correlate with tic severity in Tourette syndrome, and these genes appear to be differentially spliced in patients [49].

No similar analyses have been reported in OCD. However, the comorbidity between TS and OCD in youth has been reported to be as high as 50% [50], and the two conditions appear to have overlapping pathophysiology [51]. Tic and OCD comorbidity is 60% in PANDAS [7].

In conjunction with our findings, this literature suggests that antibodies that recognize striatal cholinergic interneurons may have a pathogenic role in PANDAS. It is noteworthy that tics were not prominent in the subjects we characterized: two had mild tics at baseline, and three had none. Therefore, the elevated binding of IgG to CINs documented here is not specific to tic symptoms, but rather is associated with PANDAS more generally.

Elevated binding of IgG in PANDAS serum to CINs is specific: there is no differential binding to other interneuron types examined. This specificity is in contrast to post mortem evidence that both PV^+ fast spiking interneurons and the nNOS-expressing GABAergic interneurons have also been shown to be affected in adults with severe tics [36–38]. IgG levels were slightly higher in controls, which represents a weakness in our study; however, the specificity of binding increases confidence that differential binding to cholinergic interneurons is not a consequence of this or of other nonspecific abnormalities in the serum, but rather a reflection of specific populations of antibody.

Cholinergic interneurons are well positioned to regulate information processing in the basal ganglia. They differentially regulate D1- and D2-expressing medium spiny neurons through activation of metabotropic ACh receptors [52, 53] and modulate activity of GABAergic interneurons [54]. Cholinergic dysregulation in the dorsal striatum may thus produce an imbalance between the direct and indirect pathways through the basal ganglia, leading to disinhibition of off-target behaviors [55, 56]. Our previous studies of the consequences of cholinergic interneuron depletion *in vivo* show that functional perturbation of these cells can produce relevant phenomenology [39]. We hypothesize that antibody deposition on cholinergic interneurons leads to functional abnormalities, and thereby to PANDAS symptomatology. How antibody binding to these cells might perturb their function, however, remains unclear. Cholinergic interneurons express D2 dopamine receptors, and antibodies in PANDAS have been reported to bind to these receptors in some studies [32]{Morris-Berry, 2013 #39;Cox, 2013 #37}; this represents one possible molecular target that might explain the preferential binding to these interneurons seen here. *In vitro*, antibodies from PANDAS patients can activate calcium-calmodulin kinase-II in immortalized human neuron-like cells [11, 33, 35]. However, this has not been demonstrated in cholinergic cells.

Our model rests on the assumption that antibodies that recognize cholinergic interneurons in mice will have similar binding in the human brain; this is a limitation. An additional limitation is that binding to tissue does not permit ready identification of the molecular targets to which PANDAS antibodies are binding; this is an important focus of future work. However, the approach employed here has unique advantages. While inflammation and microglial activation can be visualized *in vivo* in humans [30], IgG deposition cannot, and cell-type specificity of binding is difficult to characterize. Analysis of post-mortem tissue

can be used to characterize abnormalities in chronic illness, and post-mortem analysis of individuals with severe TS has revealed microglial activation [38]; but tissue for post-mortem analysis is scarce and often heterogeneous, and repeated characterization after treatment, such as we present here, is obviously not possible. Furthermore, the use of human post-mortem tissue to evaluate autoantibodies in human serum samples is limited by numerous technical challenges, including interference from formaldehyde tissue preservatives and non-specific brain IgG deposition. Thus, the use of murine brain tissue for the characterization of human anti-brain antibodies may represent an important “first-pass” tool for antibody identification. A similar approach has been used, with success, in the identification of antibodies in autoimmune encephalitis, particularly anti-NMDA receptor encephalitis [57]. An additional advantage is that we can investigate binding by cell type, rather than molecular target, which may be a more sensitive approach if antibody targets are heterogeneous.

IVIG treatment has been reported to ameliorate symptoms in PANDAS in some cases [20–22]. In the recent clinical trial from which our clinical samples are drawn, patients who received blinded IVIG treatment did not separate from controls in the primary analysis of the blinded phase, but a majority of patients responded after receiving IVIG during either the blinded or open-label phase [22]. Because our goal here was to identify potential pathophysiological mechanisms, and not to establish characteristics of the full clinical cohort, we selected subjects who demonstrated a robust clinical response to IVIG. This clinical response was paralleled by reduced binding of antibodies to striatal cholinergic interneurons. This correlation supports the conclusion that IVIG may produce clinical response by reducing pathogenic antibodies. Similar declines in antibody reactivity have been reported in convalescent serum from PANDAS [15] and Sydenham’s chorea [12], though not previously to our knowledge after IVIG treatment.

These results do not exclude the possibility of other pathogenic cellular targets in PANDAS serum. PANDAS is likely to be etiologically heterogeneous. D1- and D2-expressing medium spiny neurons represent the majority of striatal neurons; and several studies have suggested that they may be targeted by autoantibodies in PANDAS, in at least some cases [32–35].

In addition, our results do not establish that this abnormality is unique to PANDAS. We have examined a small number of extensively characterized patients, selected so as to maximize the *a priori* likelihood of identifying antibody-mediated pathophysiology. Future examination of larger cohorts and a more diverse set of patients, including individuals with Tourette syndrome, non-PANDAS pediatric-onset OCD, and Sydenham’s chorea would better demarcate the clinical correlates of antibody reactivity against CINs and might reveal different patterns of antibody deposition in distinct populations of interneurons in other groups of patients. The approach described here is extremely laborious; now that the CINs have been identified as a cellular target of interest, it may be possible to do more focused analyses in these broader clinical cohorts using *ex vivo* methods.

In summary, our *in vivo* approach to characterizing antibody reactivity in patients with PANDAS has identified a novel candidate pathophysiology: specific autoantibody binding to striatal cholinergic interneurons. This focus on cholinergic interneurons fits well with the

developing appreciation of the role of these cells in tic disorders. Identification of the specific antigens on these cells and the functional consequences of antibody binding may open new avenues for the understanding and treatment of PANDAS and related conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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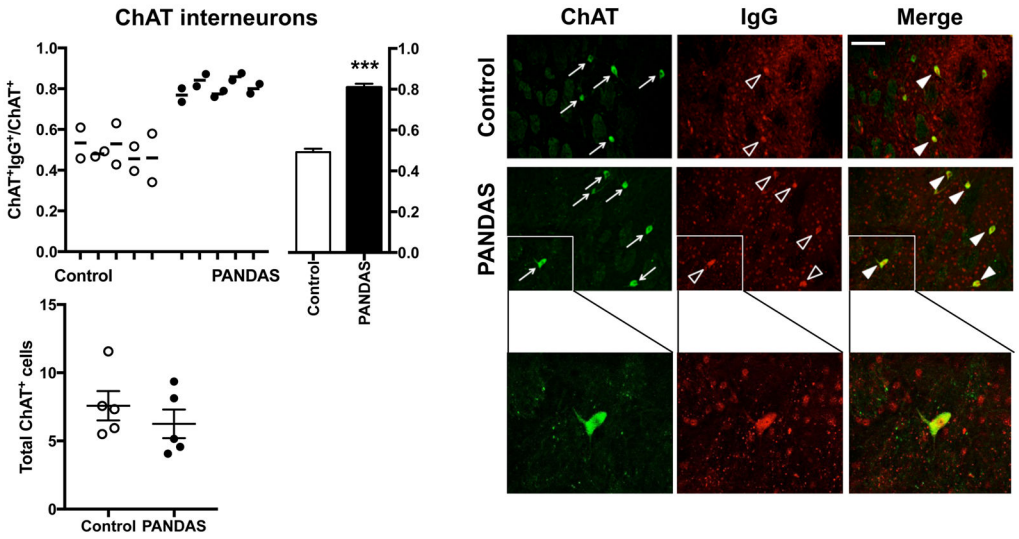

Figure 1. PANDAS autoantibodies differentially bind to ChAT interneurons in the basal ganglia

Deposition of antibodies from control and PANDAS sera (collected at baseline from highly symptomatic patients) onto ChAT-positive interneurons (CINs) *in vivo* was evaluated by double immunofluorescent staining and confocal microscopy, using anti-human IgG (red) and anti-ChAT antibodies (green). Antibodies from both control and PANDAS serum were deposited onto CINs, but a significantly larger number of $ChAT^{+}IgG^{+}$ cells (arrowheads), as a fraction of total $ChAT^{+}$ cells, was observed in PANDAS samples, compared to healthy controls (N=5 samples per group, 2 mice per sample; two-tailed t-test: $t[8] = 12.90$; $p < 0.0001$). Insets illustrate binding to a single $ChAT^{+}$ cell at higher magnification. Scale bar = 100 μm.

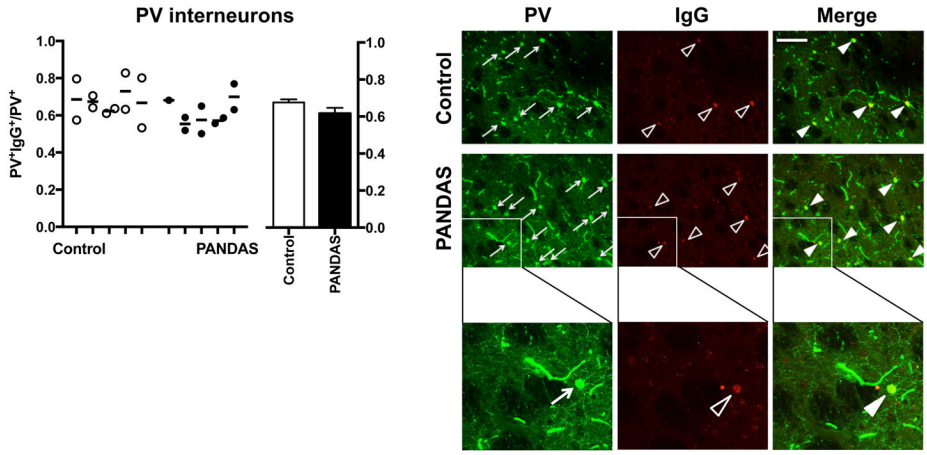

Figure 2. PANDAS autoantibodies do not show elevated binding to parvalbumin-positive GABAergic interneurons

No differences between PANDAS and control serum in antibody deposition onto PV-positive interneurons were observed (two-tailed t-test: t[8] = 1.7, p = 0.13). Scale bar = 100 μm.

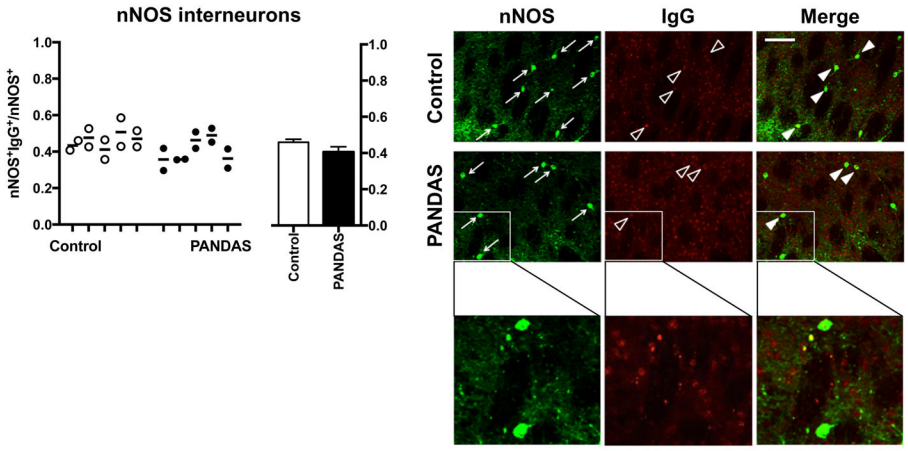

Figure 3. PANDAS autoantibodies do not show elevated binding to nNOS-positive GABAergic interneurons

No differences between PANDAS and control serum in antibody deposition onto nNOS interneurons were observed (two-tailed t-test: $t[8] = 1.6$, $p = 0.15$). Scale bar = 100 μm.

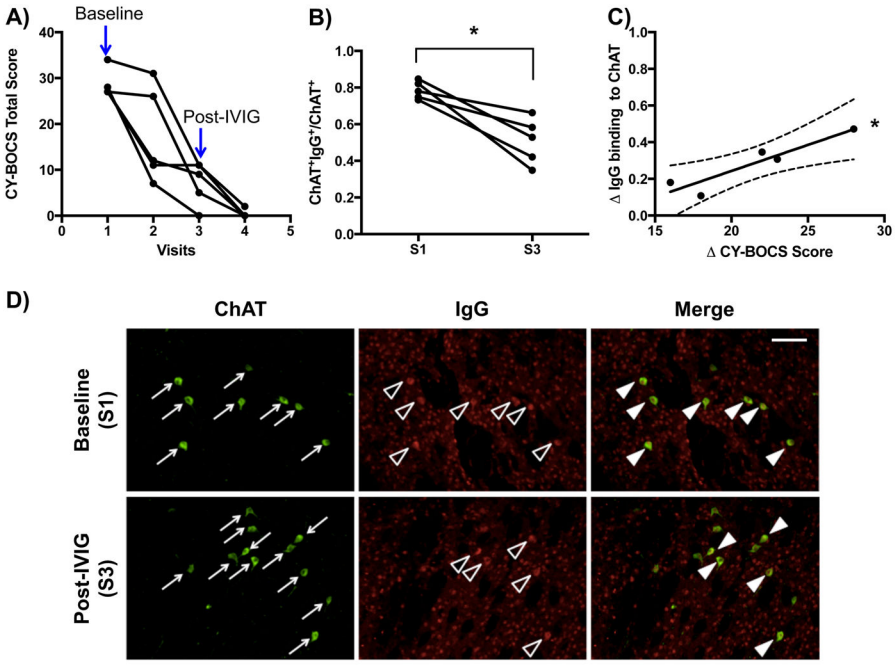

Figure 4. Resolution of elevated ChAT binding in post-IVIG serum

A. These patients all showed a significant clinical response to IVIG treatment. Individual patients' changes in the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) score across four evaluation sessions are shown (see also Table 1). Antibody binding to interneurons was assayed at baseline (Visit 1; Figures 1–3) and 12 weeks later (Visit 3). **B.** IgG binding to $ChAT^+$ interneurons was markedly reduced in serum collected from all five PANDAS patients after IVIG treatment (evaluation 3), relative to baseline (paired t-test: $t[4] = 4.4$; $p = 0.012$). **C.** Change in IgG binding to CINs correlated significantly with improvement in CY-BOCS after IVIG treatment ($r^2 = 0.86$, $p = 0.023$). Scale bar = 100 μm.

Table 1

Subjects

Patients were selected from the larger group who participated in a clinical trial of IVIG for the treatment of PANDAS [11], on the basis of clinical response to IVIG treatment and positive ANA at baseline. Healthy controls were previously screened by the same clinical site, at the NIMH.

Subjects	SEX	AGE (at trial initiation)	RACE/ETHNICITY	TIME OF IVIG (weeks)	Baseline CY-BOCS	Week 12 CY-BOCS	Baseline IgG (mg/dL)	ANA prior to IVIG
PANDAS								
1	F	9.5 y	M/NH	6	27	5	1250	+
2	F	8.9 y	W/NH	0	28	0	898	+
3	F	6.7 y	W/NH	0	27	11	1160	+
4	M	8.2 y	W/NH	6	34	11	1120	+
5	M	7.5 y	W/NH	0*	27	9	1200	+
CONTROL								
1	F	5.1 y	W/NH	-	-	-	896	+
2	F	8.0 y	W/NH	-	-	-	963	+
3	M	8.1 y	M/NH	-	-	-	768	−
4	M	7.8 y	M/H	-	-	-	877	−
5	F	7.3 y	W/NH	-	-	-	1040	−
P-value		0.26					0.02	NS

Race/ethnicity (parental report): M - mixed; W - white; H – Hispanic or Latino; NH – not Hispanic or Latino. Patients received a single IVIG treatment, either during the blinded clinical trial (at 0 weeks) or during a subsequent open-label phase (at 6 weeks).

* This patient was not randomized due to an unrelated adverse event (anxiety during a lumbar puncture) but was treated open-label with IVIG and followed up on the same schedule as those who were randomized.

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Differential binding of antibodies in PANDAS patients to cholinergic interneurons in the striatum

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Abstract

Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus, or PANDAS, is a syndrome of acute childhood onset of obsessive-compulsive disorder and other neuropsychiatric symptoms in the aftermath of an infection with Group A beta-hemolytic *Streptococcus* (GABHS). Its pathophysiology remains unclear. PANDAS has been proposed to result from cross-reactivity of antibodies raised against GABHS with brain antigens, but the targets of these antibodies are unclear and may be heterogeneous. We developed an *in vivo* assay in mice to characterize the cellular targets of antibodies in serum from individuals with PANDAS. We focus on striatal interneurons, which have been implicated in the pathogenesis of tic disorders. Sera from children with well-characterized PANDAS (n = 5) from a previously described clinical trial (NCT01281969), and matched controls, were infused into the striatum of mice; antibody binding to interneurons was characterized using immunofluorescence and confocal microscopy. Antibodies from children with PANDAS bound to ~80% of cholinergic interneurons, significantly higher than the <50% binding seen with matched healthy controls. There was no elevated binding to two different populations of GABAergic interneurons (PV and nNOS-positive), confirming the specificity of this phenomenon. Elevated binding to cholinergic interneurons resolved in parallel with symptom improvement after treatment with intravenous immunoglobulin. Antibody-mediated dysregulation of striatal cholinergic interneurons may be a locus of pathology in PANDAS. Future clarification of the functional consequences of this specific binding may identify new opportunities for intervention in children with this condition.

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Disclosure

Dr. Pittenger has served as a consultant in the past year for Biohaven Pharmaceutical Holding Company, Ltd., and is working under a contract with Blackthorn Therapeutics, Ltd., on unrelated projects. All other authors report no competing interests.

INTRODUCTION

Obsessive-compulsive disorder (OCD) and tic disorders often first appear in childhood [1–3]. In a minority of pediatric OCD cases, onset is unusually abrupt and is accompanied by a range of comparably severe associated neuropsychiatric symptoms. This syndrome has been named Pediatric Acute-onset Neuropsychiatric Syndrome (PANS) [4, 5]. In some instances, this abrupt onset is seen with or after resolution of an infectious illness, suggesting an immune-mediated pathogenesis [6]. Temporal association with infection by group A beta-hemolytic *Streptococcus* infection (GABHS, or *Streptococcus pyogenes*) has been noted with particular frequency; this association has been termed Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus, or PANDAS [7–9].

By analogy with the pathophysiology of Sydenham's chorea, a neuropsychiatric disorder that also occurs following GABHS infection, it was proposed that infection in susceptible children triggers an autoimmune reaction through molecular mimicry, a process in which host antibodies directed against *Streptococcus pyogenes* cross-react with human proteins [5, 8, 10–12]. In Sydenham's chorea (SC), for example, antibodies from patients have been found to cross-react both against neuronal lysoganglioside and streptococcal N-acetyl-beta-D-glucosamine [12]. Other antibody targets have been described in SC, including the dopamine D2 receptor [13, 14]. Numerous studies have sought to better characterize the PANDAS clinical subgroup, clarify the associated pathophysiology, and identify the brain targets of the autoantibodies [8]. Studies in animals have confirmed the ability of anti-Streptococcal antibodies to produce neural and behavioral abnormalities, further justifying the pursuit of antibody targets that may explain pathogenesis [15–19]. Despite this progress, the PANDAS diagnosis remains somewhat controversial, and its pathophysiology remains to be clearly elucidated [9].

Based on the hypothesized autoimmune etiology, a variety of immunomodulatory therapies have been investigated in children with PANDAS [8]. An early controlled study indicated efficacy of both plasmapheresis and intravenous immunoglobulin (IVIG), compared to placebo [20]. Subsequent clinical experience has continued to suggest benefit from these approaches in some cases [21]. A recent two-site study, performed at Yale and the National Institute of Mental Health, identified children with PANDAS by particularly stringent criteria and treated them with IVIG or placebo (NCT01281969). While IVIG did not separate from placebo during the blinded phase, response rates were robust after the administration of open-label IVIG, which all participants were offered, if their symptoms remained severe after completion of the double-blind phase [22].

Functional and structural abnormalities of the cortico-basal ganglia circuitry have been described in both OCD and tic disorders and are central to most current thinking about their pathophysiology [23–26]. Pathological abnormalities in the striatum have been also reported in PANDAS. Giedd and colleagues [27, 28] found enlarged striatal volume in patients with PANDAS, similar to that seen in those with acute Sydenham's chorea. Striatal abnormalities have been reported to resolve in conjunction with symptoms, either after plasmapheresis [28] or spontaneously [29]. More recently, inflammation of the striatum has been reported in

PANDAS and Tourette syndrome patients, as measured by positron emission tomography using a marker of microglial activation [30].

The hypothesis that PANDAS derives from molecular mimicry implies the presence of antibodies in PANDAS patients that cross-react with brain antigens, as has been documented in Sydenham's chorea [12]. Indeed, such reactivity has been documented *ex vivo* [31]. Reactivity of antibodies from individuals with PANDAS against several neuronal proteins, including tubulin, lysoganglioside, and dopamine receptors, has been reported, as has antibody-mediated activation of CaM kinase II; these findings have not been consistent across all studies, perhaps due to etiological heterogeneity [11, 32–35].

The current investigation draws upon recent evidence implicating striatal interneuronal abnormalities in the pathophysiology of tic disorders. *Post mortem* analyses have identified reduced density of specific populations of striatal interneurons in patients with Tourette syndrome; these include cholinergic interneurons (CINs) and GABAergic interneurons expressing the markers parvalbumin and nNOS [36–38]. Our laboratory has shown that recapitulation of these post-mortem findings in mice, using experimental depletion of cholinergic or parvalbumin-expressing interneurons in the dorsal striatum, produces tic-like phenomenology [39–41]. This suggests that striatal interneuronal dysfunction may play a causal role in tic pathophysiology. The contribution of interneuronal pathology to OCD is less clear.

To date, no studies have investigated reactivity of antibodies from individuals with PANDAS with epitopes present on interneurons. We investigated interneuron targets of PANDAS antibody reactivity using an *in vivo* model. We infused serum from children with PANDAS into the striatum of mice and characterized the cellular targets of serum antibodies using double immunofluorescence with a panel of cell-specific markers. Samples were drawn from the treatment trial described above, NCT01281969; cases matched particularly stringent clinical criteria for PANDAS (not just PANS), including acute onset of OCD symptoms, presence of characteristic associated symptoms, and documented GABHS infection [22]. Individuals who responded clinically to IVIG treatment were selected for analysis, as responders may be most likely to have antibody-mediated pathophysiology. We report the first evidence for interneuron-reactive antibodies in the pathogenesis of PANDAS.

