Impact Objectives

- Better understand the pathophysiology of epilepsy and its consequences
- Develop a preventive strategy for epilepsy
- Develop new therapeutic targets to block or otherwise modify epileptogenesis in humans

The search for epilepsy biomarkers

*The **EPISTOP** project seeks to develop new biomarkers of epilepsy and to identify new therapeutic targets to block or otherwise modify epileptogenesis in humans. The findings could significantly improve the disease burden of epileptic patients around the world*

Epilepsy is one of the most common neurological diseases, known to impact around 1 per cent of the global population. Six million people have epilepsy in Europe alone. It can begin at any age, but most often occurs in childhood or people over 60, with the highest incidence happening in the first year of life. Epilepsy in infants and children is a major health issue, with 50 per cent of children suffering significant comorbidities such as developmental delay, learning disabilities and autism spectrum disorders that are part of the same neurodevelopmental process.

Tuberous sclerosis complex (TSC) is a syndrome increasingly recognised as an important etiology of epilepsy. TSC is of special interest to epilepsy researchers because it is considered a clinical model of severe focal epilepsy. Between 70 and 90 per cent of TSC patients have epilepsy and unfortunately, in many TSC patients, the seizures are drug-resistant, so the usual anti-seizure medications have limited benefit. TSC shares its molecular basis with many other conditions associated with epilepsy, so, advances in TSC treatments may also benefit patients with epilepsy.

Research has shown that before seizures occur, there is a period during which the brain gradually develops a hyperexcitable state, known as epileptogenesis. Recently, more research is targeting the earliest stages of epileptogenesis to prevent the development of epilepsy. In TSC, more patients receive the diagnosis before seizures occur, rendering them good candidates to monitor in the silent phase of epileptogenesis. Given the high frequency of epilepsy in TSC, its pathophysiology and molecular biology is considered of greater overall importance in studying epileptogenesis and considering treatment aimed at preventing epilepsy. The observational study published by project coordinator Professor Sergiusz Jóźwiak in 2011, showed that preventive antiepileptic treatment reduces the risk of severe epilepsy and its neuropsychological comorbidities in infants with TSC.

MULTIDISCIPLINARY SYSTEMATIC APPROACH

An extensive international research collaboration involving scientists from 16 hospitals and laboratories from 10 countries was set up to further epilepsy research. Funded by the European Union's Seventh Framework Programme for research, technological development and demonstration, the multidisciplinary project, 'Long-Term, Prospective Study Evaluating Clinical and Molecular Biomarkers of Epileptogenesis in a Genetic Model of Epilepsy – Tuberous sclerosis Complex' (EPISTOP) is a five-year project comprising a clinical and a molecular component. The chief aim was to improve understanding of the development of epilepsy. A multicentre, prospective, randomised and controlled trial was performed to compare the clinical outcomes of preventive versus standard antiepileptic treatment in TSC infants. The inter-disciplinary research team worked to identify new molecular biomarkers of epilepsy and develop new targets to block or modify epileptogenesis in humans.

The EPISTOP project was divided into eight work packages, each with a leader responsible for performing the package's associated tasks. As the project spanned 10 different countries globally, the establishment of efficient coordination was essential from the outset. Work Package (WP) 1 was therefore focused on managing the overall project and coordinating the work of everyone involved in the project. 'The study proved that preventive treatment of epilepsy in TSC successfully decreases the number of patients with epilepsy, percentage of TSC children with drug-resistant epilepsy and the percentage of TSC children with intellectual disability,' highlights Jóźwiak.

'The reduced risk of seizures and drug-resistant epilepsy will significantly improve the quality of life of children with TSC and their families'

SUCCESSFUL OUTCOMES

Many important findings resulted from this substantial and collaborative effort, including the filling of many knowledge gaps throughout the five-year period. One major finding related to the clinical study showed that the risk of early seizures and severe epilepsy is reduced with preventive treatment when compared to standard management in TSC infants. 'I believe that the reduced risk of seizures and drug-resistant epilepsy will significantly improve the quality of life of children with TSC and their families,' explains Professor Katarzyna Kotulska, WP6 Leader.

Another important finding related to electroencephalograms (EEG), which are recordings of brain activity and the focus of WP2, led by Lieven Lagae. Small sensors are attached to the scalp to pick up the electrical signals that are produced when brain cells send messages to each other. The main use of an EEG is to detect and investigate epilepsy, so the procedure was of obvious interest to the team. They were able to prove that EEG can reliably predict epileptogenesis in TSC and may be used for identification of children with high risk of seizures. Ultimately, this means that use of serial EEG preventive treatment is feasible for this group of patients.

WP5, led by one of the principal investigators, Eleonora Aronica, looked at biomarkers in brain specimens. Their efforts focused on the validation of biomarkers of epilepsy and molecular targets for novel therapies in human TSC. 'Over the course of the investigations, this work package was able to support the hypothesis that other developmental brain diseases can share the same hallmarks of immaturity leading to intractable seizures observed in TSC,' comments Aronica.

