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Relationship of Head Circumference and Epilepsy Severity in Infants with Tuberous Sclerosis Complex

Faculty Supervisor: Michele Lemons, PhD

Natural Sciences

A Thesis Submitted to Fulfill the Requirements of the Honors Program at Assumption College

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**Abstract**

Tuberous sclerosis complex (TSC) is a rare genetic disorder, characterized by high incidence of seizures and tubers (benign cellular abnormalities that appear on brain imaging). Current literature reports an increased head circumference within the TSC population, but the implications, exact rate, or how big the increase is, remains unknown. We hypothesized that increased head circumference would correlate with epilepsy severity in infants with TSC. We examined clinical data from 121 infants diagnosed with TSC who were enrolled in the multi-site, prospective TSC autism center for excellence network (TACERN) study. We calculated each infant’s head circumference z-score from each study visit by comparing collected values with those reported by the World Health Organization of healthy child head circumference growth. The overall mean head circumference z-score across all study visits of all TACERN participants was 0.97 with no significant sex differences. The number of seizure types correlated with head circumference z-score are as follows: infants with no seizures had a mean z-score of 0.57, those with 1 seizure type had a mean z-score of 0.88, and those with 2 or more seizure types had a mean z-score of 1.31. Thus, our hypothesis was partially supported. This data suggests that a larger head circumference may serve as a biomarker for epilepsy in children diagnosed with TSC, enabling earlier treatment for epilepsy, and thus improved neurodevelopment.
**Introduction**

Developmental disabilities are diagnosed at alarmingly high rates. For example, autism spectrum disorder (which will be referred to as “autism” here) was present in one in every 150 births in the year of 2000; today, it is present in one in every 68 children (Center for Disease Control and Prevention (CDC), 2018). TSC is a genetic disorder that occurs in one of every 6,000 births and is associated with a high rate of cognitive disabilities and autism (Davis *et al.*, 2015). Having a child with a disability can put a lot of strain on a family, because that child requires additional care, time, and attention. Thus, any research that can improve the neurological outcomes of TSC, or any childhood disability may help those affected by the disease.

Individuals with TSC have an approximate 50% chance of developing autism (Curatolo *et al.*, 2015), making TSC one of the most frequent genetic comorbidities, or co-occurring diseases, of autism (CDC, 2018). While there is no definitive cause of the autism present with (or without) TSC, the autism that occurs with individuals with TSC presents similarly to autism alone (Davis *et al.*, 2015). For example, stereotypical behaviors seen in autism, such as hand flapping, are seen with autism in TSC. While autism is not a major focus of this thesis, if we are able to show that head circumference could be a biomarker, or indicator of risk, of severe epilepsy and therefore treat individuals sooner, we could preventively treat for autism (i.e.: applied behavioral analysis therapy) as well and perhaps lessen the severity of autism-associated symptoms. Additionally, by preventively treating for epilepsy alone, we may be preventing damage to healthy brain tissue, which could result in autism-associated symptoms. Epilepsy occurs in 20% of all cases of autism (Besag, 2017); thus, epilepsy alone may not convey
an increased risk of autism, severe epilepsy that is associated with changes in neurodevelopment, may increase the risk of autism in patients with TSC.

**Increased head circumference in individuals with TSC**

Interestingly, a common symptom seen in patients with autism alone and TSC (with or without autism as a comorbidity) is macrocephaly, or an enlarged head above 2 standard deviations above the mean, or in the 97th percentile (Lainhart *et al.*, 2006). The rate of macrocephaly in autism is 15.7% as compared to a 3% risk in the typically developing population (Viginoli *et al.*, 2015). Macrocephaly occurs at a rate of 14-29.7% in TSC and other developmental disorders together, but an exact rate of macrocephaly in the TSC population alone was not specified (Sacco *et al.*, 2015). While the literature often notes that there is an increased head circumference associated with TSC, the literature does not go any further to investigate how macrocephaly relates to the other symptoms that occur with TSC.