METHODS AND MATERIALS

Samples from patients diagnosed with PANDAS

Sera from children with PANDAS were obtained from a recent IVIG trial, NCT01281969 [22]. This randomized, double-blind trial investigated the efficacy of IVIG on PANDAS symptoms. Subjects in the clinical trial were required to meet all diagnostic criteria for PANDAS, including a positive test for beta hemolytic *Streptococcus*, and to have moderate to severe OCD symptoms, as assessed by the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS [42]). Ratings were performed by trained clinicians, blinded to the treatment status [12].

For the pilot study (Supplementary Figure 2), three children with PANDAS were selected from this cohort, without applying further selection criteria. To enhance the probability of identifying subjects with antibody-mediated pathophysiology in the main experiment (Figures 1–3), we selected subjects who had a positive clinical response to IVIG, defined as a >35% decrease in the Y-BOCS score (see Figure 4A). One subject from the pilot experiment met these additional criteria and was included in the main experiment (subject B in the pilot, #3 in the main experiment). Two subjects in the main experiment received IVIG during the blinded treatment phase; two received placebo during the blinded phase and IVIG during the subsequent open-label phase, 6 weeks later. The fifth subject received open-label IVIG infusion and follow-up on the same schedule as the others after being excluded from the trial due to anxiety associated with a lumbar puncture. Serum samples used in this analysis were obtained at baseline and at 12 weeks (time points 1 and 3 in the clinical trial; see Figure 4A). The second time point was thus either 6 (2 subjects) or 12 weeks (3 subjects) after IVIG infusion. Of the five subjects in the main experiment, two had mild tics at baseline; three had no tics.

Control subjects for the pilot experiment were drawn from healthy control samples collected at Yale. For the main experiment, well-screened healthy subjects matched to the PANDAS sample for age and gender, also evaluated at the NIMH and stored under equivalent conditions and for equivalent time, were used as controls (see Table 1). None of these healthy controls had clinically significant OCD or tic symptoms.

Serum samples were stored at −80°C until use. These investigations were approved by the Human Investigations Committees of Yale University and the National Institute of Mental Health. All subjects provided assent and parental informed consent. Samples were fully anonymized at the clinical site at NIMH before being sent to the laboratory at Yale for analysis.

Mice

All experiments were conducted under the auspices of the Yale Institutional Animal Care and Use Committee, in accordance with NIH guidelines. Adult female and male C57Bl/6 wild-type or bitransgenic BAC transgenic mice [43], aged 2–4 months, were used for all experiments; there were no significant differences between results from male and female mice. (These BAC transgenic mice were used to identify binding to D1- and D2-expressing medium spiny neurons; this staining was uninformative and is not reported here.) 16 mice were infused in the pilot experiment (2–3 mice/serum sample); 20 mice were infused in the first experiment (2 mice with each of 10 sera), and 20 were used in the follow-up treatment experiment (5 patients; 2 mice with serum from before IVIG treatment and 2 mice with serum from after IVIG treatment).

Surgery and serum infusions

Custom bilateral cannulae were manufactured to target the striatum (3.25 mm long; 4.6 mm space between guides; Plastics One, Roanoke, VA). Cannulae were chronically implanted using standard stereotaxic technique, targeting +0.50 mm anteroposterior, +/−2.3 mm mediolateral, relative to bregma [44]. Animals were anesthetized during surgery with

ketamine:xylazine 100:10 mg/kg. Guide cannulae were affixed to the skull using C&B-Metabond® Quick! Cement System (Parkell Inc.). Guide cannula patency was ensured with customized dummy cannulae that projected 0.5 mm beyond the end of the guide. Animals recovered for at least 5 days prior to first serum infusion.

Undiluted serum was infused into the striatum simultaneously in the two hemispheres at a fixed rate of 0.1μl/min through customized injectors projecting 0.5 mm beyond the end of the guide cannulae, using a PHD Ultra Pump (Harvard Apparatus).

Pilot experiments determined optimal infusion parameters. Piloting was performed with pooled human serum (Sigma: H4522). Serum was infused unilaterally, varying the volume and number of infusions and the time between the last infusion and animal sacrifice; saline was infused on the contralateral side (Supplementary Figure 1). IgG deposition was identified using immunohistochemistry with rabbit anti-human IgG (1:200 Abcam, Cambridge, MA) and visualized using diaminobenzidine (ABC Kit; Vector Laboratories, Burlingame, CA, USA). Optical density was quantified using ImageJ (NIH, Bethesda, MD, USA), as previously described [45]. Optimal IgG deposition was achieved when animals were infused for 5 consecutive days with 0.5 μl serum and then sacrificed 5 days after the last infusion; these conditions were used for all subsequent experiments.

Immunofluorescence and confocal microscopy

Mice were euthanized without perfusion. Brains were quickly dissected, fixed in 4% paraformaldehyde for 48h at 4°C, and then transferred into a 30% sucrose solution and allowed to equilibrate at 4°. Brains were sectioned at 30 μm using a cryostat; sections were stored in cryoprotectant solution (30% glycerin + 30% ethylene glycol in PBS) at −20°C until use.

Free-floating sections were washed with PBS, treated with Bloxall Solution (Vector Laboratories), washed again in PBS, incubated in 5% normal donkey serum solution in PBS + 0.3% Triton X100 for 1 hour at room temperature, and then overnight at 4° C in the same solution with rabbit anti-human IgG 1:200 (Abcam), together with goat anti-choline acetyltransferase (ChAT; 1:1000, Sigma, St. Louis, MO, USA), mouse anti-neuronal nitric oxide synthase (nNOS; 1:100, Santa Cruz Biotechnology, Dallas, TX), or mouse anti-parvalbumin (PV; 1:500; Sigma). For the anti-nNOS and anti-PV antibodies, an additional blockade with Mouse-on-Mouse (MOM) reagent (Vector Laboratories) was included to prevent reactivity of the secondary antibody with endogenous mouse immunoglobulin, following the manufacturer's instructions. Slices were washed 3X with PBS and then incubated with fluorescent secondary antibodies (1:400, Invitrogen, Carlsbad, CA, USA) for one hour at room temperature. Secondary antibodies were: for ChAT immunostatining, Alexa 555-conjugated donkey anti-rabbit and Alexa 633-conjugated donkey anti-goat; for nNOS and PV immunostaining, Alexa 555-conjugated donkey anti-rabbit and Alexa 488-conjugated donkey anti-mouse. Samples were washed again and mounted in Vectashield HardSet Mounting Medium (Vector Labs).

Confocal imaging was performed by sequential scanning of slices on an Olympus Fluoview FV-1000 confocal microscope, using a Kalman filter with an acquisition speed of 4 pixel/sec. 15 μm z-stacks were generated by using a fixed step size of 0.5 μm.

Quantification of serum antibody binding to interneurons

Two images were obtained from each slice (one per side); several slices per mouse were used to provide a representative sample of the entire mouse striatum, for a total of 8–12 images per mouse. Single- and double-positive cells were counted throughout all images, blind to experimental condition. Cell counts were averaged to produce a single value for each animal. Two mice were infused with each serum; technical variation in infusion and cell counting was very low.

Statistical analysis

Data were analyzed using Prism 6.0 (GraphPad), except for linear mixed model analysis, which was performed using SPSS 24 (IBM). The unit of analysis was the patient/serum sample, not the slice or the animal. Each data point in the final analysis averages cell counts from ~20 slices (8–12 slices from each of 2 animals).

We have examined a small number of rigorously characterized samples, rather than a larger but potentially more heterogeneous clinical group. With an N of five samples per group in the main experiment, we therefore have 80% power to detect only very large effects ($d > 1.7$). We reasoned that examination of a small, homogeneous sample would lead to smaller variance and thus to greater power than examination of a larger but more heterogeneous group.

RESULTS

Pilot study

In a small pilot study, we infused serum from three individuals with PANDAS and three controls into the striatum of adult mice. Binding to ChAT-positive interneurons was elevated after infusion of PANDAS serum, relative to controls; binding to PV-positive interneurons was unchanged (Supplementary Figure 2). These pilot data motivated a larger experiment with more carefully selected PANDAS subjects and optimally matched controls.

Serum infusion produced microgliosis and astrocytosis in the affected striatum. However, this glial activation was qualitatively equivalent after infusion of control and PANDAS serum (Supplementary Figure 3).

Main experiment: Subjects

Five subjects with PANDAS were selected from the participants in the clinical trial [22] on the basis of their robust clinical response to IVIG; one of these overlapped with the smaller group used in the pilot experiment. These subjects had no history of acute rheumatic fever or Sydenham's chorea, autism, or schizophrenia, or a history of previous immunomodulatory therapy. They were matched for age and gender with 5 healthy controls (see Table 1).

PANDAS subjects had slightly but significantly higher IgG levels at baseline than controls ($p = 0.02$).

Elevated binding of IgG from PANDAS serum to cholinergic but not GABAergic interneurons

IgG deposition onto ChAT-positive cholinergic interneurons (CINs) was seen after infusion of both PANDAS and control serum. A larger number of ChAT-positive interneurons, expressed as a fraction of total $ChAT^+$ cells, was bound by antibodies in serum collected at baseline from PANDAS patients, compared to controls (Figure 1). This increased binding had a very large effect size of $d = 8.1$. The total number of ChAT-positive interneurons was not altered by PANDAS serum infusion, indicating that this difference does not derive from a non-specific toxic effect ($t[8] = 0.89$, $p > 0.4$).

We pooled these CIN binding data with data from the pilot experiment (Supplementary Figure 2) using a linear mixed model, coding serum number as a random variable to account for the fact that one sample was included in both experiments. This confirmed a highly significant effect of diagnosis ($F[1,23] = 75.8$; $p < 0.001$).

In contrast, binding of IgG to PV^+ and $nNOS^+$ interneurons did not differ between PANDAS and control samples (Figure 2, 3). The nominal trend was towards decreased binding in both cases.

Reversal of CIN antibody deposition after IVIG treatment

These five patients were selected in part because they all improved substantially following IVIG treatment (Figure 4A). In an independent experiment, we infused sera from the same patients, collected at baseline and 6–12 weeks after IVIG treatment. Infusion was again into 2 mice per serum sample. Binding of IgG to CINs was significantly reduced in convalescent serum (Figure 4B). Improvement in CY-BOCS after treatment correlated significantly with reduction of IgG binding to cholinergic interneurons (Figure 4C).

DISCUSSION

We have identified elevated binding of serum antibodies to cholinergic interneurons (CINs) in the striatum in children with stringently defined PANDAS that responded to IVIG treatment. This represents the first time that immunoreactivity against interneurons in the striatum has been examined in PANDAS and identifies CINs as a potentially important locus of pathology in the disorder.

Several lines of evidence implicate CINs in tic disorders. In postmortem brain from patients with Tourette syndrome, ChAT-positive CINs are reduced in number by approximately 50% in the caudate and the putamen [37, 38]. Moreover, selective virus-mediated depletion of CINs in the dorsolateral striatum of mice, which reproduces the ~50% loss seen post-mortem, leads to tic-like stereotypies after either acute stress or psychostimulant challenge [39]. Mutations in the Slit and Trk-like family member 1 (SLITRK1) gene have been associated with Tourette syndrome in rare cases [46, 47]; in the adult striatum, expression of this gene is restricted to CINs [48]. Finally, expression of a number of acetylcholine-related

genes in blood has been reported to correlate with tic severity in Tourette syndrome, and these genes appear to be differentially spliced in patients [49].

No similar analyses have been reported in OCD. However, the comorbidity between TS and OCD in youth has been reported to be as high as 50% [50], and the two conditions appear to have overlapping pathophysiology [51]. Tic and OCD comorbidity is 60% in PANDAS [7].

In conjunction with our findings, this literature suggests that antibodies that recognize striatal cholinergic interneurons may have a pathogenic role in PANDAS. It is noteworthy that tics were not prominent in the subjects we characterized: two had mild tics at baseline, and three had none. Therefore, the elevated binding of IgG to CINs documented here is not specific to tic symptoms, but rather is associated with PANDAS more generally.

Elevated binding of IgG in PANDAS serum to CINs is specific: there is no differential binding to other interneuron types examined. This specificity is in contrast to post mortem evidence that both PV^+ fast spiking interneurons and the nNOS-expressing GABAergic interneurons have also been shown to be affected in adults with severe tics [36–38]. IgG levels were slightly higher in controls, which represents a weakness in our study; however, the specificity of binding increases confidence that differential binding to cholinergic interneurons is not a consequence of this or of other nonspecific abnormalities in the serum, but rather a reflection of specific populations of antibody.

Cholinergic interneurons are well positioned to regulate information processing in the basal ganglia. They differentially regulate D1- and D2-expressing medium spiny neurons through activation of metabotropic ACh receptors [52, 53] and modulate activity of GABAergic interneurons [54]. Cholinergic dysregulation in the dorsal striatum may thus produce an imbalance between the direct and indirect pathways through the basal ganglia, leading to disinhibition of off-target behaviors [55, 56]. Our previous studies of the consequences of cholinergic interneuron depletion *in vivo* show that functional perturbation of these cells can produce relevant phenomenology [39]. We hypothesize that antibody deposition on cholinergic interneurons leads to functional abnormalities, and thereby to PANDAS symptomatology. How antibody binding to these cells might perturb their function, however, remains unclear. Cholinergic interneurons express D2 dopamine receptors, and antibodies in PANDAS have been reported to bind to these receptors in some studies [32]{Morris-Berry, 2013 #39;Cox, 2013 #37}; this represents one possible molecular target that might explain the preferential binding to these interneurons seen here. *In vitro*, antibodies from PANDAS patients can activate calcium-calmodulin kinase-II in immortalized human neuron-like cells [11, 33, 35]. However, this has not been demonstrated in cholinergic cells.

Our model rests on the assumption that antibodies that recognize cholinergic interneurons in mice will have similar binding in the human brain; this is a limitation. An additional limitation is that binding to tissue does not permit ready identification of the molecular targets to which PANDAS antibodies are binding; this is an important focus of future work. However, the approach employed here has unique advantages. While inflammation and microglial activation can be visualized *in vivo* in humans [30], IgG deposition cannot, and cell-type specificity of binding is difficult to characterize. Analysis of post-mortem tissue

can be used to characterize abnormalities in chronic illness, and post-mortem analysis of individuals with severe TS has revealed microglial activation [38]; but tissue for post-mortem analysis is scarce and often heterogeneous, and repeated characterization after treatment, such as we present here, is obviously not possible. Furthermore, the use of human post-mortem tissue to evaluate autoantibodies in human serum samples is limited by numerous technical challenges, including interference from formaldehyde tissue preservatives and non-specific brain IgG deposition. Thus, the use of murine brain tissue for the characterization of human anti-brain antibodies may represent an important “first-pass” tool for antibody identification. A similar approach has been used, with success, in the identification of antibodies in autoimmune encephalitis, particularly anti-NMDA receptor encephalitis [57]. An additional advantage is that we can investigate binding by cell type, rather than molecular target, which may be a more sensitive approach if antibody targets are heterogeneous.

IVIG treatment has been reported to ameliorate symptoms in PANDAS in some cases [20–22]. In the recent clinical trial from which our clinical samples are drawn, patients who received blinded IVIG treatment did not separate from controls in the primary analysis of the blinded phase, but a majority of patients responded after receiving IVIG during either the blinded or open-label phase [22]. Because our goal here was to identify potential pathophysiological mechanisms, and not to establish characteristics of the full clinical cohort, we selected subjects who demonstrated a robust clinical response to IVIG. This clinical response was paralleled by reduced binding of antibodies to striatal cholinergic interneurons. This correlation supports the conclusion that IVIG may produce clinical response by reducing pathogenic antibodies. Similar declines in antibody reactivity have been reported in convalescent serum from PANDAS [15] and Sydenham’s chorea [12], though not previously to our knowledge after IVIG treatment.

These results do not exclude the possibility of other pathogenic cellular targets in PANDAS serum. PANDAS is likely to be etiologically heterogeneous. D1- and D2-expressing medium spiny neurons represent the majority of striatal neurons; and several studies have suggested that they may be targeted by autoantibodies in PANDAS, in at least some cases [32–35].

In addition, our results do not establish that this abnormality is unique to PANDAS. We have examined a small number of extensively characterized patients, selected so as to maximize the *a priori* likelihood of identifying antibody-mediated pathophysiology. Future examination of larger cohorts and a more diverse set of patients, including individuals with Tourette syndrome, non-PANDAS pediatric-onset OCD, and Sydenham’s chorea would better demarcate the clinical correlates of antibody reactivity against CINs and might reveal different patterns of antibody deposition in distinct populations of interneurons in other groups of patients. The approach described here is extremely laborious; now that the CINs have been identified as a cellular target of interest, it may be possible to do more focused analyses in these broader clinical cohorts using *ex vivo* methods.

In summary, our *in vivo* approach to characterizing antibody reactivity in patients with PANDAS has identified a novel candidate pathophysiology: specific autoantibody binding to striatal cholinergic interneurons. This focus on cholinergic interneurons fits well with the

developing appreciation of the role of these cells in tic disorders. Identification of the specific antigens on these cells and the functional consequences of antibody binding may open new avenues for the understanding and treatment of PANDAS and related conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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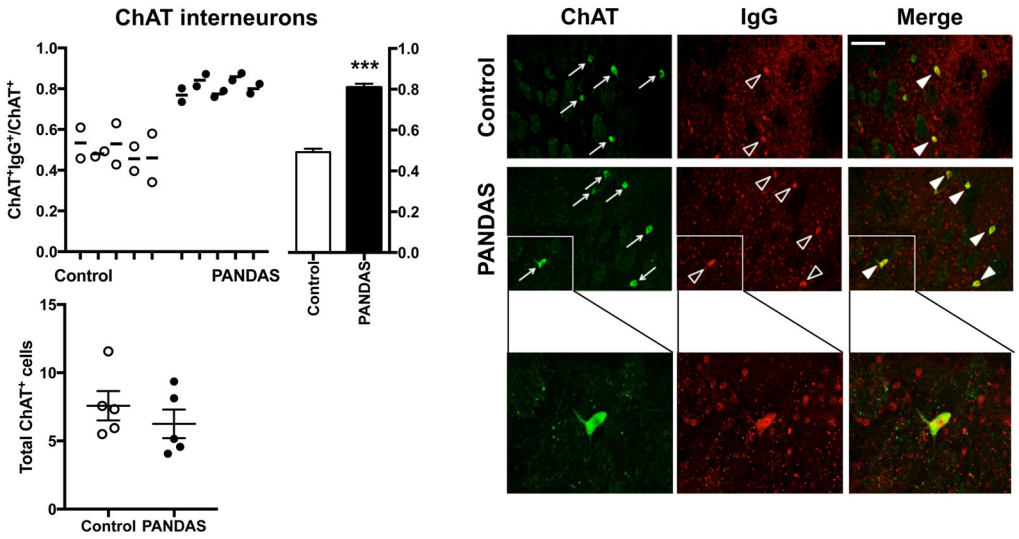

Figure 1. PANDAS autoantibodies differentially bind to ChAT interneurons in the basal ganglia

Deposition of antibodies from control and PANDAS sera (collected at baseline from highly symptomatic patients) onto ChAT-positive interneurons (CINs) *in vivo* was evaluated by double immunofluorescent staining and confocal microscopy, using anti-human IgG (red) and anti-ChAT antibodies (green). Antibodies from both control and PANDAS serum were deposited onto CINs, but a significantly larger number of $ChAT^{+}IgG^{+}$ cells (arrowheads), as a fraction of total $ChAT^{+}$ cells, was observed in PANDAS samples, compared to healthy controls (N=5 samples per group, 2 mice per sample; two-tailed t-test: $t[8] = 12.90$; $p < 0.0001$). Insets illustrate binding to a single $ChAT^{+}$ cell at higher magnification. Scale bar = 100 μm.

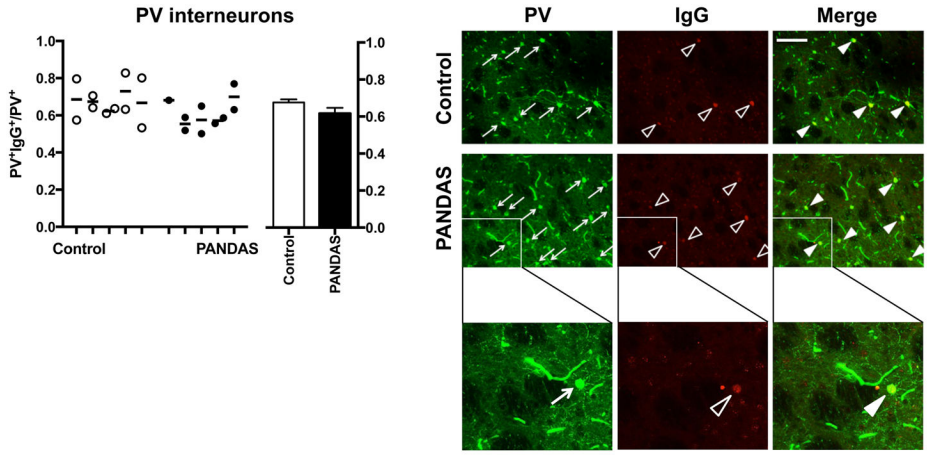

Figure 2. PANDAS autoantibodies do not show elevated binding to parvalbumin-positive GABAergic interneurons

No differences between PANDAS and control serum in antibody deposition onto PV-positive interneurons were observed (two-tailed t-test: t[8] = 1.7, p = 0.13). Scale bar = 100 μm.

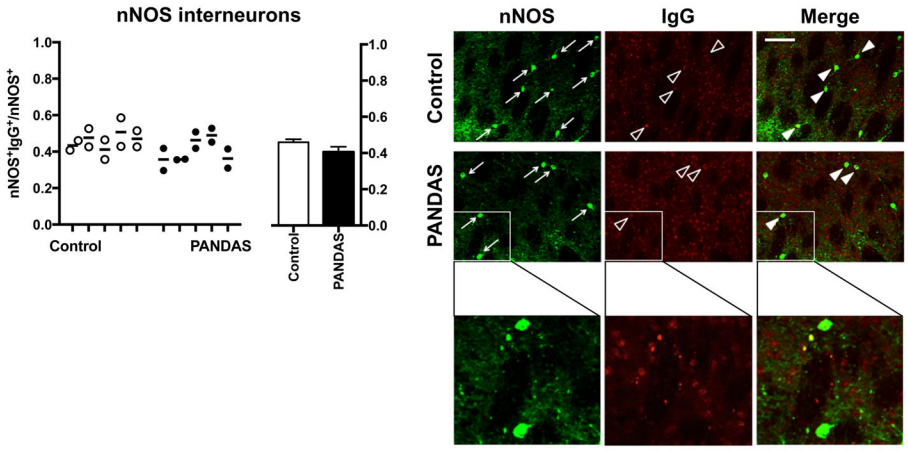

Figure 3. PANDAS autoantibodies do not show elevated binding to nNOS-positive GABAergic interneurons

No differences between PANDAS and control serum in antibody deposition onto nNOS interneurons were observed (two-tailed t-test: $t[8] = 1.6$, $p = 0.15$). Scale bar = 100 μm.

