Mycoplasma pneumoniae IgG positivity is associated with tic severity in chronic tic disorders

Schnell, Jaana; Bond, Molly; Moll, Natalie; Weidinger, Elif; Burger, Bianka; Bond, Rod; Dietrich, Andrea; Hoekstra, Pieter J; Schrag, Anette; Martino, Davide

Published in:
Brain, Behavior, and Immunity

DOI:
10.1016/j.bbi.2021.10.012

Publication date:
2022

Brain, Behavior, and Immunity 99 (2022) 281–288

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Mycoplasma pneumoniae IgG positivity is associated with tic severity in chronic tic disorders

Jaana Schnell [a,2], Molly Bond [b,2], Natalie Moll [c], Elif Weidinger [a], Bianka Burger [d], Rod Bond [e], Andrea Dietrich [f], Pieter J. Hoekstra [f], Anette Schrag [g], Davide Martino [h,i,1], Markus Schwarz [c], Ute-Christiane Meier [c,j], Norbert Müller [a,*]

[a] Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Germany
[b] Blizard Institute, Queen Mary University of London, Barts and The London School of Medicine and Dentistry, Department of Neuroscience and Trauma, UK
[c] Institute of Laboratory Medicine, University Hospital, LMU Munich, Germany
[d] Marion von Tessin Memory-Zentrum, Munich, Germany
[e] University of Sussex, Brighton, UK
[f] University of Groningen, University Medical Center Groningen, Department of Child and Adolescent Psychiatry, Groningen, The Netherlands
[g] University College London, Institute of Neurology, London, UK
[h] Department of Clinical Neuroscience, Cumming School of Medicine, University of Calgary, Calgary, Canada
[i] Hotchkiss Brain Institute, Calgary, Canada
[j] Department of Psychological Medicine, Institute of Psychiatry, King's College London, London, UK

ARTICLE INFO

Keywords:
Tourette syndrome
Chronic tic disorder
Mycoplasma pneumoniae
Infection
Tic severity

ABSTRACT

Infectious pathogens may represent an environmental risk factor for chronic tic disorders (CTD). This cross-sectional study aimed to determine whether *Mycoplasma pneumoniae* (*M. pneumoniae*) IgG positivity is associated with the presence or severity of tics. We compared *M. pneumoniae* IgG positivity across three groups: children and adolescents (3–16 years) with CTD (CTD group; n = 302); siblings (3–10 years) of people with CTD who developed tics within a seven-year follow-up period (tic onset group; n = 51); siblings (4–10 years) who did not develop tics within the study period and were ≥10-years-old at their last assessment (unaffected group; n = 88). The relationship between *M. pneumoniae* IgG positivity and the presence and severity of tics was analysed using multilevel models controlling for site, family relatedness, sex, age, presence of comorbid obsessive–compulsive and/or attention-deficit/hyperactivity disorder and use of psychotropic medication. *M. pneumoniae* IgG positivity was not associated with the presence of CTD, or the first onset of tics as compared to siblings who remained unaffected. *M. pneumoniae* IgG positivity was associated with a higher tic severity score within the CTD group ($\beta = 2.64$, s.e. = 1.15, $p = 0.02$). It is possible that *M. pneumoniae* infection influences tic severity in CTD or, that having more severe tics, increases the risk of infection. However, it is more likely that the association observed in this study reflects a propensity toward enhanced immune responses in people with CTD and that, rather than a causal relationship, infection and greater tic severity are indirectly linked via shared underlying immune mechanisms.

1. Introduction

Chronic tic disorders (CTD) are common neurodevelopmental conditions characterised by the presence of motor tics, vocal tics or the combined presence of both known as Tourette syndrome (TS) (American Psychiatric Association, 2013). TS has an estimated worldwide prevalence of 0.3–0.9% (Knight et al., 2012). The aetiology of CTD is not fully understood. While there is a strong genetic component, with estimates of heritability between 0.25 and 0.77 (Mataix-Cols et al., 2015; Zilhão et al., 2017), environmental factors also play a substantial role (Hoekstra et al., 2013; Robertson et al., 2017).

Prior studies have suggested infectious agents may be involved in the pathogenesis of at least a subgroup of patients with CTD (Krause and Müller, 2012; Martino et al., 2020; Martino et al., 2015). Research

* Corresponding author.
[1] The EMTICS collaborative group.
[2] Joint first authors.

https://doi.org/10.1016/j.bbi.2021.10.012
Received 27 May 2021; Received in revised form 23 September 2021; Accepted 19 October 2021
Available online 24 October 2021

focused on infections with Group A β-haemolytic streptococci (GABSH) given their apparent role in related movement disorders such as Sydenham's chorea and the post-streptococcal syndrome of tics and/or obsessive–compulsive disorders (OCD) termed PANDAS (paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) (Murphy et al., 2010; Orefici et al., 2016; Swedo et al., 1998). However, Martino and colleagues conducted a prospective cohort study of 715 children and adolescents diagnosed with CTD and could not find any association between recent exposure to Group A streptococcus and clinically relevant exacerbations of tics (Martino et al., 2021).

Associations between tics and other infectious pathogens including *Mycoplasma pneumoniae (M. pneumoniae)* (Dehning et al., 2009; Müller et al., 2004; Müller et al., 2000), *Borrelia burgdorferi* (Riedel et al., 1998), *Toxoplasma gondii* (Akaltun et al., 2018), and *Chlamydia trachomatis* (Krause et al., 2012) have been described in case reports and a few small-scale case-control studies. *M. pneumoniae* has also been linked to obsessive–compulsive (OC) symptoms (Ercan et al., 2008) and to movement disorders and/or basal ganglia lesions following *M. pneumoniae* encephalitis (Beskind and Keim, 1994; El Hafidi et al., 2012). In addition, one case-control study (n = 29) and case reports have suggested that infection with *M. pneumoniae* is associated with tics (Dehning et al., 2009; Müller et al., 2004; Müller et al., 2000). However, the salience of these observations is yet to be elucidated. *M. pneumoniae* is a ubiquitous infectious agent that generally causes a mild acute respiratory infection (Atkinson et al., 2008). The prevalence of *M. pneumoniae* IgG positivity, which is most likely due to past infection, is around 12% in early childhood and increases to 50–60% in adolescence with no significant difference between sexes (Tuuminen et al., 2000). *M. pneumoniae* infection tends to be either asymptomatic or a self-limiting illness. However, it is also a common cause of community-acquired pneumonia and extrapulmonary manifestations can affect any organ, including the brain (Korppi et al., 2004).

The mechanisms underpinning a possible role for infections in CTD are not fully understood. A number of studies have reported altered innate and adaptive immune responses in people with CTD (Martino et al., 2015) with increased serum levels of pro-inflammatory cytokines (Leckman et al., 2005), changes in regulatory T-cells responses (Kawikova et al., 2007) and activation of microglial cells (Lennington et al., 2016). It may be that infections facilitate an immune activation that enhances behavioural changes through direct effects on the central nervous system, such as via cytokines (Leckman et al., 2005) or antibodies (Bombaci et al., 2009; Krause et al., 2010; Martino et al., 2011; Martino et al., 2015). An autoimmune mechanism induced by molecular mimicry, with a similar aetiology to Sydenham's Chorea, has also been hypothesized for a subset of those with CTD (Hoekstra et al., 2002; Snider and Swedo, 2003). Alternatively, individuals with CTD may be more susceptible to infections because of a primary immune dysregulation (Martino et al., 2020; Martino et al., 2015).

