Lack of association of group-A streptococcal infections and Onset of Tics: European multicentre Tics in Children Study (EMTICS)

Abstract

Objective

To investigate the association between Group-A streptococcal (GAS) infections and tic incidence among unaffected children with a family history of chronic tic disorders (CTD).

Methods

In a prospective cohort study, children with no history for tics aged 3 to 10 years with a first-degree relative with CTD were recruited from the European Multicentre Tics in Children Study (EMTICS) across 16 European centres. Presence of GAS infection was assessed using throat swabs, serum Anti-streptolysin O titres (ASOT) and Anti-DNAse B (ADB) titres blinded to clinical status. GAS exposure was defined using four different definitions based on these parameters. Cox regression analyses with time-varying GAS exposure were conducted to examine the association of onset of tics and GAS exposure during follow-up. Sensitivity analyses were conducted using Cox regression and logistic regression analyses.

Results

A total of 260 children were recruited whilst one subject was found to have tic onsets before study entry and therefore was excluded. 61 children (23.6%) developed tics over an average follow-up period of 1 (SD 0.7) year. There was a strong association of sex and onset of tics, with girls having an approximately 60% lower risk of developing tics compared to boys (HR: 0.4, 95% CI 0.2-0.7). However, there was no statistical evidence to suggest an association of any of the four GAS exposure definitions with tic onset (GAS exposure definition 1: HR=0.310, 95% CI: 0.037-

2.590; definition 2: HR=0.561, 95% CI: 0.219-1.436; definition 3: HR=0.853, 95% CI: 0.466-1.561; definition 4: HR=0.725, 95% CI: 0.384-1.370).

Conclusion

These results do not suggest an association of GAS exposure and development of tics.

Classification of Evidence

This study provides Class I evidence that Group-A streptococcal exposure does not associate with the development of tics in children with first-degree relatives with chronic tic disorder.

Introduction

The aetiology of chronic tic disorders (CTDs) and Tourette syndrome (TS) is still unclear, despite significant advances in genetics[1] and neuroimaging.[2] There are clear contributions from genetic factors,[3-5] but environmental factors, including noxious exposures during prenatal and perinatal stages, e.g. maternal smoking, exposure to certain drugs such as amphetamines and other central nervous system (CNS) stimulants as well as psychosocial stress have also been speculated to contribute[6-9]. Since the description of the first 50 cases of tic-like behaviours in the context of Group-A streptococcal (GAS) infections,[10] there has been an ongoing controversy regarding the possible role of GAS infections in tic disorders. Several cross-sectional studies have found elevated anti-streptococcal antibody titres in patients with tics.[11,12] Findings from one case-control study indicated a correlation between levels of anti-streptococcal antibodies and tic severity,[13] in contrast to results from another case-control study.[14] Retrospective population studies based on data from healthcare registries from the US, Denmark, and Taiwan reported associations between the onset of tics and GAS exposure.[15-18] On the other hand, longitudinal studies based on clinical data did not suggest a temporal link between a recent GAS exposure and onset or clinical worsening of tic disorders.[19-25] Previous studies have been retrospective, register-based or had limited sample sizes. Considering the average age of onset of TS is 7 years (and the prevalence and severity reach a peak at around 9-12 years of age),[26] and GAS throat infections are common in this age group, clear associations are difficult to establish in small samples. Laboratory-confirmed prospective studies in this field are difficult to conduct as GAS infections are frequently not documented with laboratory tests and may go undiagnosed. In addition, tic onset is insidious and tics can be unnoticed outside a specialist setting for many years.[27] We set out to prospectively study the association of

onset of tics, assessed bi-monthly, with GAS infections detected using throat swabs and serology (serum anti-streptolysin O titre (ASOT) and anti-DNAseB (ADB) antibody titre), in a large high-risk sample of 3- to 10- years old children, namely first-degree relatives of patients with TS or CTD who were followed up for up to 48 months. Therefore, for the current study, the primary research question is to explore whether there is an association between GAS infections and development of tics in children with first-degree relatives with chronic tic disorder, independent of age, sex and parental education level.

Methods and materials

Study design

The European Multicentre Tics in Children Studies (EMTICS) is a prospective cohort study exploring the role of environmental and genetic factors in paediatric CTD. The methods of this study have been described previously.[28] The main objective of the ONSET arm of the study was to investigate the association between environmental and genetic factors and onset of tics in children who are first-degree relatives of patients with an established CTD.

Participants

A total of 260 children aged 3-10 years who were first-degree relatives of individuals with a CTD (criteria according to the Diagnostic and Statistical Manual fourth edition, text revision),[29] but themselves free of tics, were recruited between 2013 and 2016 from 16 (child- and adolescent) psychiatry and paediatric neurology outpatient clinics (one of the EMTICS centres did not collect data for the current study, and one subject was removed as he had tics before study entry). Children were excluded if at baseline

they were having a serious medical or neurological illness or being unable to understand and comply with study procedures. Children were allowed to receive treatment for mental health problems. The detailed inclusion and exclusion were published elsewhere. [28, 30]

Standard protocol approvals, registrations, and patient consents

All local Ethics Committees of the participating centres provided approval to the study. Parents and their child(ren) provided written informed consent and assent as appropriate according to ethical regulations.

