

ESF Exploratory Workshop on

**Neuropsychiatric disorders
associated with streptococcal
infection in Europe**

London (United Kingdom) October 22 - 23, 2009

Convened by:
**Gavin Giovannoni, Pieter Hoekstra
and Davide Martino**

SCIENTIFIC REPORT

1. Executive summary

The ESF-sponsored workshop “Neuropsychiatric disorders associated with streptococcal infection in Europe” was held in London (United Kingdom) on October 22-23, 2009, convened by prof. Gavin Giovannoni (Centre for Neuroscience and Trauma, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK), dr. Pieter Hoekstra (Department of Psychiatry, Faculty of Medical Sciences, University of Groningen, the Netherlands), and dr. Davide Martino (Department of Neurological and Psychiatric Sciences, Faculty of Medicine, University of Bari “Aldo Moro”, Italy). The main goal of this workshop was to bring together a multidisciplinary group of European psychiatrists, child psychiatrists, neurologists, epidemiologists, microbiologists, geneticists, and immunologists with a research interest in Neuropsychiatric disorders associated with Group A Streptococcal (GAS) infection, with the aim of establishing a research consortium to study this group of disorders.

Tourette syndrome (TS) and obsessive-compulsive disorder (OCD) are commonly underdiagnosed paediatric neuropsychiatric disorders, and few specialist centres in each country exist. In addition, expertise in the multiple factors that could lead to the development of these disorders is specialised and requires collaboration between laboratories with expertise which are geographically separate and are located in multiple European countries. For this reason a single centre or national approach is not possible to solve this complex problem. A European collaboration offers the opportunity to bring together the expertise from several centres and countries and to provide sufficient numbers of clinically diagnosed patients to allow for such a study. In order to undertake such a study, we plan to apply for funding from the European Commission.

Participation numbered 25 people from the United Kingdom, Italy, Spain, the Netherlands, Germany, Hungary, France and Turkey.

Surroundings permitted additional informal interaction between the workshop participants, favouring useful exchange of ideas and friendly contact during the social event. General atmosphere was very pleasant and allowed participants to gain mutual acquaintance.

The workshop was structured in topics, in 3 theme sessions, and each session was chaired/co-chaired by an expert in the respective theme. Sessions comprised presentations, followed by ample discussions.

In the final Discussion that concluded the Workshop, a detailed action plan concerning the organization of the project was elaborated. Agreement was reached on the main deliverable objective to be reached by an integrated project, consisting in drawing a conclusion on the relationship between onset of TS/OCD and prior exposure to an immune-mediated process triggered by GAS infections. We aim at pursuing this by following up in time potentially at-risk subjects, identified among first-degree unaffected relatives of patients suffering from these conditions. The general implication of this objective is the possibility to identify a priori at risk subjects who might benefit from vaccine or antibiotic prophylaxis.

2. Scientific content of the event

In the first two sessions, the workshop comprised a review and discussion of the current literature and research on neuropsychiatric disorders associated with streptococcal infection. The research update was meant to provide the back drop to discuss what further research is needed in this area.

The first session, chaired by prof. G. Giovannoni, provided a research update on epidemiology, biology and immunology of GAS, in relationship to TS and OCD. An update on streptococcal biology and immunology was meant to stimulate discussion on how we can incorporate new technologies in relation to streptococcal research into our proposed research programme.

