ADVANCED NEUROIMAGING STUDIES IN STURGE-WEBER SYNDROME: CLINICAL CORRELATES

Ph.D. thesis

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I. BACKGROUND OF THE STUDIES

I.A. The Sturge-Weber syndrome

The Sturge-Weber syndrome (SWS), also known as encephalofacial angiómatosis, is a sporadic neurocutaneous disorder, one of the diseases that are classified under the phakomatoses. It is a rare congenital syndrome with an estimated incidence of 1 per 50000 live births with equal gender distribution. Although the genetic and environmental factors leading to the disorder are not clarified, a somatic mutation has been suspected due to the sporadic and localized nature of SWS. It has been suggested that a vascular developmental disruption occurs during the first trimester *in utero* which manifests in functional and morphological abnormality of vessels of the facial skin, eye and brain. The classic hallmark of the disease is the facial cutaneous naevus (the so-called naevus flammeus or port-wine stain) which is usually located in the territory of the trigeminal nerve. The leptomeningeal angióma, the classic intracranial sign of SWS is thought to be the result of the failure of the primitive cephalic venous plexus to regress and properly mature in the first trimester of pregnancy. The close embryological proximity of the facial ectoderm and the portion of the neural tube eventually forming the parieto-occipital region of the brain provide a feasible explanation for the association of the classical facial port-wine stain and posterior brain involvement in SWS.

The leptomeningeal angiomatosis is considered to be the primary intracranial abnormality resulting in secondary intracranial pathologies, like brain atrophy and cortical calcifications. The pathological changes usually involve unilateral brain areas, however, bilateral intracranial involvement may be seen in 7.5-15% of cases. Importantly, the chronic damage of the affected brain region leads to neurological manifestations of variable degree, including early onset seizures, stroke-like episodes, visual field cut, motor and cognitive deficits and migraine. Neurological manifestations of SWS are often progressive, ultimately leading to profound neuro-cognitive decline. However, disease progression varies widely. Importantly, the factors influencing the variable outcome are incompletely understood and conventional clinical or imaging modalities are insufficient to predict clinical outcome at the early stage of the disease.

Histopathological studies of brains affected by SWS demonstrate abnormally developed, tortuous, thin-walled vascular structures in the thickened leptomeninges. The underlying cerebral cortex is often atrophic and shows neuronal loss, gliosis, calcification and malformations of cortical development. Subcortical and cortical calcifications, which can also be seen on brain CTs *in vivo*, are thought to be the result of anoxic injury of endothelial, perithelial and glial cells. Abnormal permeability of cerebral vessels and consequently increased passage of proteins and calcium has also been proposed as putative factors in calcification. Cortical vessels adjacent to the angióma are also thin and narrowed by subendothelial proliferation. Impaired innervation, as well as altered extracellular matrix protein expression have also been implicated in the pathogenesis of structurally and functionally abnormal vessels. It has been hypothesized that impaired superficial venous drainage through the abnormal leptomeningeal vessels leads to venous stasis and chronic hypoxia of the underlying gray matter and white matter. Often there are additional, partly compensatory changes, such as the development of prominent transmedullary collateral veins, hypoxia induced angiogenesis and remote functional reorganization. These complex mechanisms are poorly understood and need to be further elucidated. Chronic cortical ischaemia is associated with various pathological features including altered blood-brain barrier

function and local metabolic dysfunction. Abnormal autoregulation of blood flow coupled with the temporary increases in oxygen and metabolic demand during seizures may also contribute to the progressive brain injury. Recent data provided evidence that, similar to facial port-wine stains, leptomeningeal angiomas of SWS may also be not static lesions but can undergo a proliferative process after birth. Intriguingly, in addition to enhanced endothelial cell turnover, increased expression of the hypoxia-inducible factor (HIF-α) and vascular endothelial growth factor (VEGF) has been shown in surgical angióma specimens. Future studies are needed to elucidate whether ongoing angiogenesis might be a therapeutic target in SWS. Furthermore, it is important to emphasize that malformations of cortical development (such as polymicrogyria and focal cortical dysplasia) are commonly seen in surgical specimens of SWS patients with intractable, severe epilepsy. Early ischemic insults can contribute to the development of these highly epileptogenic lesions, even in brain areas distant from the angióma.

I.B. Neurological implications of SWS

Neurological manifestations of SWS are highly variable with variable progression; some patients develop properly, while others have severe seizures and progressive clinical course. Seizures are often the presenting neurological symptoms in children with SWS. Seizures occur in about 80-85% of patients and the onset of the first episode is usually (in 75% of the cases) within the first year of life. It has been also demonstrated that the occurrence of epilepsy is higher and seizure onset may occur earlier in patients with SWS and bilateral leptomeningeal angiómatosis than in those with unilateral lesions. The predominant seizure types are partial motor and complex partial, but infantile spasms may also occur. Epilepsy can be benign in SWS, however, infantile onset is frequently associated with severe, catastrophic epilepsy and cognitive impairment. Importantly, later onset of seizures, as well as early and long-term control of epilepsy have been associated with better neurological outcome.

Abnormal vascular autoregulation during seizures (especially when frequent and prolonged) has been suspected as a major mechanism contributing to the exacerbation of brain injury and cognitive decline. Seizures are controlled with medication in about 40-50% of the patients. If pharmacological treatment fails to control seizures, surgical techniques such as hemispherectomy, focal resection or corpus callosotomy are the treatment of choice. Not surprisingly, early resective surgery after careful evaluation, and complete resection of the affected brain region are related to better cognitive and seizure free outcome. It is important to note that successful epilepsy surgeries have been reported also in a few patients with bilateral intracranial abnormality.