'Importantly, evidence of shared cellular and molecular mechanisms underlying the mammalian target of rapamycin (mTOR)-related epileptogenesis was provided during the studies.' It can be concluded from this work that intellectual disability, neurobehavioral and psychiatric disorders are intrinsically related to the disease process.

Their study of the TSC structural and molecular networks has uncovered evidence for different cellular and molecular mechanisms that underlie the neurological and neuropsychiatric phenotypes. These include myelin pathology, which is associated with impaired oligodendroglial turnover and impacts both white and grey matter and GABAergic and glutamatergic neurotransmitter receptor functions that both retain features typical of an immature brain, supporting the hypothesis that developmental immaturity may be the underlying reason behind severe paediatric epilepsies and their associated treatment challenges. Furthermore, the prominent activation of innate and adaptive immune responses characteristic of mTOR pathway-related malformations have been highlighted in the team's analysis of the coding and non-coding gene regulatory networks in TSC, leading to the identification of critical pathways and possible new treatment targets. EPISTOP researchers have also identified that small non-coding microRNA has modifiers of neuroinflammation, neurogenesis and oxidative stress, providing an epigenic-driven therapeutic tool for both epilepsy and cognitive disabilities in TSC.

EARLY DIAGNOSIS AND MONITORING

WP6, which was the clinical study looking at preventive versus standard treatment, also delivered valuable lessons. Implementation of early surveillance improved the neuropsychological outcome in all TSC infants participating in the project, in comparison to published data on children in whom epileptogenesis had not been tracked. 'I was pleased with our results showing the beneficial effect of early clinical and EEG monitoring on epilepsy outcomes. Ultimately, the investigations showed that early diagnosis and monitoring in the first two years of life is crucial in TSC,' explains Kotulska.

WP4 looked at neuroimaging biomarkers of epileptogenesis. 'We found a significant relationship between MRI characteristics and age at first abnormal EEG, presence of clinical seizures or refractory epilepsy,' says WP4 leader, Floor Jansen. MRI characteristics were also related to cognition and language indexes at two years of age, however, age at seizure onset appeared to be the stronger predictor for cognitive outcome.

WP7, led by Professor Paolo Curatolo, was focused on the identification of early clinical predictors of neurodevelopmental trajectory deviation during the first two years of life. 'Our results showed that toddlers at higher risk of autism and cognitive impairment can be correctly identified already at 12-18 months of age,' he explains.

The project has delivered a significant amount of data and findings and has demonstrated that preventive anti-seizure treatment improves clinical outcomes with respect to epilepsy characteristics in TSC. Signs of neurodevelopmental problems can be detected at a very young age, paving the way for early intervention in the future and improve outcomes for patients. The challenge now will be to take the lessons learned and apply these to real-world settings.

Collaborative effort to improve epilepsy outcomes

An international consortium of researchers has come together to better understand the pathophysiology of epilepsy and develop preventive strategies. They discuss their respective work packages, some of the challenges they have faced and the next steps for the project


Kasia Kotulska


David Kwiatkowski


Lieven Lagae


Floor Jansen


Paolo Curatolo


Sergiusz Jóźwiak


Anna Jansen


Eleonora Aronica

Can you introduce yourselves and describe some of the key findings from your specific work package and how these have supported the overall objectives of the EPISTOP project?

Sergiusz Jóźwiak: I am based in the Department of Paediatric Neurology at the Warsaw Medical University and am the Project Coordinator. I am pleased to see that the obtained results are confirming our first studies carried out at The Children's Memorial Health Institute in Warsaw. We enjoyed a good collaborative relationship between partners and all European WP leaders had the opportunity to host other partners of the consortium during our regular steering committee or annual assembly meetings. These meetings enabled better communication and understanding between partners, which was very important in such a multi-national project.

Katarzyna Kotulska: I am based at the Children's Memorial Health Institute in Poland and the leader of WP6. I was responsible for writing the study protocols, the preparatory work for the clinical part of the project and for patient recruitment and data collection. Overall, WP6 was very successful and very rewarding. As the consortium, we achieved the targeted recruitment goal of 100 patients and very few discontinued the study. WP6 provided data on the clinical safety and efficacy of early versus standard treatment of epilepsy in children with TSC. Standard antiepileptic treatment is given when the first clinical seizures appear in TSC infants. Even when implemented immediately after the first clinical seizures, this treatment is associated with high risk of drug resistant epilepsy and the development of severe intellectual disability and other neuropsychological comorbidities. Our team previously carried out the study that suggested that preventive treatment, introduced before the onset of clinical seizures, but after registration of epileptiform EEG activity, might improve epilepsy and neurocognitive outcome of children with TSC. With WP6, we confirmed that preventive treatment reduces the risk of early seizures and severe epilepsy in infants with TSC.

