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CNS autoimmune disease after *Streptococcus pyogenes* infections: animal models, cellular mechanisms and genetic factors

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Abstract

Streptococcus pyogenes infections have been associated with two autoimmune diseases of the CNS: Sydenham's chorea (SC) and Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus* infections (PANDAS). Despite the high frequency of pharyngeal streptococcus infections among children, only a small fraction develops SC or PANDAS. This suggests that several factors in combination are necessary to trigger autoimmune complications: specific *S. pyogenes* strains that induce a strong immune response toward the host nervous system; genetic susceptibility that predispose children toward an autoimmune response involving movement or tic symptoms; and multiple infections of the throat or tonsils that lead to a robust T_H17 cellular and humoral immune response when untreated. In this review, we summarize the evidence for each factor and propose that all must be met for the requisite neurovascular pathology and behavioral deficits found in SC/PANDAS.

Keywords

autoantibodies; autoimmunity; blood–brain barrier; gene set analysis; major histocompatibility complex; pathway-based genomic studies; PANDAS; *Streptococcus pyogenes*; Sydenham's chorea; T_H17 cell

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The infectious agent *Streptococcus pyogenes*, or Group A β -hemolytic streptococcus, is a major cause of short- and long-term morbidity for both infants and adults worldwide [1]. This is due not only to direct primary intranasal infections causing childhood pharyngitis or toxin-mediated pathology [2], but also secondary autoimmune sequelae including rheumatic fever (in the heart) [3], glomerulo-nephritis (in the kidney) and CNS autoimmune diseases, which are the focus of this review [4]. *Streptococcus pyogenes* is an airborne pathogen with tropism for both the olfactory epithelium (OE) and nasal-associated lymphoid tissues (NALT), which in children include the palatine and tonsillar adenoids and secondary lymphoid tissue within the nasal cavity [5,6]. Sydenham's chorea (SC) and PANDAS are the two CNS autoimmune sequelae of *S. pyogenes* infections. SC is characterized by uncoordinated, jerking movements with behavioral components and occurs in 20–30% of children with rheumatic fever [7,8]. PANDAS affects a small subset of children with GAS infections who exhibit abrupt onset of obsessive-compulsive behavior, verbal or motor tics, anorexia nervosa, separation anxiety and other abnormal behaviors [8–12]. The causal connection between bacterial infection and SC is well established, however a similar link to PANDAS is still under debate; multiple findings are consistent with an autoimmune mechanism for both diseases [13,14], albeit a few have failed to find evidence for autoimmunity in PANDAS patients [15]. The CNS sequelae of *S. pyogenes* infections are thought to be mediated by autoantibodies that recognize neuronal targets located primarily in the basal ganglia, which range from neurotransmitter receptors (D1 and D2 dopamine receptors) and signaling kinases to ion channels that may be responsible for the neurological and behavioral deficits exhibited by patients [16–18]. The autoimmune mimicry hypothesis, namely that antibodies generated from an aberrant immune response to *S. pyogenes* infections recognize host-specific proteins due to epitope mimicry of bacterial and host antigens, has been proposed to underlie all secondary sequelae of *S. pyogenes* infections, from rheumatic fever to CNS disorders [4,19]. Therefore, autoimmune consequences of infection represent an aberrantly targeted immune response, in which circulating antibodies recognize and attack host antigens to initiate an autoimmune reaction. Although intranasal *S. pyogenes* infections in children are almost ubiquitous, SC and PANDAS are relatively rare, thus additional factors likely play an important role in initiation or persistence of an aberrant CNS immune response. Here, we review some insights that have been obtained from animal models and human studies about the neuroimmune response and neurovascular pathology, and discuss future research directions aimed at understanding both the genetic basis of susceptibility for the host immune system and the pathogenesis of CNS autoimmune complications to *S. pyogenes* infections from a molecular and neuropathological standpoint.

Animal models for SC & PANDAS

Following the pioneering work of Lipkin, Hornig and colleagues over a decade ago [20], several studies have sought to recapitulate SC or PANDAS symptoms in rodents in order to develop reliable models to study the pathological basis of the aberrant immune response as well as neuropathological and behavioral deficits [21–25]. The majority of rodent models have been developed by multiple subcutaneous (sq.) immunizations of either SJL/J mice or Lewis rats with heat-killed bacterial homogenates, often in conjunction with agents known to breach the blood–brain barrier (BBB) such as lipopolysaccharide (LPS) or *Pertussis* toxin,

or by passive transfer of anti-*S. pyogenes* antisera from immunized to naive rodents (see Tables 1 & 2). We focus here on four recent studies that shed light on the mechanisms of humoral and cellular immune responses to repeated bacterial infections and their associated neurovascular pathologies and behavioral deficits.

