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Name, title and organisation of the project's coordinator:

Prof. Dr. Pieter Hoekstra, Academisch Ziekenhuis Groningen (UMCG)

Tel: +31 (0) 50 3681 100
Fax: +31 (0) 50 3681 120
E-mail: p.j.hoekstra@umcg.nl

Project website address: www.emtics.eu

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Final publishable summary report

1 Executive summary

Genetic predisposition, autoimmunity and environmental factors (e.g. pre- and perinatal difficulties, Group A Streptococcal (GAS) and other infections, and stress-inducing events) might interact to create a neurobiological vulnerability to the development of tics and associated behaviours. However, the existing evidence for this relies primarily on small prospective or larger retrospective population-based studies, and is therefore still inconclusive. The EMTICS study was designed to shed more clarity. EMTICS consists of a longitudinal observational European multicentre study involving 16 clinical centres, with the following objectives: (1) to investigate the association of environmental factors (GAS exposure and psychosocial stress, primarily) with the onset and course of tics and/or obsessive–compulsive symptoms through the prospective observation of at-risk individuals (ONSET cohort: 260 children aged 3–10 years who are tic-free at study entry and have a first-degree relative with a chronic tic disorder) and affected individuals (COURSE cohort: 715 youth aged 3–16 years with a tic disorder); (2) to characterise the immune response to microbial antigens and the host's immune response regulation in association with onset and exacerbations of tics; (3) to increase knowledge of the human gene pathways influencing the pathogenesis of tic disorders; and (4) to develop prediction models for the risk of onset and exacerbations of tic disorders. The EMTICS study is, to our knowledge, the largest prospective cohort assessment of the contribution of different genetic and environmental factors to the risk of developing tics in putatively predisposed individuals and to the risk of exacerbating tics in young individuals with chronic tic disorders. EMTICS was supplemented by a preclinical work package, complementing the human studies by: (i) providing a proof-of-concept of the theoretical rationale behind the whole project, i.e. that repeated streptococcal infections may contribute to the symptoms observed in TS patients; (ii) testing, through prospective hypothesis-driven studies, the influence of physiological and psychosocial stressor on the course of streptococcus-mediated TS symptoms. The most significant result is that we found no indication for a role of new GAS exposures in relation to exacerbations of tic disorders. Also, we saw no improvement after treatment of GAS colonization. We also failed to find evidence for immune activation in patients with a tic disorder. We did see differences in serum concentrations between children with a tic disorder) and unaffected relatives for cytokines, with lower pro-inflammatory cytokines IL-6 and TNF-alpha cytokine, lower levels of the soluble TNF-receptor and higher immunoglobulin levels soluble monocytes activation marker CD14 among patients.

A very important achievement is the unique database that we have created. We now have a fully integrated database, containing more than 7000 visits, with prospective collection of fluctuations in severity of tics and related clinical variables, coupled with genetic information, information on psychosocial stress, perinatal complications and various immune markers. The EMTICS study therefore provides the unique opportunity to analyse data on a broad set of environmental exposures and biological markers related to chronic tic disorders. Indeed, we have now > 50 fully developed paper plans (see appendix) that will be written over the following 1-2 years.

2 Summary description of project context and objectives

Tic disorders are common, childhood-onset neuropsychiatric conditions characterised by the presence of sudden, rapid, recurrent, non-rhythmic motor movements (motor tics) and/or vocalisations (vocal tics). Tic disorders are diagnosed when motor tics and vocal tics, either alone (chronic motor or vocal tic disorder) or in combination [Tourette syndrome (TS), manifesting with multiple motor tics and at least one vocal tic], begin before age 18 and last more than 1 year, in the absence of tics being attributable to a substance or another medical condition. The prevalence of tics during childhood/adolescence is close to 3%, and that of TS is approximately 0.8% between the ages of 6 and 18 years. Tics and their associated neuropsychiatric comorbidities [attention-deficit/hyperactivity disorder (ADHD), obsessive–compulsive disorder (OCD), anxiety and depressive disorders, autism spectrum disorders] often affect quality of life of patients and families, as well as social and academic functioning of patients.

Our knowledge of the pathophysiological mechanisms involved in TS and other chronic tic disorders is still limited. Pharmacological and behavioural treatment options for TS represent the mainstay of treatment for tics, but both have limitations related to patients' access to care, tolerability, and efficacy. In addition, at least 5% of patients with TS attending specialized clinics may have a very severe form that is refractory to non-invasive treatments. There is still a major need for new treatments and effective preventative methods, potentially fostered by a better understanding of disease pathophysiology.

Tourette syndrome is viewed as a complex neuropsychiatric disorder, which is likely to be related to an as yet poorly understood interaction between genetic and environmental susceptibility factors. While the heritability of TS has been estimated to be as high as 0.77 in a large-scale multigenerational family study, a recent twin-family study found much lower heritability estimates, ranging between 0.25 and 0.37, indicating a substantial role for environmental factors. The complex trait of tic disorders is polygenic, similar to most psychiatric disorders. Over the past decade, genetic factors associated with TS have been explored primarily through genome-wide approaches including genome-wide association studies (GWASs), analysis of copy number variants (CNVs), and whole exome sequencing (WES) approaches. GWASs in TS have to date failed to identify highly genome-wide significant loci, likely due also to limited sample sizes, which were smaller than in other major psychiatric GWASs. The contribution of rare structural variation to the genetic architecture of TS is supported by recent analyses of rare CNVs, which indicate that approximately 1% of TS cases carry one of these CNVs, highlighting also genome-wide significant loci increasing TS risk, i.e. NRXN1 deletions and CNTN6 duplications. Like GWASs, WES studies in TS are also limited by their small sample sizes compared to other complex psychiatric traits; an association with de novo damaging variants has been reported for a dozen candidate genes and needs to be confirmed by studies with larger sample sizes. There is also a striking paucity of gene expression studies in tic disorders. Studies in this area focused on biological pathways related to neurotransmitters and immune regulation, but were based on small sample sizes, did not clarify whether the observed changes were causes or consequences of the behavioural phenotype, and were never adequately combined to genomic data. Finally, the exploration of epigenetic modifications associated with TS and other chronic tic disorders is still in its dawning.

Some pre- and perinatal factors potentially interfering with normal brain development have been explored also in association with TS, although the related evidence differs for quality and methodology used across the different variables explored. During the past decade, a limited number of studies have also explored the contribution of psychosocial stress with overall inconclusive results, even if clinical experience does seem to support a role of stress in patients with TS. A prospective evaluation based on questionnaires suggested an effect of psychosocial stress as a short-term predictor of tic severity. Another study showed increased cortisol responses during acute stressors. Obvious limitations of the existing literature are the limited sample sizes and the lack of longitudinal data exploring biological markers of acute and chronic stress, as well as direct measures of hypothalamic–pituitary–adrenal (HPA) axis activation.

An important research area, relevant to environmental influences in relation to tic disorders, is the involvement of abnormal innate and adaptive immune responses in the pathogenesis of tics and related behavioural symptoms. A dysfunctional neural-immune cross-talk has been observed in patients with TS, in analogy to other neurodevelopmental disorders (e.g. autism spectrum disorder). Recent data from prospective population-based cohorts have demonstrated a 30% increased incidence rate of TS in male offspring of women with an autoimmune disease, and a higher risk of any autoimmune disease among first-degree relatives of patients with OCD and chronic tic disorders. Clinical studies have documented increased proliferation and activation of B and TH1 lymphocytes, increased pro-inflammatory cytokine levels, a decreased number of TREG lymphocytes, dysregulated immunoglobulin synthesis, supporting the existence of adaptive immune responses skewed towards an inflammatory state TS. Furthermore, both post mortem data and in vivo molecular imaging suggest microglial activation in TS. Microglia cells are key players of the immune system in the central nervous system and play an increasingly recognized role in brain infections, neuroinflammation and neurodegeneration.

Alongside stress, infectious pathogens are obvious potential culprits for the over-activity of immune responses documented in tic disorders. A specific interest in a role for common infections (pharyngotonsillitis) caused by group A streptococcus (GAS or *Streptococcus pyogenes*) has been drawn by the description in 1998 of Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS), a putatively autoimmune syndrome manifesting with obsessive–

compulsive symptoms, tics, emotional lability, anxiety and regressive behaviour triggered by this pathogen. In 2012, the broader syndromic entity of Paediatric Acute Neuropsychiatric Syndromes (PANS) has been proposed, which encompasses PANDAS but includes other possible aetiologies. Both PANS and PANDAS are viewed as a subtype of paediatric OCD and/or TS that present with an abrupt onset or exacerbation of neuropsychiatric symptoms. An autoimmune mechanism triggered by molecular mimicry between GAS bacterial and host neural antigens has been proposed for PANDAS, and putatively pathogenic biomarkers, e.g. autoantibodies directed against dopamine D2 receptors and antibody-mediated calcium/calmodulin-kinase II activation in cell-based assays, have been reported and included in a proposed diagnostic panel (the Cunningham panel). The diagnostic accuracy of this panel is, however, still discussed, and the potential usefulness of these biomarkers in highlighting an 'autoimmune' subgroup of patients with tic disorders is unexplored. More recent work has highlighted the rationale of exploring immune regulatory markers in this group of patients, and decreased systemic levels of vitamin D have been documented in an Italian cohort of patients with PANDAS. Preliminary evidence reported the presence of anti-D2 dopamine receptor antibodies in a small proportion of individuals with TS, supporting the rationale for further exploration of autoantibody markers in this condition.

Although tics are now considered an associated rather than a core feature of PANDAS and PANS, researchers have tried to address, during the past decade, the still unanswered question of whether and how infections, and above all GAS infections, are associated with the onset and/or the exacerbation of tics and related behavioural symptoms. Large, retrospective, population-based cohort studies have provided some degree of evidence that GAS infections may be associated with the onset of tics although discrepancies across studies exist. At the same time, smaller, prospective studies have failed to show a clear association between GAS infections and tic exacerbations. Nevertheless, one of these prospective studies reported a multiplicative interaction between GAS infections and psychosocial stress in predicting tic and obsessive-compulsive symptom severity in the short term. Overall, these previous studies are limited by small sample sizes and some ambiguities in design, which justify the conduction of larger, more ambitious, prospective observations that consider the complexity of the clinical phenomenology of tic disorders, the genetic diversity of GAS, and the high inter-individual variability of GAS-induced immune responses. An adequately sized clinic-based prospective study that collects behavioural, microbiological, immune-endocrinological, genomic and transcriptomic data, provides a unique opportunity to advance the field by tackling some of the unanswered questions on the aetiopathogenesis of chronic tic disorders. The objectives and design of the multi-centre pan-European collaborative study EMTICS were conceived to address several of the knowledge gaps that are summarized above.

Core objectives of EMTICS

Human observational studies:

1. To investigate the association between putatively relevant environmental factors, genome-wide genetic factors, and gene expression patterns upon the risk of developing clinically relevant exacerbations of tics and/or OCD symptoms in youth with an established chronic tic disorder (COURSE cohort).
2. To investigate the association between putatively relevant environmental factors, genome-wide genetic factors, and gene expression patterns upon the risk of new onset of tics in children who are first-degree relatives of patients with an established chronic tic disorder (ONSET cohort).

For the first and second objectives, the explored environmental exposures comprise: recent GAS infection, GAS carriage status, other recent infections, fluctuations of psychosocial stress, cortisol as a marker of chronic stress and pre- and perinatal adversities.

3. To characterise patterns of the host innate and adaptive immune responses that are associated with clinical events of interest, i.e. onset of tics and clinically relevant exacerbations of tics and/or OCD symptoms in the two clinical cohorts. This will comprise analyses of immune effectors (e.g. cytokines, immunoglobulins, acute phase reactants, other effector molecules including those belonging to the tryptophan/kynurenine pathway and vitamin D) and immune cell phenotyping.

4. To characterise patterns of the host antibody response to GAS and other pathogens previously reported in association with chronic tic disorders. The anti-GAS antibody patterns will be investigated with state-of-the-art microarray technology.

5. To develop multimodal prediction models for the risk of onset of tics in first-degree relatives of patients with chronic tic disorders, as well as for the risk of clinically relevant exacerbations of tics and/or OCD symptoms in youth with an established chronic tic disorder.

One Work Package was directed studying the efficacy of treatment with antibiotics in reducing severity of tics and associated neuropsychiatric symptoms in patients with a tic disorder colonised by GAS.

The human studies were complemented by a series of experiments conducted in laboratory mice, aimed at (i) providing a proof-of-concept of the theoretical rationale behind the whole project, i.e. that repeated streptococcal infections may contribute to the symptoms observed in TS patients; (ii) testing, through prospective hypothesis-driven studies, the influence of physiological and psychosocial stressor on the course of streptococcus-mediated TS symptoms.

The specific objectives of these preclinical studies were:

1. To develop a mouse test battery aimed at mimicking behavioural (stereotypical, compulsive and perseverative patterns), immune and brain abnormalities isomorphic to clinical symptoms of TS, as a function of induced auto-immunities.

2. To expose candidate mouse strains to active transfer of streptococcus-induced antibodies, characterised by neuronal cross-reactivity (from both humans and animals) and validate its consequences on the test battery developed under objective 1.

3. To identify the prototypical mouse strain that shows the highest isomorphism with TS clinical symptoms in response to streptococcus-induced antibodies.

4. To perform active immunisation with GAS homogenates and/or pools of streptococcal antigens showing high antibody responses in patients with TS.

5. Evaluate whether the abnormalities identified under objective 4 are aggravated by psychosocial stress.

3 Description of the main science & technology results / foregrounds

3.1. Summary of the main results / foregrounds of EMTICS

The most significant result from the human cohort studies of EMTICS is that we found no indication for a role of new GAS exposures in relation to exacerbations of tic disorders. We did find that new GAS exposures are very frequently occurring. Our data indicate that exposure to GAS at some point during childhood is nearly universal. Thus, it is quite understandable that a part of the children who come to clinics with a tic exacerbation have signs of a recent GAS exposure. Clinical observations of the co-occurrence of recent GAS exposures and tic exacerbation have led to the PANDAS concept. However, the EMTICS study indicates that the co-occurrence of tic exacerbations and recent new GAS exposures is most likely due to chance. This has also important clinical implications: our findings suggest that assessing recent GAS exposure in children with tic disorders is not clinically meaningful. Of note, anti-GAS responses in patients with tics did not increase after tic exacerbations, suggesting that tic enhancement was not preceded by re-exposure to the pathogen. We also found no indication for a temporal association between new GAS exposures and the onset of tics. Again, new GAS exposures are very frequent but most children do not subsequently develop tics. In those who do develop tics, this was not clustered around a recent new GAS exposure. However, anti-GAS antibody responses were higher in sera from children with a tic onset during the follow-up period versus those who did not develop tics.