Therefore, this study sought to determine whether past infection with one infectious agent of interest, *M. pneumoniae*, was associated with the presence of CTD, the first onset of tics or tic severity. This is a cross-sectional study that used sub-samples of the European Multicentre Tics in Children Studies (EMTICS) cohorts based on available serum samples for analysis of *M. pneumoniae* IgG positivity. EMTICS is a large prospective European multicentre study of children and adolescents with CTD (Schrag et al., 2019). This study investigated whether: (A) children with CTD have higher rates of *M. pneumoniae* IgG positivity compared to unaffected siblings; (B) the rate of baseline *M. pneumoniae* IgG positivity is higher in siblings who developed tics compared to those who did not develop tics; and (C) *M. pneumoniae* IgG positivity is associated with higher tic severity in participants with CTD. To our knowledge, this is the largest study to investigate *M. pneumoniae* IgG in CTD and is unique in its assessment of serum IgG positivity prior to the development of tics in an at-risk cohort.

2. Participants and methods

2.1. Participants

Participants were drawn from the EMTICS study, a prospective observational cohort study that aimed to assess the contribution of genetic and environmental risk factors in CTD (Schrag et al., 2019). Data were collected by 16 (child and adolescent) psychiatry and paediatric neurology outpatient clinics across Europe and in Israel. The EMTICS project was based on two separate cohort studies: the COURSE study, including children and adolescents (3–16 years) with a diagnosis of CTD according to DSM-IV-TR (American Psychiatric Association, 2000), and the ONSET study, an at-risk cohort of first-degree relatives, mostly siblings, of children with CTD (3–10 years) without tics, OCD or trichotillomania. The ONSET cohort was followed up bimonthly for up to three years to assess the onset of tics according to study protocol. All unaffected children were recontacted and the majority were re-assessed via a brief telephone interview after the study ended. The regular study period took place between 2013 and 2018, with the final telephone reassessment concluded in May 2020.

The mean age of tic onset in the ONSET cohort was 7.9 years (SD 2.0, range 3.5–13.0); we therefore excluded those who were younger than 10-years-old at the time of their last assessment and had not developed any tics from the unaffected at-risk comparison group (unaffected group) to reduce the likelihood of including children who might still develop tics.

Exclusion criteria for participants in both COURSE and ONSET cohorts included treatment with antibiotics during the last month (since a separate antibiotic study was part of the research plan), a serious medical or neurological illness or an inability to understand and implement given study procedures (Schrag et al., 2019).

The current cross-sectional study used data from sub-samples of the EMTICS cohorts (COURSE and ONSET) at a single time point: at baseline for those with an existing tic disorder and for first-degree relatives who did not develop tics and at the time of tic onset for those who developed tics within the study. Schrag and colleagues described the sample size calculation for the EMTICS project for both the COURSE and ONSET cohorts (Schrag et al., 2019). The assessment of *M. pneumoniae* IgG was included in the original research plan of EMTICS as a secondary measure. Therefore, the sub-samples in this study comprised all participants for whom we had serum available to measure *M. pneumoniae* IgG. Rates of *M. pneumoniae* IgG positivity were compared across three groups: 302 participants with CTD from the COURSE cohort (CTD group); 51 first-degree relatives (all siblings) who developed tics within the seven-year total study period from the ONSET cohort (tic onset group); and 88 unaffected first-degree relatives (all siblings) who were ≥10 years old at time of their last assessment from the ONSET cohort (unaffected group). The study was approved by the Institutional Review Boards of all clinics involved. Furthermore written informed consent and assent was collected by the parents and their children.

2.2. Clinical measures

Participants in the COURSE cohort had an established diagnosis of CTD (TS, chronic motor tic disorder or chronic vocal tic disorder), which was confirmed by trained clinicians using DSM-IV-TR criteria at baseline (American Psychiatric Association, 2000), as was the possible presence of comorbid OCD and attention-deficit/hyperactivity disorder (ADHD). Baseline tic severity was measured using the Yale Global Tic Severity Scale (YGTSS) (Leckman et al., 1989), which provides the Total Tic Severity Score (range 0–50) comprised of two subscales: YGTSS Motor Tic Severity Score (range 0–25) and YGTSS Vocal Tic Severity Score (range 0–25). In addition, the Clinical Global Impression Severity (CGI-S) Scale (Guy, 1976) was used to assess tic severity during the previous week. Participants in the ONSET cohort suspected of having developed tics were assessed by study clinicians and the onset of tics was confirmed

according to DSM-IV-TR criteria (American Psychiatric Association, 2000). Use of psychotropic medication during the last two weeks was documented by the study clinicians.

2.3. Laboratory measures

Samples were sent to the Department of Laboratory Medicine, Munich (LMU) and stored at −80 °C until analysis. Serum IgG against *M. pneumoniae* were measured in the ISO 15189 accredited lab on a DiaSorin Liaison analyser using Chemiluminescence Immunoassay with paramagnetic microparticle solid phase (CLIA) technology; the analyser range was 1–200 AU/ml. *M. pneumoniae* elicits antibody responses after around one week of illness. This is initially an IgM response before seroconversion to IgG; serum IgG tends to peak at 3–6 weeks, before it gradually declines (Atkinson et al., 2008). IgG tends to remain elevated in children for around four years following infection but can also persist indefinitely (Daxboeck et al., 2003; Jacobs et al., 1986; Foy et al., 1977). Serum IgG ≥10 AU/ml was considered positive for previous infection with *M. pneumoniae* at any point in time and <10 AU/ml as negative (Waris et al., 1998; Almasri et al., 2011). IgG positivity is most likely due to past, rather than acute, infection with *M. pneumoniae* and does not provide information on how recently the participant was infected.

2.4. Statistical analysis

Clinical data of the three groups were described by mean values and standard deviations or percentages, according to the type of variable. The primary predictor variable for all our analyses was baseline serum *M. pneumoniae* IgG positivity (0 = absence, 1 = presence). We used generalised linear mixed models to assess whether IgG positivity was associated with i) having a CTD compared to the unaffected group; or ii) tic onset compared to the unaffected group. In addition, we used linear mixed-effects models to determine whether iii) IgG positivity was associated with tic severity within the CTD group. Since this is a multicentre study involving siblings, we used multilevel models with both site and family relatedness as cluster variables. Sex, age, the presence of a comorbidity (OCD and/or ADHD) and the use of psychotropic medication in the past two weeks were entered as covariates. We controlled for comorbid OCD and ADHD as these are common comorbidities in CTD (Freeman et al., 2000) and have both also been linked to infections (Ercan et al., 2008; Leslie et al., 2008) and immune dysfunction (Gabbay et al., 2009; Hoekstra, 2019). Likewise, we controlled for psychotropic medication use as some psychotropic medications have anti-inflammatory properties that could affect seropositivity (Lind and Kristiansen, 2000).