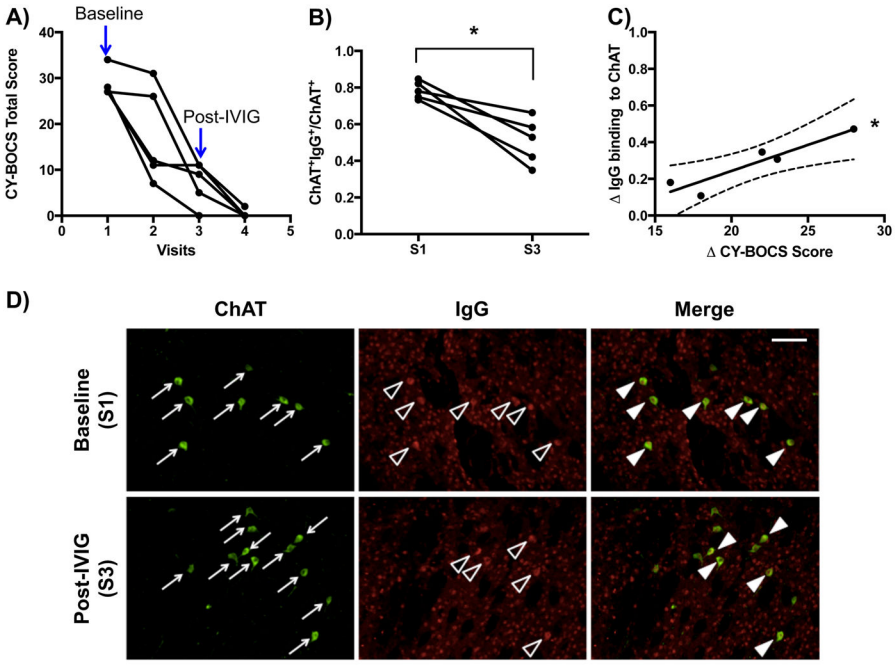

Figure 4. Resolution of elevated ChAT binding in post-IVIG serum

A. These patients all showed a significant clinical response to IVIG treatment. Individual patients' changes in the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) score across four evaluation sessions are shown (see also Table 1). Antibody binding to interneurons was assayed at baseline (Visit 1; Figures 1–3) and 12 weeks later (Visit 3). **B.** IgG binding to $ChAT^+$ interneurons was markedly reduced in serum collected from all five PANDAS patients after IVIG treatment (evaluation 3), relative to baseline (paired t-test: $t[4] = 4.4$; $p = 0.012$). **C.** Change in IgG binding to CINs correlated significantly with improvement in CY-BOCS after IVIG treatment ($r^2 = 0.86$, $p = 0.023$). Scale bar = 100 μm.

Table 1

Subjects

Patients were selected from the larger group who participated in a clinical trial of IVIG for the treatment of PANDAS [11], on the basis of clinical response to IVIG treatment and positive ANA at baseline. Healthy controls were previously screened by the same clinical site, at the NIMH.

Subjects	SEX	AGE (at trial initiation)	RACE/ETHNICITY	TIME OF IVIG (weeks)	Baseline CY-BOCS	Week 12 CY-BOCS	Baseline IgG (mg/dL)	ANA prior to IVIG
PANDAS								
1	F	9.5 y	M/NH	6	27	5	1250	+
2	F	8.9 y	W/NH	0	28	0	898	+
3	F	6.7 y	W/NH	0	27	11	1160	+
4	M	8.2 y	W/NH	6	34	11	1120	+
5	M	7.5 y	W/NH	0*	27	9	1200	+
CONTROL								
1	F	5.1 y	W/NH	-	-	-	896	+
2	F	8.0 y	W/NH	-	-	-	963	+
3	M	8.1 y	M/NH	-	-	-	768	−
4	M	7.8 y	M/H	-	-	-	877	−
5	F	7.3 y	W/NH	-	-	-	1040	−
P-value		0.26					0.02	NS

Race/ethnicity (parental report): M - mixed; W - white; H – Hispanic or Latino; NH – not Hispanic or Latino. Patients received a single IVIG treatment, either during the blinded clinical trial (at 0 weeks) or during a subsequent open-label phase (at 6 weeks).

* This patient was not randomized due to an unrelated adverse event (anxiety during a lumbar puncture) but was treated open-label with IVIG and followed up on the same schedule as those who were randomized.

The PANDAS Physicians Network (PPN) flowcharts for diagnosis and treatment will help clinicians evaluate their patients and determine the best course of treatment. Guidelines and workflows were approved by practitioners of the PANDAS Physicians Network Scientific Advisory Board. More detailed resources are available at www.pandasppn.org. Diagnosing and treating should be done by a licensed healthcare provider.

Primary care providers play important, ongoing roles in the diagnosis, treatment, and recovery of children with PANS/PANDAS. Children with a moderate or severe/life threatening onset or a complex presentation may require treatment by an experienced multi-disciplinary team of specialists or a PANS/PANDAS specialist. Additional resources can be found at www.pandasppn.org/practitioners.

References:

PANDAS Physicians Network website:
www.pandasppn.org

Seeing Your First Child with PANDAS/PANS:
www.pandasppn.org/seeingyourfirstchild

Treatment providers:
www.pandasppn.org/practitioners

PANS/PANDAS Diagnostic Flowchart

Experiencing an acute and dramatic onset of obsessions and compulsions

Experiencing an acute and dramatic onset of eating restrictions[1]

Experiencing an acute and abrupt onset of motor and/or vocal tics

1. Take a careful history of symptoms and clinical course.
2. Check for Group A Beta-Hemolytic Streptococcus (GABHS) with throat culture with 2 swabs.

Was rapid positive?

Yes → Was patient pre-pubescent at time of initial abrupt onset?

Yes → Evaluate if there are **at least 2** concurrent symptoms with similarly abrupt and severe onset from the yellow COMORBID SYMPTOM BOX.

No → 1. Send 2nd swab for 72 hour culture.
2. Order ASO and AntiD-Nase-B.
3. Check if history of GABHS infection or exposure to GABHS through siblings, parents, or close contacts.

Positive for GABHS?

Yes → Was rapid positive? (Yes)

No → Are tics primary symptom?

Yes → Evaluate for a tic disorder.

No → Evaluate if there are **at least 2** concurrent symptoms with similarly abrupt and severe onset from the yellow COMORBID SYMPTOM BOX.

Are 2+ comorbid symptoms present?

No → Possible OCD or ARFID. Assign individual diagnosis and treat as appropriate.

Yes → Order CBC with differential to check for other infections, check anti-nuclear antibody titers, and order quantitative immunoglobulins (IgG, IgA, IgM, IgE) with IgG subclasses.[3]

Determine if symptoms are better explained by a known neurological or medical disorder.

Yes → Likely not PANS. Perform appropriate clinical evaluation (e.g., MRI or other blood work) and treat condition.

No → PANS

Are 2+ comorbid symptoms present?

Yes → PANS/PANDAS

No → Provisional PANDAS[2]

Determine mild, moderate or severe/extreme case based on the severity of primary symptoms and concurrent symptoms. Treat per appropriate PANS/PANDAS treatment chart.

COMORBID SYMPTOM BOX

Evaluate if there is a concurrent presence of additional neuropsychiatric symptoms with similarly abrupt and severe onset from **at least 2** of the following categories:

1. Elevated anxiety or separation anxiety
2. Emotional lability and/or depression
3. Irritability, aggression, and/or severe oppositional behaviors
4. Behavioral (developmental) regression
5. Sudden deterioration in school performance (dramatic handwriting changes, decline in orthographic memory, and/or math difficulties)
6. Motor (including tics) or sensory abnormalities
7. Somatic signs and symptoms, including sleep disturbances, enuresis, or urinary frequency

MILD CASE
Symptoms are significant and cause disruptions at home and/or school. They occupy a few hours a day.

MODERATE CASE
Symptoms are distressing and interfere with daily activities. They occupy 50%–70% of waking hours.

SEVERE/EXTREME CASE
Symptoms are incapacitating, life threatening, or occupy 71%–100% of waking hours.

[1] If your patient is experiencing severe restrictive eating and/or ARFID (Avoidant/Restrictive Food Intake Disorder), determine if hospitalization is needed due to dramatic weight loss or if intravenous hydration is required.
[2] An official diagnosis of PANDAS includes an episodic course; however delaying treatment until a second onset is not recommended.
[3] PANS does not exclude the possibility that the patient has or had strep. Approximately 35% of pediatric patients will not generate ASO or Anti-DnaseB titers and therefore can be a false negative for strep (Chet 2003). Throat cultures are only reliable to the extent of the rigor and approach of the practitioner and both vary greatly.

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PANS/PANDAS Treatment Guidelines: Mild Case

Mild: Symptoms are significant and cause disruptions at home and/or school. They occupy a few hours a day.

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Initial evaluation and treatment

1. Perform a comprehensive laboratory and clinical evaluation.
2. Look for infections (Throat swab/culture child and family members for strep, check for exposure to Group A Streptococcus through close contacts, inquire about perianal redness or itching which may indicate perianal strep, and check for mycoplasma or other infections, e.g., yeast).
3. While waiting for lab results:
   a. Prescribe 14 days of antibiotics (Penicillin/amoxicillin,[1] azithromycin, cefdinir, Augmentin, or others).
   b. Consider a 5-7 day course of NSAIDs at immunomodulatory dose for 24 hour coverage.[2] (see resource page)
   c. Ensure the family has access to CBT/ERP (Cognitive Behavior Therapy/Exposure and Response Prevention) and parent support.
4. Schedule a follow up appointment.

First follow-up assessment

If significant improvement:

1. No further intervention is needed at this time.
2. Schedule a follow-up appointment within 30 days (or earlier if symptoms return).

If no improvement:

1. Look again for infection (i.e., swab/culture child and family members, check for mycoplasma or other infections).
2. Check for sinusitis and consider a perianal strep swab.
3. Consider changing antibiotic (change to azithromycin, cefdinir, or Augmentin).
4. Consider a 5 day prednisone burst[3] or extend course of immunomodulatory dose of a NSAID.[2] (see resource page)
5. Ensure the family has access to CBT/ERP. If the child is not able to engage in CBT/ERP due to the severity of symptoms, learning parent management techniques may be beneficial for the family.
6. Consider a referral with a psychiatrist to help with symptom management.
7. Schedule a follow-up appointment.

Second follow-up assessment

If there was significant improvement between visits, but active symptoms:

1. Recheck for active infection and exposure from siblings, parents, and close contacts.
2. Restart antibiotics for 14 days and schedule a follow up appointment.
3. If child has 2+ recurrences, consider prophylactic antibiotics.[1] (see resource page)

If no improvement:

1. Consider a 5 day prednisone burst[3] or extend course of immunomodulatory dose of a NSAID.[2] (see resource page)
2. Determine if symptoms have worsened to the point of being moderate or severe/life threatening.
3. Ensure the family has access to CBT/ERP. If the child is not able to engage in CBT/ERP due to the severity of symptoms, learning parent management techniques may be beneficial for the family.
4. Consider a referral with a psychiatrist to help with symptom management.
5. Re-evaluate for possible alternative diagnosis.

PANS/PANDAS Treatment Guidelines: Moderate Case

Moderate: Symptoms are distressing and interfere with daily activities. They occupy 50%–70% of waking hours.

Initial evaluation and treatment

1. Perform comprehensive laboratory and clinical evaluation.
2. Look for infections (Throat swab/culture child and family members for strep, check for exposure to Group A Streptococcus through close contacts, inquire about perianal redness or itching which may indicate perianal strep, and check for mycoplasma or other infections, e.g., yeast).
3. Perform additional laboratory testing to rule out other conditions and guide treatment.
4. While waiting for lab results:
   a. Prescribe antibiotics (Penicillin/amoxicillin,[1] azithromycin, cefdinir, Augmentin, or others). Consider an initial 3-4 week course.
   b. Prescribe a prednisone burst[3] or a 5-7 day course of NSAIDs at immunomodulatory dose.[2] (see resource page)
   c. Ensure the family has access to CBT/ERP. If the child is not able to engage in CBT/ERP due to the severity of symptoms, learning parent management techniques may be beneficial for the family.
   d. Consider a referral with a psychiatrist to help with symptom management.
5. Schedule a follow-up appointment.

First follow-up assessment

If significant improvement:

1. Schedule another follow-up appointment and monitor for recurrence.

If no improvement:

1. Prescribe alternate antibiotic (change to azithromycin, cefdinir, or Augmentin).
2. Check for sinusitis and consider a perianal strep swab.
3. If not tried, prescribe a 5 day prednisone burst[3] or 6 weeks of a NSAID at immunomodulatory dose.[2] (see resource page)
4. Consider MRI and EEG study.
5. Consider checking antinuclear antibody titers, cross-reactive antineuronal antibodies, and CaM Kinase II activation.[4] (see resource page)
6. Schedule a follow-up appointment.

Second follow-up assessment

If no current symptoms:

1. Continue to monitor for subsequent exacerbations.
2. Advise parents to continue with CBT/ERP.
3. If child has 2+ recurrences, consider prophylactic antibiotics.[1] (see resource page)

If active symptoms, but significant improvement between visits:

1. Recheck for active infection and exposure from siblings, parents, and close contacts.
2. Restart antibiotics for 14 days and schedule follow-up appointment.
3. Consider 30 days of prednisone with taper.[3] Continue antibiotics while the patient is taking prednisone. (see resource page)
4. Schedule IVIG (1.5-2g/kg over 2 days) and return visit 30 days post IVIG. Continue a treatment dose of antibiotics until IVIG treatment is completed. Prescribe prophylactic antibiotics post IVIG treatment.[1] (see resource page)
5. Confirm the child is receiving CBT/ERP and/or psychiatric care; and discuss expectations of clinical outcomes.

If no improvement:

1. Determine if symptoms have worsened to the point of being severe or life threatening.
2. Consider an emergency referral with a psychiatrist to help with symptom management.
3. Continue antibiotics. Total duration of antibiotic treatment is at least 30 days.
4. Try 30 days of prednisone with taper.[3] Continue antibiotics while the patient is taking prednisone. (see resource page)
5. Refer patient to ENT for evaluation of tonsils and adenoids.
6. Schedule IVIG (1.5-2g/kg over 2 days) and return visit in 30 days post IVIG. Continue a treatment dose of antibiotics until IVIG treatment is completed. Prescribe prophylactic antibiotics post IVIG treatment.[4] (see resource page)
7. Confirm the child is receiving CBT/ERP and/or psychiatric care; and discuss expectations of clinical outcomes.

Post IVIG appointment

If substantial improvement or fully improved:

1. Advise parents to continue with CBT/ERP for any residual symptoms.
2. Prescribe long-term prophylactic antibiotics post IVIG treatment.[1] (see resource page)

If modest improvement:

1. Advise parents to continue with CBT/ERP.
2. Prescribe long-term prophylactic antibiotics post IVIG treatment.[1] (see resource page)
3. Continue to monitor for symptom flares.

If no improvement:

1. Re-run diagnostic tests and determine if symptoms have worsened to the point of being severe or life threatening.
2. Consider a second IVIG or a referral to a center to evaluate for plasmapheresis. Prescribe prophylactic antibiotics while coordinating care.[1] (see resource page)
3. Advise parents to continue with CBT/ERP.
4. Re-evaluate for possible alternative diagnosis.

PANS/PANDAS Treatment Guidelines: Severe/Extreme Case

Severe/Extreme: Symptoms are incapacitating, life threatening, or occupy 71%–100% of waking hours.

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A Division of The Foundation for Brain Science and Immunology

Initial evaluation and treatment

1. Perform comprehensive laboratory and clinical evaluation.
   a. Look for infections (Throat swab/culture child and family members for strep, check for exposure to Group A Streptococcus through close contacts, inquire about perianal redness or itching which may indicate perianal strep, and check for mycoplasma or other infections, e.g., yeast).
   b. Order additional laboratory testing to rule out other conditions and guide treatment.
   c. For patients experiencing ARFID (Avoidant/Restrictive Food Intake Disorder), evaluate to determine if hospitalization is needed due to dramatic weight loss or if intravenous hydration is required.
2. Prescribe a prednisone burst[3] or a 5-7 day course of NSAIDs at immunomodulatory dose.[2] (see resource page)
3. Begin scheduling for IVIG (1.5-2g/kg over 2 days).
4. Prescribe antibiotics (Penicillin/amoxicillin,[1] azithromycin, cefdinir, Augmentin, or others). Consider an initial 3-4 week course.
5. Consider a referral with a psychiatrist to help with symptom management.
6. Schedule telephone check-in and schedule a follow-up visit.

Phone check-in

Monitor progress approximately 5 days after initial evaluation via a phone check-in.

If significant improvement:
1. Ensure the family has access to CBT/ERP. If the child is not able to engage in CBT/ERP due to the severity of symptoms, learning parent management techniques may be beneficial for the family.
2. Confirm a follow-up visit is scheduled.

If no improvement at phone check-in:
1. Consider prolonged steroids (30 days) with taper.[3] Continue antibiotics while the patient is taking steroids. (see resource page)
2. Consider switching antibiotics (to azithromycin, cefdinir, or Augmentin).
3. Refer patient to ENT for evaluation of tonsils and adenoids.
4. Consider proceeding with IVIG treatment (1.5-2g/kg over 2 days).
5. Based on the situation/safety, consider inpatient hospitalization or a center specializing in neuroimmune disorders.
6. Confirm a follow-up visit is scheduled.

Follow-up assessment

If significant improvement:
1. Consider long-term prophylactic antibiotics[1]. (see resource page)
2. If NSAIDs were prescribed, continue at immunomodulatory dose for a total of 6-8 weeks.[2] (A Proton Pump Inhibitor (PPI), such as omeprazole, lansoprazole, pantoprazole, or oresomeprazole, should be considered at prescribed dosages throughout the course of NSAIDs to prevent GI complications.) (see resource page)
3. If improved, but not back to baseline, schedule IVIG (1.5-2g/kg over 2 days) and follow-up visit at 30 days. Continue a treatment dose of antibiotics until IVIG treatment is completed. Prescribe prophylactic antibiotics post IVIG treatment.[1] (see resource page)

If no improvement:
1. Change antibiotic (to azithromycin, cefdinir, or Augmentin). Total duration of antibiotic treatment is at least 30 days.
2. Check for sinusitis and consider a perianal strep swab.
3. Prescribe prolonged steroid (30 days) with taper.[3] Continue antibiotics while the patient is taking steroids. (see resource page)
4. Consider checking antinuclear antibody titers, cross-reactive antineuronal antibodies, and CaM Kinase II activation.[4] (see resource page)
5. Based on situation/safety refer for inpatient help or a center specializing in neuroimmune disorders.
6. Schedule IVIG (1.5-2g/kg over 2 days) and follow-up visit at 30 days. Continue a treatment dose of antibiotics until IVIG treatment is completed. Prescribe prophylactic antibiotics post IVIG treatment.[1] (see resource page)

Post IVIG appointment

If fully improved:
1. Refer to a PANS knowledgeable therapist for CBT/ERP treatment for residual symptoms and for managing flare-ups.
2. Prescribe long-term prophylactic antibiotics post IVIG treatment.[1] (see resource page)

If modest improvement (reduction of <50% of symptoms):
1. Refer to a PANS knowledgeable therapist for CBT/ERP for treatment for residual symptoms.
2. Prescribe long-term prophylactic antibiotics post IVIG treatment.[4] (see resource page)
3. Consider scheduling second IVIG for 90 days from initial IVIG.

If no improvement:
1. Consider a referral to a center for plasmapheresis. Prescribe prophylactic antibiotics while coordinating care.[1] (see resource page)
2. Consider other treatment options (see text on www.pandasppn.org).
3. Refer to a center specializing in neuroimmune disorders.
4. Re-evaluate for possible alternative diagnosis.

PANS: Pediatric Acute-onset Neuropsychiatric Syndrome
PANDAS: Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections
CBT: Cognitive Behavioral Therapy
ERP: Exposure Response Prevention
NSAID: Nonsteroidal anti-inflammatory drugs
IVIG: Intravenous immunoglobulin
PEX: Plasmapheresis / Plasma Exchange

Reference: Journal of Child & Adolescent Psychopharmacology. Sept 2017. 27(7)
pandasppn.org/jcap2017

[1] Bacteriologic and clinical treatment failures can occur with any antibiotic (i.e., The failure rate for penicillin therapy for strep is approximately 30%.*). If your patient is prescribed a prophylactic antibiotic and experiencing a subsequent onset of neuropsychiatric symptoms, consider the possibility of breakthrough strep. *Pichichero ME et al. Penicillin failure in streptococcal tonsillopharyngitis: causes and remedies, Pediatr Infect Dis J.2000 Sep;19(9):917-23.

[2] NSAID dosing can be found in Clinical Management of Pediatric Acute-Onset Neuropsychiatric Syndrome: Part II—Use of Immunomodulatory Therapies, Appendix Table A1. Use of Nonsteroidal Anti-Inflammatory Drugs in Pediatric Acute-Onset Neuropsychiatric Syndrome https://www.liebertpub.com/doi/full/10.1089/cap.2016.0148. A Proton Pump Inhibitor (PPI), such as omeprazole, lansoprazole, pantoprazole, or oresomeprazole, should be considered at prescribed dosages throughout the course of NSAIDs to prevent GI complications. Ensure proper hydration during the course of NSAIDs.

[3] Long term corticosteroids are contraindicated for some patients. Screen for TB, Lyme, parasites, and fungi before prescribing. If patient responds to a 5 day steroid burst, this indicates an inflammatory/autoimmune process. Lack of significant improvement does not dismiss the possible correlation.

[4] Chain JL, et al. (2020) Autoantibody Biomarkers for Basal Ganglia Encephalitis in Sydenham Chorea and Pediatric Autoimmune Neuropsychiatric Disorder Associated With Streptococcal Infections. Front. Psychiatry 11:564. doi: 10.3389/fpsyt.2020.00564. For testing information, visit www.moleculeralabs.com.

Guidelines and workflows were approved by practitioners of the PANDAS Physicians Network Scientific Advisory Board. The diagnostic flowchart and treatment guidelines should be considered "living documents" that are open for revisions and updates as new research is published.