Animal models for SC and PANDAS are largely aimed at recapitulating three features of human disease: the presence of serum IgGs directed against *S. pyogenes* (i.e., humoral response to immunization), regions of the CNS where IgGs (autoantibodies) cross the BBB and recognize cognate antigens such as dopamine receptors and behavioral anomalies ranging from motor coordination and ability impairments, verbal or motor tics, anxiety, compulsion and stereotypy to exploratory, aggressive and social deficits; these behaviors mirror the complex neurological and behavioral deficits present in SC and PANDAS patients. Although immunization routes are similar for most models, with the exception of Dileepan *et al.* [26] (see below and Tables 1 & 2), the neurovascular pathologies, regions of the brain showing IgG deposition and behavioral deficits nevertheless vary significantly. For example, in mouse models, serum IgG predominantly deposits within the hippocampus, deep cerebellar nuclei or rostral diencephalon, and very little IgG is found in the basal ganglia (Tables 1 & 2) [20,21,25]. In contrast, there is significant IgG deposition in the prefrontal cortex and basal ganglia in the rat model (Tables 1 & 2) [22,23]; the latter more closely resembles human disease in which there is strong evidence for neuroinflammation in the basal ganglia and thalamus, from imaging studies [27,28]. Furthermore, there is some evidence that both glutamatergic and serotonergic synapses are aberrant in mice after multiple GAS sq. immunizations, whereas disrupted dopaminergic and serotonergic circuits are more prevalent in the rat model with recurrent immunizations [21–25]. While dopaminergic transmission has been associated with motor deficits (chorea or choreiform movements) that are common to both SC and PANDAS, and involve the cortex-to-basal ganglia-to-thalamus circuits [13,29–31], defects in serotonergic neurotransmission have been implicated in both motor inhibition and obsessive-compulsive behaviors, two features that are unique to PANDAS patients. Although no glutamatergic synapse dysfunction has been reported for either SC or PANDAS, deficiencies in presynaptic proteins (e.g., vGluT2) that regulate exocytosis of glutamate-filled vesicles are found in the cortex, amygdala or subiculum of patients diagnosed with other neuropsychiatric diseases (reduced anxiety and some behavioral correlates of schizophrenia) [32,33]. Moreover, mouse strains mutant for *SAPAP3*, which is linked to obsessive-compulsive disorder (OCD) in humans [34,35], have defects in metabotropic glutamate receptors and exhibit OCD-like behaviors (e.g., compulsive grooming) [36,37]. Because glutamatergic projections play an important role in regulating the basal ganglia and have been implicated in tic disorders such as Tourette syndrome (TS) [38], the glutamatergic deficits reported for mouse models after multiple *S. pyogenes* immunizations may represent a very useful and understudied aspect of a conserved functional circuit between rodents and humans that is relevant for disease pathogenesis.

One unifying feature of all rodent models for SC and PANDAS is that animals develop motor deficits as well as compulsive, stereotyped or anxious behaviors and abnormal social interactions. These are reminiscent of motor symptoms seen in children afflicted with SC/PANDAS, however distinct behavioral deficits predominate in mouse versus rat models. For example, compulsive induced grooming and marble burying, two behavioral manifestations

of OCD, as well as motor deficits such as narrow beam crossing and food manipulation are consistently found in rat models having high IgG deposition in the prefrontal cortex and caudate regions [22,23]. On the other hand, the rearing behavior and beam walking deficits are more prominent in mouse models, for which IgG deposition is largely confined to the hippocampus and piriform cortex [20,21,25]. Variability in both IgG deposition sites in the CNS and behavioral manifestations among reported studies could be due to factors such as: the strain of *S. pyogenes* used; the sex of the animals and the species (rat vs mouse; see Tables 1 & 2). It is also noteworthy that, despite the importance of demonstrating behavioral deficits following *S. pyogenes* immunization, the underlying disease-relevant cells and neural circuitry responsible for mediating these deficits in animal models remains undefined, as well as the degree of conservation of the affected neural circuits between animal models and human patients. An especially puzzling component that most animal models have failed to address is how anti-Streptococcus antibodies gain access to the brain, because CNS blood vessels prevent free diffusion of large molecules (including antibodies) by virtue of the BBB [39].