There was also no striking difference in the serotype distribution, virulence and clonal relationships between GAS strains isolated from the ONSET and COURSE children. GAS strains isolated from the COURSE study presented a wider distribution of serotypes compared to those isolated in the ONSET study. All *emm* types identified from the ONSET strains were present also in the COURSE study. Nevertheless, in particular situations such as exacerbation of tic symptoms, severe tic symptoms presentation, and persistent bacterial throat colonisation in COURSE children, an enrichment of certain serotypes and superantigen alleles was noted. The limited role for GAS exposures was underlined by the lack of a trend of improvement of the clinical and biological measures after the treatment of GAS colonization with antibiotics.

In order to elucidate the evidence for an immune-related origin of Tourette's syndrome and obsessive-compulsive disorders (TS/OCD) following a streptococcal or non-streptococcal infection, we performed a broad array of serological and immunological investigations. Interestingly, we found a significant association of higher obsessive-compulsive symptom severity with high ASO antibody titres, indicating a recent group A streptococcus infection. Antibodies cross-reacting with the dopaminergic neurotransmitter receptor D2 significantly increased at exacerbation in both COURSE and ONSET patients; this finding underlines the hypothesis of the involvement of anti-neuronal antibodies in the pathophysiology of TS/OCD. The significantly altered levels of soluble signals of the immune cells like pro-inflammatory cytokines may indicate an abnormal immune responsiveness in those patients. The pronounced lack of Vitamin D found in one third of patients may play a crucial role in that abnormal immune responsiveness. Altogether our findings underline the proposed hypothesis of an altered post-infectious immune response associated with Tourette's syndrome and obsessive-compulsive disorders.

The genetic part of EMTICS has identified genes that warrant further investigation in relation to the aetiology of TS. Our top hit was the MARK3 gene which plays a role in the establishment of cellular polarity and cell cycle control. The EMTICS study is, to our knowledge, the largest prospective cohort assessment of the contribution of different genetic and environmental factors to the risk of developing tics in putatively predisposed individuals and to the risk of exacerbating tics in young individuals with chronic tic disorders. A major environmental trigger that we addressed was the role of psychosocial stress. Two possibilities might be assumed: (a) psychosocial stress might lead to an exacerbation of tics because it increases cortisol release followed by changes in neurotransmission or immunology and (b) patients with TS might show altered neurotransmission or immunology which, in turn, results in a higher vulnerability of affected patients to respond to psychosocial stress with strong cortisol release. The animal studies provided experimental data strengthening the role of psychological and physiological stress reactivity as a potent regulator of individual reactivity to autoimmunity. This aspect is highly relevant whereby it offers new avenues towards the treatment of TS. More definitive conclusions will become available over the next months.

The infrastructure and database that has been built through EMTICS will help elucidate how the genetic background determines the molecular/cellular pathways underlying tic disorders and may shed light on how the genetic factors interact with environmental factors influencing the onset and clinical course of tic disorders. We now have a fully integrated database, containing more than 7000 visits, with prospective collection of fluctuations in severity of tics and related clinical variables, coupled with genetic information, information on psychosocial stress, perinatal complications and various immune markers. We have now > 50 fully developed paper plans (see appendix) that will be written over the following 1-2 years.

3.2. Main results / foreground of each work package

WP 01 Longitudinal ONSET and COURSE Cohort Studies

Background

Tic disorders are common, childhood-onset neuropsychiatric conditions characterised by the presence of sudden, rapid, recurrent, non-rhythmic motor movements (motor tics) and/or vocalisations (vocal tics). Tic disorders are diagnosed when motor tics and vocal tics, either alone (chronic motor or vocal tic disorder) or in combination [Tourette syndrome (TS), manifesting with multiple motor tics and at least one vocal tic], begin before age 18 and last more than 1 year, in the absence of tics being attributable to a substance or another medical condition. The prevalence of tics during childhood/adolescence is close to 3%, and that of TS is approximately 0.8% between the ages of 6 and 18 years. Tics and their associated neuropsychiatric comorbidities [attention-deficit/hyperactivity disorder (ADHD), obsessive–compulsive disorder (OCD), anxiety and depressive disorders, autism spectrum disorders] often affect quality of life of patients and families, as well as social and academic functioning of patients.

Our knowledge of the pathophysiological mechanisms involved in TS and other chronic tic disorders is still limited. Pharmacological and behavioural treatment options for TS represent the mainstay of treatment for tics, but both have limitations related to patients' access to care, tolerability, and efficacy. In addition, at least 5% of patients with TS attending specialized clinics may have a very severe form that is refractory to non-invasive treatments. There is still major need for new treatments and effective preventative methods, potentially fostered by a better understanding of disease pathophysiology.

Tourette syndrome is viewed as a complex neuropsychiatric disorder, which is likely to be related to an as yet poorly understood interaction between genetic and environmental susceptibility factors. While the heritability of TS has been estimated to be as high as 0.77 in a large-scale multigenerational family study, a recent twin-family study found much lower heritability estimates, ranging between 0.25 and 0.37, indicating a substantial role for environmental factors. Some pre- and perinatal factors potentially interfering with normal brain development have been explored also in association with TS, although the related evidence differs for quality and methodology used across the different variables explored. Alongside stress, infectious pathogens are obvious potential culprits for the over-activity of immune responses documented in tic disorders. A specific interest in a role for common infections (pharyngotonsillitis) caused by group A streptococcus (GAS or *Streptococcus pyogenes*) has been drawn by the description in 1998 of Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS), a putatively autoimmune syndrome manifesting with obsessive–compulsive symptoms, tics, emotional lability, anxiety and regressive behaviour triggered by this pathogen. In 2012, the broader syndromic entity of Paediatric Acute Neuropsychiatric Syndromes (PANS) has been proposed, which encompasses PANDAS but includes other possible aetiologies. Both PANS and PANDAS are viewed as a subtype of paediatric OCD and/or TS that present with an abrupt onset or exacerbation of neuropsychiatric symptoms. An autoimmune mechanism triggered by molecular mimicry between GAS bacterial and host neural antigens has been proposed for PANDAS. Although tics are now considered an associated rather than a core feature of PANDAS and PANS, researchers have tried to address, during the past decade, the still unanswered question of whether and how infections, and above all GAS infections, are associated with the onset and/or the exacerbation of tics and related behavioural symptoms. Large, retrospective, population-based cohort studies have provided some degree of evidence that GAS infections may be associated with the onset of tics although discrepancies across studies exist. At the same time, smaller, prospective studies have failed to show a clear association between GAS infections and tic exacerbations. Nevertheless, one of these prospective studies reported a multiplicative interaction between GAS infections and psychosocial stress in predicting tic and obsessive–compulsive symptom severity in the short term. Overall, these previous studies are limited by small sample sizes and some ambiguities in design, which justify the conduction of larger, more ambitious, prospective observations that take into account the complexity of the clinical phenomenology of tic disorders.

Overall objectives

- Establish clinical cohort studies ONSET and COURSE
- Establish whether the childhood onset of tics and/or obsessive-compulsive symptoms in first-degree relatives of patients with TS or another chronic tic disorder is associated with pharyngeal GAS carriage or infection.

- Develop prediction models for the risk of onset of symptoms in first-degree relatives of patients with TS or another tic disorder, integrating information on demographic and clinical information, psychosocial stress, perinatal factors and environmental exposures.
- Examine the effects of exposure to GAS in the form of pharyngeal carriage or infection upon the risk of developing clinically relevant exacerbations of tics in children affected by TS or chronic tic disorder.

Results

Out of the 260 ONSET participants, 61 (23.4%) developed onset of tics; in 19 of these 61 children there was lacking information on new GAS exposures due to missing throat swabs and missing sera; of the remaining 42 children, 26 children (61.9%) had developed tics without any evidence for a new GAS exposure. This is an important finding that indicates that a GAS exposure is not a prerequisite for developing a tic disorder. Only 16 of the children in the ONSET study who developed onset of tics had a new GAS exposure at some point during the ONSET study; in 6 of these children did the onset of tics coincide with a *recent* new GAS exposure (i.e. a new GAS exposure newly detected at the time of the onset of tics). Although the numbers are low, there is no indication that new GAS exposures are temporally associated with the onset of tics: while new GAS exposures were present in 18.8% of visits in which onset of tics had been documented, this was the case in 24.9% of ONSET study visits in which no tic onsets were notified.

There were 205 prospectively identified new GAS exposures in the COURSE study participants, representing 13.2% of all COURSE study visits with information on possible new GAS exposures (i.e. throat swab and/or sera collected). Moreover, we saw a total of 506 tic exacerbations in the COURSE study participants. There is no indication that new GAS exposures are temporally associated with tic exacerbations: 14.1% of tic exacerbations were preceded by a recent new GAS exposure (i.e. a new GAS exposure occurring within the past 4 months), a percentage which was not significantly higher than the 13.0% of visits without a tic exacerbation that were preceded by a recent new GAS exposure. Put in another way: a recent new GAS exposure was followed by a tic exacerbation in 15.9% of all new GAS exposures, whereas exacerbations were occurring in 14.7% of times when there had been no new GAS exposure (again not a statistically significant difference). When expanding the time horizon from 4 to 6 months after the assessment of a new GAS exposure, we saw a tic exacerbation occurring after 20.3% of new GAS exposures, versus 20.2% of times when there had been no new GAS exposure; again, not pointing to a statistically significant difference.

Papers focusing on prediction models will be completed at a later stage following initial univariate analyses.

Conclusions

The most significant result is that we found no indication for a role of new GAS exposures in relation to exacerbations of tic disorders. We did find that new GAS exposures are very frequently occurring. In our study they occurred in 13.2% of the 4 monthly visits of COURSE participants and in 23.9% of the visits in the ONSET cohort. The difference in percentage between ONSET and COURSE participants was age related (ONSET participants were significantly younger). These figures indicate that exposure to GAS at some point during childhood is nearly universal. Thus, it is quite understandable that a part of the children who come to clinics with a tic exacerbation have signs of a recent GAS exposure. Indeed, in the EMTICS COURSE study we identified 33 COURSE participants with a significant tic exacerbation preceded by a recent new GAS exposure. Clinical observations of the co-occurrence of recent GAS exposures and tic exacerbation have led to the PANDAS concept. However, the EMTICS study indicates that the co-occurrence of tic exacerbations and recent new GAS exposures is most likely due to chance. This has also important clinical implications: our findings suggest that assessing recent GAS exposure in children with tic disorders is not clinically meaningful.

We also found no indication for a temporal association between new GAS exposures and the onset of tics. Again, new GAS exposures are very frequent but most children do not subsequently develop tics. In those who do develop tics, this was not clustered around a recent new GAS exposure. We have to acknowledge though that the numbers were low.

WP 02 Microbiological Characterisation

Background

The main hypotheses of the EMTICS project that regarded Work Package 2 were to verify if particular serotypes of Group A streptococci (GAS) were prevalent in children with Tourette syndrome and if specific clones, carrying a peculiar repertoire of proteins (superantigens) were associated with the presence of Tourette syndrome. Furthermore, the EMTICS study hypothesised that the onset and/or exacerbation of tic and comorbid obsessive-compulsive disorders in children could be associated with increased preceding occurrence of GAS exposure or infection of specific serotypes.

Overall objectives

GAS strains were isolated from the throat of children enrolled in the ONSET and COURSE studies, swabbed both at the time of recruitment and according to a planned time schedule by clinical partners. Throat swabs were processed by the microbiology laboratories associated to the clinical centres following a common validated microbiological protocol, based on the pour plate method.

Bacterial strains were de-personalised in the laboratory and sent to ISS-RC partner for storage and microbiological analyses.

The microbiological characterisation included the following tasks:

- To assess the distribution of GAS serotypes from ONSET and COURSE studies by using the *emm* sequence typing method. It is the gold standard in epidemiological studies of group A streptococci and it has replaced the classical serotyping method. It consists in the assignment of the *emm* type by the analysis of the nucleotide sequence of the portion of gene encoding the hypervariable N-terminal portion of the M protein, a fibrillary surface protein of which more than 100 types are recognised⁽¹⁾.
- To determine the GAS genetic population structure in a two-step analysis. The first step implied the genotyping of all isolates by using the Multiple-Locus Variable-number of tandem repeats Analysis (MLVA). MLVA is a sequence-based typing method, recently developed for GAS, which determines the number of tandem repeats or copy unit, at multiple variable number of tandem repeat (VNTR) loci within the genome. In particular, the assay consists in a seven-loci typing scheme and has proved to have great discrimination power⁽²⁾. Along with *emm* typing, this method allowed the determination of the genetic relatedness of the bacterial strains (comparative typing). In the second step, selected representative isolates within each genetic group have been typed by the sequence-based Multi Locus Sequence Typing (MLST) method (definitive typing). The technique is based on the sequencing of internal fragments of about 400-500 base pairs from seven selected housekeeping genes. Each gene sequence is assigned as an allele number providing each strain with an allelic profile. The comparison of the seven codes combination with those deposited in the MLST data bank provide a sequence type (ST), enabling the analysis of evolutionary interrelationships among STs and the assignment to clonal complexes, ancestral genotypes, and clonal variants⁽³⁾. In conclusion, MLVA and *emm* typing schemes provided a picture of the circulating clones and of lateral gene transfer events over a short period of time; MLST enabled to trace the clonal diversification and radiation of GAS strains over time.
- To evaluate the distribution of specific bacterial virulence (*speA* and *speC* genes) including their allelic variants by Polymerase Chain Reaction (PCR) screening and DNA sequencing and their potential association with tic symptoms⁽⁴⁾.

Results

In total, 309 throat swabs resulted positive for group A Streptococcus (GAS) and 296 bacterial isolates were received for analysis (85 strains from the ONSET study, 207 strains from the COURSE study and 4 strains from the TREATMENT study). Moreover, 43 GAS strains from individuals with pharyngitis (outside of the EMTICS study) were received and typed.

A wider distribution of GAS serotypes in the COURSE study compared to the ONSET study was observed, but the ranking of the most prevalent serotypes was comparable between studies. The most diffuse GAS serotypes from individuals with pharyngitis were instead different from the EMTICS study.

The distribution of prevalent serotypes isolated from children who never developed onset of tics did not differ from that observed in children who developed onset of tics. Conversely, the most prevalent serotypes isolated from children that experienced exacerbation of tic symptoms differed from the prevalent serotypes isolated from children who never experienced exacerbations of tics. Moreover, in case of persistent throat colonization or severe tic symptoms of the COURSE children, an enrichment of particular serotypes and virulence factors was noted (5-6).

The MLVA analysis demonstrated a deeper discrimination power over *emm* typing but the same serotypes shared the same clonal groups; independently they were isolated from ONSET, COURSE or pharyngitis. The MLST analysis, performed on the more diffuse serotypes, showed as well that the association between the *emm* type and MLST type was independent from the source of the study.

Conclusions

There was not a striking difference in the serotype distribution, virulence and clonal relationships between GAS strains isolated from the ONSET and COURSE children. GAS strains isolated from the COURSE study presented a wider distribution of serotypes compared to those isolated in the ONSET study. All *emm* types identified from the ONSET strains were present also in the COURSE study. The prevalent serotypes of GAS strains collected in the EMTICS study differed from those isolated from pharyngitis.

Nevertheless, in particular situations such as exacerbation of tic symptoms, severe tic symptoms presentation, and persistent bacterial throat colonisation in COURSE children, an enrichment of certain serotypes and superantigen alleles was noted.