**The presence and connection between tubers and seizures in individuals with TSC**

In individuals with TSC, many organ systems are affected by the presence of hamartomas, collections of abnormal cells. In the brain, these hamartomas are referred to as tubers. Tubers are often identified by magnetic resonance imaging (MRI) that may have been originally performed to evaluate seizures in an individual with TSC. This is a common way that infants are diagnosed with TSC, if there is not a genetic risk of TSC in the family. Due to advances in imaging techniques, some fetuses are diagnosed before birth because tubers in the brain or hamartomas in the heart are sometimes seen during routine prenatal anatomy scans. When this is not the case, as previously mentioned, the onset of epilepsy usually leads to a diagnosis. Tubers should not be confused with
cancerous tumors, which are large malignant masses of excess tissue that can grow and directly cause damage to the body. Tubers are static structures and do not tend to grow over the lifetime.

Tubers can be thought of as a type of benign tumor, that represent areas of a high concentration of abnormally developed cells that are produced instead of typical cells. Abnormal cells within tubers take the place of healthy cells, rather than the abnormal cells adding to the total number of cells in the brain as reviewed by Crino et al. (2010). While the exact mechanism of tuber formation is not known, the mechanism of formation of subependymal giant cell astrocytomas (SEGAs) (a tumor commonly found in patients with TSC) is known, and some researchers have proposed applying the formation mechanism of SEGAs to tuber formation as reviewed by Crino et al. (2010). The SEGA formation mechanism, when applied to tuber formation, proposes a type of “double hit mechanism.” A TSC gene mutation would occur in a neuroepithelial progenitor cell in the developing brain, and this mutation would be in conjunction with somatic mutations of one of the TSC genes elsewhere, and both lead to dysfunctional proteins produced by the TSC genes (reviewed by Crino et al., 2010). The TSC genes are regulators of cell development in the mechanistic target of rapamycin (mTOR) pathway (a cell development pathway). When this pathway is activated, the mutated protein is not able to regulate the activity of mTOR. Thus, cytomegaly occurs in the original progenitor cell such that giant cells are created, forming a tuber (reviewed by Crino et al., 2010). This information is important, because it suggests that there are cellular changes in the brain in those with TSC beyond what we see as tubers; these changes could be contributing to
epilepsy, as we know that there is a link between tubers and the type and frequency of seizures in those with TSC.

90% of all TSC patients experience seizures (Vignoli et al., 2015). Tuber burden, or the number of tubers in the brain, is correlated to the frequency (i.e.: daily) of seizures. Tuber location is known to play a role in the type of seizure seen in an individual with epilepsy and TSC. For example, infantile spasms are the most common type of seizures seen in infants with a diagnosis of TSC, and it has been demonstrated that a high tuber burden in any lobe of the brain conveys a high risk of having infantile spasms (Doherty et al., 2005). Infantile spasms are characterized by onset, or start, in infancy and may present with a subtle head drop that may go unrecognized as a seizure to a caregiver. In the TSC population, 74.5% of individuals who had infantile spasms in infancy later developed refractory, or uncontrollable, epilepsy (Chu-Shore et al., 2010). The longer infantile spasms go unrecognized, and therefore untreated, the more likely the child is to develop refractory epilepsy and developmental disorders (Shields, 2018). Additionally, when seizures are left untreated, individuals are at risk for epileptic encephalopathy, or damage to healthy tissue, caused by seizures. Since we know this relationship exists, it is important to prevent this from happening; if we are able to show that head circumference is related to epilepsy severity, we can use head circumference as a biomarker and identify which infants may be at risk of the consequences of epilepsy. Eventually, we may even be able to treat these high-risk infants preventively, so their epilepsy does not reach the refractory status, and thus, decrease the damage to their brains caused by seizures.

In recent years, there have been vast developments in the research of TSC. However, TSC, unlike autism, is not well known to the public, and does not have the
immense interest and immediacy of research that autism does. Many researchers have reviewed the symptoms associated with TSC and some do note that there is an increased rate of macrocephaly, but we have been unable to find any literature investigating the role of macrocephaly in the symptomatology, specifically the epilepsy, associated with TSC. In fact, the literature has prompted us to examine about the link between head circumference and epilepsy in individuals with TSC, but to our knowledge, none has been reported to date. Here, we aim to characterize the relationship between head circumference and epilepsy severity within infants and toddlers diagnosed with TSC, in order to determine if head circumference could be used as a biomarker in the future for severe epilepsy in TSC.