Dr. Anette Schrag (Royal Free Hospital, University College London) summarized the epidemiological data on the association between GAS infections and TS/OCD, which included a retrospective UK population-based study, conducted and recently published by dr. Schrag on Neurology. TS and OCD are common in the general population, affecting approximately 1% and 3% of school children, with a childhood/adolescence onset of these conditions. GAS infections typically occur in children aged 5-15 years old. There is considerable overlap between TS and OCD, with high rates of obsessive-compulsive behaviours in children with TS, higher rates of OCD in relatives of patients with TS and higher rates of tics or TS in first-degree relatives of patients with OCD. TS appears to be a genetic disorder but no single gene has been identified despite numerous large scale studies. The genetic mechanisms are likely to be complex and other, environmental factors are thought to also be important, including gestational, perinatal, toxic, psychosocial, and immunological disorders, including infections. Outbreaks of neuropsychiatric disorders with tics and obsessive compulsive behaviour have been reported following GAS, associated with immunological abnormalities similar to Sydenham's chorea (PANDAS syndrome). Studies to assess whether streptococcal infections are related to onset and/or exacerbations of TS and/or OCD in clinic-based studies have suggested that GAS infections may also play a role in the aetiology of more typical TS or OCD. On a community-basis, only three studies have assessed this association in the wider population. The results from these studies are conflicting, with two studies suggesting a small increase in risk of previous streptococcal throat infections before onset of TS or OCD and one negative study. However, these studies have important limitations, in particular the lack of diagnostic confirmation of GABHS infection with laboratory investigations and unstructured primary care diagnosis of TS or OCD. Prospective studies to assess the risk of development of TS/OCD following GABHS infection in at-risk individuals are urgently needed.

Dr. Guido Grandi (Novartis Vaccines, Siena, Italy) and dr. Graziella Orefici (Istituto Superiore di Sanità, Rome, Italy) presented an update on their recent research on immunological response to GAS exploiting a very recent multi-array approach developed by Novartis, and recently published on PlosOne. In the attempt to shed light on the contribution of GAS infections to the onset of neuropsychiatric or behavioral disorders affecting as many as 3% of children and adolescents, dr. Grandi and dr. Orefici, among other collaborators, tested the antibody response of tic patient sera to a representative panel of GAS antigens. In particular, 102 recombinant proteins were spotted on nitrocellulose-coated glass slides and probed against 61 sera collected from young patients with typical tic neuropsychiatric symptoms but with no overt GAS infection. Sera from 35 children with neither tic disorder nor overt GAS infection were also analyzed. The protein recognition patterns of these two sera groups were compared with those obtained using 239 sera from children with GAS-associated pharyngitis. This comparative analysis identified 25 antigens recognized by sera of the three patient groups and 21 antigens recognized by tic and pharyngitis sera, but poorly or not recognized by sera from children without tic. Interestingly, these antigens appeared to be, in quantitative terms, more immunogenic in tic than in pharyngitis patients. Additionally, a third group of antigens appeared to be preferentially and specifically recognized by tic sera. These findings provide the first evidence that tic patient sera exhibit immunological profiles typical of individuals who elicited a broad, specific and strong immune response against

GAS. This may be relevant in the context of one of the hypothesis proposing that GAS antigen-dependent induction of autoantibodies in susceptible individuals may be involved the occurrence of tic disorders.

The second session, chaired by dr. Norbert Muller (University of Munich, Germany) and by dr. D. Martino, focused on physiological and pathological aspects of host immunology with regard to post-streptococcal immune-mediated conditions.

Dr. P. Hoekstra provided an overview on cell-mediated and antibody-mediated mechanisms in TS/OCD. Increased activation of immune responses in TS is suggested by changes in gene expression profiles of peripheral immune cells, relative frequency of lymphocyte subpopulations, and synthesis of immune effector molecules. Increased activity of cell-mediated mechanisms is suggested by the increased expression of genes controlling natural killer and cytotoxic T cells, increased plasma levels of some pro-inflammatory cytokines which correlate with disease severity, and increased synthesis of anti-neuronal antibodies. Important methodological differences might account for some inconsistency among results of studies addressing autoantibodies in TS. Finally, a general predisposition to autoimmune responses in TS patients is indicated by the reduced frequency of regulatory T cells, which induce tolerance towards self-antigens. Although the pathogenic role of immune activation in TS has not been definitively proven, a pathophysiological model was proposed to explain the possible effect of immunity upon dopamine transmission regulation and the generation of tics.