We and colleagues have recently described a novel mouse model for SC and PANDAS [26] that features multiple intranasal (in.) infections of mice by passive inhalation of live bacteria. This route of infection mimics closely the human scenario, and induces a robust *S. pyogenes*-specific T_H17 immune response in host nasal tissue that depends on the TGF- β 1 and IL-6 cytokines [40–42]. These cytokines are required for expansion, maturation and migration of *S. pyogenes*-specific T_H17 cells to lymphoid tissues [40–42]. We find that Streptococcus-specific T_H17 cells are generated in the mouse nasal-associated lymphoid tissue (NALT), an analog of human tonsils, and migrate into the olfactory bulb (OB), the most anteroventral part of the mouse brain that receives innervation from the olfactory sensory neurons, before spreading to other brain regions. *S. pyogenes*-specific T_H17 cell entry into the brain is associated with impairment of both structural and functional barrier properties (e.g., degradation of tight junctions between endothelial cells and increased paracellular permeability), which in turn permits circulating serum IgGs and CD4⁺ T cells to enter the brain, recognize their cognate neuronal targets, promote neuroinflammation by activating microglia and ultimately alter synaptic connectivity and function [43]. Production of TNF- α , a pro-inflammatory cytokine produced by activated microglia, can exacerbate disease and enhance neurovascular damage. It is noteworthy that the route of immunization plays an key role in producing a T-cell response, because intravenous (iv.) immunization and challenge with heat-killed GAS does not promote T-cell entry into the brain [43]. Therefore, brain infiltration by T cells requires substantial expansion of effector memory T_H17 cells in close proximity to the olfactory mucosa. Based on this mouse model, we propose that recurrent upper respiratory GAS infections both induce antibodies that mimic and cross-react with CNS epitopes and prime a T_H17 cellular response. In some children, both autoantibody production levels and the T_H17 effector memory pool exceed a threshold that allows migration from the nasal epithelium into the OB along the olfactory nerve. Within the brain, the inflammatory response induced by IL-17A, activated microglia and macrophages then breaks down the BBB, which enables autoantibodies to enter into the brain.

Our mouse model for SC and PANDAS has features in common with others. First, the basal ganglia display only a modest pathology. Second, IgG infiltrates are found in the

hippocampus, cerebellum and piriform cortex. Third, there is no artificial opening of the BBB, and finally, there is significant disruption of glutamatergic synapses. Furthermore, our model has several features to promote understanding of SC/PANDAS, such as: striking neurovascular dysfunction with BBB leakage, due to the presence of *S. pyogenes*-specific T_H17 cells in the brain after recurrent infections; a robust neuroinflammatory response indicated by activated microglia, which correlates well with recent findings from human PET imaging using a probe for microglial activation [27]; and reproducible and severe BBB leakage and IgG deposition in brain regions involved with the sense of smell (OB, anterior olfactory nucleus, olfactory tubercle and piriform cortex), hunger (lateral hypothalamus), emotional learning, aggression, fear and anxiety (amygdala). Taken together, these novel phenotypes provide an excellent paradigm to understand changes in neural circuitry in the parts of the brain that underlie behavioral deficits reliably characteristic of PANDAS patients [10,11,44]. Although *S. pyogenes* is not a pathogen for mice, it can produce an aberrant T_H17 immune response that mimics the response of the human immune system to this pathogen [26,41,42]. However, it is possible that some aspects of the human disease cannot be recapitulated in any current animal models. One goal for the future will be to ascertain whether in. inoculations alone produce sufficient anti-*S. pyogenes* autoantibodies and behavioral deficits, or rather combined routes of inoculation (in. and sq.) are required to recapitulate the cellular and humoral responses. Alternatively, mouse strains bearing humanized MHC class I or II subtypes [45], which are able to recognize *S. pyogenes* proteins, may provide a more robust model system to study the pathogenesis of SC/PANDAS.