Lieven Lagae: I am the leader of WP2 and am Professor of Paediatric Neurology at KU Leuven, Belgium. It is well known that the large majority of children with TSC will develop epilepsy in the first year of life and that epilepsy in these children has a major impact on overall psychomotor development. In this prospective study, we looked at the role of EEG, taken every four to six weeks during the first two years. We found that EEG changes occur before the onset of clinical seizures and early occurrence of severe epileptiform discharges on the EEG correlates to worse developmental outcomes. Preventive treatment before the start of clinical seizures is beneficial to the children in terms of epilepsy and development. Our data shows EEG can be used to predict epilepsy and developmental outcome in infants with TSC.

Floor Jansen: I am based in the Department of Child Neurology at the Brain Center UMC Utrecht, the Netherlandsand leader of WP4. When the diagnosis of TSC is made, patients and caregivers are confronted with a very high risk of developing epilepsy. Many factors are related to the onset of seizures and the severity of epilepsy. The main objectives of the work package were to investigate neuroimaging findings as biomarkers of epilepsy risk and epileptogenesis in TSC patients. We classified and quantified structural MRI abnormalities in TSC infants before the age of four months. We related the MRI characteristics to epilepsy characteristics and neuropsychological assessments at the age of two years. Our results show that MRI characteristics significantly contribute to the development of epileptogenesis. This finding will improve our care and guidance in infants with TSC.

Paolo Curatolo: I am based at the Tor Vergata University in Rome and am a principal investigator of the EPISTOP project and leader of WP7. The objective of my work package was to identify the biomarkers of epilepsy comorbidities: autism and neurodevelopmental delay. While intellectual disabilities and autism ASD are frequent comorbidities in children with TSC and epilepsy, there are no clinical or molecular biomarkers to distinguish whether a patient will develop autism and cognitive impairment.

The stratification of patients according to their neuropsychological and neurocognitive profile at the age of two years is crucial in interpreting the effectiveness of preventive therapy versus standard therapy. Furthermore, identification of clinical neuropsychological and neurocognitive markers leads to the implementation of preventive therapeutic strategies, lowering the incidence of neuropsychiatric comorbidities of childhood epilepsy, including autism and neurodevelopmental delay in infants and young children.

David Kwiatkowski: I am Professor of Medicine at Harvard Medical School, Senior Physician at Brigham and Women's Hospital and one of the three investigators who originally conceived the project. I lead WP3, which is focused on the identification of molecular biomarkers of epilepsy risk and epileptogenesis in TSC patients. Our analysis of molecular biomarkers is still incomplete, but we have tentative evidence that certain markers, especially microRNA, may be able to identify epileptogenesis in TSC and forecast future epilepsy development and neurodevelopmental outcomes.

Eleonora Aronica: I am a principal investigator and the leader of WP5. We performed a detailed neuropathological evaluation of cortical autopsy and surgical specimens from patients with TSC at different developmental ages and a systematic evaluation of TSC cortical tubers, perituberal cortex for biomarkers of epileptogenesis and molecular targets of antiepileptogenesis emerging from gene expression studies in TSC and different animal models. We identified genes and proteins of interest and peripheral epilepsy biomarkers were evaluated and validated in human brain tissue. TSC brain tissue was useful in identifying and validating several genes and proteins as potential therapy targets and peripheral epilepsy biomarkers and represents a valuable tool to better understand the complex network changes associated with epilepsy and related cognitive and behavioural comorbidities in TSC.

What are the next steps for the group?

Floor Jansen: We will finalise the results and hope to develop a model that predicts the risk of epilepsy and neurodevelopmental disorders, integrating a variety of clinical and molecular characteristics. We intend to publish many scientific papers, disseminate our findings at national and international meetings and aim to improve the standard of care for infants with TSC.

David Kwiatkowski: During the next year, we will complete our analyses of molecular biomarkers, integrating findings from proteomics, metabolomics and DNA, RNA, and miRNA analyses to identify a comprehensive set of biomarkers that correlate with clinical events in TSC infants. We will use advanced statistical methods including machine learning. We will also validate these findings in other cohorts of TSC infants, ideally leading to broad adoption of molecular markers for the care of TSC infants and improvement in seizure prevention and neurodevelopmental outcomes.

Sergiusz Jóźwiak: I am pleased to observe that since the beginning of EPISTOP, many sites worldwide, including a major US National Institutes of Health (NIH)-funded trial, are introducing EEG surveillance and attempting preventive treatment in TSC infants. Thus, EPISTOP has already impacted on the care of infants with epilepsy before our data is fully analysed and reported. The EPISTOP consortium has applied for a new European Commission project. We hope to work on epilepsy in other conditions to validate the results obtained in EPISTOP.

EPISTOP dissemination

Anna Jansen is the dissemination manager and one of the principal investigators for EPISTOP. She is based at the Vrije Universiteit Brussel, Belgium. Translating the findings from the project into real-world applications requires consideration of how best to communicate the project's progress and what can be expected in the future. With that in mind, the team has created several channels through which information and updates are found.

Website: http://www.epistop.eu/
Facebook: https://www.facebook.com/epistop.TSC/
Instagram: https://www.instagram.com/epistop_fp7/?hl=nl
Twitter: https://twitter.com/EPISTOP_TSC
YouTube: https://www.youtube.com/watch?v=Y4QfOlHRhnk

Project Insights

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