Age and sex varied substantially between groups and both were significant predictors of tics, though not *M. pneumoniae* IgG positivity. We therefore also conducted a sensitivity analysis with age and sex matched subgroups (CTD group versus unaffected group n = 88; tic onset group versus unaffected group n = 51) using propensity score matching in R (Randolph et al., 2014).

3. Results

3.1. Study cohort

Baseline demographic and clinical characteristics of participants are shown in Table 1. The vast majority of the CTD cohort was diagnosed with TS (n = 274, 90.73%), 26 (8.61%) had a chronic motor tic disorder and 2 (0.66%) a chronic vocal tic disorder. The average time between baseline and tic onset in the tic onset group was 1.1 years (range 0.1–5.4 years) and therefore elevation of IgG would be expected to persist during this time. Increasing age was significantly associated with *M. pneumoniae* IgG seropositivity in the tic onset group ($p = 0.04$) but not in the CTD or unaffected groups and there was no significant association with sex among any of the groups (Supplementary materials, Table 2

Table 2
GLM results comparing rates of seropositivity in CTD group and unaffected group.

Dependent variable (covariates)	OR	95% CI	*p*
CTD group versus *unaffected group*			
M. pneumoniae IgG positivity	0.71	0.29–1.75	0.45
Age	1.94	1.58–2.38	<0.01*
Sex	0.17	0.08–0.36	<0.01*
Comorbidity (OCD and/or ADHD)	3.23	1.23–8.49	0.02*
Psychotropic medication	3.89	0.82–18.41	0.09

Note. GLM (generalised linear mixed model) with age, sex, comorbidity (OCD and/or ADHD) and psychotropic medication as covariates was used. The presence of either or both ADHD and/or OCD was entered as a single covariate (absence = 0, presence = 1). CTD group, Chronic tic disorder group: COURSE children; Unaffected group: ONSET children who did not develop tics by end of the study or re-assessment and ≥10 years old. *$p < 0.05$.

Table 1
Baseline Demographics and Clinical Characteristics.

	CTD group (n = 302)		Tic onset group (n = 51)		Unaffected group (n = 88)	
Age (range) (mean ± SD)	4.5–16.99	11.0 ± 2.7	3.2–10.6	6.9 ± 1.9	4.2–10.9	7.7 ± 1.8
Sex n (%)						
Male	235	77.8%	30	58.8%	36	40.9%
Female	67	22.2%	21	41.2%	52	59.1%
Chronic tic disorder (CTD) n (%)						
Tourette syndrome (TS)	274	90.7%				
Chronic motor tic disorder	26	8.6%				
Chronic vocal tic disorder	2	0.7%				
Tic severity (range) (mean ± SD)						
YGTTS Total	0–44	20.1 ± 8.3				
YGTTS Motor	0–23	12.8 ± 4.5				
YGTSS Vocal	0–21	7.4 ± 5.4				
CGI	1–6	3.6 ± 0.9				
Psychotropic drug treatment during the previous 2 weeks n (%)	108	35.8	2	3.9	2	2.3
Comorbidities n (%)						
OCD	86	28.5	2	3.9		
ADHD	76	25.2	4	7.8	10	11.4
M. pneumoniae IgG positivity n (%)	48	15.9	9	17.1	15	17.0

Note. Unaffected group, did not develop tics and ≥10 years old at last assessment; OCD, obsessive–compulsive disorder; ADHD, attention-deficit/hyperactivity disorder; *M. pneumoniae* IgG, ≥10 AU/ml was considered positive.

and Figs. 1–3 histograms show the distribution of seropositivity by age for each group).

3.2. Previous infection is not associated with higher odds of tics

M. pneumoniae IgG positivity was not associated with either having CTD in the CTD group when compared to the unaffected group ($p > 0.05$) (as shown in Table 2), or tic onset in the tic onset group when compared to the unaffected group ($p > 0.05$) (as shown in Table 3). These results remained non-significant ($p > 0.05$) when we conducted a sensitivity analysis with age and sex matched groups (Supplementary material in Table 1).

Older age ($p < 0.01$), male sex ($p < 0.01$), and the presence of a comorbidity (OCD and/or ADHD; $p < 0.05$) were all significantly associated with having a CTD compared to the unaffected group (Table 2), whilst only older age was significantly associated with the onset of tics compared to the unaffected group ($p < 0.05$) (Table 3).

3.3. M. pneumoniae IgG positivity was associated with tic severity in CTD group

The association between seropositivity and tic severity is shown in Table 4. The presence of IgG antibodies against *M. pneumoniae* was associated with a higher YGTSS Total Tic Severity Score ($\beta = 2.64$, s.e. $= 1.15$, $p = 0.02$). There was a significant association between *M. pneumoniae* IgG positivity and the YGTSS Vocal Tic Severity Score ($\beta = 1.02$, s.e. $= 0.62$, $p = 0.03$) but no association with the CGI-S (see Table 4).

4. Discussion

The main finding of this multicentre study in children and adolescents with tic disorders was that *M. pneumoniae* IgG positivity was not associated with a diagnosis of CTD or tic onset in a prospective at-risk cohort. *M. pneumoniae* IgG positivity, however, was associated with higher tic severity in children and adolescents with established CTD.

In the current study, infection rates of *M. pneumoniae* were no higher among those with tics than those without. Seropositivity rates among all groups in this study were lower than those reported elsewhere in the general population (Atkinson et al., 2008, Tuuminen et al., 2000). The reasons for this observation are not clear. Spread of *M. pneumoniae* among children is thought to increase with early day-care and school attendance; in spring and autumn; as well as during cyclic epidemics that tend to occur every 3–5 years (Atkinson et al., 2008). Reduced school attendance because of medical appointments and fewer regional epidemics may partially account for the lower rates seen in our study population but neither fully explains the marked difference we observed. Another unexpected finding was that rates *M. pneumoniae* IgG positivity were evenly distributed by age across the groups (see Supplementary materials Figs. 1–3) and seropositivity was only associated with age in the tic onset group and not the CTD or unaffected groups. *M. pneumoniae* IgG persists in the serum for a long-time following infection (estimates of around 4 years but can be indefinite (Daxboeck et al., 2003; Jacobs et al., 1986; Foy et al., 1977)), therefore, IgG positivity rates tend to increase with age during childhood: one population-based study found the incidence of IgG positivity to increase from 12% in pre-school children to 50–60% in adolescents (Tuuminen et al., 2000). One reason why we may not have observed an association with age in this study might have been because only a relatively small number of participants from each group were found to be seropositive, thus reducing the power and likelihood of observing a significant association. Nonetheless, given the strong association between age and seropositivity reported in the literature and a significant association in the tic onset group, we adjusted for age in all our models and conducted sensitivity analysis with age and sex matched subgroups.