Study procedures

Participants were evaluated every 2 months, alternating between scheduled hospital visits and telephone interviews. Parents were also instructed to communicate any possible sign of tic onset to the study centre as soon as possible (e.g. by phone or email). All symptoms indicative of a possible onset of tics were explained to parents at the baseline visit. If parents reported possible onset of tics outside of planned visits, an "unscheduled tic onset evaluation telephone interview" was held by the study clinician to investigate whether possible onset of tics had occurred. Data collection was structured on three levels of observation: (1) through a weekly diary in which parents were asked to indicate possible symptom onset, aimed at the earliest possible detection of onset of tics throughout the whole study duration. Parents were instructed to immediately contact the study clinician whenever they suspected the onset of tics; (2) scheduled telephone interview once every 4 months with review of the weekly diaries since the last assessment and clinical evaluations of possible tic onset performed by the study clinician to parents; and (3) visits in hospital every 4 months

over the 3-year duration study period, which comprised clinical evaluation and collection of biological samples (i.e. throat swab and ASOT and ADB titres).

Tic onset was defined as the first occurrence of any sudden, rapid, recurrent, non-rhythmic involuntary movement and/or vocalization noticed on at least three separate days within a period of 3 weeks. If the evaluation pointed to a possible tic onset, in any case, an "onset of tics hospital visit" was scheduled preferably within 1 week or at the earliest opportunity for extended clinical evaluation including the Yale Global Tic Severity Scale (YGTSS)[31] to confirm the onset of tics and collect biological material. If an onset of tics was confirmed, no further planned assessments were conducted until a final follow-up visit at 1 year after the tic onset visit. Otherwise, the originally scheduled visits were continued. Please refer the detailed follow-up process in study protocol.[28] Moreover, to establish the possible onset of tics after the end of the study period, further follow-up telephone calls were made two years of the end of the study to 200 unaffected participants.

Laboratory measures

The main microbiological measures were GAS colonisation by throat swabbing and processing using a standardised methodology. To ensure homogeneity in laboratory procedures, the protocol was harmonised, and all centres participated in cross-centre training and external quality control co-led by two microbiological units in the EMTICS consortium. Exposure to GAS in study participants was also investigated by measuring ASOT and ADB. A significant rise of ASOT was identified when ASOT > 200 AND [log10 (ASOT*current visit*) – log10(ASOT*prior visit*)] $\geq$ 0.2 (variation between log10 for two consecutive measurements is higher than or equal to 0.2); a significant rise of ADB was identified when ADB > 300 AND [log10 (ADB*current*

visit) – log10(ADB*prior visit*)] ≥ 0.2 (variation between log10 for two consecutive measurements is higher than or equal to 0.2). ASOT and ADB titres were centrally measured in the laboratory of the University Hospital Munich (Ludwig-Maximilians-Universität; LMU). For determination of ASOT, the Immuno-turbidimetric test from Beckman Coulter (Brea, California) was used with a lower limit of quantification of 100 IU/ml. For determination of ADB titres, an immunonephelometric method performed on a BN Prospec analyser by Siemens Healthineers (Erlangen, Germany) was used, where the lower limit of quantification was 71 U/ml. A detailed summary of laboratory measurements was listed in the protocol paper.[28] Laboratory analyses were performed blinded to clinical status.

Four combinations of measures were used to classify GAS exposure: (1) *new definite GAS exposure*, characterised by a newly positive throat swab regardless of serological test results; (2) *new possible GAS exposure*, characterised by negative or missing throat swab but significant rise of anti-streptococcal antibody titres, i.e. ASOT and/or ADB; (3) *ongoing definite GAS exposure*, characterised by persistently positive throat swab over at least two time points, regardless of serological test results; (4) *ongoing possible GAS exposure*, characterised by significant rise of either of the two anti-streptococcal antibody titres and negative or missing throat swab but positive throat swab at the previous time point. Based on these classifications, we used four definitions of varying stringency for analysis with *definition 1* being the most conservative one and *definition 4* being the most lenient definition. *Definition 1* included only a new definite GAS exposure; *definition 2* included either a new definite or a new possible GAS exposure; *definition 3* included either a new (definite or possible) GAS exposure or an ongoing definite GAS exposure and *definition 4*

included either a new (definite or possible) GAS exposure or an ongoing (definite or possible) GAS exposure.

Other Measurements

Covariates measured at baseline were age in years, sex and parental education level. Parental education level was based on the highest education level of the two parents and consisted of two levels: low-level vs. high-level. This was dichotomised at whether the parents have received a college degree (i.e. low-level parental education: maximum education level was a level or two years college degree, and high-level parental education: at least have four-year college/university degree). Clinical site was categorised by geographical region, i.e. Northern (UK, Denmark), Central (Germany, Netherlands, Switzerland, Hungary) and Southern Europe (Spain, Italy, Israel); Psychotropic medications included first, second/third generation antipsychotics as well as alpha agonists and were checked two-weeks prior to each follow-up time point by clinicians (results are listed in eTable 1 – eTable 3).