**Methods:**

**Subject recruitment:**

Participants in this study were enrolled in the TSC Autism Center of Excellence Network (TACERN) at five sites across the United States (Boston Children’s Hospital, Cincinnati Children’s Hospital Medical Center, University of Alabama at Birmingham, University of California at Los Angeles, and University of Texas at Houston). Infants were eligible for the TACERN study if they were diagnosed with TSC before 12 months of age. Study visits were at 3, 6, 9, 12, 18, 24, and 36 months of age; however, infants could be enrolled into the TACERN study anytime before their first birthday, thus some were missing the early study visits. At each study visit, EEG, clinical history, seizure diaries, and head circumference measurement were collected. Yearly MRIs on each participant were also collected. Full study design, inclusion and exclusion criteria and collected information is reported in Davis et al. (2017). Of the 166 TACERN
participants, 121 infants were selected for data analysis in this study. Subjects were excluded based on the following criteria: premature birth (before 37 weeks estimated gestational age), lack of documented epilepsy history, or lack of head circumference measurements. All other participants in the cohort were included. Lastly, as the TACERN study is longitudinal in design, we were able to compare the same participants over time.

**Measures:**

**World Health Organization’s typical head growth data**

Because the TACERN study did not have healthy controls, we used the World Health Organization (WHO) head circumference data for healthy boys and girls from birth to age five was used as healthy controls (WHO Multicentre Growth Reference Study Group, 2007). The WHO collected data on 8,440 healthy infants who were from diverse ethnic backgrounds in order to create typical growth curves (World Health Organization, 2007).

This data was also used to calculate head circumference z-scores for each subject’s head circumference measurement using MATLAB R2017a (The MathWorks Inc., Natick, MA) using the following z-score formula:

$$Z_{i,d} = \frac{HC_{i,d} - \mu_d}{\sigma_d}$$

Where $Z_{i,d}$ is the head circumference z-score for subject $i$ at age $d$ days, $HC_{i,d}$ is the head circumference measurement in cm for subject $i$ at age $d$ days, $\mu_d$ is the WHO population mean head circumference measurement in cm at age $d$ days and $\sigma_d$ is the standard deviation of the WHO head circumference measurement at age $d$ days.
Before z-scores were calculate, all head circumference measurements were manually reviewed for measurement errors. Study sites were queried for any measurement that showed a standard deviation change greater than 1, from one measurement to the next, as well as any that appeared clearly erroneous (i.e.: a smaller head circumference at older age, or a single outlying z-score in a sequence of consecutive measurements). If the site could not confirm that the measurement was accurate or provide a corrected measurement, that data point was excluded from analysis.

Head circumference was measured at 3, 6, 9, 12, 18, 24, and 36 months of age, although many children did not yet have a 36 month measurement, so the analysis was stopped at 24 months. Z-score of head circumference was calculated for each participant at each age as well as an average z-score that spanned the first two years of life. In order to compare any individual regardless of age and, as boys and older children generally have larger heads than girls and younger children, respectively, z-score was used instead of head circumference in centimeters.

In order to directly compare the TACERN and WHO participants, the t-score formula, as noted below, was used. We could not use standard t-tests because the WHO only publishes average head circumferences for each age, by sex; thus, we compared the WHO population to the TACERN sample. Additionally, when comparing the WHO participants to the TACERN participants we used centimeters instead of z-score because the WHO individuals would always have a z-score of 0; thus, centimeters allows for an easier visualization of any potential differences.

\[
t = \frac{\bar{x} - \mu_0}{s/\sqrt{n}}
\]
Where $\overline{x}$ is the TACERN sample mean, $\mu_0$ is the WHO population mean, $s$ is the TACERN sample standard deviation, and $n$ is the TACERN sample size. This formula generates a t-score, or a critical value, then by using that value, the degrees of freedom (n-1), and $\alpha = 0.05$, a p-value can be determined by using a t-distribution table.