We observed no association between *M. pneumoniae* IgG positivity and the presence of a CTD or the first onset of tics. The only previous study of *M. pneumoniae* in TS found elevated IgA, but not IgG, in 29 individuals with TS (6–60 years of age) compared to healthy controls (Müller et al., 2004). The authors proposed that this may be because *M. pneumoniae* IgA is particularly elevated in extra-pulmonary manifestations of *M. pneumoniae* infection (Müller et al., 2004). Due to lack of available serum for analysis, IgA was not measured in this study and should be considered in future research of suspected *M. pneumoniae* involvement in neuropsychiatric diseases. Nonetheless, our findings do not suggest that children with tics or children who will later go on to develop tics are particularly susceptible to *M. pneumoniae* infection or mount an unusually sustained response to the pathogen compared to people without tics.

M. pneumoniae IgG positivity was, however, associated with a higher YGTSS Total Tic Severity Score (range 0–50) of 2.64 points on average among participants with CTD after adjustments for age, sex, the presence of comorbid OCD and/or ADHD and use of psychotropic medication. This association between seropositivity and greater tic severity could reflect either a direct or indirect relationship. As this is a cross-sectional study that only measured IgG antibodies, indicative of past infection, at a single point in time, we cannot determine whether previous infection is associated with tic onset; whether infection coincides

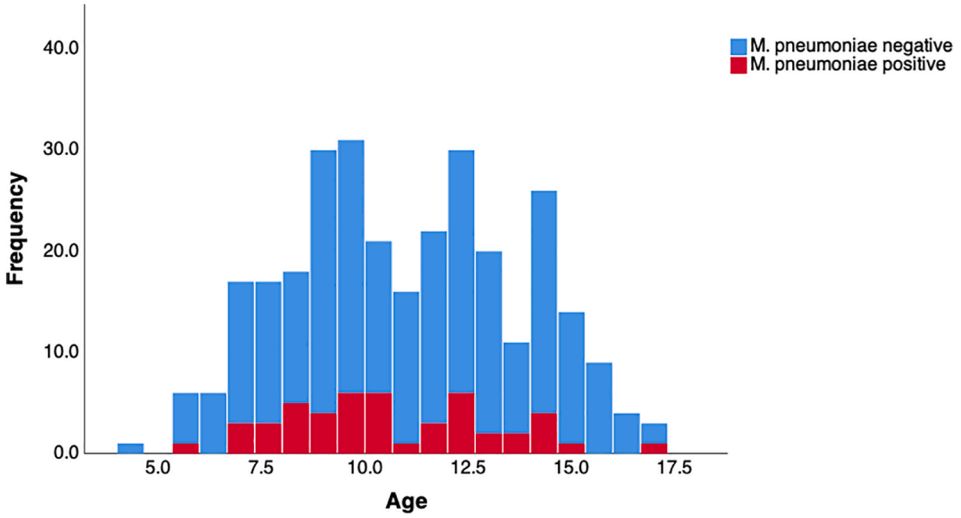

Fig. 1. Histogram of seropositivity by age in CTD group. Note. CTD group, chronic tic disorder group: COURSE children.

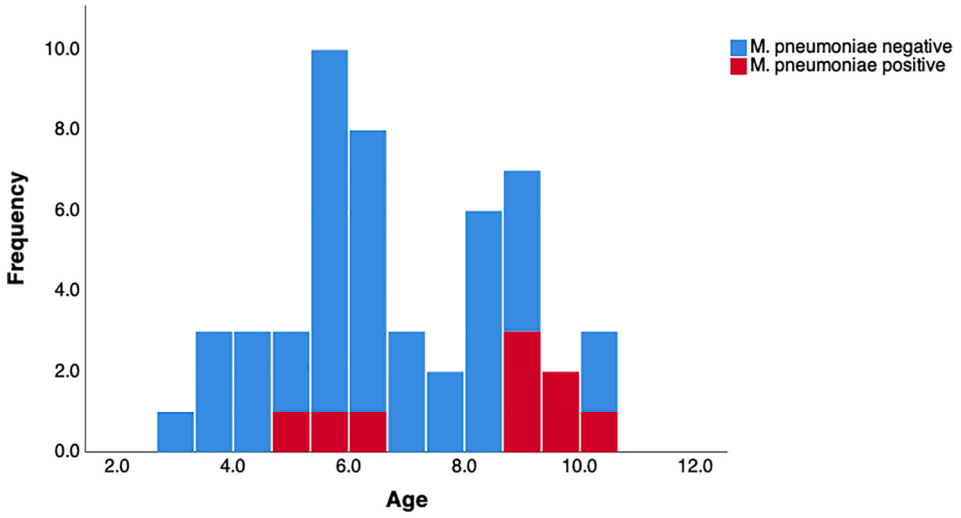

Fig. 2. Histogram of seropositivity by age in tic onset group. Note. Tic onset group: ONSET children with tic onset.

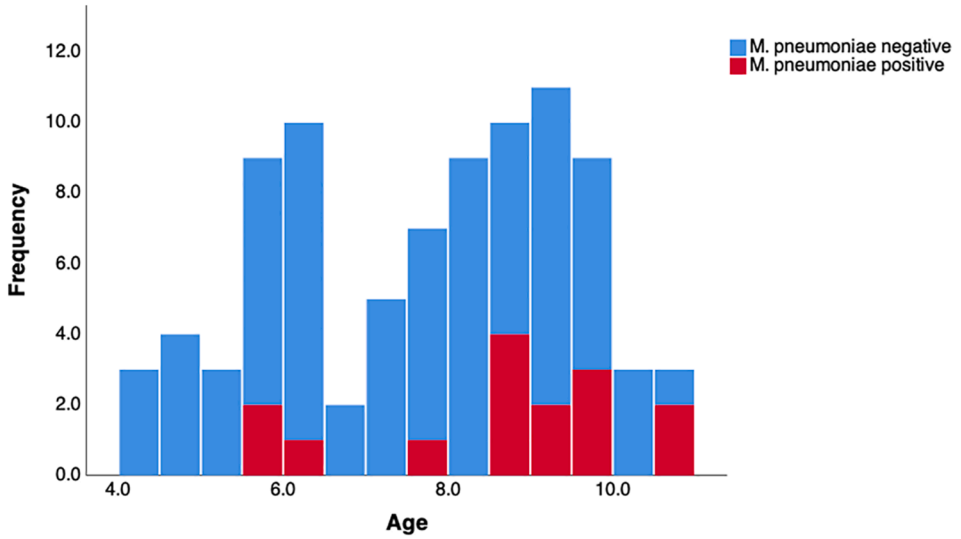

Fig. 3. Histogram of seropositivity by age in unaffected group. Note. Unaffected group: ONSET children who did not develop tics by end of the study or re-assessment and ≥10 years old.

Table 3
GLM results comparing rates of seropositivity in tic onset group and unaffected group.