Power Calculation

The current study originally aimed to recruit 500 participants who were aged 3-10 years old and were first-degree relatives of patients with a tic disorder. The finally achieved sample size of 260 still provides 80% power to detect an odds ratio of 2.85 for GAS carriers compared to non-carriers with respect to the event "onset", assuming an estimated GAS carriage rate of 15% in childhood,[32]and an estimated risk of 30%[33] for a first-degree relative of patients with TS or other chronic tic disorders to be affected by tics at α=0.05 (two-sided). Detailed information on power analysis was published elsewhere.[28]

Statistical Analyses

Participants' characteristics were summarised using descriptive statistics. Continuous variables were expressed as mean and standard deviation (SD). Categorical variables were reported as counts and percentages. For each of the different definitions of GAS exposure, the following analyses were performed: The main analysis used was a Cox regression model with time to tic onset as outcome and GAS exposure as a time-varying risk factor; this allowed us to take an individual's changes of GAS exposure over time into consideration. For this analysis, missing data on GAS exposure was imputed using the technique of the last observation carried forward (LOCF). To test the impact of missing GAS exposure on the outcome of interest, a sensitivity analysis was carried out by excluding visits with missing data on GAS exposure. We also ran additional sensitivity analyses testing possible associations of GAS exposure and tic onset: a Cox regression analysis with time to tic onset as outcome and baseline GAS exposure was conducted to examine the relationship of GAS exposure at baseline with subsequent tic onset and a logistic regression was performed to test the association between tic onset and GAS exposure at any time during follow-up. For each above analysis, we first present univariable results and subsequently adjusted for age, sex and parental education. In additional analyses we also adjusted for site and psychotropic medication use. Results of the sensitivity analyses are listed in eTable 4 – eTable6. All statistical tests were two-sided, and a p value <0.05 was considered statistically significant. Statistical tests were implemented in STATA version 16 (StataCorp LP, College Station, TX, USA).

Data availability

De-identified participant data related to all demographic, clinical, and laboratory variables will be shared following request made by any qualified investigators to the study authors.

Results

Sample Descriptive

The mean age of the 259 participants at baseline was 6.8 (SD 2.1, range: 2.8-10.9) years, and over half were female. About 57% of participants' parents had received at least college/university level education (Table 1). Follow up time was on average 1.6 (SD 1.0, range: 0-3.8) years. Overall, there were 61 onset tic cases during the study period and the average time from baseline until tic onset was 1 (SD 0.7) year. At baseline, a total of 44 (17.0%) participants tested positive on GAS, and 204 (78.8%) participants tested negative, while there was no throat swab available from 11 (4.2%) participants. Blood samples were collected from 207 participants at baseline to examine ASOT and/or ADB titres (eTable 1).

During the study follow-up period, there were a total of 1944 visits including 939 telephone interviews (928 scheduled and 11 unscheduled, respectively) and 1005 clinical visits. Throat swab and serum ASOT/ADB analyses were available for 422 (42%) and 564 (56%) of 1005 study visits, respectively. The number of confirmed positive GAS exposure during follow-up was 59, 102, 125 and 138 relating to definition 1, 2, 3 and 4, respectively. Detailed distribution of GAS exposure across clinic visits by tic onset visits without any missing data on GAS exposure can be found in Table 2.

Results from regression analyses

There was no evidence of an association of tic onset with GAS exposure in univariable Cox regression analysis using time-varying GAS exposure for any definitions of GAS exposure (Table 3). Adjustment for age, sex and parental education level did not reveal any significant associations between tic onset and GAS exposure either (Table 3). However, there was a strong association in all analyses between tic onset and sex with girls being 60% less likely to develop tics compared to boys (all *p-values* <0.01).

The sensitivity analysis using Cox regression analysis to examine the association of tic onset with GAS exposure at baseline also showed no evidence of an association of tic onset with baseline GAS exposure (eTable 4), and neither did the logistic regression analysis show an association of tic onset with GAS exposure (eTable 5). Results from the analysis after excluding visits with missing data on GAS exposure were consistent with the main findings (eTable 4). Analyses with further adjustment for clinical site and psychotropic medication use were also in line with the main findings (eTable 6).

During the additional 2-year follow-up after the end of the study, 7 patients were reported to have had onset of tics. Replication of all analyses with these additional cases did not change the results (data not shown).

Classification of Evidence

The study provides Class I evidence that Group-A streptococcal exposure does not associate with the development of tics in children with first-degree relatives with chronic tic disorder.

Discussion

In this large cohort of children at risk of tics, GAS infection was not associated with tic onset in either univariable or multivariable time-varying Cox-regression analyses adjusting for age, sex and parental education level. The results from a series of sensitivity analyses confirmed the results from the main analyses. On the other hand, our finding confirms the strong sex difference in terms of tic onset after controlling for age, GAS exposure and parental education level, with boys being significantly more likely to develop tics in this cohort. This is in line with previous studies.[21, 34,35]

The association between GAS exposure and tic onset remains controversial, with some studies reporting a significant association,[15-17] and others not.[20-22] Our results do not support an association between GAS exposure and onset of tics. One possible explanation for differences between our study and others is that there are substantial study variations with regard to study population, design and GAS measurements. For example, most studies reporting a significant association between GAS exposure and tic onset were based on health insurance data.[15-17] The identification of GAS infection and the diagnosis of tic disorder in these studies were based on information from routine care, where a number of factors related to healthcare systems and healthcare seeking need to be considered, rather than standardised prospective assessments in an at-risk population. Therefore, it is possible that the relation found between GAS infection and onset of tics was influenced by different healthcare seeking behaviours of patients and differences in diagnostic procedures for diagnosis of GAS-related throat infections. Moreover, information from studies using health records might be subject to misclassification, and the recorded dates of disease onset may differ from the true timing of disease onset. In our study, we were able to prospectively follow children who had first-degree relatives with a CTD but were free of tics at baseline

and conduct standardised examinations for GAS infection, independent of healthcare practices, and examination by experienced clinicians following standardised procedures.