Stroke and stroke-like episodes are also characteristic features of SWS and manifest as acute, temporary episodes of motor, sensory disturbances or visual field defects not directly related to epileptic activity. Weakness may even persist for a long time or permanently after a severe stroke-like episode. These episodes are attributed to recurrent thrombosis which contribute to saltatory neurological decline; however, the exact pathomechanism remains elusive.

About 50-60% of children with SWS will show developmental delay or mental retardation, or both; progressive cognitive decline is commonly associated with early onset, intractable seizures. As the incidence of cognitive dysfunction (i.e., below-average cognitive function) is considerably higher in SWS patients than in children with epilepsy unrelated to SWS (~50-80 % vs. ~25 %), other neurological features, unique to SWS most likely also contribute to mental retardation. For instance, a recent study demonstrated that white matter volume loss ipsilateral to the angióma may play a major role in cognitive impairment in children with SWS.

In addition to cognitive issues, children with SWS show a broad range and variable degree of neuropsychological dysfunction including mood disorders, noncompliance, attention–deficit hyperactivity disorder and oppositional behaviors.

Other common neurological features include migraines, hemiparesis and hemianopia; importantly, the latter ones can worsen after severe seizures or stroke-like episodes.

Currently, SWS cannot be prevented or cured. Management of neurological complications is largely limited to seizure control. Since seizures, along with complicated migraines and stroke-like episodes may lead to further brain ischemia and metabolic injury, an aggressive antiepileptic therapeutic approach is warranted. In addition, low-dose acetylsalicylic acid may reduce the number of stroke-like episodes, however, further studies are needed to substantiate this. As early cortical resection is associated not only with good seizure outcome, but also with improved cognitive function, it would be essential to identify patients who likely undergo unfavorable progression without surgery. Better understanding of the mechanisms underlying the progressive disease and the wide variability in neuro-cognitive outcome may identify future therapeutic targets. In vivo examination of the brain affected by SWS using advanced neuroimaging tools is a crucial part of this research.

I.C. Neuroimaging in Sturge-Weber syndrome

Magnetic resonance imaging (MRI) in SWS. Conventional MRI techniques, including T1-weighted post-gadolinium MRI, are often used to establish the diagnosis of SWS and to assess the extent and severity of intracranial structural involvement as well as progressive brain damage during the course of the disease. For clinical purposes, conventional MR imaging protocols include at least native spin-echo T1-weighted and T2-weighted images, as well as post-gadolinium T1-weighted images. The latter sequence provides a gold standard for delineation of the characteristic findings of leptomeningeal angioma. Further typical neuroimaging features of SWS include microcalcifications, brain atrophy (involving both gray matter and white matter), enlargement of the choroid plexus and ipsilateral hypertrophy of the cranium. Notably, *gradient-recalled echo* (GRE) sequences are well suited for the detection of calcium-rich regions.

Since conventional brain MR sequences may miss subtle structural abnormalities (e.g., small angiomas), and are not suitable for assessing functional aspects of brain involvement, advanced MRI methods have been recently implemented mostly for research purposes. For instance s*usceptibility weighted imaging (SWI)* can depict fine details of the abnormal deep venous network, choroid plexus, as well as calcified gyriform abnormalities. Furthermore, studies utilizing *perfusion weighted imaging (PWI)* have demonstrated decreased blood perfusion in areas with meningeal enhancement, however, perfusion abnormalities may extend beyond the area of leptomeningeal angioma. During the last decade, *diffusion tensor imaging* (DTI) emerged as a sensitive MRI application to measure microstructural changes in white matter as well as gray matter. DTI is sensitive to the diffusion of water molecules which is known to be higher along fiber pathways. By fitting a model (diffusion tensor model) to the diffusion measurements in each image voxel, it is feasible to estimate useful parameters such as the fractional anisotropy (FA), which is a measure of the degree of diffusion directionality (range: 0-1). Another important DTI parameter is the mean diffusivity (MD) which represents the overall mobility of water molecules. Possible data processing methods include region of interest (ROI) based and voxel-wise analysis of extracted FA, MD or other parameter maps. FA and MD values may be altered (usually FA decrease and/or MD increase) in pathological processes affecting tissue integrity that can occur as a result of various pathologic processes. Previous DTI studies in SWS have demonstrated white

matter microstructural damage extending beyond the apparent cortical abnormalities. Diffusion abnormality in posterior white matter has been associated with worse cognitive function. Another DTI study, using tractography, showed impairment of the corticospinal tract integrity ipsilateral to the angioma even before clinical motor symptoms. DTI may also be useful in detecting accelerated myelination during the early course of the disease, when other, conventional MRI techniques may not show abnormalities. In summary, DTI is a sensitive tool to detect early microstructural changes in the brain of patients with SWS, even before macrostructural abnormalities become apparent. *MR volumetry*, an objective quantitative approach for measuring global brain as well as segmented gray and white matter volume loss using high resolution MR. A study using semi-quantitative scores for the characterization of hemispheric atrophy in patients with SWS showed a good correlation between more severe brain atrophy and lower overall clinical severity scores as well as hemiparesis subscores. In a more sophisticated, segmentation-based analysis white matter but not gray matter volume loss ipsilateral to the angioma proved to be an independent predictor of cognitive functions.

Importantly, results of DTI as well as MR volumetry studies further highlight the importance of white matter integrity during the course of the disease. These data indicate that successful protection from white matter injury during the early phase of the disease would be a powerful approach to prevent poor clinical outcome in SWS.