Dependent variable (covariates)	OR	95% CI	*p*
Tic onset group versus *unaffected group*			
M. pneumoniae IgG positivity	1.74	0.61–4.97	0.30
Age	0.75	0.60–0.94	0.01*
Sex	0.54	0.24–1.17	0.12
Comorbid ADHD and/or OCD	0.54	0.13–2.17	0.38
Psychotropic medication	3.02	0.30–30.06	0.34

Note. GLM (generalised linear mixed model) with age, sex, comorbidity (OCD and/or ADHD) and psychotropic medication as covariates was used. The presence of either or both ADHD and/or OCD was entered as a single covariate (absence = 0, presence = 1). Tic onset group: ONSET children with tic onset; Unaffected group: ONSET children who did not develop tics by end of the study or re-assessment and ≥10 years old. *$p < 0.05$.

Table 4
Results from LMM on an association between *M. pneumoniae* IgG positivity and tic severity scales.

Independent variable (dependent variable)	*β*	SE	95% CI	*p*
M. pneumoniae IgG				
(YGTSS Total)	2.64	1.15	0.38–4.89	0.02*
(YGTSS Motor)	1.02	0.62	−0.21–2.24	0.10
(YGTSS Vocal)	1.71	0.78	0.18–3.24	0.03*
(CGI-S)	0.17	0.13	−0.09–0.44	0.20

Note. Linear mixed-effects models with age, sex, comorbidity (OCD and/or ADHD) and psychotropic medication as covariates were used. The presence of either or both ADHD and/or OCD was entered as a single covariate (absence = 0, presence = 1). YGTSS, Yale Global Tic Severity Scale [Total = Motor + Vocal Tic Severity Score (range 0–50)]; CGI-S, Clinical Global Impression Severity Scale; *$p < 0.05$.

with a worsening of symptoms; or whether a worsening of symptoms increases the risk of infection.

In the context of the existing literature on anti-infectious immune responses in CTD (Martino et al., 2020), it is more likely that seropositivity and greater tic severity are epiphenomena of a shared underlying mechanism, with neither directly influencing the other. Several studies have indicated that individuals with tic disorders have enhanced

immune-inflammatory responses, including stronger antibody responses (Bombaci et al., 2009; Krause et al., 2010; Martino et al., 2011), as well as positive correlations between greater tic severity and interleukin-2 (IL-2) (Bos-Veneman et al., 2011). Increased tumor necrosis factor (TNF)-α and interleukin-12 (IL-12) during tic or OC symptom exacerbation, regardless of pharmacological treatment, have been reported (Leckman et al., 2005). An upregulation in the expression of genes that control immune-modulating neurotransmitters has also been demonstrated (Tian et al., 2011a; Tian et al., 2011b; Gunther et al., 2012). In a recent review of immunological mechanisms in brain development and tic disorders, Martino and colleagues suggest that abnormal immune priming, most likely driven by a genetic predisposition interacting with environmental factors, may alter both the maturation of neural networks and immune regulatory mechanisms (Martino et al., 2020). Thereby, resulting in the co-occurrence of neurodevelopmental disorders (such as tic disorders) and hypersensitive immune responses to pathogens (such as to *M. pneumoniae*) (Martino et al., 2020).

A prospective longitudinal study described that a multiplicative interaction between GABHS infections and psychosocial stress can enhance tic and OC symptom severity (Lin et al., 2010). Psychosocial stress, a strong predictor of greater tic severity, may also influence immune responses to infection via activation of the hypothalamic–pituitaryadrenal axis (Martino et al., 2020), although this mechanism is yet not fully understood (Buse et al., 2021). Finally, behavioural patterns, which may be accentuated in young people with greater tic severity and/or a higher comorbid neuropsychiatric burden, could increase the risk of exposure to infectious pathogens (Martino et al., 2020) and, thus, also indirectly contribute the association observed in thus study.

5. Limitations and future directions

This study has a number of strengths: EMTICS recruited a large population of children and adolescents with CTD from multiple centres across Europe and in Israel. It is unique in its assessment of first-degree relatives as prospective at-risk cohort and these children were extensively followed up for a period of up to seven years to assess the possible onset of tics.

However, there were also some notable limitations. The main limitation is the lack of longitudinal data, which preclude any analysis of a temporal relationship or any inference regarding causality. As mentioned, measurements of *M. pneumoniae* IgA, in addition to IgG antibodies, would be useful given previous reports of particular elevation of IgA in extra-pulmonary manifestations of *M. pneumoniae* infection (Müller et al., 2004). There was also only a small number of available serum samples for the tic onset and unaffected cohorts and only a small proportion of each group were *M. pneumoniae* IgG positive, which reduced the likelihood of observing significant differences between groups. To elucidate the direction of causality, longitudinal data from patients with CTD and a healthy age- and sex-matched control group would be needed, ideally with regular (e.g., 4-monthly) clinical assessments and serum samples of pathogen titers (IgG, IgM, IgA), cytokines, and other immune effector molecules.

6. Conclusion

This is the largest cross-sectional study of *M. pneumoniae* infection in children and adolescents with CTD to date and the first to prospectively investigate *M. pneumoniae* infection in siblings in relation to the onset of tics. We found no evidence of an association between previous *M. pneumoniae* IgG positivity and the presence of CTD or first onset of tics. However, we did observe an association between *M. pneumoniae* IgG positivity and greater tic severity. It is possible that *M. pneumoniae* infection influences tic severity in CTD. Alternatively, greater disease severity may increase the risk of infection. However, it seems more likely that this observation reflects a propensity toward enhanced immune responses in people with CTD (as has been previously reported) and that greater tic severity and *M. pneumoniae* seropositivity share underlying mechanisms rather than a causal relationship. This is the first study to report this finding and further research into the relationship between infections and CTD may shed light on the mechanisms underlying disease onset, severity, and course.

Funding

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant agreement no. 278367. This research was further supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London (Heyman); the Guys and St Thomas' NHS Foundation Trust (Hedderly, Turner); the Spanish Ministry of Science and Innovation [RTC2019-007150-1], the Instituto de Salud Carlos III-Fondo Europeo de Desarrollo Regional [ISCIII-FEDER] [PI14/01823, PI16/01575, PI18/01898, PI19/01576], the Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía [CVI-02526, CTS-7685], the Consejería de Salud y Bienestar Social de la Junta de Andalucía [PI-0471-2013, PE-0210-2018, PI-0459-2018, PE-0186-2019], and the Fundación Alicia Koplowitz (Mir); the EU [FP7-HEALTH-2011No. 278367, FP7-PEOPLE-2012-ITN No. 316978] (Fremer and Müller-Vahl); the German Research Foundation [DFG: GZ MU 1527/3-1], the German Ministry of Education and Research [BMBF: 01KG1421], the National Institute of Mental Health [NIMH], and the Tourette Gesellschaft Deutschland e.V., the Else-Kroner-Fresenius-Stiftung (Müller-Vahl); the Possehl-Stiftung [Lübeck, Germany], Margot und Jürgen Wessel Stiftung [Lübeck, Germany], Tourette Syndrome Association [Germany], Interessenverband Tourette Syndrom [Germany], CHDI, Damp-Stiftung [Kiel, Germany], Academic research support: Deutsche Forschungsgemeinschaft (DFG): projects 1692/3-1, 4-1, SFB 936, and FOR 2698 [project numbers 396914663, 396577296, 396474989]; and European Reference Network – Rare Neurological Diseases [ERN – RND; Project ID No 739510] (Münchau); and the National Institute for Health Research UCLH Biomedical Research Centre (Schrag).