Our results do not support an association between GAS throat infection and onset of tic disorders. Interestingly, a large population-based cohort study reported that regardless of streptococcal test results, children who had testing for streptococcal status because of throat infections had a higher risk of tic disorders than those who were not being tested for streptococcal infections. However, the risk of any mental disorder and OCD was more elevated after a streptococcal throat infection than after a non-streptococcal infection.[36] Another recent large Danish population-based cohort study found that children with infections requiring hospitalisations had an increased risk of mental disorders, including tic disorders, OCD, and ADHD, but not those without hospitalisation.[18] Taken together, these studies suggest that either pathogens other than GAS, or infection-induced inflammatory mechanisms are linked to development of tics and other mental disorders in children. Future studies into other pathogens and immunological factors are needed to investigate whether these play a specific role in development of tics.

The key strength of this study is the prospective evaluation of unaffected children at risk of developing tics not relying on healthcare seeking behaviour. Further strengths of the study include the comprehensive evaluation of GAS exposure and tic onset. We used multiple definitions of GAS exposures varying in stringency in order to minimise false negative findings. A three-level observation and data collection scheme were performed to allow for an accurate diagnosis of tic onset in a timely

manner (and therefore reduce the rate of misclassification) and minimise recall bias. The timely examination of participants with GAS exposure was particularly important as findings from a previous study suggested the impact of GAS exposure on tic development might be influenced by the time window between GAS infection and tic onset.[15] To account for the potential influence of the time between GAS exposure and tic onset, we used time-varying Cox regression models taking into account changes of GAS exposure status over time and performed several sensitivity analyses analysing the association of tic onset with GAS exposure at baseline and during follow-up.

One of the potential limitations is that our participants were from 16 study centres across Europe, which could result in a great heterogeneity in terms of clinical and microbiological assessments. However, we used several strategies in the study design to mitigate this limitation including clinical procedure harmonization, across-centre clinical training and external quality control co-led by two microbiological units in the EMTICS consortium, as well as correction of the analysis for site. Furthermore, there were missing data for laboratory tests largely as a result of insufficient volume or haemolysis of the collected specimens or unavailability of participants for specimen collection. However, we performed sensitivity analyses with complete cases only (i.e. excluding visits with missing data on GAS exposure), and the results from sensitivity analyses were consistent with the main findings. The width of 95% confidence intervals of the hazard ratio estimates in primary analyses was relatively large, suggesting a type II error may exist. However, based on our power analysis, the size of study population was sufficient for detection of a moderate association between GAS exposure and tic onset.

In summary, this prospective study did not find evidence for an association between prospectively studied GAS exposure and tic onset in children who are the first-degree relatives of patients with CTD. This finding may have implications for both clinical and pathophysiological aspects of tic disorders. From a clinical perspective, as GAS exposure was not found to be associated with tic onset, our study does not support the widespread ongoing clinical practice by many primary care physicians of ordering throat swabs and antibody tests for GAS or treating with antibiotics when a child presents with a new onset of tics. Moreover, as our companion EMTICS study[25] reported no significant association between GAS exposure and tic exacerbations, investigation or recommendation of active management of GAS infection is unlikely to help modify the course of tics. Since the study participants were recruited from a high-risk population of first-degree relatives, results from this study may suggest that GAS exposure at least in those with genetic risk factors do not play an important role in the occurrence of tics. The lack of association between GAS exposure and tic onset suggests that future research needs to examine the relationships between tic onset and a wider range of factors, including other pathogens.

Appendix 1. Authors

Name	Location	Contribution
Schrag A. MD, PhD	Department of Clinical Neuroscience, UCL Institute of Neurology, University College London, London, UK	Design and conceptualized study; analysed the data; drafted the manuscript for intellectual content; corresponding author
Martino D. MD, PhD	Department of Clinical Neurosciences, Cumming School of Medicine & Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada	Design and conceptualized study; revised the manuscript for intellectual content
Wang H. PhD	Department of Clinical Neuroscience, UCL Institute of Neurology, University College London, London, UK	Interpreted the data; revised the manuscript for intellectual content
Ambler G. PhD	Department of Statistical Science, University College London, London, UK	Interpreted the data; revised the manuscript for intellectual content
Benaroya-Milstein N. MD, PhD	Child and Adolescent Psychiatry Department, Schneider Children's	Major role in the acquisition of data

	Medical Centre of Israel, Petah-Tikva. Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Israel	
Buttiglione M. PhD	Department of Biomedical Sciences and Human Oncology, University of Bari “Aldo Moro”, Bari, Italy.	Revised the manuscript for intellectual content
Cardona F. MD	Department of Human Neurosciences, University La Sapienza of Rome, Rome, Italy	Major role in the acquisition of data
Creti R. PhD	Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy	Major role in the acquisition of data, participated to design of microbiological analyses and advised on results’ interpretation
Efstratiou A. PhD	WHO Global Collaborating Centre for Reference and Research on Diphtheria and Streptococcal Infections, Reference Microbiology,	Participated to design of microbiological analyses and advised on results’ interpretation

