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# A distinct microRNA expression profile is associated with α[$^{11}$C]-methyl-L-tryptophan (AMT) PET uptake in epileptogenic cortical tubers resected from patients with tuberous sclerosis complex

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ABSTRACT

Tuberous sclerosis complex (TSC) is characterized by hamartomatous lesions in various organs and arises due to mutations in the *TSC1* or *TSC2* genes. TSC mutations lead to a range of neurological manifestations including epilepsy, cognitive impairment, autism spectrum disorders (ASD), and brain lesions that include cortical tubers. There is evidence that seizures arise at or near cortical tubers, but it is unknown why some tubers are epileptogenic while others are not. We have previously reported increased tryptophan metabolism measured with α[$^{11}$C]-methyl-L-tryptophan (AMT) positron emission tomography (PET) in epileptogenic tubers in approximately two-thirds of patients with tuberous sclerosis and intractable epilepsy. However, the underlying mechanisms leading to seizure onset in TSC remain poorly characterized. MicroRNAs are enriched in the brain and play important roles in neurodevelopment and brain function. Recent reports have shown aberrant microRNA expression in epilepsy and TSC. In this study, we performed microRNA expression profiling in brain specimens obtained from TSC patients undergoing epilepsy surgery for intractable epilepsy. Typically, in these resections several non-seizure onset tubers are resected together with the seizure-onset tubers because of their proximity. We directly compared seizure onset tubers, with and without increased tryptophan metabolism measured with PET, and non-onset tubers to assess the role of microRNAs in epileptogenesis associated with these lesions. Whether a particular tuber was epileptogenic or non-epileptogenic was determined with intracranial electrocorticography, and tryptophan metabolism was measured with AMT PET. We identified a set of five microRNAs (miR-142-3p, 142-5p, 223-3p, 200b-3p and 32-5p) that collectively distinguish among the three primary groups of tubers: non-onset/AMT-cold (NC), onset/AMT-cold (OC), and onset/AMT-hot (OH). These microRNAs were significantly upregulated in OH tubers compared to the other two groups, and microRNA expression was most significantly associated with AMT-PET uptake. The microRNAs target a group of genes enriched for synaptic signaling and epilepsy risk, including *SLC12A5*, *SYT1*, *GRIN2A*, *GRIN2B*, *KCNB1*, *SCN2A*, *TSC1*, *and MEF2C*. We confirmed the interaction between miR-32-5p and *SLC12A5* using a luciferase reporter assay. Our findings provide a new avenue for subsequent mechanistic studies of tuber epileptogenesis in TSC.

## 1. Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder that occurs due to mutations in either *TSC1* or *TSC2* genes, leading to altered expression or dysfunction of hamartin or tuberin proteins, respectively. These two proteins form a complex that modulates the activity of mechanistic target of rapamycin (mTOR). TSC is characterized by hamartomatous lesions in multiple organs, including brain, skin, kidney, and lung. Brain lesions include cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas (SEGAs). Approximately 60% of TSC patients suffer from medically intractable seizures that are resistant to pharmacological treatment

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**Table 1**
Demographics and clinical categories for TSC patients and tissue samples. All tubers were visible on MRI and characterized for seizure onset status (onset or non-onset) by ECoG. AMT-PET uptake was classified as 'hot' or 'cold.' Samples used in microRNA arrays and qPCR validations are indicated by 'Mi' and 'P' respectively. A representative subset of these samples was used for gene expression microarrays, indicated by 'Ge.'

| Sample | Patient ID | Age at surgery | Gender | Hemisphere | Mutation | Tuber | Sz Onset | AMT | Group | Location | Analyses |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 82506 | 4 y 9 m | F | Right | TSC2 | Tuber | Onset | Hot | OH | Frontal | Mi, P |
| 2 | 83002 | 3 y | F | Left | TSC2* | Tuber | Non onset | Cold | NC | Temporal | Mi, GE, P |
| 3 | | | | | | Tuber | Onset | Hot | OH | Parietal | Mi, GE, P |
| 4 | | | | | | Tuber | Onset | Cold | OC | Temporal | Mi, GE, P |
| 5 | 111400 | 6 y | M | Right | TSC1* | Tuber | Non onset | Cold | NC | Temporal | Mi, P |
| 6 | 81603 | 8 y | M | Left | TSC2 | Tuber | Non onset | Cold | NC | Temporal | Mi, P |
| 7 | | | | | | Tuber | Onset | Hot | OH | Frontal | Mi, P |
| 8 | | | | | | Tuber | Non onset | Cold | NC | Temporal | Mi, P |
| 9 | 92804 | 7.5 y | F | Left | unknown | Tuber | Onset | Cold | OC | Frontal | Mi, P |
| 10 | | | | | | Tuber | Non onset | Cold | NC | Frontal | Mi, P |
| 11 | F0508 | 9 m | M | Left | TSC2 | Tuber | Onset | Hot | OH | Central frontal | Mi, GE, P |
| 12 | | | | | | Tuber | Onset | Hot | OH | Occipital | Mi, P |
| 13 | | | | | | Tuber | Onset | Hot | OH | Occipital | Mi, P |
| 14 | | | | | | Tuber | Onset | Hot | OH | Central frontal | Mi, P |
| 15 | G2710CC | 2 y | M | Right | TSC2 | Tuber | Non onset | Cold | NC | Temporal | Mi, P |
| 16 | | | | | | Tuber | Onset | Cold | OC | Occipital | Mi, P |
| 17 | I1710AC | 5 y | M | Left | TSC2 | Tuber | Onset | Cold | OC | Frontal | Mi, GE, P |
| 18 | | | | | | Tuber | Non onset | Cold | NC | Frontal | Mi, GE, P |
| 19 | I1914MC | 13 m | F | Left | TSC2 | Tuber | Onset | Hot | OH | Occipital | Mi, P |
| 20 | J1513AB | 11 y | F | Left | TSC2 | Tuber | Onset | Cold | OC | Frontal parietal | Mi, P |
| 21 | L1412RK | 2 y | M | Right | TSC2* | Tuber | Onset | Hot | OH | Frontal | Mi, P |
| 22 | | | | | | Tuber | Non onset | Cold | NC | Frontal | Mi, P |