Prof. Norbert Muller (Ludwig-Maximilians-Universität, München, Germany) expanded upon dr. Hoekstra's presentation and summarized all the case reports and systematic studies pointing out to an involvement also of other pathogens in the pathogenesis of TS/OCD, among which *Borrelia burgdorferi* and *Mycoplasma pneumoniae*.

Following an overview on the role of streptococcal superantigens and their potential role in autoimmunity, given by dr. Shiranee Sriskandan (Imperial College, London), dr. Brian Henderson (University College London) gave an interesting talk on the biochemical and functional nature of a category of proteins, moonlighting proteins, which are among the most likely 'candidate antigens' for autoimmune cross-reactivity between GAS and human brain. Over the past 15 years it has been discovered that many of the common metabolic enzymes of the cell, particularly the glycolytic pathway and the Krebs' cycle, have multiple functions. Proteins with more than one function have been termed moonlighting proteins. Other proteins that commonly moonlight are the highly conserved molecular chaperones and evidence was presented for the intimate involvement of certain molecular chaperones in the pathogenesis of tuberculosis caused by the pathogen, *Mycobacterium tuberculosis*. One of the major bacteria to use glycolytic enzymes as moonlighting virulence factors is GAS. Indeed, it is now established that most of the streptococci have glycolytic enzymes on their cell surfaces. Indeed, it looks like the whole glycolytic pathway may exist on the surfaces of these bacteria. What possible function can these cell surface glycolytic enzymes play. It is now established that cell surface glyceraldehyde-3-phosphate dehydrogenase (GAPD) on the surface of GAS functions as a cell signalling molecule allowing entry into pharyngeal cells and protecting the bacterium from neutrophil phagocytosis. The role of the cell surface enolase on GAS is to bind plasminogen and the conversion of this zymogen to the active protease enables the bacterium to gain entry into the body past the extracellular matrix barriers. It is likely that all the other cell surface glycolytic enzymes have similar pathogenic actions. A fascinating mirroring of this cell surface expression of glycolytic enzymes on streptococci is the finding of glycolytic enzymes on the outer surface of certain neurons. In both the bacterium and the neuron a likely function for these surface proteins is as part of a signalling mechanism. Thus it is possible that glycolytic enzymes or their products function, in both bacteria and neurons, as cell-cell signalling molecules with key homeostatic functions. One sequela of the presence of cell surface glycolytic enzymes on streptococci is that these proteins are inappropriately immunogenic and antibodies to them can cross-react with cell surface glycolytic proteins on neurons to cause psychopathology. This is a fascinating hypothesis that needs to be actively tested.

Prof. David Baker (Queen Mary, London) summarized potential avenues of animal model research to be followed to increase our understanding on the mechanisms of post-streptococcal pathology. Dr. Nikolai Schwabe (Promega, UK) updated participants on novel commercial high-throughput methodologies to perform epitope mapping in human immune-mediated diseases, presenting all the potential areas of interest of this new technology for the development of an integrated research project on post-streptococcal brain disorders. Finally, dr. Ute Meier (Queen Mary, London) summarized available knowledge on innate immune mechanisms in the pathophysiology of TS/OCD. Adaptive immune responses are immune responses tailored to a specific pathogen, leading to immunological memory. Innate immune responses on the other hand are thought to be unspecific inflammatory responses, activated within hours of pathogen encounter, which help mount functional adaptive immune responses thus pivotal for pathogen clearance. A major component of innate immunity is a series of sentinel peripheral cells that act as “trouble detectors”, e.g. natural killer cells, dendritic cells and macrophages but innate cells can also be found in the brain, e.g. microglia, astrocytes and oligodendrocytes. These cells display innate receptors, which recognize pathogen associated “stranger and danger signals”. The most studied pattern recognition receptors are Toll like receptors and scavenger receptors. Many pathogens have acquired elaborate strategies to escape innate immune recognition including GAS. Alterations of innate responses have been reported in a subgroup of patients with Tourette’s syndrome, e.g. over-activity of innate/inflammatory responses. Dr. Meier proposed a detailed analysis of “innate immune status” in Tourette’s syndrome, suggesting potential new research objectives.