Declaration of Competing Interest

Davide Martino has received honoraria for lecturing from the Movement Disorders Society, Tourette Syndrome Association of America and Dystonia Medical Research Foundation Canada; honoraria for advisory board attendance from Sunovion Pharmaceuticals Canada Inc.; research and education funding support from Dystonia Medical Research Foundation Canada, the University of Calgary, the Michael P Smith Family, the Owerko Foundation, Ipsen Corporate, the Parkinson Association of Alberta and the Canadian Institutes for Health Research; royalties from Springer-Verlag. Kirsten Müller-Vahl has received financial or material research support from Abide Therapeutics, Almirall Hermal GmbH, GW pharmaceuticals, Lundbeck, Syneos Health, and Therapix Biosciences Ltd. She has received consultant's honoraria from Abide Therapeutics, Allmiral, Boehringer Ingelheim International GmbH, Bionorica Ethics GmbH, CannaMedical Pharma GmbH, Canopy Grouth, Columbia Care, CTC Communications Corp., Eurox Deutschland GmbH, Global Praxis Group Limited, IMC Germany, Lundbeck, Resalo Vertrieb GmbH, Sanity Group, STADAPHARM GmbH, Synendos Therapeutics AG, and Tilray. She is/was a consultant or advisory board member for Abide Therapeutics, The Academy of Medical Cannabis Limited, Alirio, Aphria Deutschland GmbH, CannaMedical Pharma GmbH, Bionorica Ethics GmbH, CannaXan GmbH, Canopy Growth, Columbia Care, CTC Communications Corp., IMC Germany, Leafly Deutschland GmbH, Lundbeck, Nuvelution TS Pharma Inc., Resalo Vertrieb GmbH, Sanity Group, Syqe Medical Ltd., Therapix Biosciences Ltd., Tilray, Wayland Group, and CTC Communications Corporation.

She has received speaker's fees from Aphria Deutschland GmbH, Cogitando GmbH, Emalex, Eurox Deutschland GmbH, Ever pharma GmbH, PR Berater, Spectrum Therapeutics GmbH, Tilray, and Wayland Group. She has received royalties from Medizinisch Wissenschaftliche Verlagsgesellschaft Berlin, Elsevier, and Kohlhammer. Alexander Münchau received Royalties for the book Neurogenetics (Oxford University Press). Veit Roessner has received payment for consulting and writing activities from Lilly, Novartis, and Shire Pharmaceuticals, lecture honoraria from Lilly, Novartis, Shire Pharmaceuticals, and Medice Pharma, and support for research from Shire and Novartis. He has carried out (and is currently carrying out) clinical trials in cooperation with the Novartis, Shire, and Otsuka companies. Anette Schrag has received consultancy or advisory board honoraria from Biogen, Abbvie, Bial and Neurotechnology, research support from the National Institute of Health Research, Parkinson's UK and the Economic and Social Research Council and the European Commission, and Royalties from Oxford University Press. Susanne Walitza has received in the last 5 years royalties from Thieme Hogrefe, Kohlhammer, Springer, Beltz. Her work was supported in the last 5 years by the Swiss National Science Foundation (SNF), diff. EU FP7s, HSM Hochspezialisierte Medizin of the Kanton Zurich, Switzerland, Bfarm Germany, ZInEP, Hartmann Müller Stiftung, Olga Mayenfisch, Gertrud Thalmann, Vontobel, Unicentia, Erika Schwarz Fonds. Outside professional activities and interests are declared under the link of the University of Zurich www.uzh.ch/prof/ssl-dir/interessenbindungen/client/web/. On behalf of all other authors, the corresponding author declares that the other authors have no conflicts of interest.

Acknowledgements

The authors are deeply grateful to all children and their parents who willingly participated to make this research possible. We thank all colleagues at the various study centers who contributed to data collection: Julie E. Bruun, Judy Grejsen, Christine L. Ommundsen, Mette Rubæk (Capital Region Psychiatry, Copenhagen, Denmark); Stephanie Enghardt (TUD Dresden, Germany); Stefanie Bokemeyer, Christiane Driedger-Garbe, Cornelia Reichert (MHH Hannover, Germany); Jenny Schmalfeld, Jennifer Tübing (Lübeck University, Germany); Franciska Gergye, Margit Kovacs, Reka Vidomusz (Vadaskert Budapest, Hungary); Silvana Fennig, Ella Gev, Matan Nahon, Danny Horesh, Chen Regev, Tomer Simcha (SCMCI, Tel Aviv, Petah-Tikva, Israel); Martin Woods (Evelina London Children's Hospital GSTT, United Kingdom); Marieke Messchendorp, Anne Marie Stolte, Deborah Sival and the Stichting Gilles de la Tourette (UMCG Groningen, Netherlands); Maria Teresa Cáceres, Fátima Carrillo, Laura Vargas, Pilar Gómez-Garre (Seville, Spain); Giuseppe Gagliardi, Anna Marotta (ASL, Bari, Italy); Marina Redondo (FRCB, Barcelona, Spain); Thomas Duffield (LMU Munich, Munich, Germany); Nathan Keller, Gill Smollan (Sheba Medical Centre, Tel Aviv, Israel); Elena Michaelovsky, Miri Carmel, Avi Weizman (Tel Aviv University, Israel) and all who may not have been mentioned.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.10.012.

References

Akaltun, İ., Kara, T., Sertan Kara, S., Ayaydın, H., 2018. Seroprevalance Anti-Toxoplasma gondii antibodies in children and adolescents with tourette syndrome/chronic motor or vocal tic disorder: A case-control study. Psychiatry Res. 263, 154–157.

Almasri, M., Diza, E., Papa, A., Eboriadou, M., Souliou, E., 2011. Mycoplasma pneumoniae respiratory tract infections among Greek children. Hippokratia 15, 147–152.

American Psychiatric Association, 2000. Diagnostic and statistical manual of mental disorders: DSM-IV-TR. DC, American Psychiatric Association, Washington.

American Psychiatric Association, E., 2013. Diagnostic and statistical manual of mental disorders: DSM-5. D.C., American Psychiatric Association, Washington.

Atkinson, T.P., Balish, M.F., Waites, K.B., 2008. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. FEMS Microbiol. Rev. 32, 956–973.

Beskind, D.L., Keim, S.M., 1994. Choreoathetotic movement disorder in a boy with Mycoplasma pneumoniae encephalitis. Ann. Emerg. Med. 23, 1375–1378.