(Chu-Shore et al., 2010). In these cases surgical intervention to resect epileptogenic tubers is often successful in reducing seizure frequency (Weiner et al., 2006; Jansen et al., 2007; Ibrahim et al., 2015; Arya et al., 2015).

Positron emission tomography (PET) serves an important role in understanding brain development and neurodevelopmental disorders (Kumar and Chugani, 2008). The PET tracer α[$^{11}$C]-methyl-L-tryptophan (AMT) measures tryptophan metabolism via serotonin and/or kynurenine pathways (Kumar et al., 2011). Increased AMT uptake has been demonstrated to identify epileptogenic tubers in approximately two-thirds of patients with tuberous sclerosis and intractable epilepsy (Kumar et al., 2011; Kagawa et al., 2005; Fedi et al., 2003; Asano et al., 2000). AMT PET detected epileptogenic tubers with 74% sensitivity, 100% specificity, and 82% accuracy, as defined by seizure-free surgical outcomes (Kagawa et al., 2005). Therefore, AMT serves as an important biomarker of epileptogenic foci in TSC patients (Kumar et al., 2011; Chugani, 2011). Inflammatory response signaling is believed to be involved in AMT PET uptake in seizure onset lesions (Juhasz et al., 2013). Increased AMT uptake reflects increased tryptophan metabolism due to activation of the kynurenine pathway (Zitron et al., 2013). Indoleamine 2,3-dioxygenase (*IDO1*) is the key rate-limiting enzyme in the kynurenine pathway, and elevated *IDO1* expression is correlated with AMT uptake in epileptogenic tubers (Chugani, 2011). However, ~25% of seizure onset tubers do not have increased uptake of AMT, suggesting multiple mechanisms involved in tuber seizure propensity.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that are important regulators of gene expression at the post-transcriptional level. In their mature form of 18–22 nucleotides, miRNAs anneal to complimentary sites in the 3′-untranslated region of target transcripts, causing either degradation of the transcript or inhibition of protein synthesis. A single miRNA can target multiple transcripts, and a single gene can be targeted by multiple miRNAs (Ebert and Sharp, 2012). MiRNAs have been implicated in numerous biological and pathological processes, including several neurodegenerative disorders (Szafranski et al., 2015; Nelson et al., 2008; Meza-Sosa et al., 2012). Among human organs, the brain is most enriched with microRNAs, likely owing to its functional complexity (Kosik, 2006). Several studies have demonstrated the role of miRNAs in epilepsy (Risbud and Porter, 2013; Liu et al., 2010; Jimenez-Mateos et al., 2011; Aronica et al., 2010; Schouten et al., 2015; Roncon et al., 2015). We recently reported aberrant miRNA expression in epileptogenic TSC tubers, compared to patient matched non-tuber tissue (Dombkowski et al., 2016). To further discern microRNA activity that is specifically associated with seizure onset in tubers, with and without increased tryptophan metabolism measured by AMT PET, as opposed to microRNAs that may be involved in tuber formation, we performed the present study to directly compare microRNA expression profiles in epileptogenic and non-epileptogenic tubers. This approach allowed us to focus on microRNAs that are likely involved in seizure onset. Additionally, we performed gene expression analysis in a subset of samples to identify epilepsy-risk genes targeted by the differentially expressed miRNAs.

## 2. Material and methods

### 2.1. TSC patients and tissue classification

All human brain tissue used in this study was removed as a part of planned surgery for refractory epilepsy in patients diagnosed with TSC. To obtain brain specimens following surgery for research purposes, informed consent was obtained from parents or legal guardians for all minors undergoing treatment. Collection and analysis of specimens was carried out in accordance with an approved Institutional Review Board protocol.

The 13 patients in this study underwent either a one-stage or two-stage surgery with subdural electrodes to identify regions responsible for generating habitual seizures. Anti-epileptic medications were withheld or reduced until a sufficient number of habitual seizures (typically not less than three events) were captured. Seizure onset zones were marked, with visual assessment, by a board-certified clinical neurophysiologist (E. Asano). Seizure onset was defined as a sustained rhythmic change on electrocorticography (ECoG) clearly distinguished from the background activity and accompanied by the habitual semiology not merely explained by state changes (Asano et al., 2009). Additional preoperative diagnostic assessment for identification of TSC lesions included, but was not limited to, MRI, FDG PET, and AMT PET scans (AMT PET was obtained under a clinical research protocol). Initial scalp EEG analysis was performed blinded to PET analysis. Together, along with other imaging (MRI) and clinical data, these results were used to lateralize the seizure onset region and guide the ECoG grid placement. The ECoG grid typically covered a region larger than the