Serological studies & involvement of T_H17 cells & cytokines

The mechanisms that mediate SC, and to a lesser extent PANDAS, pathogenesis are currently considered as antibody-mediated basal ganglia dysfunction, based on several human studies [46–49]. These can be grouped into: 1) antibodies derived from children with SC have a high affinity for the basal ganglia; 2) antisera from most children with SC and PANDAS recognize dopamine D1 or D2 receptors and other neuronal targets (anti- β -tubulin and anti-lysoganglioside) in the basal ganglia; and 3) anti-inflammatory treatments that either reduce or compete with circulating antibodies, namely plasmapheresis [50] and iv. immunoglobulin (IVIG) [51], respectively, are effective for disease treatment. Antibodies from SC and PANDAS patients that recognize the presynaptic component of neurons activate the protein kinase CaMKII using *in vitro* assays with neuronal cell lines. It has therefore been recommended that a CaMKII activation assay, along with elevated anti-neuronal and anti-dopamine receptor antibodies, be used as diagnostic tools for SC/PANDAS. The results are somewhat mixed, however, with some studies supportive while others fail to show a strong correlation between anti-neuronal antibodies (anti-tubulin and anti-lysoganglioside-G_{M1}) and increased anti-D1 or -D2 dopamine receptor activity with various symptoms of SC or PANDAS or disease severity [46–48,52]. This may be due to differences in antibody detection methods among various research groups (e.g., cell-based vs immobilized antigens) or variability among PANDAS patient cohorts in terms of acute or chronic stages of the disease, clear ascertainment of diagnosis, and the presence of choreiform movements versus other symptoms (e.g., OCD). It is at present unclear whether

these antibody panels are useful as laboratory diagnostic markers for the disease, especially in the absence of a clear *S. pyogenes* infection, because monitoring of serum antibody levels is only reliable when done over an extended time period and temporally correlated with infectious episodes.

Clinical studies comparing serologic profiles for antibodies directed against bacterial proteins (anti-streptolysin O and anti-DNAseB) between PANDAS patients and unaffected controls also provide insights into the role of autoimmunity in these diseases. For example, in a recent study Stagi and colleagues [15] evaluated the prevalence of autoimmune phenomena and reactivity of organ- versus non-organ-specific autoantibodies for 77 PANDAS patients (mean age 6.3 ± 2.5 years; 23% female) and 197 age- and sex-matched healthy controls. PANDAS patients had significantly higher anti-streptolysin O (705 ± 222 vs 125 ± 45 ; $p < 0.001$) and anti-DNAse B titers (1231 ± 913 vs 143 ± 37 ; $p < 0.001$), as well as a history of more frequent throat infections, than control patients ($p < 0.0001$). The prevalence of autoimmune disorders as well as organ- and non-organ-specific autoantibodies was, however, not significantly different between the two groups. Finally, two studies support the hypothesis that PANDAS is an autoimmune disorder, reporting anti-neuronal (anti-brain and anti-basal ganglia) antibodies in patients [53,54]. However, since the initial description of the disorder, the link between serum antibodies and PANDAS phenotype is inconsistent, mainly due to differences in ascertainment of outcome, small number of patients and precise temporal correlation of plasma antibody measurements with respect to symptomatic onset and *S. pyogenes* infections.

One clear finding from our mouse model is that T_H17 cells are a mechanism for BBB breakdown and neuroinflammation, in the form of activated microglia and macrophages, during SC or PANDAS. T_H17 cells are implicated in many autoimmune diseases including MS, where they secrete inflammatory cytokines and trigger secondary BBB breakdown [55]. IL-17A disrupts barrier function both *in vitro* and *in vivo*, through generation of reactive oxygen species in endothelial cells [56,57]. Moreover, IL-17A⁺ and IL-17A⁺ IFN- γ ⁺ T_H -cells are known to home to the CNS, in both human MS and rodent models of the disease [55]. Because tonsils from children exposed to multiple *S. pyogenes* infections contain many T_H17 cells that can be activated by the bacteria [43], it is reasonable to propose that such immune cells are present in brains of SC or PANDAS children where they might trigger inflammation by secreting specific chemokines. Establishing a 'signature profile' for T_H17 cell autoimmunity may therefore be useful for a definitive diagnosis of these diseases in the future, in combination with a panel of autoantibody neuronal biomarkers and elevated CaMKII activity. There have been no recent studies measuring cytokine levels that are present in the brains of SC or PANDAS patients, although it has been suggested that SC patients may have elevated levels of CXCL9/10 in their serum during the acute phase of the disease [58]. This is an intriguing observation, because the chemokine ligand CXCL9 binds its receptor CXCR3 to promote T-cell recruitment into the CNS. However, no data have been reported about this potential signal for immune infiltration in PANDAS or SC. Nevertheless, it is well established that inflammatory cytokines and neuroinflammation are present in the CSF from patients with TS [59], and PET neuroimaging studies in PANDAS patients have been reported with a label that recognizes activated microglia [27]. In addition, although a large number of patients respond well to IVIG or plasmapheresis treatment, many do not and

often require a more drastic immunosuppressive therapy, suggestive of a cellular neuroimmune response. In summary, human serological studies in future years should more precisely define the role of the immune system, both cellular and humoral responses, in the pathogenesis of SC and PANDAS.