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WP 03 Anti-Streptococcal Immune Response

Background

The presence of antibodies recognising Group A *Streptococcus* (GAS) antigens in human sera is indicative of recent exposure to the pathogen ⁽¹⁾. Previous studies conducted in our laboratories using protein microarrays led to the detection of higher antibody responses to GAS antigens in Tourette patients compared to the background paediatric population⁽²⁾. The data pointed towards a potential role of GAS infections/carriage in the development of Tourette syndrome/Obsessive-Compulsive Disorder (TS/OCD).

Objectives

The objective of EMTICS WP03 was to investigate whether exposure to Group A Streptococcus (GAS) could contribute to the onset of tic disorders in children. In particular, this Work Package was aimed at assessing and comparing antibody responses against GAS antigens in serum from participants to the ONSET and COURSE studies who did or did not have a tic disorders and who did and did not experience exacerbations.

Sera collected at different time points from ONSET and COURSE study participants were tested for their immunological reactivity versus a panel of GAS proteins. The analysis was carried out using the Protein Microarray technology, a faster and more sensitive approach compared to the conventional ELISA allowing high throughput analysis of antibody responses against large panels of antigens. Eighty-nine selected GAS surface proteins were expressed in *E. coli*, purified and spotted onto arrayed chips. The obtained microarrays were probed with the collected sera, and antibodies specific for each of the spotted proteins were detected by fluorescently labelled secondary immunoglobulins. The intensity of the obtained fluorescent signals revealed different anti-GAS humoral response patterns, providing information on possible previous exposure to the pathogen.

Results

The following set of sera from ONSET and COURSE participants were analysed on the GAS protein arrays:

- ONSET Study: sera collected at baseline in 68 children who did NOT develop tics during the ONSET follow-up period
- ONSET Study: sera from 39 children who DID develop Tics during the ONSET follow-up period, collected both at baseline as well as at the time of onset of tics.
- COURSE Study: sera from 75 COURSE patients who experienced Tic exacerbations during the follow-up period, collected both at baseline and during exacerbation

The main obtained results for each of the WP03 Tasks are reported below.

Task 1: Purification of recombinant GAS surface antigens

For this task we took advantage of a previously obtained collection of recombinant *E. coli* strains harbouring a large panel of genes encoding Histidine-tagged surface antigens selected through bioinformatics analysis of multiple GAS genomes. The effective presence of the selected proteins on the GAS surface was confirmed by Mass Spectrometry analysis of the peptides released by the bacteria after protease treatment (3) and by Flow Cytometry analysis of a large panel of GAS isolates using mouse specific antibodies (4). To assess the expression of each GAS antigen in the form of a soluble protein of the expected molecular weight, during WP03 Task 1 the above mentioned recombinant *E. coli* strains were grown at small-medium scale and total cell extracts were analysed on sodium dodecyl sulphate – poly acrylamide gel electrophoresis (SDS-PAGE). Then, recombinant GAS proteins were purified by affinity chromatography from bacterial lysates. The identity and purity of the obtained proteins was assessed by SDS-PAGE analysis on the basis of their expected molecular weights.

Task 2: Generation of protein microarrays containing purified GAS surface antigens

The 89 purified proteins were printed onto nitrocellulose slides using a robotic spotting station. Each protein was spotted in eight replicates, together with Standard Curves of known concentrations of fluorescently labelled control proteins for data normalisation. Samples of the prepared microarrays were subjected to control experiments where the slides were incubated with mouse monoclonal antibodies anti-His6 tag, in order to confirm that all streptococcal proteins were efficiently and reproducibly deposited and immobilised on the chips and to assess the absence of protein carryover in negative control spots printed with buffer alone.

Task 3: Protein microarray analysis of antibody responses to GAS antigens in patients with a tic disorder and their healthy relatives

Protein microarrays obtained as a result of task 2 were used to analyse the patterns of antibody responses to GAS antigens in sera from the following groups under study in WP01:

- ONSET Study: 68 relatives who did NOT develop TICs (baseline)
- ONSET Study: 39 participants at TIC onset (sera from baseline plus tic onset)

The presence of antibodies specific to each of the GAS antigens printed on the chip was detected using fluorescently labelled mouse anti-human immunoglobulin G (IgG). The mean fluorescence intensity value of the 8 replicates of each antigen (MFI) is a quantitative indication of the antibody response to that particular antigen in the serum under examination. Data were analysed using internally developed software to determine the antibody response profiles against the 89 printed GAS antigens in each serum. This allowed comparing immune responses in the different groups of sera based on the percentage of antigens yielding fluorescent signals above three established thresholds (MFI>2000; MFI>15000 and MFI≥30000).

Data analysis revealed that anti-GAS antibody responses (expressed as the percentage of antigens with MFI above the three pre-established thresholds) was higher in baseline sera from children who developed tics during the follow-up period versus those who did not develop tics after at least one year of follow-up observation. As expected, baseline responses to GAS were higher in children aged 7-12 years than those 3-6 years. The observed differences between children who developed versus those who did not develop tics were confirmed and even more pronounced at the low age interval. The analysis also highlighted 9 GAS antigens showing median MFI values >15000 and 4 of them with signals above 30000, with no significant differences in the antigen recognition profile among any of the groups of sera analysed.

Task 4: ELISA quantitative analysis of antibody responses to a subset of antigens highly immunogenic in patients with a tic disorder

As reported above, during Task 3, despite the finding that antibody response levels to GAS were higher in children who developed tics, no significant differences were detected in the antibody response profiles to any specific GAS antigens among the different examined groups. Therefore, Task 4 activities were substituted with the analysis of anti-GAS antibody responses in mice exposed to GAS homogenates, in collaboration with WP05. In this study, mice repeatedly exposed to Group-A β -Haemolytic Streptococcus showed perseverative behaviours, impaired sensorimotor gating, and immune activation in rostral diencephalon. Mouse sera have been analysed by Western Blot analysis of GAS homogenates, which confirmed the presence in these animals of antibodies directed to the pathogen (5).

Task 5: Protein microarray analysis of antibody responses to GAS antigens with the investigation of changes in antibody response patterns in relation to tic exacerbation

Protein microarrays obtained as a result of Task 2 were used to analyse the patterns of antibody responses to GAS antigens in sera from the following groups under study in WP01:

- COURSE Study: 75 patients with a TIC exacerbation (sera from baseline plus exacerbation)

The presence of antibodies specific to each of the GAS antigens spotted on the chip were detected as described in Task 2.

This allowed comparing immune responses in the two different groups of sera based on the percentage of antigens yielding fluorescent signals above three established thresholds (MFI>2000; MFI>15000 and MFI≥30000). A total of 150 sera derived from 75 COURSE study participants were analysed. Data analysis did not reveal significant differences between anti-GAS responses in COURSE study participants measured at baseline versus when experiencing an exacerbation. Conversely, the obtained fluorescent signals for all antigens were very similar for most of the patients at the two different investigated time points, suggesting that antibody levels were maintained across the follow-up observation period in all patients.

The analysis also confirmed the nine GAS antigens showing median MFI values >15000 and 4 above 30000 as in Task 4, with no significant differences in the antigen recognition profile among any of the groups of sera.

Conclusions

Several conclusions can be drawn from the obtained results. First, anti-GAS antibody responses were higher in sera from children with a tic onset during the follow-up period versus those who did not develop tics. The data confirm our previous observations (2) and could indicate more frequent exposure to GAS among patients with tics. The differences in GAS

responses between the two groups was more pronounced in younger children, possibly because GAS-specific antibody levels tend to increase with age after multiple exposures to the pathogen. Anti-GAS responses in patients with tics did not increase after tic exacerbations, suggesting that tic enhancement was not preceded by re-exposure to the pathogen.

Finally, the analysis highlighted high levels of antibodies against 9 proteins, with no significant differences in antigen recognition among any of the groups of sera analysed. The data suggest high expression and immunogenicity of these GAS antigens during pharyngeal colonization/infection, making them attractive candidates for a vaccine against GAS-related diseases.

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WP 04 Immune Measurements

Background

A number of environmental factors have been reported to be associated with tic disorders. The largest body of evidence has been gathered in support of a role of infections from Group A beta-haemolytic Streptococcus (GAS), exposure to psychosocial stress and perinatal adversities. The possible link between GAS infections and the onset of Tourette's syndrome (TS) and obsessive-compulsive disorders (OCD) has attracted wide attention over the past two decades. GAS is a major cause of common pharyngitis, but also of significant post-streptococcal non-suppurative sequelae associated with the existence of host autoantibodies against GAS antigens, including rheumatic fever, glomerulonephritis and Sydenham's chorea. The latter typically presents with chorea, but may include obsessions, compulsions and tics. In the 1990s, Swedo described a clinical phenotype, named Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS), characterized by the pre-pubertal, abrupt onset of OCD and/or a tic disorder that is preceded by a GAS infection, and that is thought to evolve into a relapsing-remitting illness in which clinical exacerbations are temporally associated with new GAS infections or enhanced immune responses directed against the pathogen. The strong clinical resemblance of PANDAS to common TS or OCD suggests that the former represents a post-infectious variant (or phenocopy) of the latter, and/or that GAS infections are involved in the aetiology and pathophysiology of common TS/OCD. Moreover, antibodies to neuronal surface proteins, e.g. NMDAR (a certain kind of glutamatergic neurotransmitter receptor), have been implicated in a range of neurological diseases, supporting the concept of PANDAS. We therefore hypothesised that onset and/or exacerbation of tic- and associated obsessive-compulsive disorders is associated with increased preceding occurrence of GAS infections of specific molecular subtypes, and that this association is based on genetic susceptibility factors and mediated through immunological mechanisms related to psychosocial stress and immunological factors in host and GAS strains. Beyond the GAS-based hypothesis of TS/OCD, there is evidence for the causal role of non-GAS infections and for abnormalities in the immune response of patients suffering from TS/OCD. Previous studies had indicated the impact of infections with *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, *Borrelia burgdorferi*, *Toxoplasma gondii*, as well as Epstein-Barr virus (EBV).

Objectives

In short there were two major objectives:

1. Establish whether the childhood onset of tics and/or obsessive-compulsive symptoms in at risk individuals is associated with exposure to specific environmental factors, including GAS subtypes and non-GAS bacterial or viral infections.
2. Characterize the host immune response in relation to disease manifestation and course an.

Sera prospectively obtained in WP1 were bio-banked and analysed covering nine major items:

- The basis of these objectives was the collection, bio-banking, sorting, and sample logistics of the serum samples.
- Identification of specific immune responses to group A streptococcal (GAS) infections by measuring the well validated serological markers, antibodies against GAS-specific streptolysine O (ASO) and antibodies against GAS-specific anti-DNase B (ADN).
- Identification of neuronal targets of cross-reacting auto-antibodies: Using immunohistology on rodent brain sections to test for binding of serum antibodies a range of known neuronal antigens (NMDAR, LGI1, CASPR2 etc.) have been investigated. We further investigated the binding of serum antibodies to the dopamine D2 receptor using a cell based assay.
- Identification of specific immune responses to non-GAS infections including *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, *Borrelia burgdorferi*, *Toxoplasma gondii*, as well as Epstein-Barr virus (EBV).
- Determination of cytokines and soluble cytokine receptors from minimal serum volume by multiplex assay. An existing multiplex assay for the simultaneous determination of sCD14, IL-6, IL-17, TNF- α , TNF-RI and TNF-RII and another one for the determination of immunoglobulins (IgA, IgM, and IgG-subclasses) was modified in order to optimize regarding minimal sample volume and detection limits in the high-sensitivity range.
- Determination of the pentameric / monomeric CRP ratio as a potential early marker of incomplete resolution of inflammation and its measurement by a quick immunoassay. First, test systems to measure monomeric and pentameric CRP had to be established a validated. Current immunological research indicates that high monomeric CRP indicates the severity of an acute inflammatory reaction, while high pentameric CRP indicates the possibility of impaired resolution of inflammation. The chronic consequences after infection or inflammation such as tics may be due to impaired resolution of inflammation. We hypothesized that either the absolute value of pentameric CRP or the ratio between monomeric and pentameric CRP in an acute state would predict the possibility of chronic consequences.
- Analysing cellular immunity by immunophenotyping (ProlImmune/QMUL) using collected peripheral blood mononuclear cells collected from 3 European sites (TUD, GSTT, MHH). Peripheral blood mononuclear cells (PBMCs) were isolated, frozen and stored. Staining by flow cytometry was performed to assess phenotype and activation status of T helper cells (CD4+), cytotoxic T-cells (CD8+), regulatory T-cells (FOXP3), natural killer cells (CD56+) and B-cells (CD19). Activation status was monitored by measuring the expression of CD69 during exacerbation and remission and in onset samples. Furthermore monocytes/dendritic cell populations were studied (CD14+CD11c+ monocytes, CD14+CD11c+CD86+ monocytes, CD14+CD11c+CD209+ monocytes. Intracellular staining (INF- γ and TNF- α production) was performed upon stimulation with an overlapping peptide pool, which consisted of 17 overlapping 15-mer peptides offset by 4 amino acids with sequence homology in part between dopamine receptor D2 (DRD2) and three different M proteins (M5, M9, M189) from the GAS *Streptococcus pyogenes*.
- Investigation of the kynurenine pathway, which has functional interconnections between the immune system and neurotransmitter system. These include serum levels of tryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine, xanthurenic acid, anthranillic acid, 3-hydroxyanthranillic acid, quinolinic acid, picolinic acid, 5-hydroxyindole acetic acid and other important intermediates of the tryptophan pathway. Several intermediates of this pathway are modulating distinct neurotransmitter receptors (e.g. NMDA receptor) and/or immunoregulatory pathways (e.g. Aryl-hydrocarbon receptor, GPR35 [G protein-coupled receptor 35], NF- κ B [nuclear factor 'kappa-light-chain-enhancer' of activated B-cells], and others).
- Investigation of serum levels of 25-hydroxy-Vitamin D, since Vitamin D deficiency is currently in the focus of autoimmune research (e.g. multiple sclerosis) due to the strong impact of Vitamin D on proper immune regulation.

Results

1. Serum samples bio-banking and logistics: Altogether, we had to handle more than 16,000 distinct serum aliquots. Bio-banking included identification and generation of an electronic data base.