	Directorate National Infection Service, Public Health England, London, UK	
Hedderly T. MD	Evelina London Children's Hospital GSTT, Kings Health Partners AHSC, London, UK	Major role in the acquisition of data
Heyman I. MBBS, PhD, FRCPsych	Psychological Medicine, Great Ormond Street Hospital NHS Foundation Trust, Great Ormond Street, London, UK	Major role in the acquisition of data and advised on results' interpretation
Huyser C. MD, PhD	[1]Levvel, Academic Center for Child and Adolescent Psychiatry, Amsterdam, The Netherlands; [2]Amsterdam UMC, Department of Child and Adolescent Psychiatry, Amsterdam, The Netherlands	Major role in the acquisition of data
Mir P. MD, PhD	[1]Unidad de Trastornos del Movimiento. Instituto de Biomedicina de Sevilla	Major role in the acquisition of data

	(IBiS). Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla. Seville, Spain; [2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain	
Morer A. MD, PhD	[1]Department of Child and Adolescent Psychiatry and Psychology, Institute of Neurosciences, Hospital Clinic Universitari, Barcelona, Spain; [2]Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [3]Centro de Investigacion en Red de Salud Mental (CIBERSAM), Instituto Carlos III, Spain	Major role in the acquisition of data

Moll N. MSc	Institute of Laboratory Medicine, University Hospital LMU Munich, Munich, Germany	Major role in the acquisition of laboratory data
Mueller N. MD	Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany	Major role in the acquisition of data
Müller-Vahl K. MD	Department of Psychiatry, Social psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany	Major role in the acquisition of data
Plessen K.J. MD	[1]Child and Adolescent Mental Health Centre, Mental Health Services, Capital Region of Denmark and University of Copenhagen, Copenhagen, Denmark; [2]Division of Child and Adolescent Psychiatry, Department of Psychiatry, Lausanne University	Major role in the acquisition of data

	Hospital, Lausanne, Switzerland	
Porcelli C. MD	ASL BA, Mental Health Department; Adolescence and Childhood Neuropsychiatry Unit; Bari, Italy	Major role in the acquisition of data
Rizzo R. MD, PhD	Child and Adolescent Neurology and Psychiatry, Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy	Major role in the acquisition of data
Roessner V. MD, PhD	Department of Child and Adolescent Psychiatry, Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany	Major role in the acquisition of data
Schwarz M. MD, PhD	Institute of Laboratory Medicine, University Hospital LMU Munich, Munich, Germany	Major role in the acquisition of laboratory data
Tarnok Z. PhD	Vadaskert Child and Adolescent Psychiatric	Major role in the acquisition of data

	Hospital, Budapest, Hungary	
Walitza S. MD, MSc	Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich, Zurich, Switzerland	Major role in the acquisition of data
Dietrich A. PhD	University of Groningen, University Medical Centre Groningen, Department of Child and Adolescent Psychiatry, Groningen, the Netherlands	Design and conceptualized study; acquisition of data; data curation; revised the manuscript for intellectual content
Hoekstra P. J. MD, PhD	University of Groningen, University Medical Centre Groningen, Department of Child and Adolescent Psychiatry, Groningen, the Netherlands	Design and conceptualized study; analyzed the data; revised the manuscript for intellectual content

Appendix 2. Co-investigators/authors

Name	Location	Contribution
Alan Apter	Child and Adolescent Psychiatry Department, Schneider Children's Medical Center of Israel, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Petah-Tikva, Israel	Participated to data acquisition for site
Baglioni V.	University La Sapienza of Rome, Department of Human Neurosciences, Rome, Italy	Coordinated data acquisition for site
Ball J.	Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich, Zurich, Switzerland	Participated to data acquisition for site
Bartolini E.	GSK, Siena, Italy	Participated to study design and advised on results' interpretation
Bodmer B.	Department of Child and Adolescent Psychiatry, Faculty of Medicine of the TU Dresden, Dresden, Germany	Participated to data acquisition for site
Bognar E.	[1]Vadaskert Child and Adolescent Psychiatric Hospital, Budapest, Hungary; [2]Semmelweis University, Budapest, Hungary	Participated to data acquisition for site
Bosch J.	Microbiology Department, CDB, Hospital Clinic, Barcelona, Spain	Participated to laboratory data

		acquisition for site
Burger B.	Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany	Participated to data acquisition for site
Buse J.	Department of Child and Adolescent Psychiatry, Faculty of Medicine of the TU Dresden, Dresden, Germany	Participated to data acquisition for site
Correa Vela M.	Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clinica. Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocio/CSIC/Universidad de Sevilla, Seville, Spain	Participated to data acquisition for site
Debes N.M.	Paediatric Department, Herlev University Hospital, Herlev, Denmark	Participated to data acquisition for site
Ferro M.C.	Child Neuropsychiatry Section, Department of Clinical and Experimental Medicine, School of Medicine, Catania University, Catania, Italy	Participated to data acquisition for site
Fremer C.	Clinic of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany	Participated to data acquisition for site