WAC 284-43-5440 Medical necessity determination. (1) An issuer's certificate of coverage and the summary of coverage for the health benefit plan must specifically explain any uniformly applied limitation on the scope, visit number or duration of a benefit, and state whether the uniform limitation is subject to adjustment based on the specific treatment requirements of the patient.

(2) An issuer's medical necessity determination process must:

(a) Be clearly explained in the certificate of coverage, plan document, or contract for health benefit coverage;

(b) Be conducted fairly, and with transparency to enrollees and providers, at a minimum when an enrollee or their representative appeals or seeks review of an adverse benefit determination;

(c) Include consideration of services that are a logical next step in reasonable care if they are appropriate for the patient;

(d) Identify the information needed in the decision-making process and incorporate appropriate outcomes within a developmental framework;

(e) Ensure that when the interpretation of the medical purpose of interventions is part of the medical necessity decision making, the interpretation standard can be explained in writing to an enrollee and providers, and is broad enough to address any of the services encompassed in the ten essential health benefits categories of care;

(f) Comply with inclusion of the ten essential health benefits categories;

(g) Not discriminate based on age, present or predicted disability, expected length of life, degree of medical dependency, quality of life or other health conditions, race, gender, national origin, sexual orientation and gender identity;

(h) Include consideration of the treating provider's clinical judgment and recommendations regarding the medical purpose of the requested service, and the extent to which the service is likely to produce incremental health benefits for the enrollee;

(i) Identify by role who will participate in the issuer's medical necessity decision-making process; and

(j) Ensure that where medically appropriate, and consistent with the health benefit plan's contract terms, an enrollee is not unreasonably restricted as to the site of service delivery.

(3) An issuer's medical necessity determination process may include, but is not limited to, evaluation of the effectiveness and benefit of a service for the individual patient based on scientific evidence considerations, up-to-date and consistent professional standards of care, convincing expert opinion and a comparison to alternative interventions, including no interventions. Cost effectiveness may be one of but not the sole criteria for determining medical necessity.

(4) Within thirty days of receiving a request, an issuer must furnish its medical necessity criteria for medical/surgical benefits and mental health/substance use disorder benefits or for other essential health benefit categories to an enrollee or provider.

[WSR 16-01-081, recodified as § 284-43-5440, filed 12/14/15, effective 12/14/15. Statutory Authority: RCW 48.02.060, 48.21.241, 48.21.320, 48.44.050, 48.44.341, 48.44.460, 48.46.200, 48.46.291, 48.46.530, 48.43.715, and Pub. L. No. 111-148, 124 Stat. 119 (Mar. 23, 2010) (PPACA), as amended by the Health Care and Education Reconciliation Act (HCERA), Pub. L. No. 111-152, 124 Stat. 1029 (Mar. 30, 2010), in particular § 1302 of PPACA, § 10104 (b)(1) (HCERA). WSR 13-15-025

(Matter No. R 2012-17), § 284-43-860, filed 7/9/13, effective 7/10/13.]

April 14, 2019
HB 2511:PANDAS/PANS Health Care Access and Awareness

Dear Oregon Legislature,
I am a physician who is also the parent of a child with PANDAS. I am writing in support of HB 2511 which requires health benefit plans, health care service contracts, and medical assistance coverage of PANDAS/PANS. It also requires the Oregon Health Authority to conduct an education and outreach campaign in collaboration with an advisory council. Increased awareness of and coverage for treatment of PANS/PANDAS is certainly needed. As a family physician with medical education and extensive resources, it took nine months to diagnose my daughter with PANDAS, despite her classic presentation. She was evaluated by four physicians and I spoke to numerous other medical colleagues. We also sought diagnostic evaluations from providers not covered by our insurance benefits, including a naturopathic physician, an acupuncturist, and an out of network psychiatrist. Finally her psychologist reached out in desperation to her listserv trying to find some guidance. Once the suggestion of PANDAS was made, we were able to quickly initiate appropriate treatment, resulting in my daughter's excellent response and recovery. However, she and my family suffered for nine unnecessary months due to lack of awareness. We are grateful to have had the resources, but this is not the case for most families.

Increased PANDAS education is needed to be able to quickly make this diagnosis. Also health benefit coverage is needed. In my daughter's case, only continued antibiotics with inexpensive penicillin is needed, however a vast majority of children with PANDAS require IVIG and/ or plasmapheresis for definitive care and recovery. These treatments are expensive and frequently not covered by insurance. Children forgo treatment while parents often must leave their employment to care for children who are severely debilitated. The economic loss both personally and as a society can be devastating.

Vast resources are wasted as children with PANS/PANDAS are inappropriately treated with psychiatric medications while a phantom mental illness is chased, when in reality following the treatment guidelines, which includes use of immunomodulatory IVIG and sometimes plasmapheresis, can result in cure. Money is saved and young lives are improved when the correct treatment is given. Children treated properly for PANS/PANDAS can recover, succeed in school, and become contributing members of society. In our country, where the numbers of children on psychiatric drugs and disability rolls continue to climb, it is more important than ever to identify treatable root causes. A suffering child does not have to be labeled mentally ill and potentially never become gainfully employed. By appropriately assisting families and ensuring that the necessary treatments are covered, the symptoms of debilitating anxiety and OCD can be cured. However, this treatment cannot and does not happen unless PANS/PANDAS is widely recognized and covered by health care benefits. This bill will improve and even save lives for Oregon's youth. Please give HR 2511 your supportive vote.

Respectfully,

Tricia T Williams, MD
366 Windy Owl Lane
Troy, PA 16947

International OCD Foundation

January 15, 2021

The Honorable Rachel Prusak, RN, FNP
Oregon State Legislature
900 Court St. NE, H-489
Salem, Oregon 97301

Dear Representative Prusak,

I write to you as the executive director of the International OCD Foundation (IOCDF), the nation's largest organization solely dedicated to advocating for everyone affected by obsessive compulsive disorder (OCD) and related disorders, including pediatric autoimmune neuropsychiatric disorders (PANDAS/PANS). The IOCDF wishes to convey our endorsement of HB 2390, which would extend insurance coverage to include the full range of evidence-based PANDAS/PANS treatments.

While we have known for many years that children can develop OCD, PANDAS/PANS has only recently been distinguished as an illness that is unique from more typical cases of childhood-onset OCD. Researchers believe PANDAS/PANS is caused by a malfunctioning immune system that mistakenly attacks the brain instead of an infection. Unlike in typical OCD, where symptoms set in gradually over months or even years, symptoms in PANDAS/PANS appear rapidly following an infection (e.g., strep throat).

The symptoms of PANDAS/PANS resemble severe OCD and tic disorders. Affected children may become extremely anxious, act out in disruptive ways, or be unable to eat. The speed with which children develop these symptoms — seemingly overnight — is truly frightening to parents, and the intense needs of a child with PANDAS/PANS can leave families reeling. When left untreated, PANDAS/PANS can be permanently disabling or even fatal.

Treatments that work for children with OCD may not necessarily work for children with PANDAS/PANS. Thankfully, there are several therapies that scientific research has shown to be effective: cognitive behavioral therapy, antibiotics, plasmapheresis (TPE), and intravenous immunoglobulin therapy (IVIG) are among the best treatments currently available for PANDAS/PANS.

In mild and moderate cases of PANDAS/PANS, cognitive behavioral therapy and antibiotics are the recommended treatments. IVIG is recommended in moderate and severe cases, per treatment guidelines published in 2017. The use of IVIG to treat PANDAS/PANS is supported by the results of multiple double-blind, placebo-controlled studies and numerous case reports., In one study, the children who received IVIG treatment experienced a 45% reduction in symptoms, and the vast majority maintained their symptom improvements when researchers followed up with them one year after treatment. TPE is also supported by research and was

shown to reduce symptoms by 58%. However, it is a riskier and more costly treatment and is recommended only in extreme or life-threatening cases.

Out-of-pocket costs for IVIG or TPE are significant: IVIG can cost up to $15,000 and TPE is even more expensive. The average family will struggle to afford the right treatment for a child with moderate to severe PANDAS/PANS if their insurer does not provide coverage. By mandating coverage for the full spectrum of available evidence-based treatments, HB 2390 will ensure that every Oregon child with PANDAS/PANS has a chance at recovery.

On behalf of our members and all Oregon families who are caring for a child with PANDAS/PANS, we respectfully ask the Oregon Legislative Assembly to pass this bill. Should you or any other Assembly members have questions about PANDAS/PANS or its treatments, please do not hesitate to contact us.

Sincerely,

Jeff Szymanski, PhD
Executive Director
International OCD Foundation

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[1] Frankovich, J., Swedo, S., Murphy, T., Dale, R. C., Agalliu, D., Williams, K., ... & Muscal, E. (2017). Clinical management of pediatric acute-onset neuropsychiatric syndrome: part II—use of immunomodulatory therapies. *Journal of child and adolescent psychopharmacology*, *27*(7), 574-593.

[2] Perlmutter, S. J., Leitman, S. F., Garvey, M. A., Hamburger, S., Feldman, E., Leonard, H. L., & Swedo, S. E. (1999). Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *The Lancet*, *354*(9185), 1153-1158.

[3] Williams, K. A., Swedo, S. E., Farmer, C. A., Grantz, H., Grant, P. J., D'Souza, P., ... & Leckman, J. F. (2016). Randomized, controlled trial of intravenous immunoglobulin for pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Journal of the American Academy of Child & Adolescent Psychiatry*, *55*(10), 860-867.

International OCD Foundation

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Oregon State Legislature
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On behalf of our members and all Oregon families who are caring for a child with PANDAS/PANS, we respectfully ask the Oregon Legislative Assembly to pass this bill. Should you or any other Assembly members have questions about PANDAS/PANS or its treatments, please do not hesitate to contact us.

Sincerely,

Jeff Szymanski, PhD
Executive Director
International OCD Foundation

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[1] Frankovich, J., Swedo, S., Murphy, T., Dale, R. C., Agalliu, D., Williams, K., ... & Muscal, E. (2017). Clinical management of pediatric acute-onset neuropsychiatric syndrome: part II—use of immunomodulatory therapies. *Journal of child and adolescent psychopharmacology*, *27*(7), 574-593.

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February 11, 2021

REGARDING HOUSE BILL 2390 INSURANCE COVERAGE FOR PANDAS/PANS CHILDREN

Dear Chair Representative Rachel Prusak and Healthcare Committee Members,

I sincerely ask for the approval of **HB 2390** that requests a mandate of Insurance Coverage for PANDAS and PANS children in the State of Oregon. Thank you for your consideration.

I urge you to be a leader on behalf of our children and the EIGHT state to cover medical treatment for our children (current coverage is allowable under law in Illinois, Maryland, Massachusetts, Minnesota, New Hampshire, Delaware and Arkansas). PLEASE pave the way for others and recognize important new, cutting edge research led by research and treatment clinics at Columbia, Yale, Stanford, Harvard, and Arizona Universities!

I have been the Executive Director of PANDAS Network since 2013, a national 501c3 organization recognized as an NIMH (National Institute of Mental Health) Outreach Partner. We represent 30,000+ children and families in the U.S. and worldwide. These children have been hurt by this pediatric autoimmune disease that damages the blood brain barrier and injures the brain. Early diagnosis and treatment prevents permanent lifelong injury.

As you read through this letter you may have many questions. I ask you to consider having a PANDAS-PANS expert researcher testify in Oregon. We are happy to help with this professional testimony if you will allow it. A consortium of experts from multidisciplinary backgrounds are investigating the most efficacious treatment of PANDAS-PANS. They have recently published guidelines for the Diagnosis and Treatment of PANDAS-PANS in the Journal of Child & Adolescent Psychopharmacology (2017). New 2020 research by Stanford, Yale and Columbia Universities prove that brain inflammation is occurring with strep and other infections in a discreet subset of children. We also believe there is a genetic component making these children more susceptible; but, they are not incurable! They can heal with treatments that are available but costly without insurance coverage.

Insurance coverage is critical because the expense of treatment together with debilitating, daily symptoms make lack of coverage inhumane and devastating financially. Early recognition of illness onset of PANDAS-PANS has the best chance of assuring children a rapid and substantial recovery. The average age of onset is between ages 4-10. **Untreated the child will often struggle with lifelong neurological and psychological problems.** *Families and social services will incur financial costs* potentially well into the hundreds of thousands of dollars and affect the social, medical, educational systems of Oregon.

Treatment guidelines have been written by experts and the prognosis for healing is excellent if these guidelines are followed. Treatment for this illness involves the use of anti-inflammatory treatments and medications which include: antibiotics/antivirals, steroids, or immune modulating treatments like IVIG or plasmapheresis. These are not exotic treatments and routinely used in other illnesses. Our children deserve coverage as well and we would be grateful to share this hopeful information with legislators.

On a personal note, both my daughter and son developed PANDAS in 2007 at the age of 7 and the other in 2016 at age 12. My very healthy boy and girl changed overnight after a strep infection. They each had intense debilitating fears, tourette-like movements, obsessions, fatigue, confusion, and more. With the use of IVIG and anti-inflammatory medication both children (now ages 16 and 20 years) have had full and, thus far, lasting remissions. Like many other families our family needed to fly out of state for costly, uninsured medical treatment.

Finally, please consider, that in our lives as adults we are presented with so many recalcitrant *illnesses and issues but* **HB 2390** can *reverse tragic illness* and *potentially STOP social and economic problems* the Oregon community as a whole will certainly face without this bill's passage.

Sincerely,

Diana Pohlman, Executive Director
PandasNetwork.org *phone: 619-370-5828*

Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) as a Post-Infectious Autoimmune Disease: Benefits of Intravenous Immunoglobulin (IVIG)

Isaac Melamed, MD, Roger Kobayashi, MD, Maeve O'Connor, MD, Ai Lan Kobayashi, MD, Andrew Schechterman, PhD, Melinda Heffron, Sharon Canterberry, RN, Holly Miranda, RN, Nazia Rashid, PharmD, MS

Introduction

- In the late 1990s, a subgroup of children who presented with obsessive-compulsive disorder (OCD) and/or tic disorders following streptococcal infections were described, and the diagnosis pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS) was developed to describe the disorder.[1]
- Due to difficulties in determining a relationship between strep infections and PANDAS symptoms, a new diagnosis, pediatric acute-onset neuropsychiatric syndrome (PANS), was developed to encompass the growing number of infectious agents potentially related to PANS onset.[2]
- Significant findings indicate a relationship between a post-infectious response and behavioral changes[3,4]; this suggests a form of post-infectious autoimmunity through molecular mimicry.[5]
- Given these findings, we hypothesized that an immune defect is the underlying mechanism leading to PANS.[5]
- Based on this hypothesis, we proposed a study to explore the efficacy of IVIG [Octagam 5%] for PANS treatment.

Study Overview/Schematic

OBJECTIVE

Evaluate the Benefit of Octagam 5% in Subjects with PANS Syndrome

PARTICIPANTS

Male and Female Children Ages 4 – 16 Years with a Diagnosis of PANS

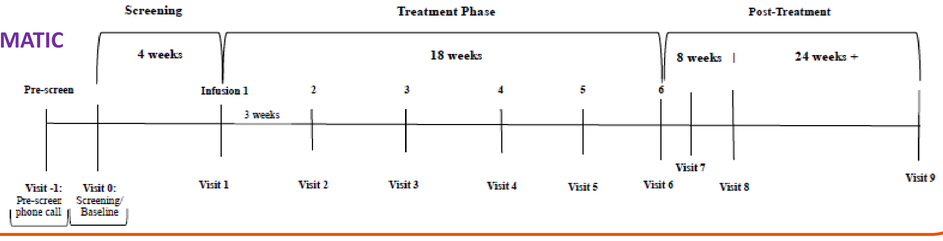
DESIGN

A Multi-site, Open-Label, Pilot Study

STUDY DRUG

Octagam 5% (1g/kg Body Weight Every 21±3 Days/6 Infusions)

STUDY SCHEMATIC



Efficacy Endpoints/

- **Changes in Psychological Evaluation Scores from Baseline to Visits 7/8/9**
  - Parent-Rated Symptom Survey
  - Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS)
  - Yale Global Tic Severity Scale (YGTSS)
  - Anxiety Disorders Interview Schedule for DSM-IV (ADIS)
  - Clinical Global Impression (CGI)
  - Pediatric Acute Neuropsychiatric Symptom Scale Phone Interview Scores
  - Parent and Patient Artifacts (various)

Results

- Total of 21 participants at 3 clinical sites.
- Mean age: 10.86 yrs; males (13 [62%]); females (8 [38%]).
- Mean follow-up time from Visit 0 to Visit 8 was 186 days (±13 days).
- Late follow-up (Visit 9) occurred 29-46+ weeks after last IVIG infusion to gather data on durability of response.
- The primary efficacy endpoints were determined by clinical observation, parent observation, validated psychometric assessments, and interviews by psychologists/psychiatrists.
  - Statistically significant improvements were demonstrated in all psychometric assessments from baseline as compared to Visit 7.

Results (continued)

- Results from the **CY-BOCS assessment (Figure 1)** demonstrate statistically significant reductions in obsessive thoughts and behavior at Visits 7/8/9 as compared to baseline.
- Results from the **Parent-Related Symptom Survey (Figure 2)** indicate significant reductions in symptoms beginning at Infusion 3 through Infusion 6 (compared to treatment initiation (baseline) at Infusion 1).

Figure 1. CY-BOCS Assessment Results

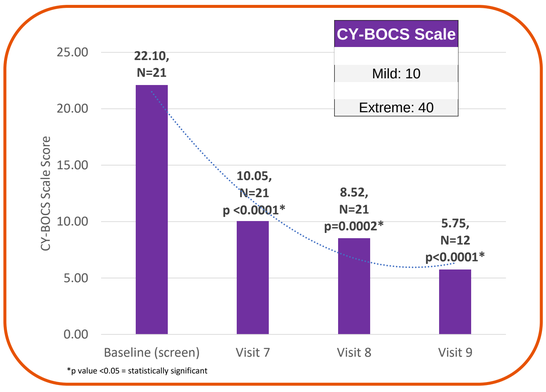
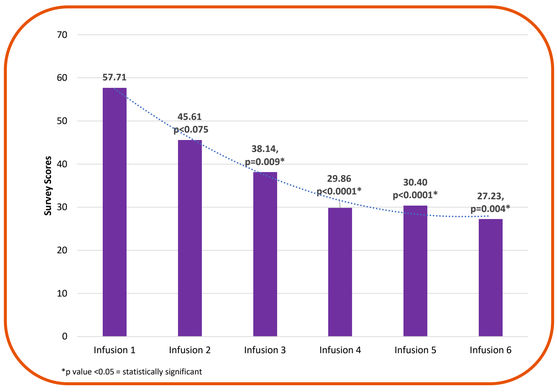


Figure 2. Parent-Rated Symptom Survey Results



Conclusions

- In PANS patients, all psychometric endpoints studied exhibited statistically significant decreases following 6 cycles (infusions) of IVIG.
- Patients with PANS can benefit from a 6-cycle course of IVIG.
  - Provisional data demonstrate durability of the positive impact of IVIG treatment.

References

1. Swedo SE, et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. Am J Psychiatry. 1998;155:265-271.
2. Swedo SE, et al. From research subgroup to clinical syndrome: Modifying the PANDAS criteria to describe PANS (Pediatric Acute-onset Neuropsychiatric Syndrome). Pediatr Therapeut. 2012;2:2.
3. Bronze MS, et al. Epitopes of streptococcal M proteins that evoke antibodies that cross-react with human brain. J Immunol. 1993;15:2820-2828.
4. Hornig M. The role of microbes and autoimmunity in the pathogenesis of neuropsychiatric illnesses. Curr Opin Rheumoltol. 2013;25:488-795.
5. Melamed I. Alzheimer's of the immune system: a new variant of immune deficiency. Immunother Open Acc. 2016;2:2.

Acknowledgments

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Cunningham Panel laboratory assessments and consulting expertise were provided by Moleculera Labs.

Dunwoody Consulting provided assistance with poster content, statistical analyses, and design.

Presented at: European Society for Immunodeficiencies, 2019, Brussels, Belgium

ASPIRE

February 7, 2021

Dear Chairman Prusak and Health Care Committee members,

I am writing to urge you to support HB 2390: Insurance Coverage For Children With PANDAS/PANS. The passage of this bill will positively impact children and their families suffering from Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS).

Early and thorough treatment is crucial to an optimal outcome. Children who are treated early and appropriately within the first year of initial onset often recover. Conversely, children who do not receive timely and complete medical treatment may require more invasive protocols to heal. Due to a lack of insurance coverage for PANS, the process of denials and appeals delays or prevents crucial immune therapy, which can lead to a worsening of symptoms and long-term disability due to untreated brain inflammation. Patients who are not appropriately treated may remain affected both neurologically and psychologically throughout their entire childhood and into their adulthood. It is fiscally more responsible to identify and treat this illness than to create a situation of life-long care. Failure to treat the patient places a significant burden on the patient and their social, educational, and family systems. We must help these families now. We must minimize the financial, emotional, and physical impact of this disease by providing insurance coverage for PANS/PANDAS.

Current research in the PANS shows there are multiple etiologies. Still, the research field is elucidating that it is a type of autoimmune encephalopathy (inflammation and swelling of the brain) typically triggered by a common infection such as strep, mycoplasma pneumonia, and others. Currently, physicians are prescribing medically necessary and effective treatments for PANS/PANDAS, which include but are not limited to: long term antibiotics, steroids, intravenous immunoglobulin (IVIG), and in rare, severe cases, plasmapheresis. Doctors consider the use of IVIG in a small subset of patients who have severe symptoms; IVIG is deemed to be disease-modifying and can halt the autoimmune process. Ideally, patients will recover with the least invasive treatment if diagnosed and treated appropriately by treating the source of inflammation and infection and halting the autoimmune process so these patients can live healthy lives.

Alliance to Solve PANS & Immune-Related Encephalopathies (ASPIRE) speaks with doctors, therapists, families, and educators every day. PANS/PANDAS may only affect a small population, as physicians and researchers still consider it rare. Still, the impact on these children, their families, as well as their community is substantial. ASPIRE sees the burden this disorder puts on families. In addition to the difficulty of caring for a child with this disorder, many families must shoulder the additional financial stress of selling their homes, incurring credit card debt, and using life savings to treat their children. This disease negatively impacts home life due to having to manage symptoms of OCD, separation anxiety, rage, restricted

eating, sleep issues, and more. Multiple siblings may have PANS. Caregivers often have to leave work to provide the required in-home support of a medically sick child. Not only does it affect the family unit, but also the repercussions of leaving children medically untreated impacts the school system. Children commonly miss a significant amount of school and require multiple supports while in school. They often require special education services (IEPs and 504 plans) as symptoms can include significant regressions in handwriting, fine motor, math skills, school refusal, and behavioral regression. When PANS is not treated, children can require special education throughout their time in school as versus a short time period.

