Can you share a little about how your research careers have developed and how this collaboration came about?

**SJ:** As a paediatric neurologist, I have worked with epilepsy patients since the late 1980s. This was the beginning of my interest in Tuberous Sclerosis Complex (TSC). The crucial moment was about 10-12 years ago, when we were disappointed with our results in the treatment of children with TSC, even when they were diagnosed prior to birth, and could be clinically monitored carefully for epilepsy development. Even in that group of infants at the Children’s Memorial Health Institute in Warsaw, we observed that half of the infants developed refractory epilepsy and intellectual disability. This led us to think about preventative treatments.

**EA:** My interest in the process of epileptogenesis began during my research as a student of medicine and at the department of neuropharmacology, and continued during my neurology/neuropathology residency and postdoctoral fellowship. I studied the mechanism of epileptogenesis using both experimental models and samples obtained from patients undergoing surgery for intractable epilepsy. As a neuropathologist, I’m particularly interested in genetic malformations of the cerebral cortex (MCD) associated with epilepsy and cognitive and behavioural problems. Understanding epileptogenesis and its relation to progressive cognitive dysfunction in these disorders is challenging, and TSC serves as an important disease model. Over 10 years ago I started to work on TSC and related MCD associated with a deregulation of the mTOR (Mammalian target of rapamycin) pathway. When Professor Jozwiak contacted me to discuss the possibility of building a research consortium focusing on the epileptogenesis of TSC, combining clinical and basic research, I was really enthusiastic. This project represented a great opportunity for me to continue to work in this field of research, together with highly specialised and dedicated TSC experts.

**DK:** I trained as a medical oncologist and then became interested in genetics. I learned about TSC, and was initially attracted to study it because of a wide variety of associated tumours. I developed an international collaboration, which identified one of the causative genes (TSC1). I met Professor Jozwiak in 1991 and this led to a lifelong collaboration between us. I also became interested in epilepsy and brain development in TSC, and developed many mouse models of TSC brain disease. More recently, I have had an active role in the National Institutes of Health’s Cancer Genome Atlas project, and this has given me experience working with large collaborations and diverse data sets, including all of the molecular markers to be studied as part of EPISODE.

Could you talk a little about the collaborative nature of the project?

**SJ:** It is a really global project, both geographically and by its scientific nature. Ten clinical sites are collecting clinical data, and molecular partners will add their molecular input. The multifactorial analysis of all obtained data will be a real challenge and will require extensive collaboration of all partners.

**EA:** I’m involved in the WPs focusing on the identification of molecular biomarkers in blood and brain. Blood and tissue samples are provided by the different collaborating clinical sites and the results of the molecular groups will be integrated with the relevant clinical, electroencephalography (EEG), neuroimaging and genetic findings provided by the other partners. Thus, all partners are contributing to the identification and validation of biomarkers.

Could you explain more about the real-world benefits of this research?

**EA:** The identification of risk factors and biomarkers of epilepsy will help provide insight for early diagnosis and early intervention (identifying a therapeutic window in TSC) and to define pathogenic mechanisms of cognitive impairment and autism. Moreover, identification of new therapeutic targets may also contribute to the development of a therapy able to modify epileptogenesis in other forms of epilepsy in humans.
Epilepsy remains a mysterious disease. Whilst certain traits of an epileptic seizure are well known, the exact mechanism remains unidentified. Equally, there is very little knowledge about how to identify those at risk other than by association with other diseases or injuries. Epileptic seizures are characterised by hypersynchronisation between excitatory neurons in the brain. In this scenario, these neurons are firing at a very high rate and show a characteristic spike and wave discharges on an electroencephalography (EEG). An EEG is the process of measuring global electrical activity in the brain, either through invasive or non-invasive electrodes positioned at different points. The difficulty in diagnosing epilepsy before a seizure occurs means preventative treatment is, as it currently stands, almost impossible. This is a particular problem in early-onset epilepsy. When epilepsy occurs in infancy, the damage on the developing brain can be significant and lasting. This will often lead to intellectual disability and development of autistic spectrum disorders in previously normal children. This is particularly concerning as 65 per cent of epilepsies begin in childhood.

In addition to the severity and difficulty in diagnosis, epilepsy is also difficult to treat. Antiepileptic drugs are not always effective, with many displaying drug resistance.

This is a particular problem for sufferers of Tuberous Sclerosis Complex (TSC), a genetic disease affecting 1 in 6,000 people, in which patients show a high rate of benign tumours. TSC patients are considered an excellent model for epilepsy, as approximately 70-90 per cent will present with epilepsy, and the vast majority will be drug resistant. Additionally, they will have their first seizures in infancy and are thus at high risk of developing TSC-associated neuropsychiatric disorders (TAND).