2. We analysed ASO and ADN titres in more than 3100 serum samples. The COURSE and ONSET children did not differ regarding titres, but the mean levels of ASO titres in both groups were above an upper limit of normal of 250 IU/ml. There was also a significantly positive correlation between ASO titres and OCD severity. Specifically, children with higher levels of ASO titre (> 400 IU/ml) showed significantly higher OCD severity than the ones with lower levels. Tic severity correlated significantly negatively with levels of ASO and ADB titres.
3. Autoantibodies against D2 dopamine receptor were significantly increased at exacerbation (compared to baseline) in both, COURSE patients as well as ONSET subjects. Further statistical analyses correlating these data with clinical and immunological variables are ongoing.
4. Identification of specific immune responses to non-GAS infections including *Mycoplasma pneumoniae*, *Chlamydia trachomatis*, *Borrelia burgdorferi*, *Toxoplasma gondii*, as well as EBV were performed in nearly 800 serum samples; these were the first and the last samples of the patients to get optimum information about a possible change of titres within the observation period. The data are currently under statistical investigation.
5. Preliminary analyses on 402 baseline cytokine samples provided evidence for a statistically significant difference in serum concentrations between cases (i.e. children with an established tic disorder) and controls (i.e. currently unaffected relatives) for six out of the twelve cytokines investigated, with lower pro-inflammatory cytokines IL-6 and TNF-alpha cytokine, lower levels of the soluble TNF-receptor and higher immunoglobulin levels soluble monocytes activation marker CD14 among patients. Levels of distinct immunoglobulin class G (IgG) sub-classes were altered as well in TS/OCD patients. Comparison between the patients who experienced a clinical event (i.e. tic exacerbation) during the course of the study and those who did not, indicated significantly lower serum concentrations for three of the investigated cytokines among those that did experience an exacerbation. Finally, significant differences in serum concentrations were also observed among the initially unaffected, but at-risk relatives that did experience a clinical event (i.e. tic onset) during the course of the study versus those that did not. Further in-depth analyses, as well as longitudinal analyses, are still going on.
6. Autoantibodies against D2 dopamine receptor were significantly increased at exacerbation (compared to baseline) in both, COURSE patients as well as ONSET subjects. Further statistical analyses correlating these data with clinical and immunological variables are ongoing. Likewise, no difference in CRP levels was observed among patients who experienced a clinical event (i.e. tic exacerbation) during the course of the study versus those who did not. Finally, no difference in CRP concentrations was found between initially unaffected, but at-risk relatives who experienced a clinical event (i.e. tic onset) during the course of the study and those who did not. Further in-depth analyses, as well as longitudinal analyses, are still ongoing.
7. Our data could not support the initial hypothesis that patients suffering from a tic disorder may present higher baseline levels of pCRP and p/m CRP ratio, compared to currently unaffected, but at risk relatives. There was no significant difference in pCRP or the ratio between pentameric and monomeric CRP.
8. Immunophenotyping did not show any significant difference between course and onset samples for the percentage of T-cells (CD4+, CD8+), B-cells and regulatory T-cells, monocytes or dendritic cells. Onset subjects have more CD56+CD16- cells, which comprise the natural killer cell population. GAS peptides with part sequence homology to human DRD2 did not stimulate cytokine secretion in COURSE or ONSET subjects.
9. Investigating the intermediates of the kynurenine pathway pre- and post-exacerbation sample pairs were analysed. Statistical analyses are currently performed and results can therefore not yet be presented.
10. We investigated the same samples as for the non-GAS infectious agents; we identified 32% of patients with 25-OH-Vitamin D levels clearly below the recommended concentration – in other words: one third of patients suffering from TS/OCD had a lack of Vitamin D levels. This Vitamin D deficiency may have significant impact on the immune response of the individuals. Further analyses to associate these results with other immune measures are ongoing.

Conclusion

In order to elucidate the evidence for an immune-related origin of Tourette's syndrome and obsessive-compulsive disorders (TS/OCD) following a streptococcal or non-streptococcal infection, we performed a broad array of serological and immunological investigations. Interestingly, we found a significant association of higher obsessive-compulsive symptom severity with high ASO antibody titres, indicating a recent group A streptococci infection. Antibodies cross-reacting with the dopaminergic neurotransmitter receptor D2 significantly increased at exacerbation in both COURSE and ONSET patients; this finding underlines the hypothesis of the involvement of anti-neuronal antibodies in the pathophysiology of TS/OCD. The field of autoantibody-related brain disorders is rapidly growing and future methods may be able to identify further neuronal structures being targeted by autoantibodies in TS/OCD patients. The significantly altered levels of soluble signals of the immune cells like pro-inflammatory cytokines may indicate an abnormal immune responsiveness in those patients. The pronounced lack of Vitamin D found in one third of patients may play a crucial role in that abnormal immune responsiveness. Altogether our findings underline the proposed hypothesis of an altered post-infectious immune response associated with Tourette's syndrome and obsessive-compulsive disorders.

References

In-depth statistical analyses are ongoing. There are no publications yet.

WP 05 Animal Model

Background

The main aim of WP 05 was to complement the activities conducted by the other WPs by: (i) providing a proof-of-concept of the theoretical rationale behind the whole project, i.e. that repeated streptococcal infections may contribute to the symptoms observed in TS patients; (ii) testing, through prospective hypothesis-driven studies, the influence of physiological and psychosocial stressor on the course of streptococcus-mediated TS symptoms.

These major aims have been achieved through experiments conducted in laboratory mice. While the use of live animals in research is frequently under scrutiny due to ethical considerations, it nonetheless allows investigating aspects that, for numerous reasons, cannot be addressed in humans.

Although animal research has to adhere to the highest ethical standards and to be performed in accordance with stringent regulations (Directive 2010/63/EU), it nonetheless allows prospective studies that, in the light of the aforementioned aspects, cannot be performed in our species. Whilst animal research has unquestionable advantages, it also presents some core limitations. First and foremost, animal research can be "lost in translation" when human-specific behavioural, emotional, and neurological symptoms have to be studied in laboratory animals. Thus, while TS nosography has been standardised in clinical practice, the identification of TS-like symptoms in laboratory rodents is more complex.

Overall Objectives

The overall objective to be achieved was the following: Develop a mouse test battery aimed at mimicking behavioural (stereotypical, compulsive and perseverative patterns), immune and brain abnormalities isomorphic to clinical symptoms of TS, as a function of induced autoimmunity (tasks 1 and 2). In other words, the first aim of our study was to devise a behavioural test battery capable of informing whether a laboratory mouse exhibited phenotypic symptoms isomorphic to TS nosography. This objective was aimed at addressing the following experimental question (Q):

Q1: How do we measure TS in mice?

Identify the prototypical mouse strain that shows the highest isomorphism with TS clinical symptoms in response to streptococcus-induced antibodies (task 3). Therefore, under this task, we screened a series of mouse strains (mice characterised by different genotypes) to isolate a candidate genotypic background upon which conducting the aforementioned (i) and (ii) activities. Here, we addressed the following experimental question:

Q2: In which mouse strain do we test whether group-A-Beta-Haemolytic Streptococcus (GAS) contributes to TS?

Expose the candidate mouse strain to active immunisation with GAS homogenates and evaluate whether this treatment results in neurobehavioural alterations isomorphic to TS. Beside these alterations, the target mouse strain (selected under task 2) was thus hypothesised to display appropriate antibody titres, T cell immunity, and brain lesions characteristic to autoimmunity (task 4). Here, we addressed the following experimental question:

Q3: Do repeated GAS administrations induce TS-like symptoms in mice?

Finally, in order to demonstrate that TS symptoms can be exacerbated by external stressors, our last task was to evaluate whether the abnormalities identified under objective 3 are aggravated by psychosocial stress. Here, we addressed the following experimental question:

Q4: Does psychophysiological stress influence GAS-mediated TS course?

The approach adopted to address these research questions and the results obtained are detailed below.

Results

Q1: How do we measure TS in mice?

While attempting to investigate whether a laboratory rodent exhibits a phenotype isomorphic (i.e. similar) to a symptom of a human disorder, it is necessary to keep into consideration its validity. Validity, in preclinical research may take different forms, including – for the scopes of this document – face and construct. Face validity indicates the morphological similarity (appearance) between the human symptom and the rodent phenotype: for example, hyperactivity can be readily measured in rodents through automated techniques and has a great degree of similarity with hyperactivity in humans. Construct validity, instead, refers to the etiological similarity of a given symptom/phenotype. For example, a given deficit in a brain structure that has been evolutionarily conserved across species (homologous) may induce different abnormalities in rodents and humans. In this case, the two phenotypes may appear different, yet have the same causative mechanism: in this case, the symptom observed in the experimental animal – albeit limited in terms of face validity – may be characterized by an elevated degree of construct validity and thereby constitute an important tool.

Based on these considerations, we identified a series of parameters, characterised by elevated face and /or construct validity, capable of informing TS preclinical research. Specifically, we systematically reviewed the available literature on animal models of TS to identify the specific dependent (readout measures) and independent (strategies to induce TS-like phenotypes) variables to be adopted throughout the project. Additionally, we constantly interacted with clinicians to evaluate the construct and face validity of these variables (in collaboration with partner 2 UNIBA and partner 14 UniROMA). The variables identified were the following: evaluation of general locomotion in baseline home-cage conditions and in response to a dopaminergic agonist; evaluation of behavioural stereotypies in baseline conditions and in response to a dopaminergic agonist; analysis of attentional set-shifting abilities and perseverative responding; evaluation of tic-like behaviours (head twitching and skin jerking) in baseline conditions and in response to a selective serotonergic agonist. These activities resulted in a review paper "Theoretical and practical considerations behind the use of laboratory animals for the study of Tourette Syndrome" (Macri et al., Neuroscience and Biobehavioral Reviews, 2013) and in the organization of a Special Issue "The multifaceted nature of Tourette syndrome: Pre-clinical, clinical and therapeutic issues", Eds. D. Martino & G. Laviola, published in a high-ranking scientific journal (Neuroscience and Biobehavioral Reviews). These activities were preparatory to the second stage of the project (Q2) in which we selected a specific mouse strain.

Q2: In which mouse strain do we test whether group-A-Beta-Haemolytic Streptococcus (GAS) contributes to TS?

Once identified the test battery, we needed to select a specific mouse strain upon which conducting the other experiments and achieving the main goals of the study. Different mouse strains can be roughly subdivided in inbred (in which subjects are genetically almost identical thanks to mating of siblings for dozens of generations) and outbred (in which genetic variations is kept through strategies aimed at avoiding mating of siblings). In order to test the role of exogenous factors (GAS infections) we preferred to minimise genetic variation and thus conduct the study on an inbred mouse strain. Furthermore, since the general hypothesis of the project rested upon autoimmune phenomena, we considered in the original battery two mouse strains that had already been extensively used in auto immunology research: SJL and ABH. These two strains were compared to another inbred

strain (C57/BL6) and an outbred strain (CD1). These four strains were tested in the behavioural test paradigms identified under Q1. These experiments ultimately served the aim to identify the mouse strain to be tested in response to GAS immunization. We observed that SJL mice, which are also characterized by elevated immune reactivity, constituted the most promising mouse strain based on several considerations: they completed all the experimental tasks, showed a remarkable response to dopaminergic and serotonergic agonists (dopamine and serotonin are two neurotransmitters involved in TS), and did not achieve maximal levels of responding (no ceiling effect, thus allowing some room to observe variations in response to active GAS immunization) in the tests performed. SJL mice were thus used in the following experiments.

Q3: Do repeated GAS administrations induce TS-like symptoms in mice?

During this stage, we tested the feasibility of the theoretical framework of the project, i.e. the possibility that repeated exposures to streptococcus contribute to the onset/exacerbation of TS. To this aim, we leveraged the information gathered during the preliminary stages of the project: identification of a phenotypic test battery to investigate TS in mice, and availability of a valid mouse strain, apt to the scopes of the project. Resting upon this information, we exposed developing SJL male mice to four injections with a GAS homogenate (devised and provided by Partner ISS-RC) and evaluated their neurobehavioural phenotype. In accordance with our predictions, GAS-exposed mice showed increased repetitive and perseverative behaviours, impaired sensorimotor gating, and reduced concentrations of serotonin in the prefrontal cortex. They also showed remarkable elevations in lactate (an indicator of inflammation) as measured *in vivo* by Magnetic Resonance Spectroscopy in the prefrontal cortex, a brain area linked to perseverative responding, impulse control, and sensorimotor gating. The presence of active inflammatory processes was further substantiated by the observation, in neuroanatomical specimens, of infiltrates and microglial activation in the white matter of the anterior diencephalon; this profile was not observed in control mice.

In summary, as predicted based on our experimental hypothesis, we observed that mice treated repeatedly with GAS exhibit increased plasma levels of autoantibodies (in collaboration with partner 11 GSK) and behavioural abnormalities analogous to the symptoms exhibited by patients with TS. Furthermore, we observed that these abnormalities are associated with deficits at the level of the central nervous system: the latter are represented by alterations in neurotransmitters and the presence of marked inflammation in brain structures that are also impaired in TS. Ultimately, in this stage of the project, we collected experimental evidence in support of the hypothesis that repeated GAS exposure may elicit inflammatory responses in brain areas involved in motor control and perseverative behaviour, and that these alterations may be causally linked to behavioural and neurological abnormalities isomorphic to TS. The experimental model developed under Q3 also served the aim to address the final question of this project.

Q4: Does psychophysiological stress influence GAS-mediated TS course?

One of the key aspects of TS is that psychosocial stressors seem to aggravate the symptoms. Leveraging the experimental model designed and refined under Q3, we addressed whether experimental stressors of different forms may modulate the course of TS. Broadly speaking, stress has two major intermingling components: a psychological and a physiological component. While the former can be considered self-explanatory, the latter is constituted by a plethora of biological events that occur upon the presentation of a stressor (a stimulus capable of perturbing individual homeostasis): these physiological responses entail the secretion and mobilization of hormones and other biological mediators. To investigate the role of physiological and psychological stressors, we exposed GAS-treated mice to different experimental paradigms stimulating physiological and/or psychological reactions.

In the first set of studies, we predominantly focused on the physiological component of stress by persistently elevating the tone of corticosterone (the key hormone involved in stress reactivity) in experimental mice. To attain a persistent elevation of corticosterone, we supplemented this hormone in the drinking water of mouse mothers while they were lactating their offspring. Mouse pups, in turn, received corticosterone through maternal milk. This experimental procedure resulted in a persistent elevation of corticosterone throughout the offspring entire lifespan (in collaboration with partner 8 TUD). Corticosterone-supplemented offspring were then repeatedly exposed to GAS (see Q3) and investigated on the test battery developed under Q1. In partial contrast with our original predictions, we observed that physiological stress mitigated rather than aggravated the phenotype of GAS-exposed mice in terms of behaviour and neurobiological rearrangements. We addressed this apparent paradox by detailing the immune reactions of experimental subjects. We demonstrated that early corticosterone administration promoted, in the long-term, the secretion of anti-inflammatory cytokines, which in turn contrasted the consequences of GAS exposure. Ultimately, this study revealed that some of the physiological mechanisms involved in the stress response may result beneficial under specific conditions. Yet, these data did not allow investigating the role exerted by psychological stressors.

To bridge the gap identified in the study described above, we adopted a form of stress which, compared to direct corticosterone administration, is characterized by a more marked psychosocial component. This stressor was constituted by the constant presence, within the cage of the experimental subject, of a potentially aggressive conspecific. Direct physical aggression and contact were, however, prevented by the presence of a perforated and transparent partition. Thanks to this procedure, GAS-treated mice were under the constant olfactory and visual presence of an aggressive mouse. While this experimental paradigm was aimed at eliciting a primarily psychological stress reaction, it nonetheless altered the underlying physiological mediators. Yet, despite this limitation, we observed that this form of stress apparently exacerbated immune reactivity to repeated GAS administrations.