A vote for this legislation will provide doctors with the ability to treat our children in the manner that their professional experiences dictate without burdening families with the additional task of fighting with insurance companies. The prolonged denial and appeal process of insurance coverage delays treatment for these children and puts them at risk of further decline and potential long-term disability. Please help our doctors make the best medical choices without worrying about the implications of insurance company denials.

Critically ill children in Oregon need your help to receive appropriate medical intervention. We must help these families now. We must minimize the financial, emotional, and physical impact of this disease by providing insurance coverage for PANS. We are hopeful that one day all insurers will cover PANS, but families in Oregon simply can not wait for this happen; it is paramount you and your fellow legislators support and pass HB 2390: Insurance Coverage For Children With PANDAS/PANS. Your constituents must be given the opportunity to access insurance coverage when confronted with PANS and the medical challenges it creates. Please join your fellow legislators who support HB 2390. This is a bi-partisan issue.

Please reach out to me for additional information via email or phone. Thank you for your time and consideration. Thank you for your continued efforts to assist these families in our community, so our children grow up and reach their full potential.

Best regards,

Gabriella True
ASPIRE, President
Email: Gabriella@aspire.care
Mobile: 562-480-7560
www.ASPIRE.care

National Association of Pediatric Nurse Practitioners℠
OREGON

January 6, 2021

The Honorable Rachel Prusak, RN, FNP
Oregon State Legislature
900 Court St. NE, H-489, Salem, Oregon 97301
Rep.RachelPrusak@oregonlegislature.gov

Dear Congresswoman Prusak:

On behalf of more than 200 pediatric nurse practitioners across the state, the Oregon Chapter of the National Association of Pediatric Nurse Practitioners is pleased to endorse the "Oregon PANDAS/PANS Insurance Coverage Bill (LC969)".

As you are well aware, this legislation would help improve access to evidence-based treatment (IVIG) for pediatric patients suffering negative effects associated with Pediatric Acute-Onset Neuropsychiatric Syndrome/Pediatric Autoimmune Neuropsychiatric Disorder Associated with Strep (PANS/PANDAS) by holding individual and commercial insurers operating in Oregon accountable to provide such coverage.

This legislation is in alignment with our National organization's commitment to ensuring that ALL children "have access to comprehensive, continuous, coordinated, compassionate, culturally responsive, sensitive and family-centered health care… to ensure physical and psychosocial health and well-being." We strive, along with your efforts, to help "address growing disparities of costs of healthcare, including insurance coverage, adequacy of benefits, [and] out of pocket expenses for… pharmaceuticals."

Our Chapter and its members are committed to supporting the "Oregon PANDAS/PANS Insurance Coverage Bill (LC969)", and its adoption to help improve quality of life for children across our State. We are eager to work with you to secure its passage and thank you for your leadership regarding this important issue.

Sincerely,

Lisa Crupi, MN, CPNP
Oregon Chapter of NAPNAP President

Stanford MEDICINE

Stanford Children's Health | Lucile Packard Children's Hospital Stanford

February 5, 2021

To: Oregon House Health Care Committee
Re: **Oregon's House Bill 2390**; Requires health benefit plan and health care service contract coverage of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections and pediatric acute-onset neuropsychiatric syndrome.

To Whom It May Concern:

We are writing to urge your legislators to support **Oregon's House Bill 2390;** advocating for health benefit and service contract coverage of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections(PANDAS) and pediatric acute-onset neuropsychiatric syndrome (PANS).

PANS/PANDAS is an abrupt-onset psychiatric disorder currently recognized, by our center and at least 15 other academic pediatric centers affiliated with Universities. Based on 3 imaging studies and at least 20 basic science publications, it has been concluded that inflammation is playing a role in the onset of symptoms of this debilitating psychiatric illness. Some cases are clearly triggered by an infection, while in other cases, the triggering agent is occult. Regardless, most cases respond robustly to medical therapy (antibiotics and/or immunotherapy) which can be curative as opposed to palliative.

Given the complexity and heterogeneity of host factors and different triggering agents, research and clinical trials are scarce; however, there is a phase 3 IVIG clinic trial that shows promise, and several additional planned trials on the horizon. Despite these efforts, the illness remains largely foreign to the medical and lay communities.

The first PANS Consortium meeting was held at Stanford in May 2013 where we (researchers and physicians from 7 University Medical Centers) met to outline the diagnostic workup of PANS and formulate research agendas. Since this meeting, we have published diagnostic and expert opinion recommendations that outline several therapy options, both antibiotics and immunotherapy, based on the host factors including documented triggers, unknown triggers, host factors, and severity of illness.

In the field of pediatric rheumatology, we treat a large number of autoimmune and autoinflammatory illnesses based on having only indirect evidence of immunological causes. Most of these diseases are managed with immunomodulation despite the absence of successful trials—and as a result, the morbidity and mortality of pediatric rheumatologic diseases have dramatically improved over the decades despite the lack of trials. Like PANS/PANDAS, rheumatologic diseases are difficult to study in trials due to insufficient numbers, high cost of trials, and vast heterogeneity of disease. Pediatric rheumatologic diseases are now being

treated according to Consensus Guidelines, and the different protocols are subsequently compared. In this model, the burden of cost goes to the insurer.

Despite the abundance of compelling basic science evidence, including both human and animal models, uninformed physicians and insurers are still turning away patients with treatable PANS/PANDAS. It is our opinion that the diagnosis and treatment of PANS/PANDAS is no longer "controversial" and now it is just a matter of refining the treatment and finding optimal regimens for the different subsets of PANS diseases.

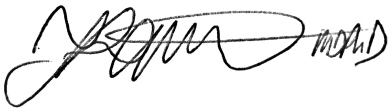
Permanent debilitating neuropsychiatric sequelae occur when PANS/PANDAs go untreated. At Stanford, we have studied 300 patients with PANS, and it is clear that in many cases, untreated flares result in a worsened baseline and many patients become disabled by permanent neuropsychiatric symptoms. The state of Oregon could significantly reduce the burden of these psychiatric sequelae by increasing coverage for both medical and psychiatric therapies including but not limited to IVIG, steroids, and Plasmapheresis.

Thank you for your consideration of this request, your decision on this piece of legislation could make a substantial impact on the lives of these patients and their families. Kindly reach out with any questions or for additional resources evidence you may need to best inform your decision.

Sincerely,

Jennifer Frankovich, MD, MS
Clinical Professor | Pediatrics – Allergy, Immunology, Rheumatology
Co-Director, Stanford Children's Immune Behavioral Health Clinic
Director, Stanford Immune Behavioral Health Research Program
Stanford University School of Medicine
700 Welch Rd, Suite 301, MC: 5896 | Palo Alto, CA 94304

Margo Thienemann, MD
Clinical Professor of Psychiatry
Stanford University School of Medicine
Director of Psychiatry Services, Stanford Children's Immune Behavioral Health Clinic
Clinic Phone: 650-497-9063 or 650-736-4364 Clinic Fax: 650-736-4344
PANSclinic@Stanfordchildrens.org



Theresa Willett, MD, PhD
Clinical Assistant Professor | Pediatrics - Allergy, Immunology, Rheumatology
Co-Director, SCH Immune Behavioral Health Clinic
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twillett@stanford.edu

Neuropsychiatric Testing Provides Objective Insight Into Beneficial Effects Of Intravenous Immunoglobulins In Patients With Pediatric Acute-Onset Neuropsychiatric Syndrome

Poster #246

Eremija J, Daines M
Division of Allergy, Immunology and Rheumatology, Department of Pediatrics, University of Arizona, Tucson

INTRODUCTION

Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS) is defined by acute onset of OCD and/or restricted eating, but other diverse neuropsychiatric manifestations are also commonly seen, presumably in the setting of underlying immune dysfunction [1-4]. Thus, immunomodulatory interventions have crucial therapeutic role, but objective post-treatment evaluations are scarce and challenging given disease complexity [5-7]. We used standardized neuropsychiatric testing to assess how intravenous immunoglobulin (IVIG) treatment impacts cognitive function in children with PANS.

METHODS

Retrospective 5-year record review was completed in Children's Postinfectious Autoimmune Encephalopathy Center at University of Arizona. We identified 12 children who were diagnosed with PANS based on well-established clinical criteria [1-3], underwent immunomodulatory IVIG therapy (1-7 courses of 2g/kg IVIG) and completed neuropsychiatric testing before/after treatment. Score improvement of 1 standard deviation in any tested domain/subdomain (e.g. intelligence, memory, learning, visual, motor, sensory, integrative functions) was considered significant and is represented as ↑ in Table 2.

Table 1: Demographic and laboratory patient characteristics

Characteristics	Mean (SD)
Age of diagnosis	8.5 (4.5)
Age at IVIG treatment	11.5 (3)
	Number (%)
Male gender	5 (42)
IVIG < 2 years from diagnosis	5 (42)
Preceding Streptococcal Infection*	7 (58)
Residence in Arizona	6 (50)
Total adverse effects on IVIG	4 (33)
Severe adverse effects on IVIG	1 (8)
Improvement on IVIG	11 (92)
Hypogammaglobulinemia**	5 (42)

*: based on positive throat swab and/or Antistreptolysin-O titers
**: low IgG levels on presentation, requiring substitution through IVIG infusion

RESULTS

Patient demographic characteristics are presented in **Table 1.** Individual test results are depicted in **Table 2**

Table 2: Individual test results

	Dg-IVIG delay (years)	Number of IVIG courses	KBIT-2	WASI-2	WISC-4/5	WRAML-2 CVLT-C	WRAT-4/5 WIAT-3	Beery VMI	WRAVMA Pegboard
Patient 1	7	3	n/a	↔	↔	↔	↔	↑	↑
Patient2	1	3	n/a	↔	n/a	↔	↔	↔	↔
Patient 3	2	4	n/a	↔	↔	↑	↔	↔	↔
Patient 4	6	1	n/a	↔	↔	↑	↔	↔	↔
Patient 5	2	7	n/a	n/a	↔	↑	n/a	↔	n/a
Patient 6	5	2	n/a	n/a	↑	n/a	n/a	↔	n/a
Patient 7	4	3	n/a	↔	↔	↔	↑	↔	↑
Patient 8	1	3	n/a	n/a	↑	↑	↔	↑	↔
Patient 9	0	3	n/a	↑	↔	↑	↔	↔	↔
Patient 10	6	2	n/a	↔	↔	↔	↔	↑	↑
Patient 11	1	3	↑	n/a	n/a	n/a	n/a	n/a	n/a
Patient 12	1	5	↑	n/a	n/a	n/a	n/a	n/a	n/a
Improved	n/a	n/a	2/2	1/7	2/9	5/9	1/8	3/10	3/8

n/a: testing not done, or not comparable ↑: significantly improved. ↔: no change

Dg-IVIG delay: delay from diagnosis to treatment
KBIT-2: Kaufman Brief Intelligence Test, 2nd edition
WASI-2: Wechsler Abbreviated Scale of Intelligence, 2nd edition
WISC-4/5: Wechsler Intelligence Scale for Children, 4th or 5th Edition
WRAML-2: Wide Range Assessment of Memory and Learning, 2nd edition
CVLT-C: California Verbal Learning Test Children's
WRAT-4/5: Wide Range Achievement Test 4th or 5th edition
WIAT-3: Wechsler Individual Achievement Test 3rd edition
Beery VMI: Beery-Buktenica Developmental Test of Visual-Motor Integration
WRAVMA Pegboard: Wide Range Assessment of Visual Motor Abilities Pegboard

CONCLUSIONS AND DISCUSSION

In our cohort, 11 of 12 patients showed significant improvement following IVIG. Treatment was tolerated well and showed efficacy in almost all participants, independently from time lapsed since disease onset, emphasizing impact of immunomodulation in PANS. Furthermore, patients benefited from different numbers of IVIG courses.

Although PANS diagnosis requires presence of hyperacute OCD and/or restricted eating, other psychiatric and neuropsychological manifestations can sometimes overshadow OCD [1-3]. Therefore, our study focused on those additional pertinent neuropsychological disorders commonly seen within PANS spectrum and provided a novel expanded insight into beneficial effects of IVIG comparing to other trials, while proving that standardized neuropsychiatric testing is a valuable tool to objectively quantify improvement in these patients. Significant presence of baseline hypogammaglobulinemia in children with PANS emphasizes the presumed role of immune dysfunction in disease pathogenesis, especially given known connection between immunodeficiency and autoimmunity [8,9].

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PANDAS—Questions and Answers

Overview

What is PANDAS?

PANDAS is short for Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections. A child may be diagnosed with PANDAS when:

- Obsessive-compulsive disorder (OCD), tic disorder, or both suddenly appear following a streptococcal (strep) infection, such as strep throat or scarlet fever.
- The symptoms of OCD or tic symptoms suddenly become worse following a strep infection.

The symptoms are usually dramatic, happen "overnight and out of the blue," and can include motor or vocal tics or both and obsessions, compulsions, or both. In addition to these symptoms, children may become moody or irritable, experience anxiety attacks, or show concerns about separating from parents or loved ones.

What causes PANDAS?

Strep bacteria are very ancient organisms that survive in the human host by hiding from the immune system as long as possible. They hide themselves by putting molecules on their cell wall so that they look nearly identical to molecules found on the child's heart, joints, skin, and brain tissues. This hiding is called "molecular mimicry" and allows the strep bacteria to evade detection for a long time.

However, the molecules on the strep bacteria are eventually recognized as foreign to the body and the child's immune system reacts to the molecules by producing antibodies. Because of the molecular mimicry by the bacteria, the immune system reacts not only to the strep molecules but also to the human host molecules that were mimicked; antibodies "attack" the mimicked molecules in the child's own tissues. These antibodies that react to both the molecules on the strep bacteria and to similar molecules found on other parts of the body are an example of "cross-reactive" antibodies.

National Institute of Mental Health

Studies at the National Institute of Mental Health (NIMH) and elsewhere have shown that some cross-reactive antibodies target the brain—causing OCD, tics, and the other neuropsychiatric symptoms of PANDAS.

Could an adult develop PANDAS?

PANDAS is considered a pediatric disorder and typically first appears in childhood from age 3 to puberty. Reactions to strep infections are rare after age 12, but researchers recognize that PANDAS could occur, though rarely, among adolescents. It is unlikely that someone would experience these post-strep neuropsychiatric symptoms for the first time as an adult, but it has not been fully studied.

It is possible that adolescents and adults may have immune-mediated OCD, but this is not known.

Signs and Symptoms

How is PANDAS diagnosed?

The diagnosis of PANDAS is a clinical diagnosis, which means that there are no lab tests that can diagnose PANDAS. Instead, health care providers use diagnostic criteria for the diagnosis of PANDAS (see below). At the present time, the clinical features of the illness are the only means of determining whether a child might have PANDAS.

The diagnostic criteria are:

- Presence of OCD, a tic disorder, or both
- Pediatric onset of symptoms (i.e., age 3 to puberty)
- Episodic course of symptom severity
- Association with group A Beta-hemolytic strep infection, such as a positive throat culture for strep or history of scarlet fever
- Association with neurological abnormalities, such as physical hyperactivity or unusual, jerky movements that are not in the child's control
- Very abrupt onset or worsening of symptoms

If the symptoms have been present for more than a week, blood tests may be done to document a preceding strep infection.

Are there any other symptoms associated with PANDAS episodes?

Yes. Children with PANDAS often experience one or more of the following symptoms in conjunction with their OCD or tic disorder:

- Symptoms of attention-deficit/hyperactivity disorder (ADHD), such as hyperactivity, inattention, or fidgeting
- Separation anxiety (e.g., child is "clingy" and has difficulty separating from his or her caregivers; for example, the child may not want to be in a different room in the house from his or her parents)
- Mood changes, such as irritability, sadness, or emotional lability (i.e., tendency to laugh or cry unexpectedly at what might seem the wrong moment)
- Trouble sleeping
- Nighttime bed-wetting, frequent daytime urination, or both
- Changes in motor skills, such as changes in handwriting
- Joint pains

What is an episodic course of symptoms?

Children with PANDAS seem to have dramatic ups and downs in the severity of their OCD and tics. OCD or tics that are almost always present at a relatively consistent level do not represent an episodic course. Many children with OCD or tics have good days and bad days, or even good weeks and bad weeks. However, children with PANDAS have a

very sudden onset or worsening of their symptoms, followed by a slow, gradual improvement. If children with PANDAS get another strep infection, their symptoms suddenly worsen again. The increased symptom severity usually persists for at least several weeks but may last for several months or longer.

My child has had strep throat before, and he has tics, OCD, or both. Does that mean he has PANDAS?

No. Many children have OCD, tics, or both, and almost all school-aged children get strep throat at some point. In fact, the average grade-school student will have two or three strep throat infections each year.

PANDAS is considered as a diagnosis when there is a very close relationship between the abrupt onset or worsening of OCD, tics, or both, and a strep infection. If strep is found in conjunction with two or three episodes of OCD, tics, or both, then the child may have PANDAS.

What does an elevated anti-strep antibody titer mean? Is this bad for my child?

The anti-strep antibody titer (i.e., the number of molecules in blood that indicate a previous infection) is a test that determines whether the child has had a previous strep infection.

An elevated anti-strep titer means the child has had a strep infection sometime within the past few months, and his or her body created antibodies to fight the strep bacteria.

Some children create lots of antibodies and have very high titers (up to 2,000), while others have more modest elevations. The height of the titer elevation doesn't matter, and elevated titers are not necessarily bad for your child. The test measures a normal, healthy response—the production of antibodies to fight off an infection. The antibodies stay in the body for some time after the infection is gone, but the amount of time that the antibodies persist varies greatly between individuals. Some children have "positive" antibody titers for many months after a single infection.

When is a strep titer considered to be abnormal, or "elevated"?

The lab at the National Institutes of Health considers strep titers between 0 and 400 to be normal. Other labs set the upper limit at 150 or 200. Because each lab measures titers in different ways, it is important to know the range used by the lab where the test was done—just ask where the lab draws the line between negative or positive titers.

What if my child's doctor does not understand or does not want to consider PANDAS?

Contact the International OCD Foundation (www.iocdf.org/find-help) or the PANDAS Physicians Network (www.pandasppn.org) to find a doctor who may be knowledgeable about PANDAS.

PLEASE NOTE: NIMH does not evaluate the professional qualifications and competence of individual health care providers listed on these websites. The resources are provided for general informational purposes only. NIMH does not intend to provide specific medical advice on its websites, but rather to help visitors better understand mental health and disorders. NIMH will not provide specific medical advice and urges you to consult with a qualified mental health or health care provider for diagnosis and answers to your personal questions.

Treatment

What are the treatment options for children with PANDAS?

Treatment with Antibiotics

The best treatment for acute episodes of PANDAS is to treat the strep infection causing the symptoms, if it is still present, with antibiotics.

- A throat culture should be done to document the presence of strep bacteria in the throat.
- If the throat culture is positive, a single course of antibiotics usually will get rid of the strep infection and allow the PANDAS symptoms to subside.

If a properly obtained throat culture is negative, the clinician should make sure that the child doesn't have an occult (hidden) strep infection, such as a sinus infection (often caused by strep bacteria) or strep bacteria infecting the anus, vagina, or urethral opening of the penis. Although the latter infections are rare, they have been reported to trigger PANDAS symptoms in some patients and can be particularly problematic because they will linger for longer periods of time and continue to provoke the production of cross-reactive antibodies.

The strep bacteria can be harder to eradicate in the sinuses and other sites, so the course of antibiotic treatment may need to be longer than that used for strep throat.

Tips for Parents or Caregivers

Sterilize or replace toothbrushes during and following the antibiotics treatment to make sure that the child isn't re-infected with strep.

It also might be helpful to ask a health care provider to perform throat cultures on the child's family members to make sure that none are "strep carriers," who could serve as a source of the strep bacteria.

How can you manage neuropsychiatric symptoms of PANDAS?

Children with PANDAS-related obsessive-compulsive symptoms will benefit from standard medications; behavioral therapies, such as cognitive behavioral therapy (CBT); or both. OCD symptoms are treated best with a combination of CBT and a selective serotonin reuptake inhibitor (SSRI) medication, and tics respond to a variety of medications.

Children with PANDAS appear to be unusually sensitive to the side effects of SSRIs and other medications, so it is important to "start low and go slow" when using these medications. In other words, clinicians should prescribe a very small starting dose of the medication and increase it slowly enough that the child experiences as few side effects as possible. If PANDAS symptoms worsen, the SSRI dosage should be decreased promptly. However, SSRIs and other medications should not be stopped abruptly, as that also could cause difficulties.

For more information about mental health medications, please visit the NIMH website at www.nimh.nih.gov/health.

What about treating PANDAS with plasma exchange or immunoglobulin (IVIG)?

Plasma exchange or immunoglobulin (IVIG) may be a consideration for acutely and severely affected children with PANDAS. Research suggests that both active treatments can improve global functioning, depression, emotional ups and downs, and obsessive-compulsive symptoms. However, there may be side effects associated with the treatments, including nausea, vomiting, headaches, and dizziness.

In addition, there is a risk of infection with any invasive procedure, such as these. **Thus, the treatments should be reserved for severely ill patients and administered by a qualified team of health care professionals**.

Should an elevated strep titer be treated with antibiotics?

No. Elevated titers indicate that a patient has had a past strep exposure, but the titers can't tell you precisely when the strep infection occurred. Children may have "positive" titers for many months after

one infection. Because these elevated titers are merely a marker of a prior infection and not proof of an ongoing infection, it is not appropriate to give antibiotics for elevated titers. Antibiotics are recommended only when a child has a positive rapid strep test or positive strep throat culture.

Can penicillin be used to treat PANDAS or prevent future PANDAS symptom exacerbations?

Penicillin does not specifically treat the symptoms of PANDAS. Penicillin and other antibiotics treat the sore throat caused by the strep by getting rid of the bacteria. In PANDAS, research suggests that it is the antibodies produced by the body in response to the strep infection that may cause PANDAS symptoms, not the bacteria itself.

Researchers at NIMH have been investigating the use of antibiotics as a form of prophylaxis to prevent future problems. However, there isn't enough evidence to recommend the long-term use of antibiotics at this time.

My child has PANDAS. Should he have his tonsils removed?

Current research does not suggest that tonsillectomies for children with PANDAS are helpful. If a tonsillectomy is recommended because of frequent episodes of tonsillitis, it would be useful to discuss the pros and cons of the procedure with your child's health care provider because of the role that the tonsils play in fighting strep infections.

Participating in Clinical Research

Clinical trials are research studies that look at new ways to prevent, detect, or treat diseases and conditions. The goal of clinical trials is to determine if a new test or treatment works and is safe. Although individual participants may benefit from being part of a clinical trial, participants should be aware that the primary purpose of a clinical trial is to gain new scientific knowledge so that others may be better helped in the future.