Positron emission tomography (PET) in SWS. PET as well as single photon emission computed tomography (SPECT) are non-invasive functional imaging tools which can detect various chemical and metabolic processes in body organs like the brain. The physical basis of PET is the emission of positrons by unstable nuclei of isotopes as they decay into more stable structures. The PET technique employs a camera with multiple pairs of oppositely situated detectors, which are used to record the paired high-energy (511 KeV) photons traveling in opposite directions (~180°) as a result of collision of electrons and positrons. In general, PET images have a spatial resolution in the mm range which is superior to that of SPECT, and also provide better signal-to-noise ratio. Tracer kinetic models that mathematically describe biochemical or physiologic reactions of compounds labeled with positron-emitting isotopes allow for the characterization of kinetics and calculating rates of the biological process being studied.

The most generally used PET tracer is 2-deoxy-2[1^8F]fluoro-D-glucose (FDG) to measure glucose metabolism of the brain. Under steady-state conditions, the measured FDG uptake reflects the utilization rate of exogenous glucose. In the brain, this rate is highly related to the synaptic density and functional activity of the brain tissue. It is important to note that absolute metabolic rates undergo major maturational changes during the first year of life; however, the overall glucose metabolic pattern of the brain is largely fixed after this early period. Therefore, focal decreases or increases in FDG uptake can be reliably identified in activity images without calculating absolute metabolic rates. In addition, in disorders, like SWS, which most often affect only one hemisphere, the contralateral hemisphere can be used as an internal control for evaluating unilateral focal abnormalities.

FDG PET is increasingly used in pediatric neurology as a tool for pre-surgical evaluation of patients with medically intractable epilepsy. Although FDG PET is currently not routinely used in the radiological evaluation of patients with SWS, it can provide useful information regarding functional imaging correlates of neuro-cognitive impairment. In SWS, FDG PET demonstrates reduced glucose metabolism of the cortex underlying the angioma, and even extending beyond the general area of vascular abnormality. Earlier studies have also shown that the extent of mild-moderate hypometabolism was larger in children with frequent seizures. Furthermore, a recent longitudinal PET study demonstated that the major progression of

hypometabolic cortex in SWS occurs during the first 3 years of life, and higher seizure frequency may be a factor in metabolic progression. Altogether, metabolic imaging data provide support for the argument for early, aggressive seizure control in children diagnosed with SWS and associated epilepsy. Interestingly, a paradoxical pattern of focal interictal glucose *hyper*metabolism has also been observed in a few infants. Similarly, increased perfusion (using SPECT) has been reported in infants with SWS who had never had seizures before, and this *hyper*perfusion appears to shift to the classic hypoperfusion with age. However, the exact mechanism and potential prognostic value of this phenomenon is currently unknown. The extent and severity of cortical hypometabolism are important imaging markers of cognitive function. Interestingly, the relationship between cortical hypometabolism and cognitive function is complex and most likely not linear. Factors like the timing of early structural and functional brain damage may influence the cognitive deterioration. This notion is also supported by a study from Lee et al., who demonstrated a paradoxical preservation of IQ in some children who underwent an early, widespread hemispheric injury, reflected by severe, extensive hypometabolism. It has been suggested that early, severe metabolic demise may facilitate effective reorganizational processes in relatively intact parts of the brain. Effective reorganization may also be influenced by the nociferous effects of repeated seizures. Importantly, PET studies can also be useful in depicting functional reorganization, for which there is a great potential in unilateral SWS, since the contralateral hemisphere is basically normal. It is important to note, however, that reorganization processes are most likely hindered in the relatively rare cases of bilateral SWS. This may be an important factor accounting for the generally worse clinical phenotype in this subset of patients.

Although FDG is the primary tracer used in clinical as well as research settings of PET imaging in SWS, amino acid tracers, like [^{11}C]methionine or [^{11}C]leucine may also provide important insights into the pathophysiology of SWS. As these PET tracers can be used to measure proliferative activity of brain tumors, they may also provide an *in vivo* marker of angioma proliferation.

II. AIMS OF THE STUDIES

Our studies aimed to investigate structural and functional brain injury in SWS using advanced imaging techniques. Particular emphasis has been given to explore clinical or prognostic values of certain neuroimaging findings.

Questions addressed:

A. Previous studies have demonstrated a relationship between brain tissue damage and clinical phenotype of SWS. Most studies to date focused on cortical abnormalities. Recent results however showed that subcortical changes, for instance the degree of cortical atrophy as well as white matter volume loss appeared to correlate well with the overall severity of neurologic impairment and cognitive performance, respectively. Nevertheless, the role of focal white matter abnormalities as well as subcortical, particularly thalamic abnormalities has not been studied previously in SWS. Therefore, initially we analyzed microstructural and metabolic alterations of subcortical (thalamic and white matter) structures. Specifically, the following questions were addressed:

1. Is the thalamus microstructurally and metabolically impaired in SWS? If yes, are these abnormalities related to cortical injury?

2. Can thalamic asymmetries provide a simple, independent imaging marker for cognitive and motor functions in unilateral SWS?

3. Which white matter regions are commonly affected in SWS?

4. Is there any detectable white matter injury contralateral to the angioma in unilateral SWS?

5. Are there any specific areas whose demise is associated with motor or cognitive dysfunction?

B. FDG PET usually depicts cortical hypometabolism of variable extent in SWS. However, a seemingly paradoxical phenomenon of increased glucose metabolism, not related to ongoing epileptic activity, has been reported in a few children. The potential clinical significance of this pattern has not been elucidated. Therefore, we addressed the following questions:

6. At which stage of the disease course may interictal cortical *hyper*metabolism occur? Is this phenomenon related to the appearance of first seizures?