Bombaci, Mauro, Grifantini, Renata, Mora, Marirosa, Reguzzi, Valerio, Petracca, Roberto, Meoni, Eva, Balloni, Sergio, Zingaretti, Chiara, Falugi, Fabiana, Manetti, Andrea G.O., Margarit, Immaculada, Musser, James M., Cardona, Francesco, Orefici, Graziella, Grandi, Guido, Bensi, Giuliano, Ratner, Adam J., 2009. Protein array profiling of tic patient sera reveals a broad range and enhanced immune response against Group A Streptococcus Antigens. PLoS ONE 4 (7), e6332.

Bos-Veneman, N.G., Olieman, R., Tobiasova, Z., Hoekstra, P.J., Katsovich, L., Bothwell, A.L., Leckman, J.F., Kawikova, I., 2011. Altered immunoglobulin profiles in children with Tourette syndrome. Brain Behav. Immun. 25, 532–538.

Buse, J., Rothe, J., Uhlmann, A., Bodmer, B., Kirschbaum, C., Hoekstra, P.J., Dietrich, A., Roessner, V., 2021. Hair cortisol-a stress marker in children and adolescents with chronic tic disorders? A large European cross-sectional study. Eur. Child Adolesc. Psychiatry.

Daxboeck, F., Krause, R., Wenisch, C., 2003. Laboratory diagnosis of Mycoplasma pneumoniae infection. Clin. Microbiol. Infect. 9, 263–273.

Dehning, S., Matz, J., Riedel, M., Kerle, I.A., Müller, N., 2009. Symptom exacerbation in tourette syndrome due to bacterial reinfection. *J. clin. Psychiatry,* 70, 1606-1606.

el Hafidi, N., Allouch, B., Benbrahim, F., Chellaoui, M., el Mahraoui, C., 2012. Mycoplasma pneumoniae encephalitis associated with basal ganglia necrosis. Rev. Neurol. (Paris) 168, 49–52.

Ercan, T.E., Ercan, G., Severge, B., Arpaozu, M., Karasu, G., 2008. Mycoplasma pneumoniae infection and obsessive-compulsive disease: a case report. J. Child Neurol. 23, 338–340.

Foy, H.M., Kenny, G.E., Sefi, R., Ochs, H.D., Allan, I.D., 1977. Second attacks of pneumonia due to Mycoplasma pneumoniae. J. Infect. Dis. 135, 673–677.

Freeman, R.D., Fast, D.K., Burd, L., Kerbeshian, J., Robertson, M.M., Sandor, P., 2000. An international perspective on Tourette syndrome: selected findings from 3500 individuals in 22 countries. Dev. Med. Child Neurol. 42, 436–447.

Gabbay, V., Coffey, B.J., Guttman, L.E., Gottlieb, L., Katz, Y., Babb, J.S., Hamamoto, M. M., Gonzalez, C.J., 2009. A cytokine study in children and adolescents with Tourette's disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 967–971.

Gunther, J., Tian, Y., Stamova, B., Lit, L., Corbett, B., Ander, B., Zhan, X., Jickling, G., Bos-Veneman, N., Liu, D., Hoekstra, P., Sharp, F., 2012. Catecholamine-related gene expression in blood correlates with tic severity in tourette syndrome. Psychiatry Res. 200, 593–601.

Guy, W., 1976. Clinical Global Impression (CGI). U.S. Department of Health, Education, and Welfare, Rockville, MD, ECDEU assessment manual for psychopharmacology.

Hoekstra, P., Kallenberg, C., Korf, J., Minderaa, R., 2002. Is Tourette's syndrome an autoimmune disease? Mol. Psychiatry 7, 437–445.

Hoekstra, P.J., 2019. Attention-deficit/hyperactivity disorder: is there a connection with the immune system? Eur. Child Adolesc. Psychiatry 28, 601–602.

Hoekstra, P.J., Dietrich, A., Edwards, M.J., Elamin, I., Martino, D., 2013. Environmental factors in Tourette syndrome. Neurosci. Biobehav. Rev. 37, 1040–1049.

Jacobs, E., Bennewitz, A., Bredt, W., 1986. Reaction pattern of human anti-Mycoplasma pneumoniae antibodies in enzyme-linked immunosorbent assays and immunoblotting. J. Clin. Microbiol. 23, 517–522.

Kawikova, Ivana, Leckman, James F., Kronig, Holger, Katsovich, Lily, Bessen, Debra E., Ghebremichael, Musie, Bothwell, Alfred L.M., 2007. Decreased numbers of regulatory T cells suggest impaired immune tolerance in children with tourette syndrome: a preliminary study. Biol. Psychiatry 61 (3), 273–278.

Knight, T., Steeves, T., Day, L., Lowerison, M., Jette, N., Pringsheim, T., 2012. Prevalence of tic disorders: a systematic review and meta-analysis. Pediatr. Neurol. 47, 77–90.

Korppi, Matti, Heiskanen-Kosma, Tarja, Kleemola, Marjaana, 2004. Incidence of community-acquired pneumonia in children caused by Mycoplasma pneumoniae: serological results of a prospective, population-based study in primary health care. Respirology 9 (1), 109–114.

Krause, D., Matz, J., Weidinger, E., Wagner, J., Wildenauer, A., Obermeier, M., Riedel, M., Müller, N., 2010. Association between intracellular infectious agents and Tourette's syndrome. Eur. Arch. Psychiatry Clin. Neurosci. 260, 359–363.

Krause, D.L., Müller, N., 2012. The Relationship between Tourette's Syndrome and Infections. Open Neurol. J. 6, 124–128.

Krause, D.L., Weidinger, E., Matz, J., Wildenauer, A., Wagner, J.K., Obermeier, M., Riedel, M., Möller, H.J., Müller, N., 2012. Infectious Agents are Associated with Psychiatric Diseases. Ment. Illn. 4, e10.

Leckman, J.F., Katsovich, L., Kawikova, I., Lin, H., Zhang, H., Krönig, H., Morshed, S., Parveen, S., Grantz, H., Lombroso, P.J., King, R.A., 2005. Increased serum levels of interleukin-12 and tumor necrosis factor-alpha in Tourette's syndrome. Biol. Psychiatry 57, 667–673.

Leckman, J.F., Riddle, M.A., Hardin, M.T., Ort, S.I., Swartz, K.L., Stevenson, J., Cohen, D. J., 1989. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. J. Am. Acad. Child Adolesc. Psychiatry 28, 566–573.

Lennington, J.B., Coppola, G., Kataoka-Sasaki, Y., Fernandez, T.V., Palejev, D., Li, Y., Huttner, A., Pletikos, M., Sestan, N., Leckman, J.F., 2016. Transcriptome analysis of the human striatum in Tourette syndrome. Biol. Psychiatry 79, 372–382.

Leslie, D.L., Kozma, L., Martin, A., Landeros, A., Katsovich, L., King, R.A., Leckman, J.F., 2008. Neuropsychiatric disorders associated with streptococcal infection: a case-control study among privately insured children. J. Am. Acad. Child Adolesc. Psychiatry 47, 1166–1172.