Genetic risk factors for developing SC or PANDAS

Shortly after the first description of PANDAS by Swedo and colleagues [60], a higher prevalence of both psychiatric disorders and auto-immune disease in close (e.g., first-degree) relatives of PANDAS patients was reported in the literature [61,62]. For example, 18% of biological PANDAS patient mothers from one study self-reported autoimmune disease such as Hashimoto's thyroiditis, as compared with a prevalence of approximately 5% in the general population [63]. In another study of 139 family members and 54 children with PANDAS, Lougee *et al.* [61] reported that rates of obsessive-compulsive disorder (OCD) and tic disorders in first-degree relatives were 12% and 15%, respectively. Although there is an inherited component, the concordance rates among siblings of PANDAS probands have not been extensively studied. The heritability of TS and OCD, a broad collection of behaviors of which PANDAS appears to be a subset, is much better understood at the level of individual risk loci. For example, twin studies suggest that, compared to dizygotic twins, monozygotic twins have a higher genetic predisposition and concordance rate for both OCD (52 vs 21% concordance) [64] and tic disorders (77 vs 23% concordance) [65], both of which share common symptoms with PANDAS.

To date, only a single, small molecular genetic study has been published for PANDAS, demonstrating a positive association between one genetic polymorphism (308 G/A – rs1800629) in the *TNF-α* promoter for PANDAS patients, using a hospital-based case-control study of 42 children with PANDAS and 58 healthy controls [66]. There was, however, no positive relationship between this 308 G/A polymorphism and anti-streptolysin O (ASO) titers, a serological indication of previous *S. pyogenes* infections. This work followed the authors' previous result on OCD patients and the same gene promoter [67]; however their PANDAS study included only a small number of patients and controls, looking at only one SNP within a single gene, and therefore has very limited statistical power for a comprehensive genetic analysis. Genetic variations in *TNF-α* and *IL-6* have been associated with several infectious diseases in children. A large case-control study in China of 816 patients with sepsis (53% of whom had severe forms of this disease) and 624 healthy controls reported a strong association of both the -308A/G (rs1800629) SNP in the promoter region of *TNF-α* and *TNF-α* serum levels with severe sepsis; patients with AA or AG genotypes had a higher odds ratio (OR) of 1.92 ($p = 0.0005$) for severe sepsis in comparison to controls [68]. In addition, a large meta-analysis of 25 studies that examined associations between the -308A/G (rs1800629) polymorphism in *TNF-α* and sepsis showed an overall positive association (OR: 2.2; $p < 0.01$), particularly in Asians (OR: 3.1; $p < 0.01$) [69]. Finally, two large studies in the USA among 6–35-month-old children also showed that polymorphisms in both *TNF-α* (-308) and *IL-6* are associated with increased risk for otitis media from streptococcal infections [70,71]. Although no other genetic studies have been conducted in PANDAS patients, the genetic landscape of other heritable neurodevelopmental disorders with a partially shared genetic etiology such as OCD and TS has been more

extensively studied. Recently, McGrath *et al.* conducted a large genetic association study of copy number variations (CNVs) among 2699 cases (1613 ascertained for OCD, 1086 ascertained for TS) and 1789 controls [72]. Using this approach, they reported that deletions in the 16p13.11 region are associated more strongly with OCD than TS.

A significant challenge for embarking upon a genome-wide search for factors predisposing to or protecting from SC/PANDAS following repeated *S. pyogenes* infections is the relative scarcity of patients, which hinders assembling a cohort size for enough statistical power to achieve a significant result. One method to address this shortcoming is to apply a pathway-based or gene set analysis to the data, which evaluates the combined effects of multiple genetic variants or loci when some knowledge exists about the cellular and molecular basis for a complex disease or syndrome. In the case of SC/PANDAS, for example, genes and pathways that have been implicated in other autoimmune pathologies, plus the T_H17 cell and BBB mechanisms arising from animal models, may prove useful for guiding a more focused approach as opposed to an hypothesis-free, genome-wide search. As an example of the utility of such an approach, de Leeuw *et al.* found a role for astrocyte metabolic coupling in TS pathogenesis by means of a pathway analysis [73]. Furthermore, by broadening a search to include ten pediatric autoimmune diseases (pAIDs) in a large meta-analysis, including common ailments such as inflammatory bowel disease and Type 1 diabetes as well as rarer conditions like psoriasis and thyroiditis, another collaborative group has identified 27 significant loci that are associated with one or more pAID [74]. While some candidate risk loci had been previously identified as autoimmune genes, several were newly-discovered based on this pooled approach. Clearly, future identification and validation of candidate risk factors will benefit from integrating genetic, cellular and molecular expression studies; most pAID genes can be assigned to regulatory networks that control either immune effector cell activation and proliferation or cytokines and their response factors.