Researchers at NIMH and around the country conduct many studies with patients and healthy volunteers. We have new and better treatment options today because of what clinical trials uncovered years ago. Be part of tomorrow's medical breakthroughs. Talk to your doctor about clinical trials, their benefits and risks, and whether one is right for you.

For more information about clinical research and how to find clinical trials being conducted around the country, visit www.nimh.nih.gov/health/trials.

Finding Help

Behavioral Health Treatment Services Locator

The Substance Abuse and Mental Health Services Administration provides this online resource for locating mental health treatment facilities and programs. Find a facility in your state at https://findtreatment.samhsa.gov. For additional resources, visit www.nimh.nih.gov/findhelp.

Questions to Ask Your Doctor

Asking questions and providing information to your doctor or health care provider can improve your care. Talking with your doctor builds trust and leads to better results, quality, safety, and satisfaction. Visit the Agency for Healthcare Research and Quality website for tips at www.ahrq.gov/patients-consumers.

For More Information

NIMH website
www.nimh.nih.gov

MedlinePlus (National Library of Medicine)
https://medlineplus.gov
(En español: https://medlineplus.gov/spanish)

ClinicalTrials.gov
www.clinicaltrials.gov
(En español: https://salud.nih.gov/investigacion-clinica)

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Article

PANS/PANDAS: Clinical Experience in IVIG Treatment and State of the Art in Rehabilitation Approaches

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Abstract: Pediatric acute-onset neuropsychiatric syndrome (PANS) is a condition characterized by the abrupt, dramatic onset of obsessive–compulsive disorder (OCD) or eating restriction accompanied by equally abrupt and severe comorbid neuropsychiatric symptoms. PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection) is a heterogeneous syndrome identified as post-Streptococcus pyogenes infection (β-hemolytic Streptococcus group A) complications regarding the central nervous system with specific involvement of neuropsychiatric and behavioral skills. In the first part of our study, we share our experience in the treatment of a group of extreme-grade (according to CY-BOCS severity scale) symptomatic patients with intravenous immunoglobulin (IVIG), following the most recent studies regarding the dosage of the drug. Our contribution is to share our experience made on a sample of 55 patients all in the highest level of a severity grade. In the second part of our study, we also analyze the literature on PANS/PANDAS rehabilitation therapy, since in the literature there is no discussion of union and comparison on this method. *Objective*: This study aims to evaluate the clinical features of the patients observed from different Italian cohorts, with the attempt at evaluating clinical response to IVIG treatment in children with an extreme severity grade of PANS/PANDAS disease. Furthermore, after having analyzed the literature, we propose rehabilitation therapy as an added value to the pharmacological treatment. *Materials and Methods*: A total of 55 patients with a diagnosis of PANS/PANDAS, who belonged to an extreme grade of disease, were enrolled. All patients were administered with IVIG treatment at 2 g/kg per day for two consecutive days. *Results*: From our study, a noticeable improvement (until complete remission) of symptoms was evident for at least one year in 47 out of 55 (85%) observed children, while 11 out of these 43 (25%) showed an evident symptoms remission in a single attempt and the remaining 32 (75%) required a second administration to notice a lasting symptomatic improvement.

Keywords: PANDAS/PANS; therapeutics; rehabilitative approaches; IVIG treatment

1. Introduction

PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection) collects a specific specter of disturbances linked to group A beta-hemolytic streptococcal infection (GABHS). It is a post-infectious complication affecting the central nervous system, similarly to Sydenham chorea or rheumatic fever, but with specific involvement of neuropsychiatric and behavioral skills. Swedo et al. firstly described these disturbances in 1998 [1], reporting five clinical features required to diagnose these disorders, including the presence of neuropsychiatric abnormalities, mainly obsessive–compulsive disorder (OCD) and tic, with onset in the pre-pubertal age (3–14 years) and a relapsing/remitting course, clearly linked to a GABHS previous infection. Hyperactivity, choreiform movements, and Tourette syndrome (TS) are also possible diagnostic elements. From the several cases that came out from literature over time [2–4], it is evident that there is a great variety of phenotypes, so allowing to widen the edges of these new disorders including CANS (childhood acute neuropsychiatric symptoms), defined by the presence of OCD with abrupt onset and PANS (pediatric acute-onset neuropsychiatric syndrome), of which PANDAS represents a subgroup of disorders [5,6].

Despite different pathogenic causes evaluated [7], such as previous infections, immune impairment, or environmental factors, a precise triggering event is not always detectable. Defining criteria for PANS [8] is the presence of sudden-onset OCD or food restriction behavior together with at least two associated manifestations including behavioral disturbances (irritability, aggression, oppositional behavior), anxiety, depression, deterioration in school performance, and other neurologic signs like a motor or sensory impairment, sleep disturbances with enuresis, or incontinence (Figures 1 and 2).

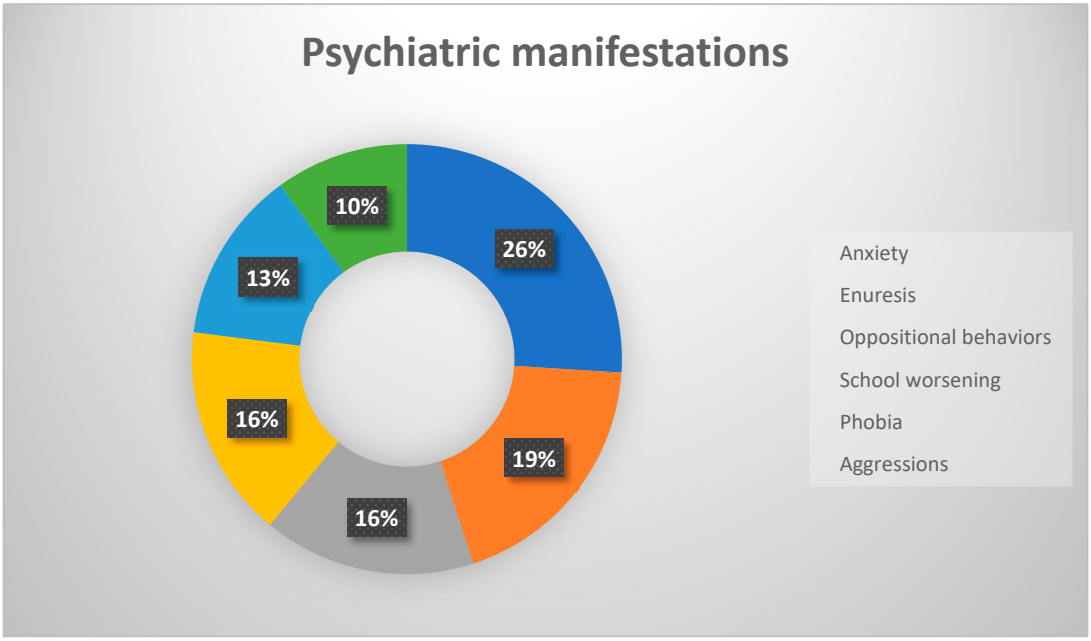

Figure 1. Percentages frequency of the main psychiatric manifestations.

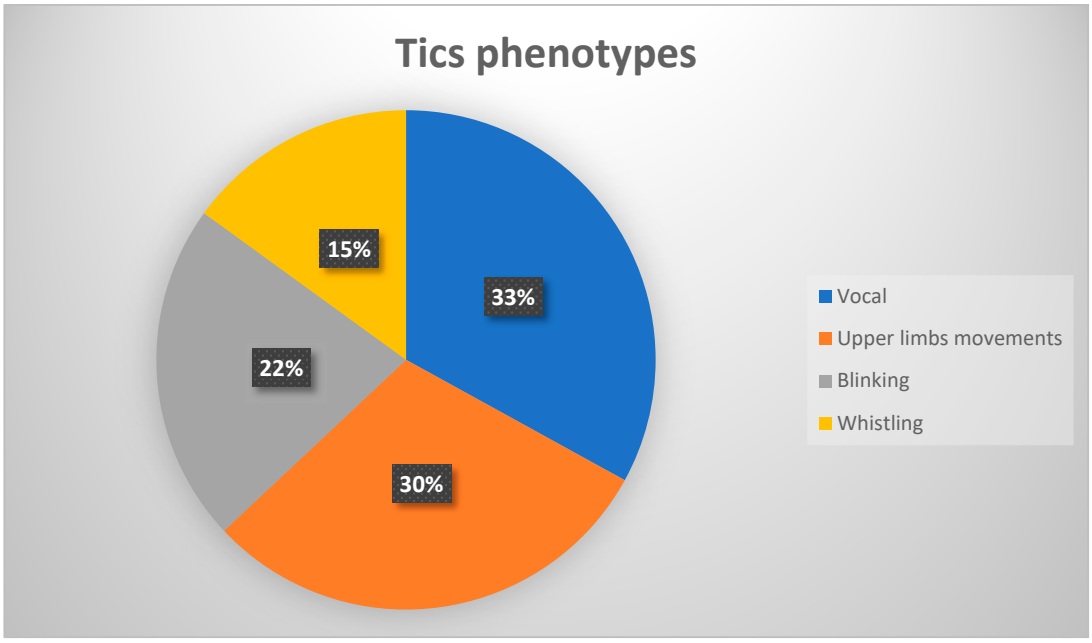

Figure 2. Percentages frequency of the main tics phenotypes.

The pathogenic mechanism is now held responsible for an autoimmune impairment, consisting of molecular mimicry between antigens in the bacterial cell and antigens. Particularly, the production of autoantibodies has been shown against neurotransmitter receptors D1 or D2 of dopamine, responsible for neuropsychiatric impairment of these patients. Also, the presence of antibasal ganglia antibodies in sera of PANDAS patients is a highly specific and sensitive marker in comparison to control, nonaffected groups. Antineuronal antibodies that may contribute to the progression of PANDAS symptoms include antipyruvate kinase antibodies, antidopamine receptor antibody, and antilysoganglioside (GM1) antibody [9]. However, these findings seem not to be a specific marker of PANS, resulting also in Sydenham chorea or TS. A clear etiologic factor is not always detectable, so it is currently di cult to establish a standardized protocol to manage the treatment of these disorders. A recent PANS Conference Consensus proposed orienting the treatment according to the grade of symptoms.

These guidelines [10] suggest an initial approach based on antibiotics together with corticosteroids for the light and mild forms, while for more severe forms, an equally severe treatment as intravenous immunoglobulins infusion (IVIG) or plasmapheresis or biologic drugs such as rituximab can be required. Another possible approach taken into consideration is the surgical removal of tonsils. However, tonsillectomy has not shown any improvement in delaying the onset of symptoms or reducing the grade, as confirmed in a previous paper of Pavone et al. [11]. Complementary therapies to suppress the autoimmune response causing the symptoms consist of the administration of corticosteroids, therapeutic plasma exchange, intravenous immunoglobulin (IVIG), or rituximab [12]. Among these different options, a surely advised treatment is represented by IVIG. One of the first e cacy evaluations of this approach was performed in 1999 by Perlmutter's team [13], who made a comparison between a subgroup of patients affected by OCD and tics treated with five single-volume plasma exchanges (PEX) and another treated with IVIG at 2 g/kg daily on two consecutive days, both compared to the same number of placebo controls; they noticed important clinical improvements either for tics or for OCD disturbances with good maintenance even 12 months after the treatment. Another study to evaluate the e cacy and safety of IVIG treatment in PANDAS children was performed by Williams et al. [14], who evaluated a group of 35 children rated as having a moderate–severe grade of OCD manifestations. The treatment was revealed to be well tolerated after a follow-up performed up to 24 months later. Further, other studies have been performed to assess the potential role of IVIG treatment for PANDAS

patients, revealing a potential role in addition to antibiotics therapy [15] to prevent reinfection from etiologic agents, with a clear improvement of symptoms and prolonging the period of clinical wellness. Nevertheless, since dealing with an expensive procedure not lacking in risk, its application should be limited to those serious–severe cases, according to precise indications [16].

This study aims to assess the clinical features of the patients observed from different Italian cohorts, and to evaluate clinical response to IVIG treatment in children with an extreme severity grade of PANDAS/PANS disease. The outcome is to verify the decrease in tics and OCD using the Children's Yale–Brown Obsessive–Compulsive Scale (CY-BOCS) severity score. We will consider a satisfactory clinical response to treatment a lowering of at least one degree of the CY-BOCS scale, which persists for at least one year of observation.

Besides, we conducted a review of disability and rehabilitation programs in PANS/PANDAS. We described the importance of rehabilitation, as occupational therapy, logopedic treatment and psychologic support, to prepare to recognize and help the child with PANS/PANDAS.

2. Materials and Methods

We enrolled a total of 55 patients with a diagnosis of PANS/PANDAS, who belonged to the extreme grade of disease and were never treated with IVIG/other therapy before, except for the antibiotic one. That group had been followed for one year since the onset of disease from our and other participating centers. Our sample (n = 55) was composed of 30 males (54%) and 25 females (46%), with an average age of 8.9 years old. At the diagnosis, all patients were assessed according to Swedo et al.'s (2012) parameters for PANS disorders [17]: abrupt, dramatic onset of OCD or eating restriction accompanied by equally abrupt and severe comorbid neuropsychiatric symptoms, which include anxiety, emotional lability, depression, irritability, aggression, oppositionality, deterioration in school performance, behavioral (developmental) regression, sensory amplification, movement abnormalities, sleep disturbance, and urinary frequency. Psychiatric manifestations were present in all patients, and all of them presented anxiety, enuresis, or oppositional behavior. OCD was present, especially in the form of food behavioral restrictions, and other psychiatric disturbances such as psychosis or self-harm were noticed as well. As concerns movement disturbances, instead, tics were the most frequent manifestation reported with wide variety among simple and complex forms, either vocal or motor type, while choreic movements were described in a smaller subgroup of patients (n = 4), with the main involvement of the upper limbs.

All of them were submitted to the CY-BOCS based on five items including questions on obsession and five items including questions on compulsion (0 to 4 points for each item); the items were administered by our team of psychologists who only enrolled the patients with a Total Severity Score ≥ 32 (Table 1). Presentation findings were assessed, including either clinical features (tics, chorea, OCD) or laboratory data (pharyngeal swab, antistreptolysin O titer, antideoxyribonuclease titer, Epstein–Barr virus, chlamydia, mycoplasma, toxoplasmosis, other infections, rubella, cytomegalovirus, and herpes simplex virus-2 and autoimmunity panel), together with brain MRI and EEG findings.

Table 1. Total CY-BOCS score: range of severity for patients who have both obsessions and compulsion.

Severity Grade	Total Score
Subclinical	0–7
Mild	8–15
Moderate	16–23
Severe	24–31
Extreme	32–40

All patients enrolled were administered, after informing their parents about the possible although rare risks of allergic reactions and after having them sign the proper consent form, with the IVIG treatment at the dosage of 2 g/kg per day for two consecutive days.

Pavone et al. (2018) [18] reported on a study conducted in 34 children affected by PANDAS, all of them treated with an IVIG dosage of 2 g/kg day for two consecutive days, having a good final result with evident clinical improvement. Following this and previous studies that used the same dosage, we decided to follow the same posology.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as revised in 2000 and was approved by the ethic committee of the University Hospital Vittorio Emanuele of Catania, Italy (nd 1394 04/04/2017).

For the review about rehabilitation feature and PANDAS, we searched PubMed for the following terms and keywords: "PANS/PANDAS" and "rehabilitation" and "PANS/PANDAS" and "disability". The database searches yielded nine references from 2000 to 2020. Thus, only nine titles and abstracts were screened and then six remained for full-text screening; of these, three met the inclusion criteria.

3. Results

3.1. Clinical

In our study, we have analyzed a total of 55 extreme-grade PANS/PANDAS children, treated with IVIG at the dosage of 2 g/kg per day for two consecutive days. From 3 to 30 days after therapy in 47 out of 55 of the cases treated (85%), a long-term (1 yr) clinical benefit was noticed (n = 12 from extreme grade to no symptoms, n = 22 from extreme to mild, n = 9 from extreme to moderate, n = 4 from extreme to severe of CY-BOCS severity ratings), whereas in a minor percentage (15%, n = 8 cases), we noticed a reappearance of symptoms within 1 to 6 months after treatment, after an only temporary improvement (Table 2).

Table 2. Distribution on severity scale after therapy (1–2 IVIG cycles) after 1 year of observation.

Severity Grade	Start	After IVIG
Subclinical	0	12
Mild	0	22
Moderate	0	9
Severe	0	4
Extreme	55 Patients	8 Patients

3.2. Laboratory

A positive result in pharyngeal swab was present in n = 37 (67%) children, versus a number of 14 (25%) negatives. In 4 (8%) cases, swab results were not available due to a laboratory error. ASO titer (measured in the whole sample) and Anti-DNase B titer (29/50 patients) provided a wide range of value, spanning from 296 to 2141 UI for ASO (982.7 ± 457.9 UI), and from 299 to 1160 UI (539.8 ± 214.1 U/mL) for DNase B. The discordance from the values here obtained and those obtained in other centers could be ascribable to the different bacterial stains involved. All the other laboratory parameters searched were negative and not worth further study. EEG and ECG were normal in all patients analyzed with not-pathological waves to the tracks, and MRI data revealed anomalies, such as bilateral enlargement in perioptic sheath in one case and mild asymmetry of the lateral ventricles in two patients; both findings had no clinical relevance and no correlation with the pathology.

All patients, affected from clinical manifestation defined nonresponsive to antimicrobial treatment, were administered intravenous immunoglobulin (IVIG) treatment at 2 g/kg per day for two consecutive days.

3.3. Other Findings

What comes out from our study is a noticeable improvement (transition from extreme symptom grade to lower grade) in 47 out 55 children, and in some cases (12 out 47), a complete remission of

symptoms for at least one year in observed children. A total of 11 patients (4 males and 7 females) out of the 47 responders required only one cycle of IVIG therapy (2 g/kg per day for two consecutive days), showing clear clinical improvement of symptoms with no further relapse, whereas in the remaining 36 patients, a second cycle led to clinical well-being for at least one year as in the following months they had manifested resumption of symptoms. In a small percentage (15%, n = 8 cases) after an initial response, symptoms reappeared requiring a third further cycle one year after the first administration, without obtaining a significant result. Of the eight nonresponder cases, five also had temporo-mandibular joint disorders. We applied exercises that consisted of 10 repetitions, twice a day, of protrusion–retrusion of the mandible with a spacer (cotton roll) between the incisors and lateralization of the jaw with a spacer between the canines. After a month of exercises, the painful symptoms improved in all of them along with the stress deriving from this condition. This was one of the reasons that prompted us to carry out a small review on rehabilitation therapies, a weapon often underestimated but that can be useful as a support to other therapies (Figure 3).

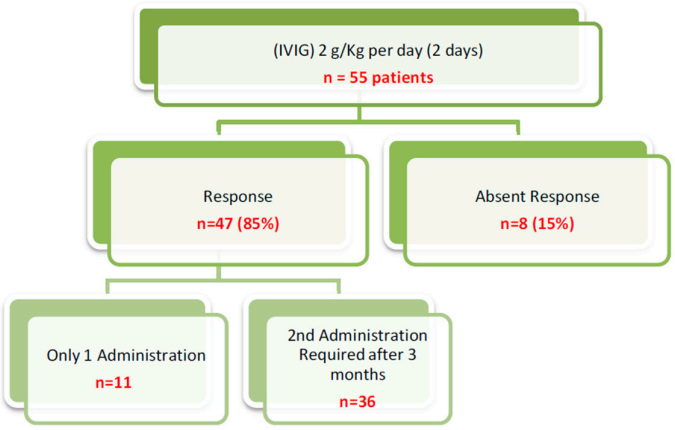

Figure 3. Outcome after administration of IVIG therapy.

4. Discussion

PANDAS are possible post-infectious disorders of pediatric age in which, after a previous pharyngeal infection of GABHS, children present with sudden-onset symptoms including behavioral disturbances together with movement disorders such as tics or OCD, with possible relapsing–remitting clinical course, consequent to new infective processes [1–4]. Similarly to Sydenham chorea [17], another condition related to GABHS infection, it is universally thought that in both conditions, a molecular mimicry between streptococcal and cellular antigens leads to an antibody production that would be responsible for the clinical manifestation, so representing the previous GABHS (or from other etiological agents, as in PANS) as a mere trigger for an autoimmune process. Sometimes the symptomatology may retrace the typical Tourette syndrome phenotype, at the point that precise differential diagnosis between the two forms is often di cult, and so leading some authors to consider the two conditions a different manifestation of the same disease. This pathogenetic hypothesis is nowadays limited by the lack of evidence regarding etiological causes of TS, nevertheless, a possible interaction has been observed by Spinello et al. [19], who documented that after a repeated GABHS infection, an immune response was generated which was associated with neurological and behavioral phenotypes similar to Tourette syndrome. Considering the wide heterogeneity of clinical manifestations and the possibility of overlap with any other neuropsychiatric disturbance, reaching the diagnosis of PANS/PANDAS can represent a real clinical challenge, requiring the support of biochemical and laboratory assays, together with complementary instrumental surveys. In the specific case of our sample, we selected a cluster of extreme-grade patients whose clinical manifestations were composed of a wide range of tics movements, together with equally severe OCD subtypes, including suicidal behavior. Quite

informative have been, in the same way, laboratory assays, which gave positive results for cultural pharyngeal swabs in 58% of cases (n = 32 out 55 patients), and negative in 14 cases in which false negativity could not be excluded due to previous antibiotics treatment assumed shortly before the patient was placed in our study.

Varying from literature evidence that admits the presence of abnormal brain activity in EEG in PANS/PANDAS patients (together with obstructive apnea syndrome, sleeping disturbances, or parasomnias) [20], in our sample, EEG pattern was normal in nearly all the observed patients.

The MRI had no significant result either; the isolated anomalies revealed in the three cases observed are probably not related to symptomatology, as in the literature there are no comparisons between the abnormalities found and the clinical finding of PANS/PANDAS. Despite morphologic imaging, a better comprehension of neuropathogenesis of clinical features could be provided by functional imaging assays. An initial approach was provided by Citak et al. [21], who performed an HMPAO-SPECT (hexamethyl propylene amine oxime single-photon missions computed tomography) on a group of patients affected by Sydenham chorea or PANDAS, revealing a hypoperfusion pattern, with special involvement of thalamus or basal ganglia (striatum); a further contribution in explaining the pathophysiology of behavioral and movement impairment of these patients come from Kumar's [22] team, who demonstrate the presence of a neuroinflammation pattern involving bilaterally lentiform nuclei and caudate nuclei in observed cases and not in controls, suggesting that this distribution pattern of neuroinflammation could be related to the clinical manifestation.