Lin, Haiqun, Williams, Kyle A., Katsovich, Liliya, Findley, Diane B., Grantz, Heidi, Lombroso, Paul J., King, Robert A., Bessen, Debra E., Johnson, Dwight,

Kaplan, Edward L., Landeros-Weisenberger, Angeli, Zhang, Heping, Leckman, James F., 2010. Streptococcal upper respiratory tract infections and psychosocial stress predict future tic and obsessive-compulsive symptom severity in children and adolescents with Tourette syndrome and obsessive-compulsive disorder. Biol. Psychiatry 67 (7), 684–691.

Lind, K., Kristiansen, J.E., 2000. Effect of some psychotropic drugs and a barbiturate on mycoplasmas. Int. J. Antimicrob. Agents 14, 235–238.

Martino, D., Chiarotti, F., Buttiglione, M., Cardona, F., Creti, R., Nardocci, N., Orefici, G., Veneselli, E., Rizzo, R., Group, I.T.S.S., 2011. The relationship between group A streptococcal infections and Tourette syndrome: a study on a large service-based cohort. Dev. Med. Child Neurol. 53, 951–957.

Martino, D., Johnson, I., Leckman, J.F., 2020. What does immunology have to do with normal brain development and the pathophysiology underlying tourette syndrome and related neuropsychiatric disorders? Front. Neurol. 11, 567407.

Martino, D., Schrag, A., Anastasiou, Z., Apter, A., Benaroya-Milstein, N., Buttiglione, M., Cardona, F., Creti, R., Efstratiou, A., Hedderly, T., Heyman, I., Huyser, C., Madruga, M., Mir, P., Morer, A., Debes, N. M., Moll, N., Müller, N., Müller-Vahl, K., Munchau, A., Nagy, P., Plessen, K. J., Porcelli, C., Rizzo, R., Roessner, V., Schnell, J., Schwarz, M., Skov, L., Steinberg, T., Tarnok, Z., Walitza, S., Dietrich, A., Hoekstra, P. J. 2021. Association of Group A Streptococcus Exposure and Exacerbations of Chronic Tic Disorders: A Multinational Prospective Cohort Study. *Neurology*.

Martino, D., Zis, P., Buttiglione, M., 2015. The role of immune mechanisms in Tourette syndrome. Brain Res. 1617, 126–143.

Mataix-Cols, D., Isomura, K., Pérez-Vigil, A., Chang, Z., Rück, C., Larsson, K.J., Leckman, J.F., Serlachius, E., Larsson, H., Lichtenstein, P., 2015. Familial risks of tourette syndrome and chronic tic disorders a population-based cohort study. JAMA Psychiatry 72, 787–793.

Müller, N., Riedel, M., Blendinger, C., Oberle, K., Jacobs, E., Abele-Horn, M., 2004. Mycoplasma pneumoniae infection and Tourette's syndrome. Psychiatry Res. 129, 119–125.

Müller, N., Riedel, M., Forderreuther, S., Blendinger, C., Abele-Horn, M., 2000. Tourette's syndrome and mycoplasma pneumoniae infection. Am. J. Psychiatry 157, 481-a-482.

Murphy, T.K., Kurlan, R., Leckman, J., 2010. The immunobiology of Tourette's disorder, pediatric autoimmune neuropsychiatric disorders associated with Streptococcus, and related disorders: a way forward. J. Child Adolesc. Psychopharmacol. 20, 317–331.

Orefici, G., Cardona, F., Cox, C.J., Cunningham, M.W., 2016. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet].

Randolph, J.J., Falbe, K., Manuel, A.K., Balloun, J.L., 2014. A Step-byStep Guide to Propensity Score Matching in R. 19.

Riedel, M., Straube, A., Schwarz, M.J., Wilske, B., Müller, N., 1998. Lyme disease presenting as Tourette's syndrome. Lancet 351, 418–419.

Robertson, M.M., Eapen, V., Singer, H.S., Martino, D., Scharf, J.M., Paschou, P., Roessner, V., Woods, D.W., Hariz, M., Mathews, C.A., Črnčec, R., Leckman, J.F., 2017. Gilles de la Tourette syndrome. Nat. Rev. Dis. Primers 3, 16097.

Schrag, A., Martino, D., Apter, A., Ball, J., Bartolini, E., Benaroya-Milshtein, N., Buttiglione, M., Cardona, F., Creti, R., Efstratiou, A., Gariup, M., Georgitsi, M., Hedderly, T., Heyman, I., Margarit, I., Mir, P., Moll, N., Morer, A., Müller, N., Müller-Vahl, K., Münchau, A., Orefici, G., Plessen, K.J., Porcelli, C., Paschou, P., Rizzo, R., Roessner, V., Schwarz, M.J., Steinberg, T., Tagwerker Gloor, F., Tarnok, Z., Walitza, S., Dietrich, A., Hoekstra, P.J., 2019. European Multicentre Tics in Children Studies (EMTICS): protocol for two cohort studies to assess risk factors for tic onset and exacerbation in children and adolescents. Eur. Child Adolesc. Psychiatry 28, 91–109.

Snider, L.A., Swedo, S.E., 2003. Post-streptococcal autoimmune disorders of the central nervous system. Curr. Opin. Neurol. 16, 359–365.

Swedo, S.E., Leonard, H.L., Garvey, M., Mittleman, B., Allen, A.J., Perlmutter, S., Dow, S., Zamkoff, J., Dubbert, B.K., Lougee, L., 1998. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. Am. J. Psychiatry 155, 264–271.

Tian, Y., Gunther, J.R., Liao, I.H., Liu, D., Ander, B.P., Stamova, B.S., Lit, L., Jickling, G. C., Xu, H., Zhan, X., Sharp, F.R., 2011a. GABA- and acetylcholine-related gene expression in blood correlate with tic severity and microarray evidence for alternative splicing in Tourette syndrome: a pilot study. Brain Res. 1381, 228–236.

Tian, Y., Liao, I.H., Zhan, X., Gunther, J.R., Ander, B.P., Liu, D., Lit, L., Jickling, G.C., Corbett, B.A., Bos-Veneman, N.G., Hoekstra, P.J., Sharp, F.R., 2011b. Exon expression and alternatively spliced genes in Tourette Syndrome. Am. J. Med. Genet. B Neuropsychiatr. Genet. 156B, 72–78.

Tuuminen, T., Varjo, S., Ingman, H., Weber, T., Oksi, J., Viljanen, M., 2000. Prevalence of Chlamydia pneumoniae and Mycoplasma pneumoniae immunoglobulin G and A antibodies in a healthy Finnish population as analyzed by quantitative enzyme immunoassays. Clin. Diagn. Lab. Immunol. 7, 734–738.

Waris, M.E., Toikka, P., Saarinen, T., Nikkari, S., Meurman, O., Vainionpää, R., Mertsola, J., Ruuskanen, O., 1998. Diagnosis of Mycoplasma pneumoniae pneumonia in children. J. Clin. Microbiol. 36, 3155–3159.

Zilhão, N.R., Olthof, M.C., Smit, D.J., Cath, D.C., Ligthart, L., Mathews, C.A., Delucchi, K., Boomsma, D.I., Dolan, C.V., 2017. Heritability of tic disorders: a twin-family study. Psychol. Med. 47, 1085–1096.