7. What is the longitudinal course of the cortical metabolic pattern of increased glucose metabolism?

C. Recent data support the notion that leptomeningeal angiomas, the intracranial hallmarks of SWS, are not static lesions but undergo proliferative changes. Using an amino acid PET tracer (C^{11}-leucine) suitable for in vivo estimation of protein synthesis, we addressed the following questions:

8. Is the uptake of ^{11}C-leucine increased in brain regions affected by the angioma?

9. If yes, what is the mechanism of increased uptake: elevated protein synthesis rate and/or elevated leucine transport?

10. Where are these PET abnormalities located, and what is their relation to glucose metabolism of the affected cortical regions?

D. Bilateral intracranial involvement is rare (~15%) in SWS, and therefore the imaging and clinical characteristics of this subset of patients have not yet been well described. We carried out a retrospective study correlating cortical FDG PET findings with gross clinical characteristics in a series of young patients with bilateral SWS, and we aimed to answer the following questions:

11. Do patients with bilateral SWS invariably have severely impaired neuro-cognitive function?

12. Can cortical pattern or severity of glucose metabolic abnormalities be markers of unfavorable neurological outcome?

13. Is homotopic bilateral cortical damage particularly unfavorable?

III. SUBJECTS

The patients involved in our studies were selected from an FDG PET database which included 110 patients (mostly children) with the clinical diagnosis of SWS. FDG PET scans were performed either at the University of California at Los Angeles (UCLA; n=50; 1986-1993) or at the PET Center, Children`s Hospital of Michigan (CHM; n=60; 1994-2010). Many children were clinically followed in our institution and/or were involved in a longitudinal clinical/imaging research study; thus a subset of patients (n=32) had multiple PET scans. MRI was routinely performed since 2003, including T1+T2 pre- and T1 post-gadolinium, and susceptibility weighted imaging (SWI) as well as DTI sequences since 2005. Quantitative analysis of PET scans and/or MRI data was carried out in a total of 35 patients with unilateral brain involvement; all of these scans were done at the CHM. Additionally, 14 patients had bilateral intracranial involvement (UCLA+CHM), whose PET images were analyzed qualitatively. Patients for specific studies were selected based on the inclusion and exclusion criteria described in the Methods sections of each article.

IV. SUMMARY OF THE STUDIES

1. The role of the thalamus in neuro-cognitive dysfunction in early unilateral hemispheric injury: A multimodality imaging study of children with Sturge-Weber syndrome

[Alkonyi et al., *European Journal of Paediatric Neurology* 2010 Sep;14(5):425-33.]

The thalamus is a major relay center of the brain and could be affected directly (by venous congestion in the deep veins draining the thalamus), as well as indirectly (by way of the cortico-thalamic connections) in SWS. Furthermore, the thalamus is part of a complex network responsible for cognitive functions. The functional and structural impairment of the thalamus in SWS and its potential role in cognitive outcome have not been described.

In this study we applied a combination of diffusion tensor imaging and FDG PET in 20 prospectively collected children (11 girls; age range: 3 years to 12.4 years) with SWS and unilateral hemispheric involvement to assess the abnormality of the thalamus on the affected side. Using an ROI method, average FA, MD values, corresponding asymmetry indices (AIs), as well as FDG metabolic AIs were measured and compared to normative data of control subjects. Furthermore, these measures were correlated with the extent of cortical hypometabolism (in the affected hemisphere) and neuropsychological variables (IQ and dexterity scores).

Glucose metabolic asymmetry of the thalamus was significantly higher in patients than in controls ($p=0.001$) indicating hypometabolism of the thalamus. Although FA and MD of either side of the thalamus did not differ from that of controls after adjusting for age, the asymmetry of FA in patients with *left* hemispheric involvement was significantly higher than in normal controls (lower FA on the ipsilateral side; $p=0.019$). In addition, there was a trend of higher MD asymmetry in patients with *left* hemispheric involvement compared to normals. Thalamic metabolic asymmetry correlated significantly with the asymmetry of MD ($r=-0.69$; $p=0.001$), extent of cortical hypometabolism ($r=-0.75$; $p<0.001$) and IQ ($r=0.59$; $p=0.006$). In the subgroup of patients with left hemispheric involvement (N=13), thalamic glucose metabolic asymmetry remained an independent predictor of IQ after controlling for age and the extent of cortical hypometabolism ($r=0.81$; $p=0.002$). Furthermore, worse fine motor function in the hand

contralateral to the affected hemisphere was associated with more severe glucose metabolic asymmetry of the thalamus (r=-0.66; p=0.002), but not with asymmetries of any DTI parameters.

Our results demonstrate that the injury of the affected hemisphere in SWS also significantly affects the structure and function of the ipsilateral thalamus. Microstructural and metabolic abnormalities of the thalamus are closely related to cognitive functions in unilateral SWS. Thalamic metabolic asymmetry (assessed by FDG PET) is a simple, but robust independent imaging marker of neuro-cognitive outcome in children with early unilateral hemispheric injury related to SWS.

2. Focal white matter abnormalities related to neurocognitive dysfunction: An objective diffusion tensor imaging study of children with Sturge-Weber syndrome
[Alkonyi et al., *Pediatr Research*, 2010 Jan;69(1):74-9.]

Earlier studies of patients with SWS suggested that the severity of subcortical white matter (WM) volume loss, ipsilateral to the angioma, may be a stronger predictor of neuro-cognitive functions than the degree of cortical atrophy. Investigation of the WM is of high clinical relevance, since specific WM regions encompassing various cortico-cortical and cortico-subcortical tracts play a crucial role in motor and neuro-cognitive functioning. In this study we aimed to identify specific WM regions with significantly altered microstructural integrity and regions whose diffusion properties correlate with cognitive or fine motor functions.