**Classifying epilepsy severity:**

Parents were taught to keep seizure diaries indicating the frequency and type (infantile spasms, focal seizures, and other seizure types) of their child’s seizures. To assess the severity of epilepsy in each case an adapted version of the early childhood epilepsy severity scale (E-Chess) was used, which specifically assesses epilepsy associated with TSC and best fit the TACERN data (Humphrey et al., 2008). The full E-Chess scale includes: seizure frequency (i.e.: daily; not seizure duration), how long seizures have been occurring, number of seizure types present, number of antiepileptic drugs (AEDs) used, and response to treatment. Many subjects had epilepsy onset prior to entering the study or did not have enough seizure diary history recorded to accurately determine portions of the E-Chess scale; therefore, an adapted version of the E-Chess scale, referred to as a “mini E-Chess” score was created. The mini E-Chess scale was based on factors that most subjects had these data recorded. The mini E-Chess score was based on number of seizure types and number of AEDs; these categories were scored according to Table 1. The data recorded in the seizure diaries was used to generate a mini E-Chess score for each participant. The total mini E-Chess severity score is attained by adding the score from each parameter. In addition to mini an E-Chess score, number of seizure types each infant had was also used as a proxy for epilepsy severity.
Table 1: Mini E-Chess scoring of epilepsy severity

<table>
<thead>
<tr>
<th>Parameter:</th>
<th>0 = no seizures</th>
<th>1 = 1 type</th>
<th>2 = 2 types (continues if more types, no upper limit)</th>
<th>3 = More than 2 AEDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of seizure types</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of AEDs</td>
<td>0 = None</td>
<td>2 = 1 or 2 AEDs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Then a series of two tailed t-tests were ran to examine differences in head circumference z-score based on the types of seizure(s) infants had and the number of seizure types each infant had. Number of seizure types TACERN participants was used as a representation of epilepsy severity in the t-tests more than the type of seizures, because the latter subcategories were very small and thus results may not have been reliable. While the number of AEDs was included in the mini E-Chess score, number of AEDs was not used as a parameter in the t-tests, because number of AEDs alone does not characterize the severity of epilepsy.

**Volumetric analysis of brain size MRIs**

Brain MRIs were analyzed clinically by radiologists at each participant’s respective site and they were analyzed volumetrically by the Warfield lab as outlined in Tomas-Fernandez and Warfield (2015). The Warfield lab calculated total brain volume, and the distribution of volume between different tissue types (white matter, subcortical and cortical grey matter, and cerebrospinal fluid) within the brain. The clinical radiologists also reported tuber burden and any other notable brain abnormalities that would be grounds for exclusion; 94% of the TACERN sample with MRI data (n=115) had cortical tubers.
Data analysis plan:

1. Compare TACERN infants’ head circumferences to WHO’s healthy infants’ head circumferences in centimeters.

2. Determine an average head circumference z-score for each subject that spans the first two years of life. Then determine the overall average of the TACERN sample using these individual averages.

3. Examine the relationship between TACERN infants’ head circumference z-score and epilepsy severity, based on the number of seizure types, the type each infant has, and mini E-Chess score using two tailed t-tests.

4. Investigate the relationship between age (6, 12, and 24 months) and head circumference z-score in TACERN infants using two tailed t-tests. Then analyze head circumference based on having epilepsy or not, by age using two tailed t-tests.

5. Assess whether or not the onset of seizures increases head circumference at any age of seizure onset by comparing the last head circumference measurement before seizures started and the first head circumference measurement after seizures started with a two tailed t-test.

6. Analyze the relationship between head circumference and brain volume using MRI data and Pearson’s correlational coefficient, r; and determine the distribution of different tissue types in relation to total brain volume.
Results:

Average head circumference of all TACERN infants is 1 standard deviation above the typically developing population

To examine the differences between the TACERN sample and WHO population, we used the t-score formula and distribution tables, which revealed there were significant differences between the two, at 6, 12, and 24 months of age (Figure 1). We used centimeters to better represent the differences, because the WHO population would always represent a z-score of zero.