Conclusions

Briefly, the activities of WP 05 complemented the investigations conducted in the clinical cohort study by providing solid evidence in support of the theoretical construct underlying TS onset/exacerbation in response to repeated GAS administrations. Additionally, we provided experimental data strengthening the role of psychological and physiological stress reactivity as a potent regulator of individual reactivity to GAS-dependent autoimmunity. This aspect is highly relevant whereby it offers new avenues towards the treatment of TS.

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WP 06 Genetics & Gene Expression

Background

Tourette syndrome (TS) is a complex neuropsychiatric disorder, which is likely to be related to an as yet poorly understood interaction between genetic and environmental susceptibility factors. Some pre- and perinatal factors potentially interfering with normal brain development have been explored in association with TS, as well as psychosocial stress and increased cortisol responses during acute stressors, the involvement of abnormal innate and adaptive immune responses in the pathogenesis of tics and related behavioural symptoms, and infectious pathogens viewed as responsible for the over-activity of immune responses documented in tic disorders, with particular focus on the role of common infections (pharyngotonsillitis) caused by group A streptococcus (GAS or *Streptococcus pyogenes*). On the other hand, while the heritability of TS has been estimated to

be high, the complex trait of tic disorders is polygenic, similar to most psychiatric disorders. Over the past decade, genetic factors associated with TS have been explored primarily through genome-wide approaches, including genome-wide association studies (GWASs), analysis of copy number variants (CNVs), and whole exome sequencing (WES). GWASs in TS have, to date, failed to identify highly genome-wide significant loci, likely due also to limited sample sizes, which were smaller than in other major psychiatric GWASs. The contribution of rare structural variation to the genetic architecture of TS is supported by recent analyses of rare CNVs, which indicate that approximately 1% of TS cases carry one of these CNVs. Like GWASs, WES studies in TS are also limited by their small sample sizes compared to other complex psychiatric traits; an association with *de novo* damaging variants has been reported for a dozen candidate genes and needs to be confirmed by studies with larger sample sizes. There is also a striking paucity of gene expression studies in tic disorders. Studies in this area focused on biological pathways related to neurotransmitters and immune regulation, but were based on small sample sizes, did not clarify whether the observed changes were causes or consequences of the behavioural phenotype, and were never adequately combined to genomic data.

Overall objectives

Within this Work Package we sought to investigate the association between genomic factors and the onset as well as physical course of TS. More specifically, we aimed to investigate the association between genome-wide genetic markers and the onset of tics and we aimed to identify gene expression patterns and gene pathways that correlate with tic exacerbation and also tic onset. In doing so, we examine the interaction between environment, autoimmunity and genetics related to the onset and clinical course of the disorder spectrum. Importantly, we also aimed to establish a European biobank of samples for the study of tic disorders and associated comorbidities.

Results

Thanks to the great efforts of the 16 clinical centres participating in EMTICS, collection of DNA samples actually exceeded the expected numbers (according to the project's milestones, the target was 615 cases). Of the 655 DNA samples collected at DUTH between May 2013 and December 2017, 625 belong to the COURSE arm and 30 belong to the ONSET arm. This helped us establish a European biobank of samples for the study of tic disorders and associated comorbidities and expand the existing collection of TS samples and family trios already available at DUTH in the context of other relevant projects, such as TSGeneSEE (The Tourette Syndrome Genetics – Southern and Eastern Europe Initiative) and the Marie-Curie Initial Training Network "TS-EUROTRAIN". Genome-wide genetic factors were investigated through whole-genome genotyping using peripheral blood-extracted DNA and an array targeting more than 700,000 common variants. Published genome wide genotyping data for individuals of European descent were used for the control dataset in order to perform GWAS and identify single-gene or gene-gene interactions associated with TS and obsessive-compulsive symptoms. Furthermore, we developed the necessary infrastructure and optimized appropriate protocols for processing and storing total RNA samples (total mRNA and miRNA), which became available at DUTH between May 2013 and December 2017. RNA was analysed on an Affymetrix Human Transcriptome Array (HTA 2.0), targeting more than 285,000 coding and non-coding and alternatively-spliced transcripts, in order to unravel biological pathways that may influence the onset and clinical course of tics activated upon symptom exacerbations and remissions.

In order to identify genes and gene pathways that influence the pathogenesis of tics and obsessive-compulsive symptoms (ONSET), we analysed the whole-genome transcriptomes of 42 individuals at baseline versus at the time of tics onset. To examine the gene pathways that influence the clinical course of tic disorders and that are activated upon symptom exacerbations (COURSE), we analysed the whole-genome transcriptomes of 165 individuals at time points of tics exacerbation versus at two months post-exacerbation, to explore how the gene expression profiles, fluctuate depending on whether the patients present with symptoms remission, worsening or remain stable. The top hits from the differential gene expression were further subjected to Gene Ontology, Pathway and Gene Set Enrichment Analysis (GSEA). For exon level analysis, we used further filters where we have removed undetected probe sets and genes utilizing the background scores generated from Affymetrix Power Tools (APT). Any probe sets that shows cross-hybridization was also removed for the downstream analysis. The exon level quality filtered probe sets were utilized to find the alternative splicing sites using splice index indicating the possibility of a splice site. The quality control of microarray data left with 87 pairs of COURSE and 26 pairs of ONSET samples for ~23,000 mapped probe sets with gene information. No probe set in either COURSE or ONSET has reached the genome-wide significant threshold. We have used the top hits with p-value < 0.01 for the Gene Ontology analysis and have found some neuronal related genes being captured. One of the reasons for this is the low sample size. We also found no alternative splicing sites in both COURSE and ONSET cohorts.

We performed a genome-wide association study (GWAS) using single nucleotide polymorphisms over 1,490 TS cases and 12,039 population-matched controls collected from different European nations, genotyped on various Illumina chips, including the 630 EMTICS participants. To account for different genotyping platform issues and potential discrepancies, we performed imputation on each platform separately before merging the data. Ancestry matching of imputed data was done by merging the cohort with 1000genomes genotype data and projecting it on top principal components as calculated from Principal Component Analysis, followed by removal of population outliers. Next, quality control-filtered, merged, imputed data were subjected to logistic regression analysis using PLINK. Of interest among the top statistically significant hits associated with TS are genes previously implicated in schizophrenia and major depressive disorder, with a biological role in neurogenesis and axonal growth and guidance of motor and sensory neurons. Among the top statistically significant hits from the association we found gene MARK3 which is involved in RET signalling pathway which encodes tyrosine kinase receptor essential for axonal growth and guidance of motor and sensory neurons. MARK3 gene also plays a role in progression of Alzheimer's disease. Furthermore, gene ZBTB20 which has been previously implicated in Schizophrenia and Major Depressive Disorder was also among our top hits. ZBTB20 plays a role in neurogenesis and postnatal growth. Hyper-methylation in the coding region of ZBTB20 was also shown to be associated with Major Depressive Disorder which has a high genetic correlation with GTS. These genomic variants warrant further investigation in order to shed light into the genetic background of TS.

Conclusions

In conclusion, we have identified genes that warrant further investigation in relation to the aetiology of TS. Our top hit is the MARK3 gene which plays a role in the establishment of cellular polarity and cell cycle control. The EMTICS study is, to our knowledge, the largest prospective cohort assessment of the contribution of different genetic and environmental factors to the risk of developing tics in putatively predisposed individuals and to the risk of exacerbating tics in young individuals with chronic tic disorders. The infrastructure and database that has been built through EMTICS will help elucidate how the genetic background determines the molecular/cellular pathways underlying tic disorders and may shed light on how the genetic factors interact with environmental factors influencing the onset and clinical course of tic disorders.

WP 07 Treatment

Background

This study was an extension of the EMTICS Course study; its aim was to investigate the effects of treatment with an antibiotic in children with a chronic tic disorder and presence of group A streptococcus (GAS) bacterium in their throat (so called GAS colonisation). We aimed to investigate the possible effects of antibiotic treatment on tic symptoms in term of severity and number of flare-ups (exacerbations).

Following the few studies performed on this topic, children affected by a tic disorder show a rate of GAS colonisation similar or slightly higher than that reported in the normal population (Cardona and Orefici, 2001; Creti et al., 2004). However, several studies have documented higher rates of elevated anti-streptolysine O (ASO) titres (i.e. a specific immune response to GAS infection or exposure) in children affected by tic disorders in comparison with different control samples (Cardona and Orefici, 2001; Morshed et al., 2001; Muller et al, 2001; Church et al, 2003; Rizzo et al, 2006; Martino et al, 2005; Martino et al, 2011). Other longitudinal studies have evidenced also that ASOT were elevated for a long time in large number of patients with tic disorder (e.g. Martino et al, 2011). Moreover, patients with tics tend to have an elevated immune response towards a broad range of streptococcal components (Bombaci et al, 2010).

All together, these observations have led to hypothesize that patients with TS colonised by GAS are not merely carriers but that this colonisation, i.e. the persistence of the bacterium in the throat, may stimulate a sustained anti-streptococcal immune response contributing to the persistence of tic symptoms. If this hypothesis would be true, the antibiotic treatment of GAS colonisation in patients affected by chronic tic disorder could modify their symptoms in term of severity and number of exacerbations.

Overall Objectives

Aim of this clinical trial was to study the efficacy of treatment with antibiotics in reducing severity of tics and other behavioural symptoms in patients with a tic disorder colonised by GAS.

Primary Objective:

- Test the hypothesis that antibiotic treatment of GAS colonisation compared to placebo is associated with a larger reduction of tic and associated neuropsychiatric symptoms in the short-term (1 month) in patients with a tic disorder colonised by GAS.

Secondary Objectives:

- Test the hypothesis that antibiotic treatment of GAS colonisation is superior to placebo in the long-term (8 months) reduction of tic and associated neuropsychiatric symptoms in patients with a tic disorder colonized by GAS.
- Investigate the factors that could play a role on treatment outcome.
- Investigate whether antibiotic treatment could modify the anti-streptococcal immune response of colonised patients.

The study was designed as a multicentre trial. Patients affected by a chronic tic disorder followed in the EMTICS-COURSE study who showed a positive throat swab for GAS at any examination during their follow-up and who agreed to participate were recruited in the trial. Patients were assigned in a random way to one of two arms of the trial. A 10-days regimen of antibiotic (amoxicillin/clavulanic acid at the dose of 25/3.6 mg/kg/day, two times a day) or placebo, both as syrup, was prescribed to participants. Neither participants nor investigators were aware of which arm any single subject belong to, until the end of study.

Results

14 patients (1 drop-out), enrolled in 4 clinical centres (9 in Rome, 3 in Catania, 1 in Seville, 1 in Zurich), participated in the study. Their mean age was 9.3 years, the male/female ratio was 10/3. In a random way, seven of them were assigned to placebo arm and seven to active arm (included the drop-out).

No adverse events or reactions were reported following drug assumption.

At post-treatment visit (1 month after the recruitment and the treatment), the primary outcome measure – the tic severity measured by the mean Yale Global Tics Severity Scale (YGTSS) score - did not show any clear difference in comparison to that of the baseline visit.

No significant difference of the mean immune parameters (ASO and Anti-DNase B titres) was found between the baseline and the post-treatment-visit.

At 8 months follow-up visit, the primary outcome measure - mean YGTSS total score - did not show any significant variation in comparison to that of the baseline visit.

No significant variation of the mean ASO and Anti-DNase B titres was found between the baseline and the post-treatment-visit.

In consideration of the low number of patients enrolled for the study (13 over 45 planned- 28%) it was impossible to further investigate the influence of possible factors on treatment outcomes.

Conclusions

The low number of patients recruited in the trial deserves some explications. Three factors played an important role in this situation:

- 1) *Discussions with the local Ethic Committees (ECs)*

The trial received the approval from the European Commission, but it was also necessary to obtain approval from local ECs. Only in a few clinical sites, this was obtained quickly; in the majority of the clinical sites, different bureaucratic issues were raised resulting in a very late approval. Moreover, in one case the local EC refused the approval. Obviously, all these problems have reduced the possibility to recruit patients for the trial or substantially shortened the time available for this.

2) *Rate of GAS positivity*

Overall, in the COURSE study there was a lower rate of GAS positivity than expected, thus the number of subjects eligible for the recruitment was reduced; importantly, there was also a wide discrepancy of GAS positivity rates among centres.

3) *Refusal to participate*

Many eligible patients refused to participate in the trial and this fact further reduced the overall patient number. The principal reason of refusal was the randomised design of the trial: many parents were afraid that their children would receive the placebo. Other hypotheses (i.e. worries about antibiotic treatment) were ruled out by the examination of data base, in particular of diaries and phone questionnaires that demonstrated many patients have taken antibiotics outside the trial.

In any case, the low number of patients recruited in the trial did not allow to investigate in depth our initial hypothesis, whose premises (high rate of GAS positivity, high rate of ASO and Anti-DNase B titres and persistently high value of both titres); however, it should be underlined that the lack of a trend of improvement of the clinical and biological measures after the treatment of GAS colonization seems to exclude a direct relationship between them.

To support this conclusion a careful examination of the clinical course of the tic patients showing GAS colonisation that have taken antibiotics (outside the trial) is on its way.

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WP 08 Psychosocial Stress

Background

Patients with Tourette Syndrome (TS) frequently report increased levels of stress as well as an association between fluctuations in stress level and tic severity. Tourette syndrome usually has a waxing and waning course with periods of tic exacerbation and periods of decreased tic severity (Leckman, 2002). Tics arise in bouts over the course of a day, and these episodes change in frequency and severity over weeks and months. Many authors have suggested that psychosocial stress is among the most relevant factors influencing this waxing and waning of tics (Bornstein, Stefl, & Hammond, 1990; Hoekstra, Steenhuis, Kallenberg, & Minderaa, 2004; Lin et al., 2007; Silva, Munoz, Barickman, & Friedhoff, 1995).

It has also been suggested that psychosocial stress might trigger the first onset of tics, but the currently available literature does not directly support this assumption. A few studies also suggested that the physiological stress response is altered in patients with TS (Corbett et al., 2008, Chappell et al., 1994, Chappell et al., 1996). In response to stress, cortisol, a steroid hormone, is released from the adrenal gland. Long-term cumulative cortisol levels can be determined by analysis of the hair cortisol concentrations (Stadler et al., 2017; Stadler & Kirschbaum, 2012). Before EMTICS, it has never been investigated if hair cortisol concentrations –as a longer-term marker of stress- are substantially changed in patients with TS.