In a world first, doctors and researchers joined forces to launch EPISTOP, a long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in TSC. This project is aimed at tackling the major problems of epilepsy — early diagnosis and treatment — using TSC as a model. Professor Sergiusz Józwiak of the Children’s Memorial Health Institute, Warsaw, is the principal investigator coordinating a large, global group of researchers to prospectively investigate molecular and genetic biomarkers, the identification of those who will develop epilepsy and the methods of treatment of these individuals. Overall, 16 institutions and companies in 10 countries are collaborating in this study. These include many of the leading experts in TSC, epilepsy and clinical treatments. ‘The idea of EPISTOP came from our previous work,’ Józwiak explains. ‘Results showed preventative treatment might reduce the incidence of epilepsy, and drug-resistant epilepsy in particular. We invited colleagues working with TSC to join us for the project.’

BUILDING BIOMARKERS
An essential component that makes EPISTOP unique is a focus on gathering a wide range of biomarkers. These studies will help with a variety of issues facing epilepsy and its treatment. First, the identification of key molecular and physiological markers of disease will allow for a quick and accurate diagnosis of those at risk. Secondly, molecular and cellular indicators will prove essential in finding or highlighting potential targets for future treatment methods. And finally, findings in this area will uncover key steps in the development of intellectual disability. Professor Eleonora Aronica of the Academic Medical Center, University of Amsterdam in the Netherlands summarises these aims: ‘The identification of risk factors and biomarkers of epilepsy will provide insights for early diagnosis and early intervention, and define pathogenic mechanisms of cognitive impairment and autism. Moreover, identification of new therapeutic targets may also help develop a therapy able to modify epileptogenesis in humans.’
Aronica is personally involved in the establishment of a database and biobank of brain tissues from TSC patients. This biobank will allow for the collection of a large number of samples from which it will be possible to identify common features. In TSC patients, this might be crucial information on how the cortex matures in comparison to ordinary development. Noting developmental differences is essential in understanding how the TSC brain is more prone to epilepsy, and therefore the underlying tissue changes that will increase the chance of developing the disease. Furthermore, these samples can be investigated at a molecular level. Looking in such detail will enable Aronica and her team to identify more biomarkers but, crucially, more potential targets for treatment. As she explains: ‘Inflammatory pathways and microRNAs related to a dysregulation of the inflammatory response could prove to be targets and an important aim will be to identify miRNAs that could be used as peripheral biomarkers of epileptogenesis in TSC.’ This histopathological work will be vital to understanding how the brain develops in epilepsy prone individuals, and how this has an impact on the disease.

Complimentary to Aronica’s work is that of Professor David Kwiatkowski of Harvard Medical School and Brigham and Women’s Hospital in the US. Kwiatkowski is responsible for overseeing a genomic, transcriptomic and proteomic analyses based on blood samples from TSC patients, as well as non-TSC volunteers. He will also be looking at establishing the miRNA profile of the samples, which will provide further information on gene expression in these individuals. Analysis of this large data set will ideally allow identification of molecular and genetic biomarkers that may be used to identify at-risk patients through a blood sample alone. In addition, this analysis will also be used to identify differences between those individuals with and without epilepsy, drug-resistant versus treatable epilepsies, and those who respond to pre-emptive treatment prior to seizure development versus those who do not. Overall, research into biomarkers will give EPISTOP crucial information that will highlight not only how to identify epilepsy, but also some of the fundamental aspects of its development and molecular characteristics.

**CLINICAL WORK**

Essential to the EPISTOP project is the close cooperation and interaction between clinical and basic scientists to integrate the molecular analyses with the clinical findings. Józwiak aims to integrate further information on the nature of preventative treatment of TSC using currently available medications. The planned trial will involve children with TSC less than four months. They will be monitored until the 24th month of life for signs of paroxysmal activity as noted by EEG, and half of those displaying this will be treated with conventional epilepsy drugs. Non-TSC children will be used as controls. Initially, the work is designed to provide further evidence for Józwiak’s work on preventative treatment. However, information about changes in biomarkers amongst the different groups will be used to offer the key link between the biomarker work of Aronica and Kwiatkowski, and its application to a clinical setting. Discoveries from the trial will also help focus the hunt for appropriate biomarkers.

The close ties between the clinical investigation and fundamental academic research, along with the idea of preventative treatment, sets EPISTOP apart from similar projects. So far, the EPISTOP team has met its first major goal – enrolment of 101 TSC infants. As Józwiak explains: ‘We are now beginning the clinical and molecular analyses, so it is a very exciting time. It is clear EPISTOP will provide huge benefits, not only to TSC patients, but also to those with all varieties of epilepsies.'