Garcia-Delgar B.	Department of Child and Adolescent Psychiatry and Psychology, Institute of Neurosciences, Hospital Clinic Universitari, Barcelona, Spain	Participated to data acquisition for site
Gulisano M.	Child Neuropsychiatry Section, Department of Clinical and Experimental Medicine, School of Medicine, Catania University, Catania, Italy	Participated to data acquisition for site
Hagen A.	[1]Levvel, Academic Centre for Child and Adolescent Psychiatry, Amsterdam, the Netherlands; [2]Amsterdam UMC, Department of Child and Adolescent Psychiatry, Amsterdam, The Netherlands	Participated to data acquisition for site
Hagstrøm J.	Child and Adolescent Mental Health Centre, Mental Health Services, Capital Region of Denmark, Copenhagen, Denmark	Participated to data acquisition for site
Imperi M.	Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy	Participated to design of microbiological analyses
Madruga M.	Neuropediatrics, Centro de Pediatría de Sevilla, Hospital Viamed Santa Ángela De la Cruz, Seville, Spain	Participated to data acquisition for site
Margarit I.	GSK, Siena, Italy	Participated to overall study design and

		advised on results' interpretation
Meier U.C.	Blizard Institute, Queen Mary University of London, London, UK	Participated to overall study design and advised on results' interpretation
Munchau A.	Institute of Neurogenetics, University of Lübeck, Lübeck, Germany	Participated to overall study design.
Nagy, P.	[1]Vadaskert Child and Adolescent Psychiatric Hospital, Budapest, Hungary; [2]Semmelweis University, Budapest, Hungary	Participated to data acquisition for site
Neri V.	University La Sapienza of Rome, Department of Human Neurosciences, Rome, Italy	Participated to data acquisition for site
Orefici G.	formerly Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy	Participated to study design and advised on results' interpretation

Pellico A.	Child Neuropsychiatry Section, Department of Clinical and Experimental Medicine, School of Medicine, Catania University, Catania, Italy	Participated to data acquisition for site
Petruzzelli O.	University of Bari "Aldo Moro", Medical School, Department of Biological Sciences and Human Oncology, Bari, Italy	Participated to design of immunological analyses
Ruhrman D.	Child and Adolescent Psychiatry Department, Schneider Children's Medical Centre of Israel, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Petah-Tikva, Israel	Participated to data acquisition for site
Schnell J.	Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany	Major role in the acquisition of data
Silvestri P.R.	University La Sapienza of Rome, Department of Human Neurosciences, Rome, Italy	Participated to data acquisition for site
Skov L.	Paediatric Department, Herlev University Hospital, Herlev, Denmark	Participated to data acquisition for site
Steinberg T.	Child and Adolescent Psychiatry Department, Schneider Children's Medical Center of Israel, affiliated to Sackler Faculty of Medicine, Tel Aviv University, Petah-Tikva, Israel	Participated to data acquisition for site

Tagwerker Gloor F.	Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich, Zurich, Switzerland	Participated to data acquisition for site
Tallon M.	IT Service, Istituto Superiore di Sanità, Rome, Italy	Designed and managed centralized database
Turner V.L.	Evelina London Children's Hospital GSTT, Kings Health Partners AHSC, London, UK	Participated to data acquisition for site
Weidinger E.	Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany	Participated to data acquisition for site

References

1. Qi Y, Zheng Y, Li Z, Liu Z, Xiong L. Genetic Studies of Tic Disorders and Tourette Syndrome. Methods Mol Biol. 2019;2011:547-71.

2. Hsu CJ, Wong LC, Wang HP, Lee WT. The multimodality neuroimage findings in individuals with Tourette syndrome. Pediatr Neonatol. 2020;61(5):467-74.

3. Carter AS, Pauls DL, Leckman JF, Cohen DJ. A prospective longitudinal study of Gilles de la Tourette's syndrome. J Am Acad Child Adolesc Psychiatry. 1994;33(3):377-85.

4. Mataix-Cols D, Isomura K, Perez-Vigil A, Chang Z, Ruck C, Larsson KJ, et al. Familial Risks of Tourette Syndrome and Chronic Tic Disorders. A Population-Based Cohort Study. JAMA Psychiatry. 2015;72(8):787-93.

5. Zilhao NR, Olthof MC, Smit DJ, Cath DC, Ligthart L, Mathews CA, et al. Heritability of tic disorders: a twin-family study. Psychol Med. 2017;47(6):1085-96.

6. Jankovic J, Kurlan R. Tourette syndrome: evolving concepts. Movement disorders. 2011;26(6):1149-56.

7. Hoekstra PJ, Dietrich A, Edwards MJ, Elamin I, Martino D. Environmental factors in Tourette syndrome. Neurosci Biobehav Rev. 2013;37(6):1040-9.

8. Dalsgaard S, Waltoft BL, Leckman JF, Mortensen PB. Maternal History of Autoimmune Disease and Later Development of Tourette Syndrome in Offspring. Journal of the American Academy of Child & Adolescent Psychiatry. 2015;54(6):495-501.e1.

9. Martino D, Johnson I, Leckman JF. What Does Immunology Have to Do With Normal Brain Development and the Pathophysiology Underlying Tourette Syndrome and Related Neuropsychiatric Disorders? Front Neurol. 2020;11:567407.