Overall Objectives

The first question to address was whether cortisol levels are elevated in patients with TS. Further, we investigated the relative impact of perceived social stress and hair cortisol concentrations on fluctuations of tics (and comorbid obsessive-compulsive disorder symptoms) in a longitudinal setting. In this context, we also investigated whether the presence of comorbid conditions as well as sex and age differences do moderate the relationship between cortisol levels and tic severity. A second aim was to describe to what extent children and adolescents, in which a tic onset has occurred, were exposed to social stress as compared to those without an onset of tics. We expected a higher level of social stress and hair cortisol concentrations in those children with tic onset. A third aim was to describe how perceived social stress, hair cortisol concentration and quality of life of the siblings enrolled in the ONSET study are affected by tic exacerbations (and remissions) of their brothers or sisters enrolled in the COURSE study. We also took exacerbations of comorbid obsessive-compulsive disorder and ADHD symptoms into account, since those comorbid psychiatric disorders are highly correlated to quality of life in patients with tics

As mentioned above, many patients with TS report that their tics are highly situation-specific. However, before the start of the EMTICS project only little experimental work has been done on the topic of tic fluctuations over the course of the day. We added one study on tic frequency during short-term psychosocial stress (Buse, Enghardt, Kirschbaum, Ehrlich, & Roessner, 2016). In this study we asked children with tics to take part in the Trier Social Stress Test for children. This is a well-established test designed to induce psychosocial stress. The children received the beginning of a story (in written form) and were told to finish the story as exciting as possible in front of a committee, which was announced as experts in judging the quality of children's stories. In order to increase the stress induction, the participants received no verbal and non-verbal feedback. A relaxation situation, in which the children listened to pieces of quiet instrumental music, and a concentration situation, in which the children accomplished a symbol search task, served as control conditions. Patients were asked either to suppress their tics or to "tic freely." Physiological measures of stress were measured throughout the experiment.

Results

Within the scope of the EMTICS project we first published a literature review on the modulating role of stress in the onset and course of TS (Buse, Kirschbaum, Leckman, Münchau, & Roessner, 2014). Our own investigation of psychosocial stress elicited a clear stress response with elevated levels of cortisol, increased heart rate, and a larger number of skin conductance responses. During relaxation and concentration, the instruction to suppress tics reduced the number of tics, whereas during stress, the number of tics was low, regardless of the given instruction. Our study suggests that the stress might even result in a short-term situational decrease of tic frequency. Over the course of the EMTICS study, we collected 2562 hair samples of patients with tics (COURSE study) and patients with high risk to develop tics (first-degree relatives, e.g. siblings or children of patients with tics, ONSET study). In collaboration with the lab run by Prof. Kirschbaum at the department of biopsychology, we analysed the cortisol concentration in the hair strands. Since hair grows approximately 1 cm per month, the cortisol concentration in a strand of hair with 4 cm length reflects the cumulative cortisol level of approximately 4 months. The last samples were analysed in May 2018 and have all been entered in the database. We expect to get the first results by the end of September 2018.

Conclusions

Within this WP we addressed the relationship between stress & tics and outline possible underlying mechanism of how stress may affect tics. We found that dopaminergic and noradrenergic neurotransmission as well as immunology might play a crucial role. Two possibilities of causal direction might be assumed: (a) psychosocial stress might lead to an exacerbation of tics because it increases cortisol release followed by changes in neurotransmission or immunology and (b) patients with TS might show altered neurotransmission or immunology which – in turn - results in a higher vulnerability of affected patients to respond to psychosocial stress with strong cortisol release. More definitive conclusions will become available over the next months.

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WP 09 Data Management

Background

The EMTICS project needed a collection of data from the participating centres, located in different countries. The goal was the computerization in which key characteristic was usability; what kind of software to use for this multi-centric study, whether to use an existing product by readjusting it or developing a specific one.

Overall Objectives

The principal objective was to have a platform ready to be adapted for further data and to be able to respond to new research questions. It was necessary to have a flexible tool to allow rapid responses: A reliable web platform in a secure environment, establishing an effective electronic data capture system for the cohort studies and the treatment study enabling the coupling of measurements with the electronic data capture system having as a final result a Europe-wide database for the longitudinal cohort studies and the associated measurements obtained from the participants.

Results

WP09 has developed an integrated e-CRF (Electronic-Case Report Form), based on a web platform with patient data and related visits to support the EMTICS project, establishing an effective electronic data capture system for the cohort studies and the treatment study. The platform is available to the accredited users, via Internet connection.

Principal environment characteristics:

- website and database available 99.9% of the time
- safe and secure environment: hosting, backup and access
- single sign-on with strong username and password
- role-based access control
- database download for authorized users
- query tools
- secure communication (HTTPS)
- back office

Main characteristics of the e-platform:

- single point-of- access for all web users
- user-friendly
- easy data entry
- customized environment
- monitoring tool
- query personalised
- database downloadable
- overview of patient-related data and follow-up

The platform was specifically developed for the project, through a continuous relationship with the WPs, carrying out periodic reviews of the activities according to requests by partners, previously approved by the EMTICS coordinator. A relational database has been used with specific technical solutions developed for this purpose. This allowed the implementation/modification of new questionnaires using only SQL queries in the back office. Through this methodology it was possible to provide a web-based data system to host all the variables necessary for EMTICS, highly customized. The platform guarantees the confidentiality of the electronic data acquisition system.



EMTICS
European Multicentre Tics Study

EMTICS
European Multicentre Tics Study

EMTICS aims to identify the genetic and environmental susceptibility factors of tic disorders and will greatly contribute to a better understanding of the underlying mechanisms, especially by elucidating the role of autoimmunity and infections. EMTICS is a project funded by the EU programme FP7 and combines 27 partners from 11 different countries.

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Training activities on the system and helpdesk were provided to the various centres, especially at the beginning of the project and in the final phase, to verify the data. During the whole period, support for the maintenance and operation was guaranteed. The web form is organized in sections and divided in a part containing the patient card and the other one with the reports of visits. To allow partial entering of data, visit data could be filled in and saved during different periods.

Quality checks were performed at different stages, according to the rules established. During the data entry phase, real time checks were performed: automatic verification of missing data, format of input data; data out of bounds, interval. When saving data, consistency checks and compatibility rules were performed. At the end of the visit, if all the mandatory fields and records had been filled in, the user could "submit" it. Lastly, we had also back office analyses: statistical checks on data to verify significant differences in relation to expected values. Interim reports were periodically performed on the data collected to verify the progress of the activities related to what was planned.

Conclusions

We have set up a web-based data capture system that has successfully collected all clinical data of the human studies. The database has also been set up for biological data, with the result of having a European database for clinical longitudinal cohort studies and measurements, including characteristics of throat swab results, blood results, immune measurements, genetic data and measures of cortisol in hair.

The database has now been finalized, export tools of raw data have been implemented according to variables as described in a codebook in excel, csv and planned for xml. The coupling of biological data was done and is available in the offline cleaned statistical master file.

WP 10 Training and Dissemination

Background

The purpose of this work package was 1) to offer training in the highest standards of clinical ratings and adherence to good clinical practice (GCP) as well as in reliable detection of GAS carrier state (followed by two external quality assessments = EQAs), 2) to guarantee that dissemination of new knowledge will take place through journals, websites and conferences, and 3) to assess and protect intellectual property and exploitable foregrounds on a regular basis.

Overall Objectives

The specific training objectives were:

1. To assure an appropriate conduct of research in accordance with Good Clinical Practice (GCP) (task 3);
2. To provide EMTICS clinicians (including young female scientists) advanced training in Clinical Rating Files (CRFs) and psychiatric assessment instruments developed by WP01 and WP08; and to check quality standards with respect to GCP (tasks 1, 2 and 3).
3. To provide EMTICS scientists advanced training in epidemiology and clinical aspects of Group A streptococcal (GAS) infections and non suppurative sequelae (task 2).
4. To harmonise and improve, through *ad hoc* training courses and two external quality assessments (EQAs), the microbiological methods used by different centres to isolate GAS from carriers with low bacterial density. This is essential to guarantee reliability of results. Particular attention was devoted to the training of young female scientists and scientists from Eastern Europe in order to encourage their participation in research projects and improve their professional development (task 2).

The specific dissemination objectives were:

Primary objective

Coordinate dissemination of project progress and results.

Secondary objectives

1. Coordinate the further development and regular updating of the dissemination and exploitation strategy.
2. Generate information kits for investigators and participants.
3. Identify relevant target group for dissemination of aims, methods and results of EMTICS project.
4. Communicate with professionals, patient organisations and the general public.

Results

- All partners have been trained in CRF clinical rating scales through training courses. All CRFs were translated into eight languages, disseminated to the project participants, and posted and shared on the EMTICS intranet.
- In addition, all clinical partners have been trained in a new microbiology method through a training course, and a detailed guideline to isolate GAS from low bacterial carriers. A new method has been established and distributed to participants. A further modification of this test has been checked and disseminated to participants.
- Fifteen microbiology laboratories participated in the second more complex EQA. Thirteen of the 15 participants displayed 100% sensitivity and specificity in the identification of GAS and two of the laboratories displayed 75%

sensitivity and specificity. These results reveal that a high accuracy in the identification of GAS in throat specimens is maintained at all sites and the training for microbiology laboratories was successful.

- The EMTICS publication plan was setup in the first project period and continuously updated. Up to date, 35 publications were published in the context of EMTICS, 24 of them were peer-reviewed, and 17 are accessible in open access journals. 57 more manuscripts are currently in preparation, likely to be submitted to high-impact, peer-reviewed journals before the end of 2019.
- Furthermore, project partners reported more than 160 dissemination activities. This occurred mainly on conferences / congresses via talks and/or posters. One example is the ECNP congress in Paris 2017, where the project flyer was distributed, a poster summarising the project was shown, and results were discussed with congress attendees.
- In addition, the EMTICS website, bi-annual newsletters, radio interviews, press releases, workshops and articles in the popular press, but mainly of course the direct contact to patients and patient organisations raised awareness towards tic-related disorders within the general public. Throughout the duration of the project we have been contacted by many of affected young patients asking to become actively involved in the study. Many were able to participate in the study.
- A general exploitation strategy was implemented according to the project results, and progress was continuously updated. Three key exploitable foregrounds are already available because of research performed during the EMTICS project (see below for details).
- Currently, the consortium is setting up a summary report on new knowledge obtained through the EMTICS studies for patients, families, patient associations, as well as for professionals and clinicians.
- Three major exploitable foregrounds have resulted from research performed over the course of the EMTICS project: Partner ProImmune is developing a novel product in the field of “immune monitoring devices” based on their gained expertise regarding the flow cytometric evaluation of immune responses in children, and Partner APD developed two separate research kits, one for the measurement of monomeric CRP and another one for the measurement of pentameric CRP. While no patents have been submitted yet, both partners intend to use the gained knowledge to bring commercially available products to the market within the next years (see chapter 4.3 for details).

Conclusions

We are very pleased with the successful methodological training of our young EMTICS scientists, including the distribution of the clinical rating files (CRFs), the standardization of the psychological evaluations, as well as the high quality and accuracy of the microbiology methods that were applied throughout the duration of the project. This was also confirmed by the two external quality assessments (EQAs). The work force statistics of EMTICS further highlight the tremendous role female scientists played within the leadership of the project (6 female WP leaders), the large number of young female scientists (43 experienced researchers, 16 PhD students, and 60 supporting personnel) who contributed exceptionally high quality of work to the project, and the valuable contribution of research performed by scientists from Eastern Europe (3 experienced researchers in Hungary, 3 in Greece, and 6 supporting personnel in Hungary). Thus, the EMTICS projects made a major effort to implement equal opportunity and actively promote the professional development of female scientists as well as researchers from Eastern Europe. The fact that more than 160 dissemination activities, not only posters and presentations during scientific gatherings, but also many interactive workshops and informative events for the general public, patients and relatives, have been realised over the course of the project is a major achievement. As expected, our 35 publications to date (24 of them peer-reviewed) are only the beginning of further dissemination of key EMTICS research results that will occur within the next 2 years in high-impact, peer-reviewed journals. That two of the enterprises that participated as partners in the project (APD and ProImmune) are currently developing commercially available products is the result of our continued assessment and valorisation of gained knowledge and exploitable foregrounds.

In summary, the training and dissemination WP was a full success, as reflected especially by the high standard of methodology, high-quality scientific publications, the large number of dissemination activities involving the general public, and the active professional engagement of young female scientists.

WP 11 Ethics

Background

The key issue when considering a research study involving children and adolescents is their vulnerability. All study procedures were designed to minimise burden on the patients. The study staffs were experienced in child and adolescent psychiatry, child neurology and paediatrics. The study followed the EU regulations in all participating countries.

Overall Objectives

- Set the highest standards for the ethical aspects of the proposed human studies complying with EU regulations and all participating countries
- All sites leaders and groups will be trained in Good Clinical Practice GCP procedures
- Allow appropriate informed consent (parent/guardian) and assent (children/adolescents)
- Protect research participants' confidentiality
- Ensure care and protection of human research participants
- Ensure Adherence to the above determined ethical standards, thus insuring the EMTICS project will be carried out in compliance with fundamental ethical principles as stated by the European Union and all s
- Ensure Animal use and experimentation (in Association with WP 05), According to the 3Rs principle

Results

The EMTICS group discussed in each and every meeting the ethical aspects in the conducted human studies. The ethical parts of the study were complying with EU regulations in all participating countries. All ethical issues were inserted to the study protocol.

Patients' safety

Researchers acted towards research participants in such a way as to minimise any potential risk, pain, distress and fear (e.g., local anaesthesia before blood withdrawal, psychological support when needed).

Ethical Approval

1. Prototyped ethical forms were prepared by WP11. The forms included informed consent forms and information leaflets for participants and their families. In addition, prototyped ethical forms for subpart of genetic testing were prepared by WP11.
2. The ethical forms were translated to all needed languages. All ethical forms were emended by all centres due to their national law. All sites leaders and groups were trained in Good Clinical Practice GCP procedures. All centres received local ethical approvals for the study.
3. Annual reports and final reports to IRBs according to local laws were delivered (once a year during five years of study).

Informed consents

Patients and their parents/caregivers were informed in written and oral form regarding the key objectives of the study, the procedures that have to be followed, and the reasonably foreseeable risks or discomforts and potential benefits. As the study subjects were children and adolescents their assent was sought where appropriate and informed consent was obtained from their legal representatives (one or both parents, according to local regulations). Further, in the case of adolescents over the age of 12 (or as defined by the rules of respective countries) their consent was obtained, through the provision of age-appropriate

information. Children and adolescents were included in the study only after all relevant consent forms have been obtained. Even after providing informed consent families remained the right to withdraw from the study at any time. Small percentage of families indeed withdrew from the study.

Data Privacy

We designed a guideline for protecting confidentiality and privacy of data of the patients. Personal information was anonymised and a code was held in the department of recruitment. Databases were and will be kept secured. Data collected, was kept treated and transferred in a confidential manner according to regulations. In accordance with regulations, each transition of information from one place to another (i.e. site, laboratory, central database) was accompanied by a different identification code for each patient. The final database is anonymous so that identification of persons is impossible.

Animal Studies

The protocol of animal use for experiments was written and approved in accordance with applicable regulations and due to EU lab animal regulations (WP 05). We have ensured animal use and experimentation according to the 3Rs principle. The animal laboratory used methods which minimised animal suffering and improved welfare (WP 05).

Conclusions

In the EMTICS project we have succeeded to set the highest standards for the ethical aspects of the studies complying with EU regulations and all participating countries. There were no infringements of the ethical procedures.