The third session focused on an update by dr. Hoekstra and prof. Giovannoni, followed by discussion regarding strategies to obtain European funding to investigate neuropsychiatric disorders associated with streptococcal infection.

In the final Discussion that concluded the Workshop, a detailed action plan concerning the organization of the project was elaborated. Agreement was reached on the main deliverable objective to be reached by an integrated project, consisting in drawing a conclusion on the relationship between onset of TS/OCD and prior exposure to an immune-mediated process triggered by GAS infections. We aim at pursuing this by following up in time potentially at-risk subjects, identified among first-degree unaffected relatives of patients suffering from these conditions. The general implication of this objective is the possibility to identify a priori at risk subjects who might benefit from vaccine or antibiotic prophylaxis.

3. Assessment of the results, contribution to the future direction of the field, outcome

The subdivision of the forthcoming project agreed upon by the participants will be in the following sections:

- 1) Clinic-based longitudinal observation study on a large cohort of subjects (500 patients and 500 control patients). This will include detailed phenotyping and impact of disease on quality of life, microbiological and immunological assessment. A yearly follow-up with active surveillance of tics and pharyngitis episodes of high-risk first-degree unaffected relatives of TS/OCD probands, and of control subjects with other post-infectious disorders, other neurological illnesses unrelated to immune-mediated mechanisms.
- 2) Microbiological study with genotyping and characterisation of GAS strains. This should include genome sequencing of certain strains. Methodologies might include conventional microbiology, whole genome sequencing, super antigens (phage), regional / lab control isolates, microbiology sensitivity, and EQA control.
- 3) Adaptive and innate immunology study, that will evaluate innate, B cell and T cell immunological responses to streptococcal proteins and putative autoantigens. This should involve biobanking, control serology (Mycoplasma, Borrelia, Chlamydia, Tetanus), total IgG, M, A & E and isotypes, epitope mapping – Proimmune; anti-strep response Novartis antigen chip; T-cell studies with auto-antigens and strep proteins; innate immune markers, peripheral super-antigen signatures – FACS transcriptome – mRNA and micro-RNA, DNA – HLA typing.
- 4) Studies investigating functional effects of anti-basal ganglia antibodies and animal models

4. Final programme

Thursday, 22 October 2009

Afternoon	<i>Arrival</i>
14.30	<i>Refreshments</i>
15.00-15.10	Welcome by Convenor Gavin Giovannoni (Queen Mary, London, UK)
	Session 1: Introduction
15.10-15.30	Presentation of the European Science Foundation (ESF) Haluk Topaloglu (ESF Standing Committee for the European Medical Research Councils (EMRC)
	Session 2: Group A Streptococcus Chairs: Gavin Giovannoni (Queen Mary, London, UK) and
15.30 -16.00	The role of Group A Streptococcal infections in paediatric neuropsychiatric disorders: epidemiology update Anette Schrag (Univesity College London, London, UK)
16.00 - 16.30	Recent advances in the detection of adaptive immune responses to streptococcal antigens Guido Grandi (Novartis Vaccines, Siena, Italy)
16.30- 17.00	Recent advances in isolation, growth, genomics and genotyping of GAS from clinical specimens : <i>the Italian experience</i> Graziella Orefici (Istituto Superiore di Sanita, Rome, Italy)
17.00-17.30	Role of other infectious agents in triggering paediatric neuropsychiatric disorders Norbert Müller (University of Munich, Germany)
17.30-18.00	<i>Coffee/ Tea Break</i>
18.00-19:30	Discussion of participants Chairs: Gavin Giovannoni (Queen Mary, London, UK) and Graziella Orefici (Istituto Superiore di Sanita, Rome, Italy)
	Which microbiological outcome measures should be analysed in order to integrate clinical research and commercial/industrial aims?
19.30	<i>Get-together, social event, informal (London)</i> <i>Dinner</i>

Friday, 23 October 2009

Morning	<i>Arrival</i>
08.30	<i>Refreshments</i>
	Session 3 Immunological responses Chairs: Norbert Muller (?, Munchen, Germany) and Davide Martino (University of Bari "Aldo Moro", Bari, Italy)