Fifteen children with SWS and unilateral hemispheric involvement (age range: 3-12.4 years) were studied and compared to 11 healthy controls (age range: 6-12.8 years). Tract based spatial statistics (TBSS) was used for objective, voxel-wise comparisons of FA and MD between the two groups using age as a covariate. Moreover, within the SWS group local white matter FA and MD values were correlated with IQ and fine motor scores using age as a covariate. Correlations with $p<0.01$ in a minimal cluster size of 3 voxels were considered significant.

Extensive (ipsi- and contralateral), multilobar areas showed decreased FA compared to control subjects (mean FA of these regions in SWS patients: 0.56 vs. 0.74 in controls), while MD changes (increases) were confined to smaller ipsilateral posterior regions (mean MD: 0.882×10^{-3} mm^2/s vs. 0.765×10^{-3} mm^2/s in controls). Clusters of voxels with FA values significantly correlating with full-scale IQ (range: 47-128; mean: 76) were identified in the ipsilateral prefrontal WM, while MD was inversely associated with IQ in ipsilateral posterior parietal WM. The strongest negative correlation between MD and fine motor function (i.e., higher MD associated with worse dexterity) was found in the ipsilateral frontal WM encompassing motor pathways.

Our analysis revealed microstructural abnormalities of WM regions ipsi- and also contralateral to the angioma in SWS. Interestingly, although conventional MRI and PET studies showed no or less injury of the frontal lobes, some frontal areas showed decreased FA on the group level, suggesting common involvement of the frontal lobe WM. Cognitive and fine motor dysfunctions are related to the injury of specific ipsilateral WM regions.

3. Transient focal cortical increase of interictal glucose metabolism in Sturge-Weber syndrome: Implications for epileptogenesis
[Alkonyi et al., *Epilepsia* 2011 Jul;52(7):1265-72.]

Interictal focal cortical glucose *hyper*metabolism has been reported in a few infants with Sturge-Weber syndrome (SWS) and recent seizure onset. To better understand the phenomenon of this unique metabolic phenomenon, we studied clinical correlates and longitudinal course of cerebral glucose *hyper*metabolism in children with SWS.

FDG PET scans of 60 children (age range: 3 months-15.2 years) with Sturge-Weber syndrome, selected from the PET database of the Children's Hospital of Michigan, were reviewed for focal hypo- or *hyper*metabolism. Thirty-two patients had two or more consecutive PET scans (most of them particpitaed in a prospective, longitudinal neuroimaging study on SWS). Age, seizure variables and the occurrence of epilepsy surgery were compared between patients with and without *hyper*metabolism. The severity of increased glucose metabolism was assessed using standardized uptake value (SUV) ratios (ipsilateral/contralateral) and correlated with seizure variables.

Interictal cortical *hyper*metabolism, ipsilateral to the angioma, was seen in 9 patients, with the most common location in the frontal lobe. Mean age as well as time between the scan and onset of the first seizure was lower in patients with *hyper*metabolism than in those without (mean: 1.9 vs. 4.5 years; p=0.022 and 1.0 vs. 3.6 years; p=0.019, respectively). Increased metabolism was transient and switched to hypometabolism in all 5 children where follow-up scans were available (**Figure 1**).

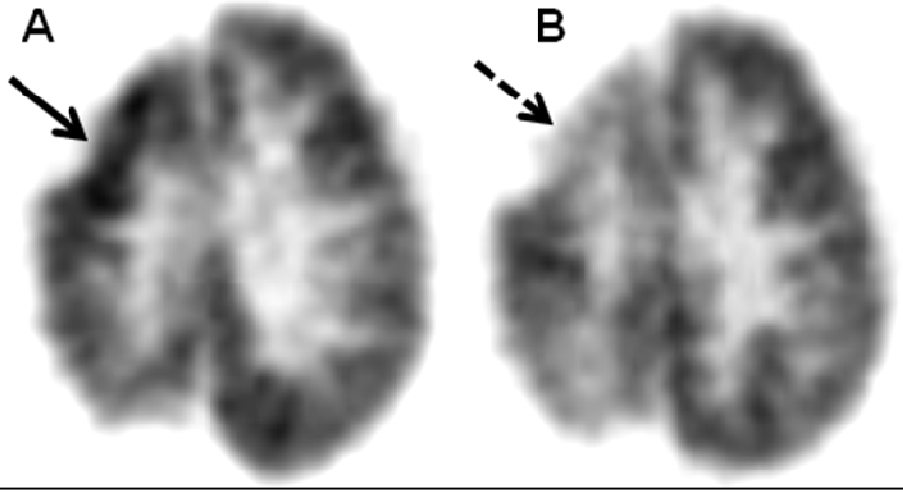

Figure 1: Representative axial slices of the FDG PET scan of a patient with SWS and right hemispheric angioma. Initial scan (**A**) at the age of 1.9 years showed right frontal *hyper*metabolism in addition to hypometabolism of the other lobes. Follow-up scan 7 months later (**B**) demonstrated an interval switch of the *hyper*metabolism to hypometabolism.

Increased metabolism occurred in 28 % of children scanned under the age of 2 years. Children showing *hyper*metabolism had higher rate of subsequent epilepsy surgery as compared to those without *hyper*metabolism (5/8 vs. 10/43; p=0.039).

Interictal focal glucose *hyper*metabolism is common in young patients with SWS and is most often seen within a short time before or after the onset of first clinical seizures. Increased glucose metabolism detected by PET may reflect ongoing cortical excitotoxic damage due to hypoxia. Early presence of *hyper*metabolism predicts later metabolic deterioration and may be associated with worse seizure outcome.