WP 12 Project Management

Effective project management is a central element of successful research. This is because large research projects often entail a lot of administrative work which needs to be dealt in an efficient and timely manner. In view of this, the purpose of WP12 was project management for the EMTICS project. This WP took care of all administrative and coordinating tasks.

To ensure compliance by beneficiaries with their obligations under the grant agreement, the project management office at concentris routinely supported the Coordinator in monitoring the partners' performance based upon the following:

- To make sure that tasks assigned to them were correctly and timely performed.
- Reports were submitted according to the guidelines and on time.
- Funds were used and claimed according to the rules.
- The partners fulfilled their obligations regarding dissemination, funding acknowledgements and intellectual property rights.
- Any changes to the work plan were communicated to the European Commission (EC) efficiently.
- Compliant to ethical regulations.

The Project management office acted as a helpdesk for all participants. It was the central node of communication on a day-to-day basis and communicated with the European Commission on behalf of the Coordinator regarding administrative and managerial issues (i.e. contract, amendments, reportings etc.).

4 Potential impact, main dissemination activities & exploitation of results

4.1. Socio-economic impact and the wider societal implications of EMTICS

The projects undertaken by EMTICS, under the supervision of leading experts from across Europe, have been directed at shedding light into the aetiology and pathology of TS and related disorders (such as OCD, ADHD). Despite the frequency of these disorders and their impact on patients' lives, the pathophysiology of these disorders is poorly understood and only symptomatic treatments with limited effectiveness are available. Existing evidence pointed to both genetic predisposition and environmental factors, namely GAS infections and psychosocial stress to possibly play an important role in the pathogenesis of these disorders. However, genetic studies had failed to fully explain the occurrence of tic disorders and the strength of association and mechanisms leading to tic disorders following these environmental factors were not clear. Tourette syndrome is a classical model for understanding developmental psychopathology. In this large European multicentre study, bringing together clinical, microbiological, immunological and genetic centres, we were able to address the shortcomings of previous studies which were hampered by the small study samples and narrow focus of the studies. This study was the first to prospectively study an at-risk population to determine the risk factors for development of tic disorders or their exacerbations and provide answers to a number of questions, including the strength of association of tic disorders with preceding GAS (and other) infections and psychosocial stressors, of a genetic profile accounting for increased risk, and bacterial microbiological and immunological and host immunological factors that determine the risk of development or exacerbations of these disorders. We also examined the mechanisms in an animal model and conducted a treatment trial to establish whether treatment of these disorders with antibiotic prophylaxis, which is postulated and practised by many, is an evidence-based treatment for clinical practice or is unlikely to be beneficial in patients with TS. The full impact of EMTICS will be outlined in the following paragraphs.

Better appreciation of the role of new GAS exposures and of the concept of PANDAS for clinical practice

As one of the major objectives, EMTICS intended to verify if and how a GAS infection can stimulate the onset and/or exacerbation of symptoms in patients with TS. Several attempts had been done to try and verify this hypothesis, but given the relatively low number of dedicated TS clinics and the difficulty of detecting the microorganism in low GAS density samples, a univocal relationship between GAS infections and TS symptoms has never been reached. The Europe wide approach of EMTICS with a large number of specialised hospitals participating did provide a sufficient number of cases coupled with accurate microbiological training and EQA, thus guaranteeing homogeneity and reliability of results.

As a major result stemming from EMTICS, we found no indication for a role of new GAS exposures in relation to exacerbations of tic disorders. We did find that new GAS exposures are very frequently occurring, to a point that exposure to GAS at some moment during childhood is nearly universal. Thus, it is quite understandable that a part of the children who come to clinics with a tic exacerbation have signs of a recent GAS exposure. Indeed, in the EMTICS COURSE study we identified a number of COURSE participants with a significant tic exacerbation preceded by a recent new GAS exposure. Clinical observations of the co-occurrence of recent GAS exposures and tic exacerbation have led to the PANDAS concept. However, the EMTICS study indicates that the co-occurrence of tic exacerbations and recent new GAS exposures is most likely due to chance. We also found no indication for a temporal association between new GAS exposures and the onset of tics. Again, new GAS exposures are very frequent but most children do not subsequently develop tics. In those who do develop tics, this was not clustered around a recent new GAS exposure. We have to acknowledge though that the numbers were low. This has important clinical implications: our findings suggest that assessing recent GAS exposure in children with tic disorders is not clinically meaningful. This should have a major impact on clinical practice. Our treatment trial was aimed at further unravelling the role of GAS colonisation in the pathogenesis of tic disorders. Results speak against antibiotic treatment for clinical practice; and thus should prevent undue use of antibiotics in patients with tics.

Better understanding of factors associated with the onset and course of TS

A very important achievement is the unique database that we have created. The database that has been designed allows the identification of environmental factors implicated in the manifestation or amelioration of symptoms. Results so far had often been contradictory, with statistical power being hampered by the relatively small sample sizes in each individual study. Analysis of the involvement of environmental factors as triggers for the onset of the disorder really demanded the collection of detailed data from large numbers of patients coupled with sophisticated statistical analysis. We now have a fully integrated database in SPSS format, containing more than 7000 visits, with prospective collection of fluctuations in severity of tics and related clinical variables, coupled with genetic information, information on psychosocial stress, perinatal complications and various immune

markers. The EMTICS study therefore provides the unique opportunity to analyse data on a broad set of environmental exposures and biological markers related to chronic tic disorders. Indeed, we have now > 50 fully developed manuscripts planned (see appendix) that will be written over the following 1-2 years. Since both tics and obsessive behaviours will be the focus of this project and since these behaviours relate to self-control and the emergence of a cohesive self, the impact of our findings should have far reaching implications for understanding some of the basic mechanism of healthy child development. Analysis of the data will provide further insight into the course of the disease will be the basis (I) for further genetic analyses, (II) for the characterisation of predictors, (III) and for improved treatment strategies and social support in order to minimise socioeconomic costs of the disease. In the future it will be used for further clinical research, e.g. neuroimaging, genetics, epigenetics, immunology, epidemiology, and treatment.

The EMTICS scientific programme helped elucidate the complex genetic background of TS, habit formation and tics. Our final data analyses will result in the identification of genetic and environmental susceptibility factors and will greatly contribute to a better understanding of the underlying mechanisms of tic disorders with and without co-occurring paediatric OCD. Through multi-modal combined behavioural and systems neuroscience studies in representative subgroups of children with TS and well-defined comorbid disorders the EMTICS database will delineate intermediate phenotypes which will represent both a prerequisite for the understanding of consequences of genetic and environmental susceptibility factors at a system level and a means to define susceptibility factors within these system networks.

To consider the role of non-streptococcal bacterial, as well as viral, and other microbial infections as potential environmental risk factors for TS/OCD, different specific antibody titres have been determined. The specific pathogen-related responses of the innate and adaptive immune systems have been identified on the level of the cellular immune system, of cytokines and soluble cytokine receptors, and of specific cross-reacting antibodies against neuronal targets. The tryptophan/kynurenine metabolism which is the link between immune changes and neurotransmitter functions have been determined in order to detect early neurochemical changes after infection before the development of tics. This gives us information about the inflammatory response and possible molecular structures for the development of therapeutic targets. An incomplete resolution of inflammation which would result in chronic low-grade inflammatory state may be one of the pathophysiological mechanisms in development of tics. The monomeric and pentameric CRP detection test to be developed is novel and may detect the incomplete resolution of inflammation in early stage so that early therapeutic strategies could be undertaken before tics develop after the infection.

Specialised laboratories from different European countries have jointly cooperated in analysing the immune response. Due to the highly differentiated and complex interactions between the humoral and the cellular immune responses and the influence on neurotransmitters such as glutamatergic neurotransmission, including the cellular immune system (SME ProlImmune), the monocytic system (QMUL), of cytokines and soluble factors (SME Cytolab), of specific antibodies (UNIBA), monomeric and pentameric CRP detection (SME APD), the influence of different pathogens (LMU), and the effects on the tryptophan/kynurenine metabolism (LMU).

Although there is expert consensus about the important role of psychosocial stress in tic disorders and/or OCD, so far details underlying this relationship were largely unknown. Limitations of previous studies in this field such as small sample size, short follow-up period and lack of biological stress measures have prevented firm conclusions. The assessment of chronic psychosocial stress by rating scales and also by cortisol level in hair as the first biomarker of retrospective chronic stress in both large and well-defined European cohorts have overcome these methodological shortcomings. The results might help to develop new strategies for targeted prevention, new therapies, and ultimately lead to disease prevention or a significant decrease in the incidence of these diseases by highlighting the role of therapies reducing chronic stress. Previous studies addressing the link between environmental factors, such as GAS infections and psychosocial stress, and the onset of tics and obsessive-compulsive symptoms in children were limited by their retrospective design and the use of community-based datasets that are liable to information bias. The ONSET cohort study has been the first clinic-based study to assess in a prospective fashion the relationship between these environmental factors and the onset of tics and/or obsessive-compulsive symptoms in a well-defined high-risk population, and has therefore the potential to provide definite and robust evidence of this association. The COURSE study has been the largest clinic-based study ever performed to evaluate prospectively the relationship between the above-mentioned environmental factors and the course over time of tics and obsessive-compulsive symptoms in children with TS or other chronic tic disorders. Moreover, the COURSE study has increased our knowledge of the pathophysiology of TS and other chronic tic disorders by providing robust data on the involvement of the immune system in determining the clinical course of these illnesses.

Our findings are significant for reasons that extend beyond the benefit of new knowledge on TS. TS can provide a model for other behavioural disorders that share clinical manifestations and therefore could be involved in a common pathway (for instance, ADHD and OCD). Thus, research in TS will undoubtedly also shed light into the aetiology of disorders that are suspected to share a common aetiological background with TS. Understanding the aetiology of TS may ultimately lead to improving treatment and increasing the quality of life for patients and their families.

A major boost to Europe-wide TS research

EMTICS has truly fuelled the field of research for TS and neuropsychiatric disorders in general with the next generation of pioneers that will further increase our understanding of these disorders, bringing us closer to improved management strategies, standardising the standards of care for affected children across Europe, and improving the quality of life of children and their families. To date, the pathogenetic mechanisms leading to the onset of the disorder remain largely unknown, despite the fact that TS represents an area of active research, engaging numerous investigators from around the world. In Europe, efforts to elucidate the aetiology of TS were largely fragmented. Research in the aetiopathogenesis and clinical management of TS in Europe is still behind compared to other neuropsychiatric disorders. EMTICS has fostered an international interdisciplinary network of European experts in psychiatry, psychology, neurology, genetics, statistics and bioinformatics, who have joined their efforts in increasing our understanding of the aetiology, pathogenesis and course of TS and related disorders. Towards these goals, the partners of EMTICS formed a highly multidisciplinary and intersectorial team, with the European experts in the study of TS. All of the partners are at the forefront of TS research and were directly involved in producing a large part of existing knowledge on TS. Partners have been drawn from both the academic and private sectors, ensuring the direct exploitation of research results. We also fruitfully collaborate in the European Society for the Study of Tourette Syndrome (ESSTS).

EMTICS has also boosted new large-scale collaborative grants in the field of TS, with major European involvement. Two particular examples are TS EUROTRAIN (a Marie Curie Initial Training Network) and TIC Genetics, a major NIMH R01 collaborative grant aimed at the genetics of tic disorders, and a direct consequence of the success of EMTICS. Efforts to collect samples for TS throughout Europe had for a long time been fragmented. The large numbers of samples which are demanded for the elucidation of the genetic basis of TS can only be collected through large-scale collaborative efforts. The same is true for efforts aiming to disentangle the relative contribution of genetic and environmental factors. It is therefore extremely important to unify national projects across Europe and create a common platform for collaboration.

EMTICS has also significantly boosted the European Society for the Study of Tourette Syndrome (ESSTS), as well as aiming to standardise clinical practices for TS across Europe, increasing the standards of care and coordinating national research efforts into pan-European initiatives. Since all clinical partners are members of the European Society for the Study of Tourette Syndrome (ESSTS), EMTICS has enabled participating centres to further strengthen and expand already existing collaborations channelling their expertise towards a large collaborative research programme. Not only in Europe, but also on a worldwide level, the network has been the first interdisciplinary and international consortium of such an extent and possibility.

Structuring research capacities in Europe

The “Helsinki Mental Health Declaration for Europe”, signed by the ministers of health of the member states in the “World Health Organisation European Region”, recognised the deficit in research infrastructure for mental health in Europe, and pledged to promote research and support evaluation and dissemination. The EMTICS studies have been designed exactly to address the pan-European need of delivering a cohort of young researchers that will advance the field of research in mental health (and particularly the field of childhood-onset disorders) and translate their expertise into practice. The partners of the EMTICS consortium constitute a team of leading experts from around Europe with knowledge in diverse but inter-connected fields, ranging from neuropsychiatry, neurology, and neuropsychology to neuroscience, genetics, epidemiology and statistics. This infrastructure has provided a network of expertise with unique synergies, leading to scientific results and expertise at a level that is far beyond the capacity of any local research programme.

Career possibilities for young researchers

Within EMTICS, leading experts from European institutions with a long-standing expertise in the multiple disciplines that are required for the study of childhood-onset psychiatric disorders provided a research environment with excellent (training) possibilities for young researchers exposing them to state-of-the-art scientific knowledge ranging from basic to applied and clinical research. These young researchers within EMTICS have thus received a multi-methodological education and a set of skills that combines clinical practice with neuroscience which helped shape their future career in research (in academia or the private sector). They have been trained how to translate clinical issues into scientific research and vice versa and have been

educated in phenomenology, psychopathology, and treatment of TS and associated psychiatric comorbidities, a wide range of research methods, epidemiology, genetics, database management and statistical analysis, research methodology, and research software. The young researchers had the opportunity to develop a holistic view of a neuropsychiatric disorder as an example for other psychiatric disorders (e.g. mood disorder, schizophrenia) as well. EMTICS combined the challenging task of excellent training with original and innovative research. The young researchers have been trained to handle ambitious scientific research projects in the exciting field of research in childhood-onset psychiatric disorders in an interdisciplinary working environment. They acquired a broad range of transferable skills such as teamwork, leadership, and, through our partners from the private sector, commercial awareness. Such skills are urgently needed for careers in academia, industry or government.

Most importantly, young researchers learned how to function and succeed in a dynamic scientific research area. They are now part of a world-class European and interdisciplinary community of researchers sharing a common scientific goal thereby gaining an appreciation of cutting-edge research across a breadth impossible within the confines of a standard research project structure. Joining EMTICS, young researchers have become members of a network of experts in the field of TS research and psychiatry in general. The training and contacts derived from EMTICS have put them in an extremely strong position to make their own career choices, and we are confident that some will become future international scientific leaders.

Finally, through our stimulating programme of public engagement and by being involved in translating scientific knowledge to the public, young researchers have learned to find a language to underline and illustrate the importance of scientific projects for advances in the understanding and care of patients. This in turn will prepare students for future science – public dialogues. The involvement of EMTICS in the planned dissemination activities has widened the perspective of researchers in particular as regards design and approach to research projects which are relevant to the society at large and has inspired them to bring change in society through their science.