09.00 -09.30	Literature update: cell-mediated and antibody-mediated mechanisms in TS/OCD Pieter Hoekstra (University Medical Centre, Groningen, Netherlands)
09.30-10.00	Streptococcal superantigens and their role in autoimmunity Shiranee Sriskandan (Imperial College, London, UK)
10.00-10.30	Moonlighting streptococcal proteins Brian Henderson (University College London, London, UK)
10.30-11.00	Animal models of post-infectious neuropsychiatric disorders David Baker (Queen Mary, London, UK)
11.00-11.30	<i>Coffee/ Tea Break</i>
11.30-12.00	Monitoring adaptive cellular immunological responses Nikolai Schwabe (Promega, UK)
12.00-12.30	Monitoring innate immune responses Ute Meier (Queen Mary, London, UK)
12.30-13.00	Discussion of participants Which immunological outcome measures should be analysed?
13.00-14.00	<i>Lunch</i>
	Session 4
	Lobbying and strategic issues
	Chairs: Gavin Giovannoni (Queen Mary, London, UK) and Pieter Hoekstra (University Medical Centre, Groningen, Netherlands)
14.00-14.30	Lobbying strategies aiming at specific FP calls: update on the lobbying activity in 2008-2009 (achievements and obstacles) Pieter Hoekstra (University Medical Centre, Groningen, Netherlands)
14.30-15.30	Review of proposal Gavin Giovannoni (Queen Mary, London, UK)
15.30-16.00	<i>Coffee/ Tea Break</i>
16.00-17.00	Discussion
17.00-17.30	Action plan and closing summary Gavin Giovannoni (Queen Mary, London, UK)
17.30	<i>End of Workshop and departure</i>

5. Final list of participants

Gavin GIOVANNONI, Queen Mary University of London, UK
Pieter HOEKSTRA, University of Groningen, Netherlands
Davide MARTINO, University of Bari "Aldo Moro", Italy
Haluk TOPALOGLU, **ESF**, **EMRC**
Anette SCHRAG, University College London, UK
Renata RIZZO, University of Catania, Italy
Andrea Eugenio CAVANNA, Amedeo Avogadro University, Italy
Pablo MIR, University of Seville, Spain
Andreas HARTMANN, University Paris VI, France
Emmanuelle DENIAU, University Paris VI, France
Francesco CARDONA, University of Rome "La Sapienza", Italy
Graziella OREFICI, Istituto Superiore di Sanità, Italy
Norbert MULLER, Ludwig-Maximilians-University, Germany
Markus Schwarz, Ludwig-Maximilians-University, Germany
Eszter KENEZLOI, Vadaskert Child and Adolescent Psychiatry Hospital, Hungary
Luca FARKAS, Vadaskert Child and Adolescent Psychiatry Hospital, Hungary
Zsanett TARNOK, Vadaskert Child and Adolescent Psychiatry Hospital, Hungary
Astrid MORER, University Hospital Clinic/ Universitat de Barcelona, Spain
Jeremy STERN, St. George's University of London and St. George's Hospital, UK

Expert External speakers:

Nikolai SCHWABE, ProImmune Limited, UK
Guido GRANDI, Novartis Vaccines and Diagnostics S.r.l, Italy
David BAKER, Queen Mary University of London, UK
Ute MEIER, Queen Mary University of London, UK
Shiranee SRISKANDAN, Imperial College London, UK
Brian HENDERSON, University College London, UK

Local Organiser:

Surinder PAL, Queen Mary University of London, UK

6. Statistical information on participants

Country of Origin		
	Core delegates	External delegates
United Kingdom	3	5
Italy	5	1
Spain	2	
Hungary	3	
Netherlands	1	
France	2	
Germany	2	
Turkey	1	
Gender		
Male	11	4
Female	8	2
Age		
20-30	3	
30-40	3	1
40-50	11	4
50-60	2	1