In order to determine the overall average standardized head circumference z-score of all TACERN infants, we first calculated individual averages that spanned the first two years of life for each participant. Then we took an average of all participants, which was determine to be 0.97 (where the WHO data would have a z-score of 0). This shows that head circumferences in TSC are about 1 standard deviation above the normal population, as seen in Figure 2A. The average head circumference in centimeters of TACERN participants was 43.41 cm. There were no significant sex differences when assessing head size by z-score; but as expected based on normal growth standards, male infants had overall large head circumferences in centimeters than females. There was a slight increase in head circumference z-score with age, which could be due to measurement errors or an atypical change in head circumference z-score over time. For very young children, measurement errors cause larger deviations due to smaller standard deviations, which is important because if errors in head circumference measurements were made, they would be more apparent at a young age, rather than an older age, such that there would appear to be an increase in z-score overtime.
In order to determine if the increase in head circumference is relative to body size, we compared head circumference and height. We did this, because we were interested in whether this potential biomarker of overgrowth was localized to the brain or if it occurred throughout the body, as represented by height. Head circumference was positively correlated to height in this sample; meaning, head size is proportional to the height of the infants in TACERN study (Figure 2B). Yet, head circumference of these infants is still above average based on age and gender (Figure 2A). This suggests that overgrowth was contained to the brain. To further support this we created a histogram (not shown) of TACERN infants’ height, and it was a normal distribution with no skewing above or below typical heights based on age and sex, suggesting that there were no extreme heights in the TACERN sample.

**Epilepsy severity of TACERN infants**

Of our 121 TACERN participants, 94 had seizures. We used the mini E-Chess score as described in the methods to determine severity of epilepsy within the group. Those who had one seizure type had an average mini E-chess score of 3.29, while those with two seizure types had an average mini E-chess score of 4.57. Our participants had an average mini E-chess of 3.85, and 54 of the 94 participants with epilepsy were above this average. Overall, we had a wide range of epilepsy severity, but also a large representation of participants with severe epilepsy. There were more TACERN individuals with epilepsy than without; for example, only had 9 of 59 total females did not have epilepsy and only 18 of the total 62 males did not have epilepsy. In addition to mini E-chess, we decided to use number of seizure type as a variable in our statistical analysis because this variable allowed similarly sized groups to make our results reliable, more than using
seizure type. Additionally, because mini E-Chess score was varied between all participants, using the number of seizure types created comparison groups that were unified by a single variable.

**Number of seizure types is proportional to degree of increase in head circumference of TACERN infants**

In order to determine if TACERN individuals with any seizure types had larger head circumference z-scores than those who did not have seizures at all, a two tailed t-test was used and revealed those with epilepsy were significantly larger than those without epilepsy (Figure 3). The average z-score for TACERN participants who did not have seizures across all ages was 0.667, therefore, even without epilepsy, head circumference is larger in infants with TSC than in healthy infants. TACERN participants with any seizures across all ages had an average head circumference z-score of 1.08. However, when assessed by seizure type, there was no significant difference in mean head circumference z-score for those with only infantile spasms (n=15) versus no seizures (n=26), only focal seizures (n=12) versus no seizures (n=26), or only a different seizure type (n=1) type versus no seizures (n=26). 67 infants were excluded from this portion of the analysis as they had more than one seizure type. This trend suggests that no one type of seizure causes the increased head circumference, but the presence of seizures in general accounts for the change. Conversely, the lack of significant differences may be due to the small sample size of participants having only one seizure type. Since there were no significant differences between any one seizure type and head circumference, we investigated the role of number of seizure types in head circumference. As portrayed in Figure 4, those with two or more types of seizures had a significantly larger head
circumference then those who did not have any seizures, as determined by a two tailed t-test. This result suggests that having two or more types of seizures, a more severe seizure phenotype, is associated with a significantly larger head circumference.