Administering the first cycle of IVIG therapy has already given us good results 3–30 days after administration. It is very important to underline that in cases of symptomatology reappearing within the year, administering the second course of therapy was important and led us to obtain a lasting improvement in symptoms (at least one year). The importance of the second cycle after the failure of the first is, therefore, an important aspect to consider. Administering the third cycle to those who did not respond to the second one did not lead us to obtain relevant clinical results, at which point we used other support therapies, such as rehabilitation, helping the patient to obtain a slight improvement in some aspects.

As regards therapeutic approaches, as support to accompany the IVIG treatment, as an initial option to control neuropsychiatric disturbances, an initial approach would consist of cognitive behavior therapy together with pharmacologic support with selective serotonin reuptake inhibitors (SSRIs) [23] for treating OCD manifestations, while for tic disorders, initial behavioral interventions are similarly recommended together with psychopharmacological treatments [24]. Nevertheless, after an initial high percentage of response, most patients showed lifelong treatment resistance [20], following the autoimmune etiology suspected, thus requiring in most cases the adoption of other therapeutic strategies.

Besides standard psychiatric treatments, an alternative therapy could include a treatment/prevention approach consisting of antibiotics or tonsillectomy [19] to prevent the recurrence of GABHS infections.

Considering the evidence that emerged from the literature and the intrinsic limitations of our study, such as the lack of a control group and a too-small period of follow-up, we cannot a rm a sure conclusion about the e cacy of IVIG treatment in PANDAS. In our opinion, anyway, it represents a safe and effective accessory approach in the clinical management of patients with serious–severe type of disturbances, agreeing with the PANS Research Consortium (PRC) [25]. Tics, movement disorders such as motoric hyperactivity, dysgraphia, speech disorders, and OCD are the main causes of disability in many aspects of daily living [26]. The performance skills in activities of daily living (ADL) of the patients, like personal hygiene and grooming, bathing, extracurricular activities, and participation in all aspects of leisure activities are other features that the physician team must evaluate.

Pharmacologic treatment is the most common treatment for the disorder. The rehabilitation could be an added value. Several studies highlight the importance of different therapeutic approaches, but without a specific description [27,28]. In patients with an abnormal neurological examination, muscle

weakness, abnormal reflexes, or chorea, a further workup is indicated. In these cases, an individualized rehabilitation treatment could avoid repetitive and nonrhythmic motor movements. In the study of Cocuzza et al. [29], PANDAS patients with temporomandibular joint disorders have undergone rehabilitation treatment. The exercises were explained in the hospital to correctly execute them, then the patients executed mirror exercises at home. The exercises consisted of 10 repetitions, twice a day, of protrusion–retrusion of the mandible with a spacer (cotton roll) between the incisors, lateralization of the jaw with a spacer between the canines positioned homolaterally to the direction of the lateral displacement, maximum opening and closing of the mouth. The four PANDAS patients with oromandibular dystonia achieved an improvement of the algic symptoms through the self-rehabilitation program, with a reduction of the hyperkinetic movements. No disturbances or worsening have been reported after the self-rehabilitation treatment was performed. Sokol [30] described eating compulsions, as an OCD, in four patients. He supposed a temporal relationship between antibiotic treatment and decreased eating disorder symptoms. According to his research, the compulsions progressively decreased with antibiotics and with rehabilitation treatment.

Tona et al. [31] described activities of daily living, math, handwriting, extracurricular activities, free play, organized sports, community and family social participation, higher-level thinking, attention, memory, sequencing, emotional coping, and energy and drive were commonly affected during exacerbations. During exacerbations, children often required assistance and adaptation to remain functional or were unable to function at a typical level.

A personalized rehabilitation program could be applied to subjects with this disorder to reduce pain, intensity and number of hyperkinetic movements, consequent awareness, and disability in ADL. Obsessive–compulsive disorders, included eating disorders, could be treated with individual therapy, group counseling and skills training, cognitive behavioral therapy, psychodynamic therapy, mindfulness-based approaches, and yoga therapy. Occupational therapy, introduced early, improves fine and gross motor skills and motor planning.

5. Conclusions

During our study, we have seen how the proposed IVIG treatment has benefited our patients, although not all of them, being in line with the studies already present in the literature from which we took inspiration and used the same immunoglobulin dosages.

Considering the variability of a disease still under study, we have taken as a good result the simple clinical improvement of at least one degree of decrease (according to the CY-BOCS), lasting at least one year. Considering the great impairment during their lives that extreme-grade patients have, moving to a lower grade brings enormous improvements in daily living. Considering the heterogeneity of the studied group, the short observation time, and the still unclear pathogenic mechanisms of PANS/PANDAS, we cannot establish the correct definitive therapy; however, the encouraging data from this and other studies can tell us that we are not far from a better understanding of this disease.

Surely, more precise knowledge about the pathogenic mechanisms at the basis of neuropsychiatric impairment involved in these disturbances could help researchers to identify more effective and shared therapeutic approaches and confirm the accuracy of IVIG treatment. However, from the review, we did try to create a clear idea on the importance of rehabilitation therapy; it is clear that this therapy is eligible to be an added value of proven utility associated with drug and other therapy in PANS/PANDAS. A rehabilitation program must be tailored to a child's specific needs, and the exercises must build specific weak skills. Personalized rehabilitation training could improve the movement disorders of patients with PANS/PANDAS.

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Relevant Studies to Establish a Medical Standard

Intravenous Immunoglobulin

IVIg for children with selective IGg deficiency/ hypogammaglobulinemia:

- ● Compagno N, Malipiero G, Cinetto F, Agostini C. Immunoglobulin replacement therapy in

  secondary hypogammaglobulinemia. Front Immunol. 2014;5:626. Published 2014 Dec 8.

  doi:10.3389/fimmu.2014.00626

- ● Garcia-Lloret M, McGhee S, Chatila TA. Immunoglobulin replacement therapy in children.

  Immunol Allergy Clin North Am. 2008 Nov;28(4):833-49, ix. doi: 10.1016/j.iac.2008.07.001. PMID:

  18940577; PMCID: PMC2585601.

IVIg efficacy in PANS patients:

- ● Chang K, Frankovich J, Cooperstock M, et al. Clinical evaluation of youth with pediatric acute-onset neuropsychiatric syndrome (PANS): recommendations from the 2013 PANS Consensus Conference. J Child Adolesc Psychopharmacol. 2015;25(1):3-13. doi:10.1089/cap.2014.0084
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- ● Pavone et al., PANS/PANDAS: Clinical Experience in IVIG Treatment and State of the Art in Rehabilitation Approaches. NeuroSci 2020;1:75-84
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Search for a **rare disease**

PANDAS *

Search

(*) mandatory field

- (•) Disease name
- ( ) OMIM
- ( ) Gene name or symbol
- ( ) ORPHAcode
- ( ) ICD-10

Other search option(s)

PANDAS

Suggest an update

Disease definition

PANDAS is an acronym for Pediatric Autoimmune Neuropsychiatric Disorders Associated with a group A beta-hemolytic Streptococcal infection and applied to a subgroup of children with obsessive-compulsive disorder (OCD) and/or tic disorders.

ORPHA:66624

Classification level: Disorder

Synonym(s):

Pediatric autoimmune disorders associated with Streptococcus infections

Pediatric autoimmune neuropsychiatric disorders associated with Streptococcus infections

Prevalence: **Unknown**

Inheritance: **Not applicable**

Age of onset: **Childhood**

ICD-10: **G96.8**

ICD-11: **8E4A.0**

OMIM: -

UMLS: **C2931429**

MeSH: **C537163**

GARD: **7312**

MedDRA: -

Summary

Epidemiology

The prevalence is unknown but the boy-to-girl ratio is 2.6:1.

Clinical description

The current diagnostic criteria for the PANDAS are: presence of OCD and/or a tic disorder, very young age at onset (prepubertal), sudden and dramatic onset of symptoms, association between streptococcal infections and episodic relapsing-remitting exacerbations manifesting as neuropsychiatric symptoms (motor hyperactivity or adventitious movements including choreiform movements or tics). The increased severity of symptoms usually persists for at least several weeks, but may last for several months or longer, followed by a slow, gradual improvement. The major distinctive feature of PANDAS is the temporal association between neuropsychiatric symptom exacerbations and streptococcal infections. Additional neuropsychiatric symptoms occur frequently: emotional lability, separation anxiety, anorexia, impulsivity, distractibility and motor hyperactivity characteristic of attention deficit hyperactivity disorder (ADHD). Comorbid disorders include major depression (36%), major dysthymia (6%) and separation anxiety disorder (20%).

Etiology

The etiology is uncertain. One theory is that streptococcal infections trigger an antibody response in some children that causes changes in the basal ganglia. No specific genetic factors have been identified.

Diagnostic methods

Diagnosis of PANDAS is clinical. Neuroimaging studies may reveal increased basal ganglia volumes.

Management and treatment

Management includes standard interventions for obsessive-compulsive and tic disorders: cognitive-behavioural therapy, reversal therapy in the case of tic disorders and pharmacologic therapy (neuropsychiatric drugs, antibiotics to prevent infections and intravenous immunoglobulin therapy).

- Last update: ***November 2006***

A summary on this disease is available in Deutsch (2006) Español (2006) Français (2006) Italiano (2006) Nederlands (2006)

Additional information

Further information on this disease

- Classification(s) (1)
- Gene(s) (0)
- Clinical signs and symptoms
- Other website(s) (1)

Patient-centred resources for this disease

- Expert centres (131)
- Networks of expert centre (5)
- Diagnostic tests (2)
- Patient organisations (59)
- Orphan designation(s) and orphan drug(s) (0)

Research activities on this disease

- Research projects (4)
- Clinical trials (0)
- Registries/biobanks (20)
- Network of experts (1)

Specialised Social Services

- Eurordis directory

Newborn screening

- Newborn screening library

PLOS ONE

RESEARCH ARTICLE

Clinical Practice Guidelines for Rare Diseases: The Orphanet Database

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Abstract

Clinical practice guidelines (CPGs) for rare diseases (RDs) are scarce, may be difficult to identify through Internet searches and may vary in quality depending on the source and methodology used. In order to contribute to the improvement of the diagnosis, treatment and care of patients, Orphanet (www.orpha.net) has set up a procedure for the selection, quality evaluation and dissemination of CPGs, with the aim to provide easy access to relevant, accurate and specific recommendations for the management of RDs. This article provides an analysis of selected CPGs by medical domain coverage, prevalence of diseases, languages and type of producer, and addresses the variability in CPG quality and availability. CPGs are identified via bibliographic databases, websites of research networks, expert centres or medical societies. They are assessed according to quality criteria derived from the Appraisal of Guidelines, REsearch and Evaluation (AGREE II) Instrument. Only open access CPGs and documents for which permission from the copyright holders has been obtained are disseminated on the Orphanet website. From January 2012 to July 2015, 277 CPGs were disseminated, representing coverage of 1,122 groups of diseases, diseases or subtypes in the Orphanet database. No language restriction is applied, and so far 10 languages are represented, with a predominance of CPGs in English, French and German (92% of all CPGs). A large proportion of diseases with identified CPGs belong to rare oncologic, neurologic, hematologic diseases or developmental anomalies. The Orphanet project on CPG collection, evaluation and dissemination is a continuous process, with regular addition of new guidelines, and updates. CPGs meeting the quality criteria are integrated to the Orphanet database of rare diseases, together with other types of textual information and the appropriate services for patients, researchers and healthcare professionals in 40 countries.

Introduction

Clinical practice guidelines (CPGs) are "systematically developed statements to assist practitioners and patient decisions about appropriate health care for specific circumstances" [1].

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Many CPGs have been developed in the last 25 years, as depicted in a recent review [2]. However most of them are aimed at common diseases and recommendations dedicated to rare diseases (RDs) remain scarce. In the European Union, a disease is considered as rare when it affects not more than 5 per 10,000 persons [3]. The rarity of randomised controlled trials puts a brake on the development of high quality guidelines for RDs, as well as the high development costs for funders who consider more prevalent diseases to be priority investment targets. In spite of these hurdles, over the last few years the development and dissemination of CPGs for RDs has garnered increasing attention [4–6]. The contribution of CPGs to shortening the time to diagnosis and improvement of the quality of care is now widely acknowledged, and several European countries have included CPG development as a priority in their respective national plans on RDs [7].

Retrieving RD guidelines from Internet searches is sometimes challenging for health professionals and patients who may not have the time or skills to search for the most relevant information [8]. Large national and international databases gathering CPGs are available [9–12] but they generally contain very few guidelines specific to RDs, which are difficult to find amongst the mass of recommendations available for more frequent diseases. In addition, in the field of RDs, a proportion of the guidelines produced by research networks, reference centres or other expert organisations is not published in international peer-reviewed journals, and thus is not retrieved from biomedical literature databases. Alternatively, Google searches may help identify RD guidelines, but the specificity of the suggested results is very low [13].

Once CPGs have been identified, another issue (not specific to RDs) might be their variable quality. To assess the quality of CPGs, several manuals have been published [14, 15]. One of the most employed and internationally validated grading systems is AGREE (Appraisal of Guidelines, Research and Evaluation) [16] and its revised version AGREE II Instrument [17]. Despite the availability of appraisal handbooks, methodological quality remains variable [2, 14, 18, 19] and needs to be verified. This might be even more important for RDs, as CPGs are sometimes developed by specialist groups who may not have the same resources as governmental bodies [2]. Methodologies for CPG development have been proposed that take into account some of the specificities of RDs, *e.g.* the lack of sound evidence, data about patients' opinion, ethical considerations and surveys of clinical practices [5, 6, 20–22].

Orphanet is an international data resource dedicated to RDs that was created in 1997 to address the scarcity and fragmentation of information on RDs. It is co-funded through the European Union's Health Programme and comprises a network of 40 countries. Orphanet endeavours to provide the community at large with a comprehensive set of information and data on RDs and orphan drugs in order to contribute to the improvement of the diagnosis, care and treatment of patients with RDs.

Orphanet is currently the most comprehensive repertoire of information and data on RDs, notably in terms of referenced documents, and the only resource that establishes a link between a classification of RDs, textual information and the appropriate services for patients, researchers and healthcare professionals.

This article describes the Orphanet workflow for the identification, evaluation and dissemination of CPGs on RDs, and provides an analysis of the resulting CPGs disseminated through Orphanet in terms of medical domain coverage, prevalence of diseases, languages and type of producer. Moreover, this study provides an insight into the variability in CPGs quality and availability, and shows the way these problems have been addressed in order to provide access to relevant, specific and good-quality recommendations for the management of RDs.

Methods

CPG identification and selection

Documents are considered as guidelines when they provide recommendations for clinical practice, in the form of consensus statements/recommendations, best practice statements or guidance recommendations. Only documents developed by expert groups are selected. Reviews providing some recommendations as concluding remarks or authors' point of views are excluded, as well as recommendation documents released by single authors. A global bibliographic survey on RD literature is performed as a continuous process using a list of selected medical journals, which allows a small number of CPGs to be retrieved. It is completed by more specific bibliographic searches for guidelines and RDs on PubMed, Google and Google Scholar, each search engine often generating different and complementary search results. For certain diseases, websites of national and international databases, research networks, foundations, learned societies, governmental institutions and expert centres are browsed if applicable. Pearl-growing (using one resource to further identify other resources) is also frequently used. Although the most frequent sources of information are in English and in other major European languages, no language restriction is applied. Documents older than 5 years are not considered unless recent publications clearly state that they are still up-to-date. Preferably, recommendations less than three years-old are selected. Of note, some CPGs older than 5 years may still be up-to-date in spite of the absence of recent publication that states so. The 5-year selection limit may therefore result in the rejection of up-to-date recommendations. This cut-off was chosen as it is often applied by national guideline producers [23–25]. The topic should be directly related to a disease listed in the Orphanet nomenclature of RDs, and for dissemination purposes, when several CPGs are available, it was decided to include only the most comprehensive one. The topics covered are also taken into account: recommendations should cover almost, if not all aspects of disease management (*e.g.* guidance addressing only surgical intervention for skeletal anomalies in Marfan syndrome or on the use of a particular drug are excluded, guidelines proposing recommendations for only neurological issues for a multisystemic disease are not selected, etc.), unless a more general CPG is not available for this disease. Attention is paid to the universality of application of the guidelines with regard to the document language. For instance, guidelines in English that propose recommendations for inhabitants in China only will not be retained, but guidelines in Chinese that target Chinese patients will be considered. For the same reason, local CPGs are excluded. CPGs discussing recommendations for all population ages are preferred over CPGs targeting only children for a disease affecting all ages. Moreover, as much as possible, CPGs specific for rare forms of diseases are selected. However, due to the scarcity of available information, more general guidelines that also include recommendations for specific forms are sometimes retained (*e.g.*, guidelines dealing with some cancers including both rare and non-rare forms). In summary, the quality evaluation procedure includes some mandatory criteria and some desirable ones (Fig 1), the latter being considered together with all the criteria in order to validate the decision to retain a document.

Evaluation and dissemination

In addition to the specific criteria mentioned above, selected documents are evaluated by an information scientist according to quality criteria derived from the AGREE II Instrument, comprising 23 items organised into six domains: scope and purpose, stakeholder involvement, rigour of development, clarity of presentation, applicability and editorial independence [17]. To better fit the evaluation process to the actual guideline quality, the original rating system of

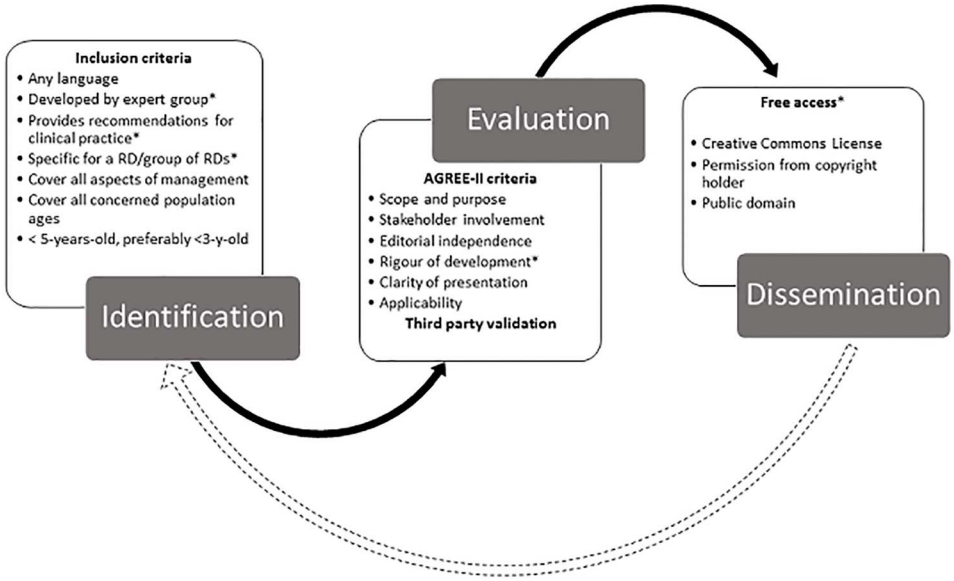

Fig 1. Methodological flow chart for CPG dissemination via Orphanet. * indicates the mandatory criteria versus the desirable ones for which exceptions can be made depending on all the other criteria.

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AGREE II that uses a 7-point scale for each item (from strongly disagree to strongly agree) was simplified by yes/no answers. Appraisal of the six domains is carried out, but methodological aspects (domain 3 of AGREE II) are given particular weight: a poor methodological description leads to guideline rejection even if all other domains are outstanding. As in AGREE II, the assessors are asked whether they would recommend the use (here adapted into whether they would recommend dissemination) of the guideline. Then, overall assessment is carried out by taking into account the combined AGREE II and Orphanet inclusion criteria (Fig 1). Evaluated guidelines are validated by a third party (medical doctor) before the decision to display via Orphanet is taken. Authorisation from the copyright holders to disseminate CPGs is a key condition to the dissemination of guidelines via Orphanet. Articles published in "Open Access" (or with equivalent labelling: Open, Unlocked, . . .) and associated with a Creative Commons (CC) licence are directly made available without the need for specific permission. For documents that do not expressly state that free dissemination for non-commercial purposes is authorised, permission to establish links on www.orpha.net are first obtained from the copyright holder.

Analysis of CPGs in the Orphanet database

The distribution of CPGs included in the Orphanet database until July 2015 was analysed by medical specialty. To do so, the linearisation of the RDs multi-hierarchical classification produced by Orphanet [26] was used. It provides a mono-hierarchical system in which each disease, usually included in multiple classifications, is allocated to a predominant body system. Specialties with no CPG were not displayed in the results. For CPGs associated with several Orphanet entries (CPGs targeting a disease with several subtypes, or a group of diseases), only the main target of the guideline was considered. Analyses of the publication languages were also carried out, either globally or by medical speciality. A Venn diagram representation [27] was used to visualise the diseases covered by CPGs in several languages.

The type of publication medium was analysed by distinguishing CPGs published in peer-reviewed journals and CPGs published by various organisations, independently of the type of authors.

Moreover, to analyse the CPG distribution as a function of disease prevalence, the Orphanet epidemiology database was used to retrieve the corresponding prevalence. As above, for CPGs associated with several entries (CPGs targeting a disease with several subtypes, or a group of diseases), only the main target of the guideline was considered for the choice of prevalence. The point prevalence was selected, and the geographic area corresponding to worldwide prevalence was used. When worldwide prevalence data were not available, Europe prevalence data were considered, then specific countries prevalence data (which was the case for only 2 diseases, for which USA prevalence was available). Diseases with no available prevalence data were not included in the analysis, which accounted for 44% of the diseases (22% with unknown prevalence and 22% with data not yet available in the Orphanet database). Diseases with CPGs in several languages were counted only once. Results are presented by class of prevalence, from the most frequent (1–10/10,000) to the rarest diseases (>1/1,000,000). Finally, an analysis of the number of CPGs downloaded in 2015 (1 January to 31 December) was carried out using Google analytics data. This analysis could be performed only on PDFs hosted on the Orphanet server, which accounted for 85 documents (31% of the disseminated CPGs). Audience analysis of the remaining 69% of guidelines was not possible due to their dissemination through direct links to the publishers' websites.

Results

Quality evaluation of CPGs

Very few RD guidelines were found to meet a majority of the AGREE II criteria. Among the least followed criteria, we often noticed insufficient information about the management of conflicts of interest, insufficient information about the methodology to establish recommendations, a lack of consideration of patients' preferences, and a lack of information about implementation, dissemination and updating procedures. Moreover, we observed variable quality depending on the dissemination medium. In general, guidelines disseminated in peer-reviewed journals were of better methodological quality than guidelines disseminated on websites of research networks or expert groups. For the latter, methodological procedures were often either incomplete, difficult to locate (presented in separate documents with no link provided in the guideline document) or missing.