Conclusion

The complex pathologies of CNS sequelae to *S. pyogenes* infections are evident from animal models as well as human patients, suggesting that a range of factors contribute to disease pathogenesis, including: a unique bacterial strain or strains that induce an autoimmune response toward the host CNS; genetic risk factors that predispose children toward an autoimmune response following infection; and repeated infections of the throat or tonsils that, when left untreated, induce a robust Th17 cellular and humoral immune response targeted toward the brain. While some progress has been made in understanding SC and PANDAS pathogenesis from both animal models and human studies, more progress is needed in understanding genetic risk factors for the disease and the contribution of specific streptococcal strains to CNS autoimmune induction. Developing novel biomarker and imaging techniques that rely on recent evidence implicating both *S. pyogenes*-specific T_H17 cells and disruption of the BBB in PANDAS pathology will lead to a wider spectrum of diagnostic tools for these enigmatic yet devastating diseases.

Future perspective

A triad-based, gene set analysis to identify risk loci for SC & PANDAS

In order to understand better the mechanistic basis for the risk of developing SC or PANDAS among the large fraction of children infected with *S. pyogenes*, we propose to undertake a family-based study to identify SNPs/genes, from a limited subset of the genome, whose biological pathways underlie specific risk factors identified from neuropathological findings in animal models and human patients. These include signaling pathways that regulate T_H17-cell differentiation and maturation or BBB formation as well as glutamatergic and dopaminergic signaling. Other known common genetic risk factors and their pathways that are associated with pediatric autoimmune diseases (OCD, chronic tic disorder, TS, multiple sclerosis, rheumatoid arthritis, acute rheumatic fever, ankylosing spondylitis, systemic lupus erythematosus) as well as the phenotypically similar neuropsychiatric disorders (OCD, chronic tic disorder, TS) will also be included in our screen for genes associated with PANDAS risk. It is now clear that inherited genetic risk for most complex neuropsychiatric diseases, including PANDAS, involves cumulatively the small or moderate effects of many SNPs/genes. Gene set (or pathway-based) analyses that test associations between an entire group of genes in a pathway and a specific phenotype (e.g., PANDAS) are a valuable approach for exploring the polygenic effects of complex traits [75–77]. These methods are designed to test the cumulative effect across multiple genes in a specific pathway, which may be the result of heterogeneous effects at the single gene/variant level. Furthermore, gene set analyses improve the power to detect statistically significant associations for two reasons: collapsing individual SNPs into gene sets necessitates fewer statistical tests be performed, and individual weak effects that are not detectable in a standard unbiased, genome-wide association study combine to produce a strong association signal [76].

For example, cell-mediated immune mechanisms, in particular genes found in the helper T-cell differentiation pathway, are highly over-represented in loci linked to SNPs implicated in multiple sclerosis [78]. Other genetic resources for human autoimmunity [79,80] are increasingly available to enable a more state-of-the-art, precision approach to defining risk factors for SC and PANDAS. Ultimately, we envision that such studies will enable physicians to counsel parents of future SC or PANDAS patients as to the likelihood of other siblings being at risk for developing an autoimmune/neuropsychiatric response from a recurrent *S. pyogenes* infection, following an initial diagnosis in the family. Knowledge of this risk can aid in an earlier and perhaps more effective treatment plan after a child presents with motor and psychiatric symptoms following *S. pyogenes* exposure. Moreover, this will aid to partition the genetic contributors to TS and OCD; it may be that some PANDAS patients are inherently at high risk for TS/OCD, and the autoimmune flare-up following multiple *S. pyogenes* infections is a final trigger for developing symptoms. We hope that such information, in the era of personalized medicine and accompanied by advances in diagnosis and treatment protocols [81], will lessen the significant disease burden for PANDAS patients and their families.

Novel biomarkers & imaging modalities as diagnostic tools for PANDAS patients

Although the timing of both symptom onset and persistence, the positive responses to immune therapy as well as the discovery of antineuronal autoantibodies are all consistent with an autoimmune mechanism for both SC and PANDAS, a major issue is the lack of reliable laboratory diagnostic tools to allow clinicians to distinguish between PANDAS and other confounding diseases that present with similar symptoms. Current biomarkers such as autoantibodies against D1 and D2 dopamine receptors, anti-tubulin and anti-lysoganglioside, as well as CamKII activity levels in neuronal cell lines, have multiple challenges (see above) and do not always correlate with disease progression or severity. The results from our animal studies implicating *S. pyogenes*-specific T_H17 cells and the concomitant BBB leakage in rodent brains after recurrent in. infections suggest that is worthwhile to test whether cytokine profiles characteristic of a T_H17 phenotype are present in CSF from PANDAS patients, as well as to develop novel dynamic contrast-enhanced MRI imaging tools for detecting barrier dysfunction during periods of disease flare-up [82] that might add to our diagnostic tool-box; such tests are common for patients suffering from multiple sclerosis and traumatic brain injury. Clinical studies in this direction would be extremely helpful, and improved diagnostic tools should guide physicians towards more accurate diagnoses for these children.