10. Swedo SE, Leonard HL, Garvey M, Mittleman B, Allen AJ, Perlmutter S, Dow S, Zamkoff J, Dubbert BK, Lougee L. Pediatric autoimmune neuropsychiatric disorders

associated with streptococcal infections: clinical description of the first 50 cases. American Journal of Psychiatry. 1998 Feb 1;155(2):264-71.

11. Murphy TK, Sajid M, Soto O, Shapira N, Edge P, Yang M, et al. Detecting pediatric autoimmune neuropsychiatric disorders associated with streptococcus in children with obsessive-compulsive disorder and tics. Biological Psychiatry. 2004;55(1):61-8.

12. Müller N, Riedel M, Straube A, Günther W, Wilske B. Increased anti-streptococcal antibodies in patients with Tourette's syndrome. Psychiatry Research. 2000;94(1):43-9.

13. Cardona F, Orefici G. Group A streptococcal infections and tic disorders in an Italian pediatric population. J Pediatr. 2001;138(1):71-5.

14. Arman S, Golmirzaei J, Naeini AE, Azhar MM. The evaluation of relationship between group A streptococcal infection with tic disorders in children. Saudi Med J. 2009;30(9):1180-5.

15. Mell LK, Davis RL, Owens D. Association Between Streptococcal Infection and Obsessive-Compulsive Disorder, Tourette's Syndrome, and Tic Disorder. Pediatrics. 2005;116(1):56-60.

16. Leslie DL, Kozma L, Martin A, Landeros A, Katsovich L, King RA, et al. Neuropsychiatric disorders associated with streptococcal infection: a case-control study among privately insured children. Journal of the American Academy of Child and Adolescent Psychiatry. 2008;47(10):1166-72.

17. Wang H-C, Lau C-I, Lin C-C, Chang A, Kao C-H. Group A streptococcal infections are associated with increased risk of pediatric neuropsychiatric disorders: a Taiwanese population-based cohort study. The Journal of clinical psychiatry. 2016;77(7):848-54.

18. Köhler-Forsberg O, Petersen L, Gasse C, Mortensen PB, Dalsgaard S, Yolken RH, et al. A Nationwide Study in Denmark of the Association Between Treated Infections and the

Subsequent Risk of Treated Mental Disorders in Children and Adolescents. JAMA Psychiatry. 2019;76(3):271-9.

19. Luo F, Leckman JF, Katsovich L, Findley D, Grantz H, Tucker DM, et al. Prospective longitudinal study of children with tic disorders and/or obsessive-compulsive disorder: relationship of symptom exacerbations to newly acquired streptococcal infections. Pediatrics. 2004;113(6):e578-e85.

20. Perrin EM, Murphy ML, Casey JR, Pichichero ME, Runyan DK, Miller WC, et al. Does Group A β-Hemolytic Streptococcal Infection Increase Risk for Behavioral and Neuropsychiatric Symptoms in Children? Archives of Pediatrics & Adolescent Medicine. 2004;158(9):848-56.

21. Murphy TK, Snider LA, Mutch PJ, Harden E, Zaytoun A, Edge PJ, et al. Relationship of Movements and Behaviors to Group A Streptococcus Infections in Elementary School Children. Biological Psychiatry. 2007;61(3):279-84.

22. Schrag A, Gilbert R, Giovannoni G, Robertson MM, Metcalfe C, Ben-Shlomo Y. Streptococcal infection, Tourette syndrome, and OCD: is there a connection? Neurology. 2009;73(16):1256-63.

23. Leckman JF, King RA, Gilbert DL, Coffey BJ, Singer HS, Dure LSt, et al. Streptococcal upper respiratory tract infections and exacerbations of tic and obsessive-compulsive symptoms: a prospective longitudinal study. J Am Acad Child Adolesc Psychiatry. 2011;50(2):108-18.e3.

24. Martino D, Chiarotti F, Buttiglione M, Cardona F, Creti R, Nardocci N, et al. The relationship between group A streptococcal infections and Tourette syndrome: a study on a large service-based cohort. Dev Med Child Neurol. 2011;53(10):951-7.

25. Martino D, Schrag A, Anastasiou Z, Apter A, Benaroya-Milstein N, Buttiglione M, Cardona F, Creti R, Efstratiou A, Hedderly T, Heyman I. Association of Group A

Streptococcus Exposure and Exacerbations of Chronic Tic Disorders: A Multinational Prospective Cohort Study. Neurology. 2021 Feb 10.

26. Scahill L, Erenberg G, Berlin CM, Budman C, Coffey BJ, Jankovic J, et al. Contemporary assessment and pharmacotherapy of Tourette syndrome. NeuroRX. 2006;3(2):192-206.

27. Greene DJ, Koller JM, Robichaux-Viehoever A, Bihun EC, Schlaggar BL, Black KJ. Reward enhances tic suppression in children within months of tic disorder onset. Dev Cogn Neurosci. 2015;11:65-74.

28. Schrag A, Martino D, Apter A, Ball J, Bartolini E, Benaroya-Milshtein N, et al. 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. 2019;28(1):91-109.

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

30. Openneer TJ, Huyser C, Martino D, Schrag A, EMTICS Collaborative Group, Hoekstra PJ, Dietrich A. Clinical precursors of tics: an EMTICS study. Journal of Child Psychology and Psychiatry. 2021 Jun 25.