In practice, depending on the scarcity of disease information, difficulty to retrieve the guidelines from Internet searches, recommendation quality, usefulness of specific information for health professionals, and the presence or absence of other documents on Orphanet covering the same topics, some exceptions were made to the coverage and target population inclusion criteria, to enable selection of relevant medical recommendations even when all disease aspects or target populations were not covered. In conclusion, limitations encountered at the CPG identification step led us to make some adjustments to the criteria used to retain or not a guideline.

Guideline representation on Orphanet

From January 2012 (beginning of this project) to July 2015, 277 CPGs were disseminated on www.orpha.net (S1 Table). They represent about half of the documents that fulfilled the content and quality criteria, the other half being rejected due to the impossibility to obtain copyright holders' permissions to make links. Guidelines targeting several closely related diseases, or diseases that include etiological, histopathological or clinical subtypes were linked to each of

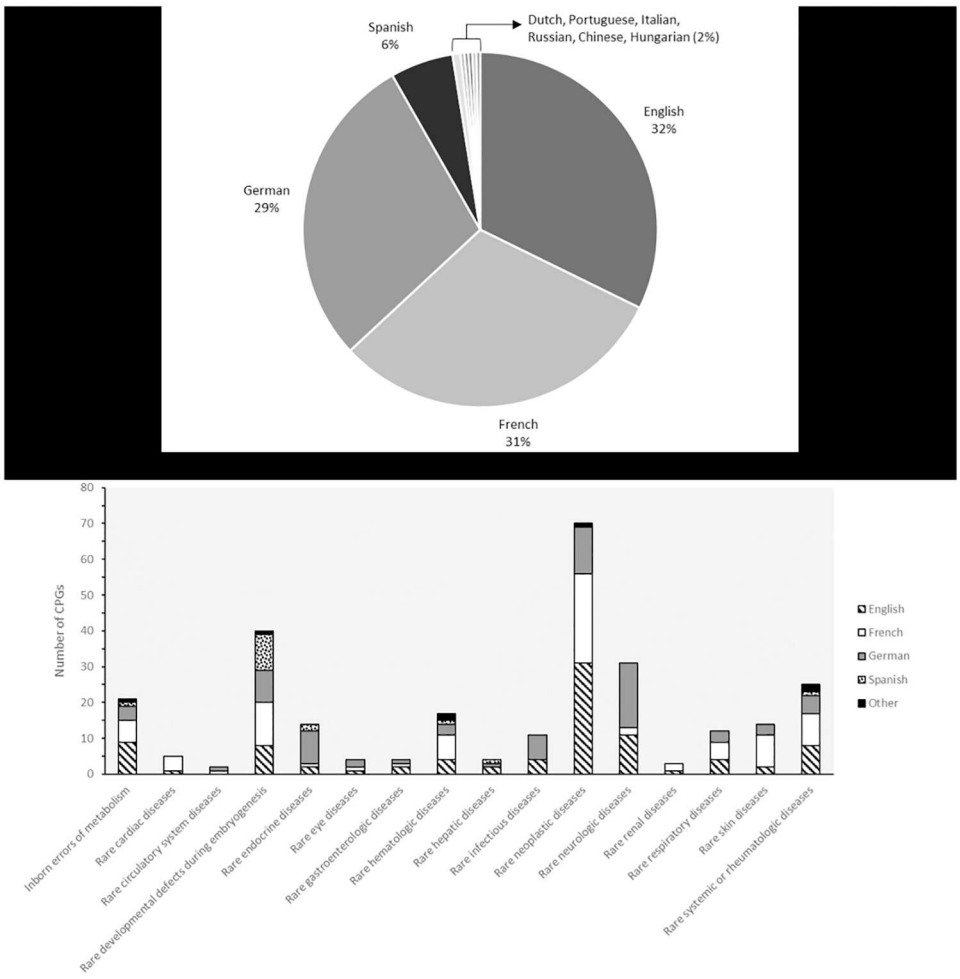

Fig 2. CPG distribution on Orphanet. (A) Proportion of CPGs available by language, (B) CPG distribution by medical specialty and language.

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the related diseases/subtypes in the database, which represents coverage of 1,122 entities. Ten languages were present, with a predominance of guidelines in English (32%), French (31%) and German (29%) (Fig 2A). About 6% of all texts were CPGs in Spanish, and the other 2% gathered CPGs in Hungarian, Italian, Dutch, Portuguese, Chinese and Russian.

Distribution of CPGs among medical specialties showed some "privileged" groups. Rare neoplastic diseases, neurologic, hematologic, rheumatologic and developmental diseases were the most represented, often with CPGs in both English, French and German (Fig 2B). It should be noted that, in the case of guidelines targeting multisystem diseases, the use of the linearised classification did not allow the involvement of a CPG covering multiple body systems to be shown. However, the use of this linearisation was chosen for the analysis in order to provide a combined picture of the exact number of CPGs, together with their distribution by medical specialty and by language of publication.

Among the French guidelines, 27% were from the French National Cancer Institute (INCa) [28], which, in addition to cancer guidelines from other sources, results in a strong representation of guidelines for neoplastic diseases. For CPGs in English, a more diverse range of author sources is observed because they were mostly published in medical journals, although

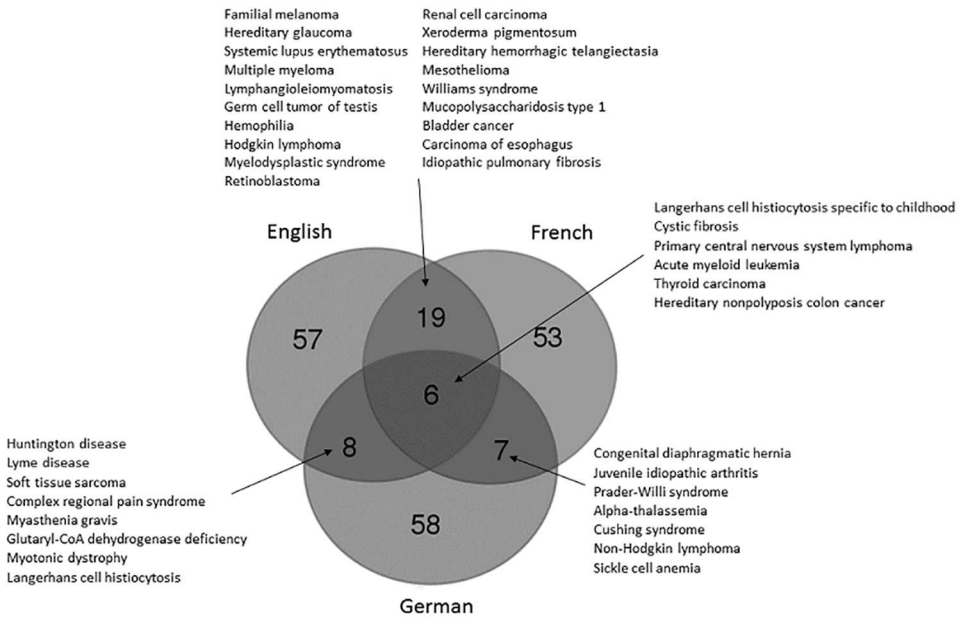

Fig 3. Repartition among diseases of CPGs in different languages. The Venn diagram representation shows the diseases covered by either one, two or three languages (English, French, German).

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guidelines on cancers were also clearly overrepresented (34% of all CPGs in English). Interestingly, very few diseases had guidelines simultaneously linked in several languages (Fig 3). Twenty-five diseases had CPGs in both English and French, 14 diseases had CPGs in both English and German, and 14 in both German and French. Only 6 diseases had CPGs in English, French and German.

Types of dissemination medium of CPGs differed depending on the language (Fig 4). CPGs in English were mostly published in international journals, and were most frequently authored by international working groups. The majority of CPGs in French were from public health organisations: the French National Authority for Health (HAS) and the INCa. The main sources of German guidelines were published by the Association of the Scientific Medical

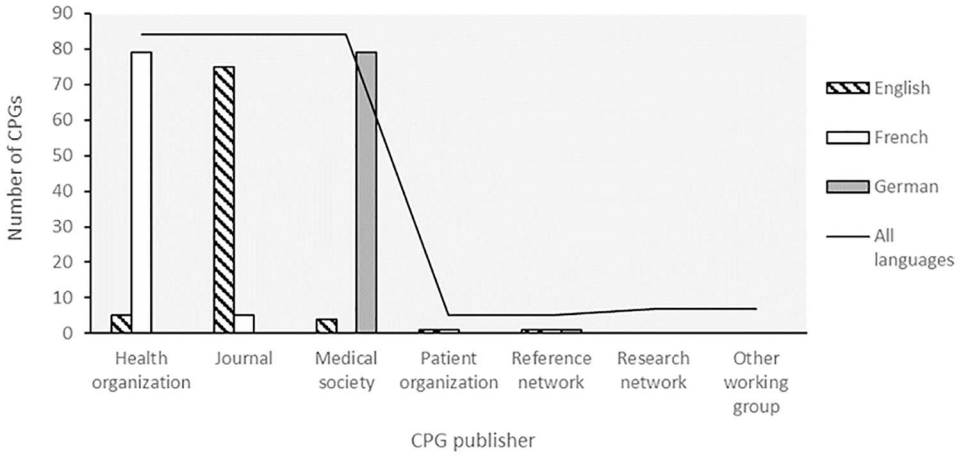

Fig 4. CPG distribution by type of publication medium and language.

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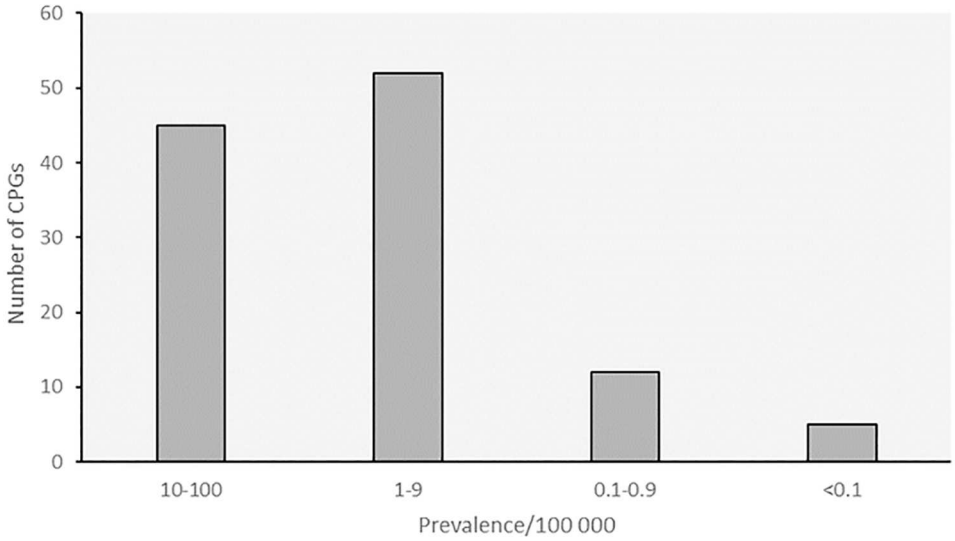

Fig 5. CPG distribution by disease prevalence.

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Societies in Germany (AWMF). Besides these three major publishers, a few of the collected guidelines were directly published by patient organisations, research networks, reference networks and other working groups on their websites. It should be mentioned that in all cases the CPGs were authored by expert working groups (which is one of the selection criteria), independently of the publication type.

About 20% of guidelines were related to diseases with higher prevalence (10–100 /100,000), while guidelines for extremely rare diseases were scarce (2% targeted diseases with prevalence <0.1/100,000) (Fig 5). In fact, some of these CPGs also include recommendations for rarer forms of the target diseases, with which they were also associated in the Orphanet database, but which were not taken into account in the analysis because only the main target of the guideline was considered for the choice of associated prevalence. Inclusion of these rarer forms in the analysis resulted in a slight shift of the number of CPGs towards diseases with lower prevalence, with about twice the amount of guidelines concerning diseases in the class of prevalence 1–9/100,000 (data not shown). Nevertheless, this shift did not impact the lowest class of prevalence (<0.1/100,000), which remained underrepresented.

Access to the guidelines on Orphanet

Guidelines on a specific disease can be found by accessing the disease page via the disease search tool on www.orpha.net. A link "Clinical practice guidelines" appears in the "detailed information" section at the bottom of the disease page when a CPG is available. Moreover, the presence of guidelines can be visualised and accessed via the "Encyclopaedia for professionals" tab, where an alphabetical list of diseases and documents linked to them is displayed.

In 2015, more than 1,334,000 downloads of PDF files (concerning 31% of the guidelines in the database) were recorded, with a monthly average of >111,000 downloads. Since 2012, an increasing number of documents have been disseminated through links to the publishers' websites and could not be included in the audience analysis. The PDF documents hosted on the Orphanet server thus represented the oldest guidelines and it is likely that these figures have underestimated the real number of consultations of all the guidelines available via Orphanet.

Discussion

The difficulty to retrieve RD guidelines from the abundance of recommendations available for non-rare diseases is well known [13, 20]. As an additional challenge, the Orphanet database comprises more than 7,800 diseases/groups of diseases (excluding disorder subtypes) that represent potential targets for guideline retrieval, and to which only a global literature survey for guidelines and RDs can be applied, yielding very few appropriate results. Some medical societies and health organisations with websites gathering guidelines for a large variety of diseases, including RDs, were useful sources. In all cases, the retrieval of a potentially interesting document through this global survey was followed by specific disease guideline search to determine whether it was the most relevant CPG. Due to the diversity of publication media, it is clear that case-by-case approaches should be applied rather than a single retrieval strategy. Moreover, different search engines may be complementary to each other (*e.g.* Pubmed and Google Scholar) and may help in retrieving different documents. This is likely due to differences in the search algorithms rather than in differences in content coverage [29, 30].

With the aim to provide a comprehensive set of information and data on RDs to contribute to the improvement of the diagnosis, care and treatment of patients, the quality control of CPGs disseminated via the Orphanet database is an important aspect to consider. Therefore, we have set up an optimised evaluation procedure to provide sufficiently trustworthy information, without applying criteria that are too strict and that would lead to the rejection of documents containing nonetheless useful information. This is of particular importance with RDs, for which clinical trial data are sometimes insufficient compared to the data available for common diseases. Based on the AGREE II criteria, some insufficiencies were frequently noticed, especially regarding rigour of development and editorial independence. This observation has been described by others for some RDs [31] but is not specific to this field, as also reported for more common diseases [2, 32, 33]. Variable quality was also noticed depending on the publication medium. This discrepancy may reflect a lack of methodological expertise of guideline developers or limited resources. While it does not preclude the high medical quality of the recommendations, insufficient or lacking methodological data may impair the credibility of these guidelines. To overcome issues with variable quality, some national health institutions and medical societies have established standardisation procedures. This is the case of the HAS that has established within the first French National Plan for RDs a procedure based on the AGREE II criteria to evaluate the methodological rigour and transparency during the guideline elaboration process [23]. This methodology is implemented in the French National Diagnostic and Treatment Protocols (PNDS guidelines) for RDs. AWMF has adopted a similar procedure based on DELBI, The German Instrument for Methodological Guideline Appraisal (AGREE II-derived) in order "to provide a tool for the scientific medical societies to create and publish up-to-date and high-quality guidelines" [24]. The discrepancy in CPG quality explains the differences in the origin of publishers represented on Orphanet according to the language, and is the direct result of the applied quality evaluation process.

No language restriction was applied but a human resources factor accounted for a bias in guideline collection in various languages. Guidelines published in less spoken languages were often fortuitously collected, rather than actively searched for. Other factors also contributed to this imbalanced language representation: impossibility of obtaining permission to make links, insufficient methodological quality of guidelines, and last but not least, the non-existence of CPGs for many countries. For some countries in Europe, the development of guidelines and their diffusion have been set-up as a major objective of National Plans or strategies for RDs, in order to improve the quality of care for RD patients [7]. The inclusion of such objectives in the French and German National Plans also explains the high number of CPGs in French and

German, in addition to CPGs in English that are most often the result of international working group publications in medical journals. A very low simultaneous coverage of diseases with guidelines in several languages was observed. This result underlines the importance of not applying language restrictions, as it allows larger guideline distribution among diseases.

Some recent initiatives to collect and disseminate RD guidelines are under development. RARE-BestPractices is an EU-funded project aiming to create a platform to share management guidelines for RDs [34]. It also aims at training stakeholders to produce and evaluate CPGs. CPGs indexed in this database undergo a quality validation process. Appraisal results are provided, but no filter based on the level of quality is applied for dissemination, and direct access to the document is not always provided, depending on the copyright conditions. The ZIPSE project aims to create an information portal for rare diseases in Germany to link existing information sources [35]. Among the collected information, links to guidelines and other documents disseminated through the Orphanet website shall be made. The Orphanet project on the collection, evaluation and dissemination of CPGs responds to the objectives of the EU's Third Health Programme (2014–2020), in particular those concerning rare diseases [36], and to the Council recommendation on an action in the field of rare diseases of 8th June 2009 that proposes to use Orphanet as a tool for information and research in order to contribute to improving the quality of care of patients, by improving the practice of health professionals and make information accessible through dissemination [37].

In addition to data quality assessment, free and direct (in one or two clicks) access to the linked information is an essential aspect of Orphanet's editorial policy. Authorisation from the copyright holders to disseminate CPGs is mandatory, even when these documents are freely accessible (*i.e.* viewable but not reusable). For research networks (mostly EU-funded) and national health institutes, it is often not an issue, as the aim of these organisations is to freely disseminate CPGs, and linking guidelines on Orphanet contributes to increasing their diffusion and thus, achieving their goal. Permission from medical societies are usually more difficult to obtain, and document access is sometimes restricted to members only. For articles published in medical journals, granting of permission by publishers to make links free of charge constitutes the major limitation to broader guideline dissemination. The articles in question represent at least half of the potentially interesting CPGs. Therefore, the major proportion of journal articles linked on Orphanet consists in documents published with a CC licence allowing diffusion without the need for specific permission. Of note, the number of articles with a CC licence has considerably increased during the last few years, which allows more CPGs to be disseminated. In addition to articles reusable without permission, some publishers may grant specific permission to link an article freely, but this concerns a very limited number of CPGs. All the other articles cannot be disseminated on Orphanet due to the impossibility of obtaining the permission to make a link, irrespective of their quality. A complementary source of permission to disseminate CPGs resides in Orphanet's active participation in the *patient* Inform collaborative program [38]. Indeed, through the production of disease summaries based on information contained in CPG articles, Orphanet is allowed to make special links to the articles, provided by the participating publishers. This allows patients and their caregivers to freely access relevant journal articles. Remarkably, while CPGs are originally dedicated to health professionals, a growing interest from patients and families for access to professional information is observed, that has paralleled the easier access to information provided by the Internet [39]. Empowerment of patients is also largely encouraged by their professional caregivers, through patient education [40].

Differences in the coverage of medical specialties were observed. Keeping in mind that guideline representation on Orphanet cannot be fully representative of all existing guidelines on RDs due to the applied selection criteria and non-exhaustive guideline retrieval, one can

however make some parallels with the orphan drug development situation. When looking at the distribution of the number of drugs with orphan designation and authorisation on the market, antineoplastic agents represent 40% of all drugs [41]. Fifty-six percent of the ongoing clinical trials registered in the Orphanet database were on rare cancers. Also observed for CPGs in general (not only for RDs), the availability of guidelines is related to conditions for which more research and investment are made [2]. In the oncology field, the availability of guidelines is likely correlated with the existence of funding programs. At the European Commission level, a European Partnership on action against cancer (EPPAC) was created in 2009 aimed at the promotion of National cancer plans in EU Member States [42]. The EU-funded RareCareNet was set to build an information network on rare cancers [43]. In France, the INCa recommendation guides on cancers are elaborated within the framework of the National Cancer Plan in order to define and disseminate the reference clinical practices to health professionals and to the public [44]. In addition, the higher representation of oncology CPGs is influenced by the fact that some of these documents do not specifically address recommendations for RDs, but encompass both rare and non-rare entities. As observed in the field of non-rare diseases in which the availability of guidelines is correlated with higher prevalence [2], the distribution of RD guidelines via Orphanet is associated with relatively prevalent diseases: most CPGs concern diseases with prevalence between 5/10,000 and 9/100,000. This is likely due to the fact that the choice of diseases to be covered often takes into account the availability of a treatment or therapeutic solution (*e.g.* surgery) and the availability of clinical trial data. In the case of the French PNDS recommendations, for instance, prioritisation criteria for the choice of diseases to be covered include controversy over diagnosis, controversy over treatment, availability of new treatments and regular use of drugs without market authorisation [45].

Conclusions

Access to information on RDs is one of the priorities adopted in most national plans in Europe [46]. Initiated in 2011, the Orphanet guideline dissemination project responds to this objective. In addition, it corresponds to a real need from both professionals and patients, as observed from our annual surveys [47] and from direct users' feedback who have expressed their interest in accessing more clinical guidelines in more languages. CPG collection, evaluation and dissemination is a continuous process, with regular addition of new guidelines. Documents included in the Orphanet database may also be replaced by updates released by a guideline development group (providing they fulfil the quality criteria), old documents may be removed when they are obviously not up-to-date or according to the expiry date set by the development group, and finally, older guidelines may be replaced by more recent ones when appropriate, *e.g.* when a new document is of higher quality or when it is of similar quality but contains additional/more recent information useful for clinical practice. Compared to general guideline databases such as G-I-N [9], besides free access and quality assessment, CPGs disseminated via Orphanet are part of an information network gathered around the disease entity: each CPG can be accessed from the specific disease page where other types of information can be found, among which the ORPHA code, epidemiological and genetic data, disease summary, classification, health care resources, data on research activities and available drugs, links to other websites, guides for patients and families, etc. Therefore, the guideline database is included into a more global project that establishes a link between diseases, the existing textual information concerning RD and the appropriate services for patients, researchers and healthcare professionals. This integrative dimension is a unique feature of the Orphanet database that can serve various stakeholders and purposes. It can be used to identify medical areas

for which CPGs are totally lacking but for which clinical research is active and/or drug development is ongoing.

Supporting Information

S1 Table. List of diseases associated with a clinical practice guideline disseminated on www.orpha.net. For each disease, the corresponding medical specialty considered for data analyses is indicated. "CPG language" refers to the language of publication of each CPG. "Source (publisher)" indicates in what medium the CPG has been published.
(PDF)

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Author Contributions

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Formal analysis: SP.

Funding acquisition: AR.

Investigation: SP KR MEMM SH.

Methodology: SP AR.

Project administration: SP AR.

Resources: VL.

Software: VL.

Validation: SP AR.

Writing – original draft: SP.

Writing – review & editing: SP KR MEMM SH VL AR.

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