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- of considerable interest

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EXECUTIVE SUMMARY

Animal models for Sydenham's chorea & Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus* infections

- Animal models for Sydenham's chorea (SC) and Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus* infections (PANDAS) recapitulate three features of human disease: serum IgGs against *Streptococcus pyogenes*, IgGs in the brain and behavioral deficits.
- Although immunization routes are similar, the neurovascular pathologies, brain regions with IgG deposition and behavioral deficits vary significantly among models.
- Variability among animal models could be due to: the strain of *S. pyogenes* used, the sex of the animals and the species (rat vs mouse).
- A novel mouse model for SC/PANDAS featuring multiple intranasal infections with live *S. pyogenes* displays bacterial-specific T_H17 cells in the brain that are associated with barrier leakage and IgG extravasation.
- A future goal in animal models will be test whether in. inoculations alone produce sufficient anti-*S. pyogenes* autoantibodies and behavioral deficits that fully recapitulate human disease.

Serological studies & involvement of T_H17 cells & cytokines

- Clinical studies comparing serologic profiles of antibodies directed against bacterial proteins (anti-streptolysin O and anti-DNAseB) between PANDAS patients and controls are not definitive.
- Antisera from SC and PANDAS patients recognize dopamine D1 or D2 receptors or other neuronal targets (β -tubulin and lysoganglioside-G_{M1}) in the basal ganglia.
- Elevated anti-neuronal and anti-dopamine receptor antibodies, in conjunction with a CaMKII activation assay, have been proposed as diagnostic tools for SC/PANDAS patients.
- There is no clear correlation between anti-neuronal or dopamine receptor antibodies and various symptomatic manifestations of PANDAS or disease severity.
- A 'signature profile' for T_H17-cell autoimmunity to ascertain diagnosis of PANDAS should be established.

Genetic risk factors for developing SC or PANDAS

- A single molecular genetic study has demonstrated a positive association between one genetic polymorphism in the *TNF- α* promoter and PANDAS patients.

- Twin studies indicate a higher genetic predisposition and concordance rates for both OCD (52 vs 21% concordance) and tic disorders (77% vs 23% concordance).
- Pathway-based or gene set analysis, which evaluates the combined effects of multiple genetic variants or loci when some knowledge exists about the cellular and molecular basis for a complex syndrome, may be beneficial for PANDAS studies.
- Genes and pathways implicated in other autoimmune pathologies, as well as T_H17-cell differentiation and blood–brain barrier (BBB) formation, should provide a more focused approach.

Conclusion

- Three main factors contribute to pathogenesis of SC and PANDAS: a unique bacterial strain that induces an autoimmune CNS response; genetic risk factors that predispose children toward an autoimmune response following infection; and repeated infections inducing a robust T_H17 cellular and humoral immune response.
- Genetic risk factors for SC/PANDAS and the contribution of specific streptococcal strains to CNS autoimmune induction in human patients are poorly understood.

Future perspective

- A triad-based (parents and children), gene set analysis to identify risk loci for SC and PANDAS focused on signaling pathways that regulate T_H17 cell differentiation and barrier formation as well as glutamatergic and dopaminergic signaling is underway.
- Clinical studies to identify novel biomarkers (cytokine signatures) and imaging modalities (BBB leakage) should provide better diagnostic tools for PANDAS patients.

Table 1

Summary of rodent models for Sydenham’s chorea and PANDAS.