Benefits for society

The focus of our studies, TS, can become a paradigm for the development of European policies for the promotion of mental health. Tourette syndrome represents one of the most common childhood-onset neuropsychiatric disorders with an estimated prevalence of about 0.4-1%. Despite of being common, TS was not so far ago considered a rare, odd disease both by professionals and the public. It has a “fringe” status, partly because it can be associated with symptoms and signs causing social misunderstanding and stigmatisation. Advantages in clinical care, epidemiology and research on aetiology and pathophysiology in TS in recent years as achieved through EMTICS have now been paralleled by increases in public awareness and understanding, at least in Europe. The invested research resources that have been dedicated to TS through EMTICS have significantly contributed to earlier recognition, and significantly reduced secondary morbidity due to the avoidance of delayed diagnosis, inadequate treatment and poor counselling. In good cooperation with patient organisations and TS societies EMTICS has contributed a great deal to improve public awareness in several European countries, and to dissemination of knowledge on TS to a broader audience throughout Europe. The outreach activities that we did within EMTICS have improved public awareness on the importance and societal impact of TS and collaborative research activities carried within EMTICS will ultimately improve quality of life for TS patients.

There is also major impact of EMTICS beyond TS as many of the findings are also relevant for neuropsychiatric disorders in general. It has been estimated that about 10 to 20% of children and adolescents suffer from mental health problems worldwide (Braddick et al., 2009). Neuropsychiatric disorders are the second cause of disability-adjusted life years (DALYs) in Europe and account for 19%. A recent publication of the European Community, surveying different European countries, revealed the fact that 1) childhood and adolescent mental health issues are not included in all relevant higher education qualifications, 5) budgets dedicated to such issues are not identifiable, specific or sufficient in the long term, and 6) stakeholders and the general public are not considered to be widely aware of the determinants of childhood and adolescent mental health. The foundation for good mental health is laid in the early years and society as a whole benefits from investing in children and families. Across the EU, one in every 5 children and adolescents suffers from developmental, emotional or behavioural problems and approximately 1/8 have a clinically diagnosed mental disorder. These disorders have a major social and psychological impact on the child and family. The financial costs arising from their occurrence are considerable and fall not only on health, but also on social, education and voluntary services and on the families themselves.

4.2. Main dissemination activities of EMTICS

Organisation of conferences

EMTICS researchers from UniROMA and ASL-Bari in Italy organized two large (about 300 attendees) conferences on tics and Tourette Syndrome in Rome, one entitled “Therapies of childhood tic disorders” in May 2016 and the other one entitled “La Sindrome die Tourette: a scuola, a casa, a Roma”, and one smaller conference (about 50 attendees) on “Thinking and Movement” in Bari in November 2014.

Organisation of workshops for scientists & for civil society

The EMTICS consortium in general has been very active in maintaining an active dialogue with patients and the public, and in training young researchers and personnel within the project’s workforce. In total, more than 20 workshops have been organised since the start of the project in 2011. Major training workshops of up to 100 attendees for EMTICS researchers and involved scientists included the three microbiological workshops by partner ISS and DH-PHE (former HPA) on a) the storage and dispatch of bacterial strains, b) typing of group A streptococci, and c) the laboratory diagnosis of streptococcal infections in Colindale, UK, in May 2012, the “Habit reversal training with tic disorders” organised by partner UZH in August 2012, a workshop on “Tourette syndrome - course, causes and therapeutic methods” by partner VCAH in May 2016, and a large workshop with about 150 attendees about “Movement disorders: a journey between thought, language and movement” by partner ASL-Bari in September 2017. Further workshops educated in the newest treatments and therapeutic approaches used for tic disorders. The latter were usually held in the language of the respective institution and country.

In addition, six interactive workshops were held for both scientists, as well as the civil society, including patients or relatives to answer questions, inform about tic disorders, Tourette syndrome, new treatment strategies, and hear first-hand about the everyday challenges of dealing with tic disorders. Special efforts in this area were made by partners VCAH, UZH and MHH; partner SCMCI (Clalit Health Services, Israel) also organised an “EMTICS site day” to discuss progress, problems and future ideas.

Flyers, press releases, media briefings/interviews & articles published in the popular press

In addition to the 35 EMTICS publications to date (24 of them peer-reviewed, and 57 more manuscripts are currently in preparation, see appendix of the confidential 4th Periodic Report), the official EMTICS project flyer (available in English and Italian) as well as EMTICS recruitment flyers were distributed amongst the partner sites and beyond, four press releases were published, seven media briefings and/or interviews were given (including a “Question time for the Minister of Health” in Italy), and five articles about EMTICS, its research, tic disorders, and its relation to streptococcal infections were published in the popular press by partners ASL-Bari, UZH and TUD. Also, feel free to click below for a link to a 25-min long radio special featuring EMTICS researchers in BR2 (German radio station):

<https://www.br.de/radio/bayern2/sendungen/iq-wissenschaft-und-forschung/tics-gehirn-hirnschrittmacher-tourette-100.html>

Websites, video clips & multimedia

The official EMTICS project website (www.emtics.eu) was updated on a regular basis. The website addresses interested parties within the general public as well as participating children and their parents or relatives. In addition, a password-protected intranet was designed for, and used frequently by the EMTICS consortium members to share files, access project-relevant updates and information, and for access to the EU documents (description of action, grant agreement, milestone lists etc.). See below for an excerpt of the EMTICS website.

Partners Prolimmune, ISS, and ASL-Bari used video-technology to communicate EMTICS-related scientific content. Of particular interest is the Hungarian dubbing of the US-American HBO-production “I have Tourette’s, but Tourette’s doesn’t have me” by partner VCAH in April 2014. A link to a brief English preview of the HBO video clip:

<http://www.tourette.org/about-tourette/tourettes-doesnt-have-me/>

In addition, partner TUD used Facebook and an online-platform for neurology and psychiatry to search for and attract study participants, and partner VCAH made psycho-educational materials for families affected by Tourette syndrome available online, and also posted several interviews and video clips of kids suffering from tic disorders online. Examples are accessible below:

1. <https://www.neurologen-und-psychiater-im-netz.org/kinder-jugend-psychiatrie/news-archiv/meldung/article/europaweite-studie-zu-erblichen-komponenten-von-tics-studienteilnehmer-gesucht/>
2. https://index.hu/tudomany/2018/04/16/meglepo_magyar_eredmeny_a_tourette-szindromas_gyerekek_jobban_tanulnak/?token=25fdf16614aeb892ce9ecee16e6e3b4
3. <http://tourette.hu/35-114/gal-petra-riportja-a-tourette-szindromarol-az-rtl-klubon.html>
4. <https://24.hu/belfold/2018/05/25/tikkel-tourette-tanulas/>

Oral presentations to a wider audience

The EMTICS consortium also ensured to organise presentations and events for the general public and a wider audience. The following outreach activities and presentations were made possible in the country's respective native language by partners AMC, FCRB, LMU, MHH, TUD, UMCG, UniROMA, VCAH and UZH (titles translated into English for this report):

- May 2013: "The EMTICS project in Switzerland" (UZH)
- July 2013: "Pharmacological treatment of Tourette syndrome" (FCRB)
- December 2013: "Tourette syndrome – an update" (UMCG)
- July, September, November 2014: "Tic disorders / Tourette syndrome", "Tic Family Day", "Support Group Tics" (TUD)
- October 2014: "Everyday life with Tourette's Syndrome" (VCAH)
- November 2014: "Tourette research in Germany – clinical studies and current research projects" (MHH)
- June 2015: "PANDAS" (AMC)
- October 2015: "Current studies on tic disorders" (LMU)
- January 2016: "Quirks, Obsessions, Tics" (TUD)
- January 2018: "Tourette syndrome in child psychiatry" (UniROMA)

Oral presentations and posters at scientific events

In total, more than 90 oral presentations and posters were disseminated amongst experts within the field at scientific events all over Europe, Israel and in the USA, such as the annual meeting of the European Society for the Study of Tourette Syndrome (ESSTS), the annual congress of the European College of Neuropsychopharmacology (ECNP), the 1st World Congress on Tourette Syndrome and Tic Disorders (June 2015, London, UK), the German Endocrine-Brain-Immune-Network (GEBIN) Congress, the annual conference of the Israel Society for Biological Psychiatry (ISBP), the annual Italian meeting of Young Researchers in Physiology (SIF), the annual meeting of the German Tourette Society (TGD), the European Society for Child and Adolescent Psychiatry (ESCAP), the BIO International Convention, the Protein Engineering Summit (PEGS), or a local event of the Tourette Syndrome Association of Barcelona. Concentris presented the EMTICS project furthermore at the annual ECNP Congress in form of a professional exhibition (booth, flyers, personal conversations and presentations).

4.3. Exploitation of EMTICS results

Three major exploitable foregrounds have resulted from research performed over the course of the EMTICS project:

- The beneficiary ProImmune Ltd. advanced general knowledge regarding the flow cytometric evaluation of immune responses in children and sample management in clinical trials involving blood samples for analysis in cell-based immunoassays. ProImmune Ltd. intends to use the gained knowledge to develop a novel product in the field of “immune monitoring devices” within the next years. The device may be exploited commercially and made available for contract research services. Implementing cell-based immune monitoring for clinical trials in children is very challenging and requires practical experience which participation in this project has provided. The experience gained through EMTICS provides ProImmune Ltd. with a long-term competitive advantage in this area.
- Advanced Practical Diagnostics (APD) developed a research kit for the measurement of monomeric CRP (mCRP), and an IVD kit for pentameric CRP (pCRP). The mCRP kit has been developed during and on behalf of the EMTICS project. In combination with pCRP, it should improve the assessment of inflammation (acute versus chronic). However, further validation on other sample panels is required because the measurement of mCRP/pCRP in the EMTICS sample panel did not reveal a significant discrimination. Thus, the timeframe for commercial exploitation will not fall in the immediate future, but likely within the next two years.

5 Address of the project’s public website & relevant contact details

The public website of EMTICS has been continuously updated, and can be found under the following address: www.emticts.eu



The screenshot shows the EMTICS website interface. At the top, there is a banner with a child's face and the text "TICS often have a favourable outcome as children grow older". The EMTICS logo is also present, with the tagline "European Multicentre Tics Study". Below the banner is a navigation menu with the following items: HOME, ABOUT EMTICS, THE EMTICS STUDY, PATIENTS AND RELATIVES (highlighted in orange), NEWS, CONTACT, and MEMBERS. Below the menu, a breadcrumb trail reads: "You are here: Home > Patients and relatives > Why should I participate in EMTICS?". The main content area is titled "Why should I participate in EMTICS?" and contains a list of bullet points:

- Tourette syndrome and other chronic tic disorders are common, childhood-onset neuropsychiatric conditions characterised by the presence of multiple sudden, rapid, recurrent, and non-rhythmic movements (motor tics) and/or utterances (vocal tics).
- Apart from tics, many individuals with Tourette syndrome experience additional neurobehavioural problems such as inattention, hyperactivity and impulsivity, and obsessive-compulsive symptoms.
- Unfortunately, our understanding of the nature of Tourette syndrome is still poor.
- The aim of EMTICS is to have a better understanding of what causes tics

On the left side of the main content area, there is a vertical list of links: "What are tics?", "Which types of tics exist?", "How can tics be treated?", "How can I participate in EMTICS?", "What is the aim of EMTICS?", "Why should I participate in EMTICS?" (highlighted in orange), and "Links/Further Readings". On the right side, there is a "QUICK LINKS" section with the following items: "CONTACT", "News & Events", "Members", and "Patient's Participant Area".

Excerpt from the official EMTICS website

Below is a list of all active beneficiaries (27 in total) and contact details of the team leaders at each of these institutions. For clarification: Beneficiary 13 (BHAM) has been terminated, and is therefore not listed anymore. Former beneficiary 19 (UKE) has been terminated, and beneficiary 28 (UZL) added as a new partner due to move of the principal investigator from UKE to UZL. Former beneficiary 07 (HPA) underwent a “Partial Transfer of Rights and Obligations” to beneficiary 29 (DH-PHE). Contact details for each beneficiary can also be found on the EMTICS website under “About EMTICS => Members”.

Beneficiary	Title	First Name	Last Name	Email
01 UMCG	Prof.	Pieter	Hoekstra	p.hoekstra@accare.nl
02 UNIBA	Dr.	Maura	Buttiglione	maura.buttiglione@uniba.it
03 UCL	Prof.	Anette	Schrag	a.schrag@ucl.ac.uk
04 LMU	Prof.	Norbert	Müller	norbert.mueller@med.uni-muenchen.de
05 Cytolab	Mr.	Adrian	Urwyler	a.urwyler@gmx.ch
06a ISS-GL	Dr.	Roberta	Creti	roberta.creti@iss.it
06b ISS-RC	Dr.	Giovanni	Laviola	gianni.laviola@iss.it
06c ISS - PR	Dr.	Paolo	Roazzi	paolo.roazzi@iss.it
08 TUD	Prof.	Veit	Roessner	veit.roessner@uniklinikum-dresden.de
07 HPA	terminated (explanation see above)			
09 DUTH	Prof.	Peristera	Paschou	ppaschou@gmail.com
10 ProlImmune	Dr.	Nikolai	Schwabe	nschwabe@proimmune.com
11 GSK (NVD)	Dr.	Immaculada	Margarit Y Ros	immaculada.x.margarit-y-ros@gsk.com
12 SCMRI	Prof.	Alan	Apter	asapter@gmail.com
13 BHAM	terminated (explanation see above)			
14 UniROMA	Prof.	Francesco	Cardona	francesco.cardona@uniroma1.it
15 APD	Dr.	Pascale	Verstappen	pascale.verstappen@apdia.be
16 QMUL	Dr.	Ute Christiane	Meier	u.meier@qmul.ac.uk
17 UNICT	Prof.	Renata	Rizzo	rerizzo@unict.it
18 concentris	Dr.	Sara	Stöber	sara.stoeber@concentris.de
19 UKE	terminated (explanation see above)			
20 VCAH	Dr.	Zsanett	Tárnok	tarnok@vadasnet.hu
21 SAS	Dr.	Pablo	Mir	pmir@us.es
22 UZH	Prof.	Susanne	Walitza	susanne.walitza@kjpzdzh.ch
23 FCRB	Dr.	Astrid	Morer	amorer@clinic.ub.es
24 RegionH	Prof.	Kerstin	von Plessen	kerstin.plessen@regionh.dk
25 MHH	Prof.	Kirsten	Müller-Vahl	mueller-vahl.kirsten@mh-hannover.de
26 GSTT	Dr.	Tammy	Hedderly	tammy.hedderly@gstt.nhs.uk
27 ASL-Bari	Dr.	Cesare	Porcelli	cesareporcelli@icloud.com
28 UZL	Prof.	Alexander	Münchau	alexander.muenchau@neuro.uni-luebeck.de
29 DH-PHE	Dr.	Androulla	Efstratiou	androulla.efstratiou@phe.gov.uk
30 AMC	Dr.	Chaim	Huyser	c.huyser@debascul.com