31. Leckman JF, Riddle MA, Hardin MT, Ort SI, Swartz KL, Stevenson J, et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. Journal of the American Academy of Child & Adolescent Psychiatry. 1989;28(4):566-73.

32. Martin JM, Green M, Barbadora KA, Wald ER. Group A streptococci among school-aged children: clinical characteristics and the carrier state. Pediatrics. 2004 Nov 1;114(5):1212-9.

33. McMahon WM, Carter AS, Fredine N, Pauls DL. Children at familial risk for Tourette's disorder: Child and parent diagnoses. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2003 Aug 15;121(1):105-11.

34. Khalifa N, von Knorring AL. Prevalence of tic disorders and Tourette syndrome in a Swedish school population. Developmental Medicine & Child Neurology. 2003;45(5):315-9.

35. Bitsko RH, Holbrook JR, Visser SN, Mink JW, Zinner SH, Ghandour RM, et al. A national profile of Tourette syndrome, 2011–2012. Journal of developmental and behavioral pediatrics: JDBP. 2014;35(5):317.

36. Orlovska S, Vestergaard CH, Bech BH, Nordentoft M, Vestergaard M, Benros ME. Association of streptococcal throat infection with mental disorders: testing key aspects of the PANDAS hypothesis in a nationwide study. JAMA psychiatry. 2017;74(7):740-6.

Table 1.Baseline characteristics of participants

		No tic onset (N=198)	Tic onset (N=61)	Total (N=259)
Age in years	**mean, (SD)**	6.9 (2.2)	6.8 (1.9)	6.8 (2.1)
Sex	**Male**	77 (38.9%)	38 (62.3%)	115 (44.4%)
	Female	121 (61.1%)	23 (37.7%)	144 (55.6%)
Parental education	**Low**	83 (43.0%)	27 (45.0%)	110 (43.5%)
	High	110 (57.0%)	33 (55.0%)	143 (56.5%)

Table 2. Distribution of GAS exposure status by tic onset visits (without any missing data on GAS exposure)

		No tic onset visit (n=874)	**Tic onset visit** (n=56)
Def 1	No GAS exposure	817 (93.5%)	54 (96.4%)
	GAS exposure	57 (6.5%)	2 (3.6%)
Def 2	No GAS exposure	777 (88.9%)	51 (91.1%)
	GAS exposure	97 (11.1%)	5 (8.9%)
Def 3	No GAS exposure	757 (86.6%)	48 (85.7%)
	GAS exposure	117 (13.4%)	8 (14.3%)
Def 4	No GAS exposure	744 (85.1%)	48 (85.7%)
	Gas exposure	130 (14.9%)	8 (14.3%)

Definition 1: *new definite GAS exposure*, characterised by a newly positive throat swab regardless of serological test results. Definition 2: *new definite GAS exposure or new possible GAS exposure*, the latter characterised by negative or missing throat swab but significant rise of anti-streptococcal antibody titers, i.e. ASOT and/or ADB titer. Definition 3: *new definite GAS exposure or new possible GAS exposure or ongoing definite GAS exposure*, the latter characterised by persistently positive throat swab over at least two time points, regardless of serological test results. Definition 4: *new definite GAS exposure or new possible GAS exposure or ongoing definite GAS exposure or ongoing possible GAS exposure*, the latter characterised by significant rise of either of the two anti-streptococcal antibody titres and negative or missing throat swab but positive throat swab at the previous time point.

Table 3. Time-varying Cox regression analyses testing the association between tic onset and GAS exposure. All analyses were run firstly with GAS exposure as only independent variable (univariable analyses) and then adjusted for all covariates including age, sex, and parental education level (multivariable analyses)

Definition of GAS exposure	**HR (95% CI)**	**p-value**
GAS exposure (Def 1)		
Univariable	0.619 (0.130 to 2.940)	0.546
Multivariable	0.310 (0.037 to 2.590)	0.279
GAS exposure (Def 2)		
Univariable	0.731 (0.272 to 1.966)	0.535
Multivariable	0.561 (0.219 to 1.436)	0.228
GAS exposure (Def 3)		
Univariable	1.062 (0.616 to 1.833)	0.828
Multivariable	0.853 (0.466 to 1.561)	0.607
GAS exposure (Def 4)		
Univariable	0.936 (0.527 to 1.662)	0.822
Multivariable	0.725 (0.384 to 1.370)	0.322

Note: HR: Hazard ratio; 95%CI: 95% confidence interval; In all multivariable analyses, sex was found to be a significant factor associating with the development of tic onset, with females were less likely to develop tics than male (HR=0.4, p-values<0.01). Definition 1: *new definite GAS exposure*, characterised by a newly positive throat swab regardless of serological test results. Definition 2: *new definite GAS exposure or new possible GAS exposure*, the latter characterised by negative or missing throat swab but significant rise of anti-streptococcal antibody titers, i.e. ASOT and/or ADB titer. Definition 3: *new definite GAS*

exposure or new possible GAS exposure or ongoing definite GAS exposure, the latter characterised by persistently positive throat swab over at least two time points, regardless of serological test results. Definition 4: *new definite GAS exposure or new possible GAS exposure or ongoing definite GAS exposure or ongoing possible GAS exposure*, the latter characterised by significant rise of either of the two anti-streptococcal antibody titers and negative or missing throat swab but positive throat swab at the previous time point.