Species	Hoffman <i>et al.</i> (2004)	Yaddanapudi <i>et al.</i> (2010)	Brimberg <i>et al.</i> (2012)	Lotan <i>et al.</i> (2014)	Macri <i>et al.</i> (2015)	Dileepan <i>et al.</i> (2016)
Rat	–	–	Male	Male	–	–
Mouse	Female	Male, female	–	–	Male	Female
<i>S. pyogenes</i> strain	M6	N/A	M18	N/A	M6	M1
Route of bacterial introduction	sc.	N/A (passive transfer of IgGs after sc. inoculations with M6 strain)	sc.	N/A (passive transfer of IgGs after sc. inoculations with M18 strain)	sc.	Intranasal
Immune response to infection	Humoral (T cell involvement unknown)	N/A (passive transfer of IgGs)	Humoral (T cell involvement unknown)	N/A (passive transfer of IgGs)	Humoral, CD3 ⁺ T cells present in rostral diencephalon	Cellular, CD4 ⁺ T cells (Th1 and Th17) present in CNS
Ref.	[20]	[21]	[22]	[23]	[25]	[26]

N/A:Not applicable; sc.: Subcutaneous.

Table 2

Neurobehavioral features of rodent models for Sydenham's chorea and PANDAS.

Phenotypes reported for SC/PANDAS models	Hoffman <i>et al.</i> (2004)	Yaddanapudi <i>et al.</i> (2010)	Brimberg <i>et al.</i> (2012)	Lotan <i>et al.</i> (2014)	Macri <i>et al.</i> (2015)	Dileepan <i>et al.</i> (2016)
Neurovascular pathology						
Synapse dysfunction:						
– Glutamatergic	–	–	+++	–	+++	+++
– Dopaminergic	–	–	+++	+++	–	–
– Serotonergic	–	–	–	+++	++	–
Neurovascular dysfunction	No artificial opening of BBB (peri-capillary IgG deposition in DCN)	Artificial opening of BBB	Artificial opening of BBB	Bypass BBB by intrastriatal infusion of IgGs	No artificial opening of BBB	Severe BBB dysfunction after recurrent infections
Neuroinflammation	–	–	–	–	Microglial activation in rostral diencephalon	Microglial activation in olfactory bulb
Brain region of IgG extravasation/binding						
Olfactory bulb						
Cortex:						+++
– Prefrontal cortex	–	–	+++	–	–	++
– Piriform cortex	–	++	–	–	–	+++
Hippocampus:						
– Dentate gyrus	–	+++	–	–	–	+
Basal ganglia:						
– Caudate	+	++	+++	+++	–	+
– Globus pallidus	+	++	–	–	–	+
Diencephalon						
– Lateral hypothalamus	–	–	–	–	–	+++
– Thalamus	+	–	+++	–	–	+
– Reticular thalamic nucleus	+	–	–	–	–	–

Phenotypes reported for SC/PANDAS models	Hoffman <i>et al.</i> (2004)	Yaddanapudi <i>et al.</i> (2010)	Brimberg <i>et al.</i> (2012)	Lotan <i>et al.</i> (2014)	Macri <i>et al.</i> (2015)	Dileepan <i>et al.</i> (2016)
– Superior/inferior colliculus	+	–	–	–	–	+
– Red nucleus	–	–	–	–	–	+++
– Amygdala	–	–	–	–	–	+++
Cerebellum						
– Deep cerebellar nuclei	+++	–	–	–	–	+++
– Pontine nuclei	–	–	–	–	–	+++
Brain stem						
– Tegmental nuclei	–	–	–	–	–	+
– Periolivary nucleus	–	–	–	–	–	+
– Other brainstem/motor nuclei	–	–	–	–	–	++
Behavioral assays						
Motor ability and coordination:						
– Beam walking	–	+++	++	+	–	–
– Dowel test	–	–	–	–	++	–
– Rotarod test	–	++	–	–	–	–
– Food manipulation	–	–	++	+++	–	–
Anxiety and stereotypy:						
– Open field test (ambulatory distance)	++	–	–	–	–	–
– Open field test (time in center)	–	–	+	–	++	–
– Elevated plus or O maze	–	–	–	–	++	–
Compulsion						
– Induced grooming	–	–	++	++	–	–
– Marble burying	–	–	–	+++	–	–
– Rearing	+++	+++	–	–	++	–
Exploratory, aggressive, social and olfactory behaviors:						
– Olfactory discrimination	–	+++	–	–	–	–
– Resident-intruder task	–	+++	–	–	–	–

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Phenotypes reported for SC/PANDAS models	Hoffman <i>et al.</i> (2004)	Yaddanapudi <i>et al.</i> (2010)	Brimberg <i>et al.</i> (2012)	Lotan <i>et al.</i> (2014)	Macri <i>et al.</i> (2015)	Dileepan <i>et al.</i> (2016)
- Spatial and reversal learning		+++				

- Not found; + Weak; ++ Moderate; +++ Strong; ++++ Very strong; BBB: Blood-brain barrier; DCN: Deep cerebellar nuclei; SC: Sydenham's chorea.