4. Increased L-[1-^{11}C]leucine uptake in the leptomeningeal angioma of Sturge-Weber syndrome: a PET study

[Alkonyi et al., *Journal of Neuroimaging* 2011 Jan 11.; Epub ahead of print]

Leptomeningeal angioma, the characteristic intracranial lesion in Sturge-Weber syndrome (SWS) is traditionally considered to be a static developmental malformation. However, recent histopathology studies demonstrated endothelial proliferation in the angioma as well as underlying cortex. L-[1-^{11}C]leucine PET is useful for the indirect *in vivo* detection of increased cell proliferation by estimating brain tissue protein synthesis rate.

In the present study, we used PET scanning with L-[1-^{11}C]leucine in 7 children (age range: 3 month-13 years) with unilateral SWS to explore if increased amino acid transport and/or protein synthesis rates can be detected in the posterior angioma region and in less affected frontal regions. Leucine uptake (expressed as SUV asymmetries, compared to contralateral homotopic brain regions showing no abnormalities on MRI or FDG PET) was also correlated with glucose metabolic abnormalities measured by FDG PET. Resected brain and angioma tissues were immunostained for Ki-67 (a proliferative marker) and VEGF-A in an infant who underwent hemispherectomy due to intractable epilepsy.

Increased leucine uptake was found in the angioma region in 6 out of 7 patients (mean increase: 15.1%) (**Figure 2**) and less pronounced increases of leucine uptake were also found in frontal cortex (mean increase: 11.5%).

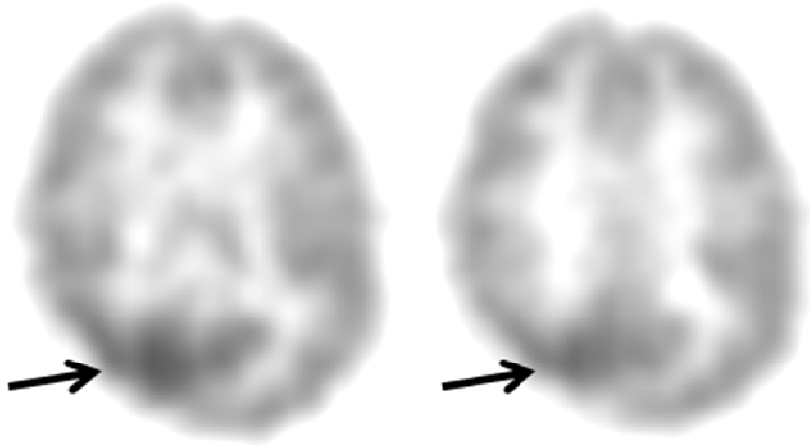

Figure 2: Axial slices of the C^{11}-leucine PET scan of a 10-year old patient with SWS and right posterior angioma. Arrows indicate temporo-parietal regions (angioma regions) with increased leucine up-take.

Increased leucine uptake was mainly driven by increased tracer transport, with milder increases of protein synthesis rates. Higher leucine uptake asymmetries were associated with more severe hypometabolism ($r=-0.82$, $p=0.047$). Signs of endothelial cell proliferation and angiogenesis (a proliferative index of 5-10% and strong VEGF-A expression in endothelial cells) were seen in resected specimens from an infant who underwent epilepsy surgery.

Increased leucine transport and protein synthesis rates in the angioma regions suggest proliferative activity, likely related to active vascular remodeling. Degree of increased leucine uptake may be a marker of disease activity contributing to brain tissue damage in children with SWS.

5. Clinical outcome in bilateral Sturge-Weber syndrome
[Alkonyi et al., *Pediatric Neurology* 2011 Jun;44(6):443-9.]

Up to 15% of patients with Sturge-Weber syndrome (SWS) have bilateral intracranial involvement and the prognosis of these patients is believed to be particularly unfavorable. The imaging and clinical characteristics of this subset of patients have not been well studied. Therefore, the aim of this retrospective study was to describe clinical and neuroimaging features and identify potential markers of unfavorable outcome in this rare patient group.

FDG PET scans of 110 patients with the diagnosis of SWS were reviewed, and cases with bilateral involvement (also confirmed by CT or MRI) were identified. Seizure variables, presence of hemiparesis and degree of developmental impairment at last follow-up were noted and qualitatively correlated with the extent and location of glucose metabolic abnormalities.

Fourteen patients (12.7%; age at PET scan: 8 months-21 years) had bilateral brain involvement. In these patients glucose metabolic abnormalities had typically an asymmetric pattern on PET. Although early onset of epilepsy was not necessarily coupled with intractability, total hemispheric hypometabolism was invariably associated with poor seizure control. Bilateral frontal hypometabolism was closely coupled with severe developmental impairment. Two children with bitemporal hypometabolism showed autistic features. Furthermore, hemiparesis was associated with superior frontal (motor cortex) hypometabolism. Three patients had resective surgery, resulting in improved seizure control and/or developmental outcome.

Degree of neurological complications and clinical course are variable and highly depend on the extent and localization of cortical dysfunction in bilateral SWS; bifrontal hypometabolism is associated with poor developmental outcome and bitemporal hypometabolism is often coupled with autistic phenotype. Importantly, good seizure control and only mild/moderate developmental impairment can be achieved in about half of the bilateral SWS patients, with or without resective surgery.

V. SUMMARY OF THESES

1. The ipsilateral thalamus is metabolically impaired in unilateral SWS and this damage is associated with the extent of cortical injury.

2. In children with left hemispheric angiomatosis, the metabolic and microstructural damage of the thalamus (measured by FDG PET and DTI, respectively) can be a simple, but robust, independent imaging marker of neuro-cognitive outcome.

3. Microstructural white matter abnormalities occur not only ipsilateral, but to a certain extent, contralateral to the angioma in patients with unilateral SWS.

4. Nevertheless, cognitive function (IQ) and fine motor function of the hand opposite to the angioma appears to be associated with microstructural damage of focal ipsilateral white matter areas.

5. A paradoxical pattern of (mostly frontal) cortical glucose *hyper*metabolism is common among young patients with SWS. Focal increases of cortical metabolism occur shortly after or before the first clinical seizures.

6. Cortical *hyper*metabolism is invariably transient and predicts subsequent metabolic deterioration of the cortex.

7. Intractable epilepsy requiring epilepsy surgery is more frequent in children with initial cortical *hyper*metabolism than in those without, which suggests that this imaging marker may predict drug resistant epilepsy.

8. The leptomeningeal angioma region of SWS patients shows increased ^{11}C-leucine uptake, which is mainly driven by increased amino acid transport.

9. Higher leucine uptake is associated with more severe glucose hypometabolism in the angioma region.

10. Increased leucine transport and protein synthesis rates in the angioma regions suggest proliferative activity, likely related to active vascular remodeling. The presence of active vascular proliferation in the angioma tissue is also supported by our histopathology data.

11. Neuro-cognitive outcome in the subset of patients with bilateral intracranial involvement depends on the extent and localization of brain injury. Early seizure onset not necessarily results in severe clinical outcome in these patients.

12. Total hemispheric or bilateral frontal brain damage appears to be coupled with unfavorable cognitive and seizure outcome in bilateral SWS; bitemporal lesion is often associated with autistic phenotype.

13. Good seizure control and only mild/moderate developmental impairment may be achieved in about half of the bilateral SWS patients, with or without resective surgery.

VI. PUBLICATIONS

- **Articles related to thesis:**

Alkonyi B, Chugani HT, Karia S, Behen ME and Juhász C: Clinical outcome in bilateral Sturge-Weber syndrome. *Pediatric Neurology;* 2011 Jun;44(6):443-9.
Impact factor (IF) (2009): **1.497**

Alkonyi B, Chugani HT and Juhász C: Transient focal cortical increase of interictal glucose metabolism in Sturge-Weber syndrome: Implications for epileptogenesis. *Epilepsia.* 2011 2011 Jul;52(7):1265-72.
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Alkonyi B, Chugani HT, Muzik O, Chugani DC, Sundaram SK, Kupsky WJ, Batista CE and Juhász C: Increased L-[1-11C]leucine uptake in the leptomeningeal angioma of Sturge-Weber syndrome: a PET study. *J Neuroimaging*. 2011 Jan 11. doi: 10.1111/j.1552-6569.2010.00565.x. Epub ahead of print
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Alkonyi B, Govindan RM, Chugani HT, Behen ME, Jeong J and Juhász C: Focal white matter abnormalities related to neurocognitive dysfunction: An objective diffusion tensor imaging study of children with Sturge-Weber syndrome. *Pediatr Res.* 2011 Jan;69(1):74-9.
IF (2009): **2.604**

Alkonyi B, Harry T. Chugani, Michael E. Behen, Stacey Halverson, Emily Helder, Malek I. Makki and Csaba Juhász: The role of the thalamus in neuro-cognitive dysfunction in early unilateral hemispheric injury: A multimodality imaging study of children with Sturge-Weber syndrome. *Eur J Paediatr Neurol*. 2010 Sep;14(5):425-33.
IF (2009): **2.007**

- **Articles not related to thesis:**

Alkonyi B, Miao Y, Wu J, Cai Z, Xuan Y, Hu J, Chugani HT and Juhász C: A perfusion-metabolic mismatch in Sturge-Weber syndrome: A multimodality imaging study. [under review]

Alkonyi B, Mittal S, Zitron I, Chugani DC, Kupsky WJ, Muzik O, Chugani HT, Sood S and Juhasz C: Mechanisms of abnormal tryptophan transport and metabolism in epileptogenic dysembryoplastic neuroepithelial tumors. [under review]

Alkonyi B, Barger GR, Mittal S, Muzik O, Chugani DC, Bahl G, Robinette NL, Kupsky WJ, and Juhasz C: Accurate differentiation of recurrent gliomas from radiation necrosis by kinetic analysis of α-[11C]methyl-L-tryptophan PET. [under review]

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Trauninger A, Alkonyi B, Kovács N, Komoly S, Pfund Z: Methylprednisolone therapy for short-term prevention of SUNCT syndrome. *Cephalalgia.* 2010 Jun;30(6):735-39.
IF (2009): **3.686**

Auer T, Janszky J, Schwarcz A, Dóczi T, Trauninger A, Alkonyi B, Komoly S, Pfund Z: Attack-related brainstem activation in a patient with SUNCT syndrome: an ictal fMRI study. *Headache*. 2009 Jun; 49(6): 909-12.
IF (2009): **2.786**

Fehér G, Koltai K, Alkonyi B, Papp E, Keszthelyi Z, Kesmarky G, Toth K: Clopidogrel resistance: Role of body mass and concomitant medications. *Int J Cardiol*. 2007 Aug 21; 120(2): 188-92.
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Fehér G, Koltai K, Papp E, Alkonyi B, Solyom A, Kenyeres P, Kesmarky G, Czopf L, Toth K: Aspirin resistance: possible roles of cardiovascular risk factors, previous disease history, concomitant medications and haemorrheological variables. *Drugs Aging*. 2006;23(7):559-67.
IF (2006): **2.200**

Cumulative impact factor: 29.2
First author articles: 17.7

- **Book Chapter:**

Csaba Juhász, Bálint Alkonyi and Harry T. Chugani: Imaging brain structure and function in Sturge-Weber syndrome. In: Roach ÉS, Bodensteiner JB. eds.: *Sturge-Weber syndrome.* The Sturge-Weber Foundation, NJ, 2010.

- **Congress presentations and posters related to thesis (first author if not otherwise indicated):**

White matter perfusion abnormalities in Sturge-Weber syndrome: relation to cortical glucose metabolism and seizure severity
[poster] (2011 Annual Meeeting of the American Academy of Neurology, Honolulu, HI, USA, April, 20111)

Focal white matter diffusion abnormalities in children with Sturge-Weber syndrome: Relation to neurocognitive dysfunction
[poster] (2010 Child Neurology Society Annual Meeting, Providence, RI, USA, October, 2010)

Increased L-[1-^{11}C]leucine Uptake in the Angioma Associated with Cortical Hypometabolism in Children with Sturge-Weber Syndrome: a PET Study
[poster] (Molecular Neuroimaging Symposium, National Institutes of Health, Bethesda, Maryland, USA, May, 2010)
Image of the month (July 2010 in SNM Molecular Imaging Center of Excellence website) reprinted from *J Nucl Med*. 2010;51:827,830.

Metabolic and diffusion abnormalities of the thalamus and their cognitive correlates in unilateral Sturge-Weber syndrome
[poster] (2010 Annual Meeeting of the American Academy of Neurology, Toronto, Canada, April, 2010)

- **Congress presentations and posters not related to thesis:**

Juhász C, Alkonyi B, Hu J, Xuan Y, Chugani HT: Neurometabolic abnormalities and epileptogenesis in Sturge-Weber syndrome
[abstract accepted for poster presentation at the 2011 Child Neurology Society Annual Meeting, Savannah, GA, USA, October, 2011]

Differentiation of recurrent gliomas from radiation necrosis by kinetic analysis of α-[^{11}C]methyl-L-tryptophan PET
[platform presentation] (2011 Annual Meeeting of the American Academy of Neurology, Honolulu, HI, USA, April, 2011)

Metabolic and molecular imaging characteristics of epileptogenic dysembryoplastic neuroepithelial tumors (DNETs) [poster] (2010 Annual Meeting of The American Epilepsy Society, San Diego, TX, USA, December, 2010)

Muzik O, Pai D, Alkonyi B, Juhász C, Hua J: Performance evaluation of an integrative software environment for analysis of multi-modality neuroimaging data [platform presentation] (SNM 57th Annual Meeting, Salt Lake City, Utah, USA, June, 2010.)

Focal cerebral increases of α-[^{11}C]methyl-L-tryptophan (AMT) uptake suggest activation of the kynurenine pathway in multiple sclerosis: a PET study
[poster] (2010 Annual Meeting of the American Academy of Neurology, Toronto, Canada, April, 2010)

Quantitative brain surface mapping of cortical hypometabolism in neocortical epilepsy [poster] (2009 Annual Meeting of the American Epilepsy Society, Boston, MA, USA, December, 2009)

Complementary MR imaging examinations in headaches of trigeminal nerve origin [platform presentation] (Conference of the Hungarian Society of Headache, Siófok, Hungary, 2008)

An ictal fMRI study of a patient with SUNCT syndrome
[platform presentation] (Conference of the Hungarian Society of Headache, Siófok, Hungary, 2007)

- **Citable abstracts based on congress presentations:**

Bálint Alkonyi, Geoffrey R. Barger, Sandeep Mittal, Otto Muzik, Gautam Bahl, Pulak K. Chakraborty and Csaba Juhasz: Differentiation of recurrent gliomas from radiation necrosis by kinetic analysis of α-[11C]methyl-L-tryptophan PET. *Neurology* 2011;76 (Suppl 4) A182. IF (2009): 8.172

Bálint Alkonyi, Yanwei Miao, Jiani Hu, Harry T. Chugani and Csaba Juhasz: White matter perfusion abnormalities in Sturge-Weber syndrome: Relation to cortical glucose metabolism and seizure severity. *Neurology* 2011;76 (Suppl 4) A301. IF (2009): 8.172

Sandeep Mittal, Ian M. Zitron, William J. Kupsky, Bálint Alkonyi, Sandeep Sood and Csaba Juhász: Cytokine-mediated inflammation in epileptogenic dysembryoplastic neuroepithelial tumors. *Neuro-Oncology* 2010;12 (suppl 4): iv32. IF (2009): 4.984

Bálint Alkonyi, Rajkumar M. Govindan, Harry T. Chugani, Michael E. Behen, Jeong-Won Jeong, Csaba Juhász: Focal White Matter Diffusion Abnormalities in Children with Sturge-Weber Syndrome: Relation to Neurocognitive Dysfunction. *Ann Neurol* 68 Oct 2010 (Suppl 14) S 104. IF(2009): 9.317

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J Nucl Med. 2010; 51 (Suppl 2) 82P. IF (2009): 6.424

Bálint Alkonyi, Harry T. Chugani, Otto Muzik, Diane C. Chugani, Senthil K. Sundaram, William J. Kupsky, Carlos Batista and Csaba Juhász: Increased L-[1-^{11}C]leucine Uptake in the Angioma Associated with Cortical Hypometabolism in Children with Sturge-Weber Syndrome: a PET Study.
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Neurology 74 March 2, 2010 (Suppl 2) A230. IF (2009): 8.172

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Gergely Fehér, Katalin Koltai, Előd Papp, Zsuzsanna Keszthelyi, Bálint Alkonyi, Péter Kenyeres, Hajnalka Rapp, Gábor Késmárky and Kálmán Tóth: Acetylsalicylic acid and clopidogrel resistance: Possible role of risk factors, medication and hemorheological variables. *Atherosclerosis Supplements* 7 (3):398.