To determine if the age of seizure onset had an effect on head circumference, we examined head circumference z-score by age of seizure onset by using a two tailed t-test, and found no correlation. Yet, the start of seizures did have an effect on head circumference z-score irrespective of age; head circumference z-score increased from pre seizure onset to post seizure onset (Figure 6A). This difference was conserved in those who only had one type of seizure, but not in those who had two or more types of seizures (Figure 6A). Instead of using age in this analysis, the last head circumference measurement when the infant did not have seizures was used as the pre seizure measurement, which was compared to the first head circumference measurement once seizures started, or the post seizure measurement. We also determined that TACERN infants without epilepsy had a significantly different z-score from their first measurement to their last, and as noted above, typically developing infants rarely change z-score over time (Figure 6B); this suggests that abnormal growth is occurring in the brains of infants who have TSC even without having seizures.

These results could be because those with 2 types of seizures had a bigger head circumference from an earlier age, while those with 1 seizure type have more abnormal head growth only after they develop seizures. This hypothesis is supported by the fact that those with 2 or more types have a significantly larger head circumference than those without epilepsy at 6 months of age, but those with only one type of seizures was not significantly different at 6 months of age. Overall, this data suggests that having seizures
could somehow change the brain and its growth trajectory, beyond the change in growth that is accompanied by having just TSC. Lastly, when head circumference was assessed by pre and post seizure onset and by individual seizure type, the post onset z-score increase was seen in only those who have infantile spasms (data not shown). As noted above, z-score should not change over time for head circumference; therefore, we are again seeing an abnormal growth rate.

Data analysis by age reveals 6 months of age may be a critical period in growth

After analyzing the sample as a whole, irrespective to age, we speculated as to whether there was a time point at which these differences appeared. This led us to analyze the data by age. Since the TACERN study was longitudinal in design, we were able to compare the same participants at 6, 12, and 24 months. We determined that head circumference was consistently and significantly above the WHO’s mean at 6, 12, and 24 months of age when assessing all TACERN participants, with no sex differences in z-score at any age (Figure 1 and 5).

In order to determine other factors that may contribute to increased head circumference, we analyzed the data based on age and seizure status (whether or not the infant had seizures at any time). This analysis revealed that at 6 months of age those who had seizures had a significantly larger head circumference z-score than TACERN participants who did not have seizures (Figure 5). However, this difference was not statistically-significant at 12 or 24 months of age; yet, there were still an apparent differences (Figure 5). As seen in Figures 1 and 5, as a population, TACERN participants have an increased head circumference consistently through their first two years of life, thus the lack of significant differences between those with and without epilepsy at 12 and

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24 months of age may be due to a small sample size. At 12 months, only 21 infants did not have seizures (average z-score= 0.667), while 82 infants did have seizures (average z-score= 1.22). At 24 months, only 19 infants did not have seizures (average z-score= 0.871), while 64 did have seizures (average z-score= 1.15). The number of infants was slightly different at each age, because some infants were seen too far before or after the age of interest. Moreover, at 12 and 24 months (as well as 6 months), by using the t-score formula and a two tailed t-distribution, the TACERN population was significantly different from the WHO data.

Since we determined that number of seizure types influences head size of TACERN participants, we next analyzed the data by age and number of seizure types using a two tailed t-test to see if there was an age affected the increase in head circumference. Again, we observed that the only significant difference was between those without epilepsy and those with 2 or more types of seizures, but now we determined this difference occurs only at 6 months of age (Figure 7). All of these results together suggest that if an infant with TSC has 2 or more types of seizures, there are brain size differences such that head circumference is increased early in the child’s life.

To further characterize the relationship between head circumference and epilepsy phenotype, we ran a series of two tailed t-tests, which detected some interesting patterns of significant differences in head circumference, by age, number (0, 1, 2 or more) of the seizure types and the type of seizure occurring. At 6 months of age, those who had infantile spasms and focal seizures had significantly larger head circumferences than those who did not have seizures and those who only had infantile spasms (Table 3). At 12 months of age, those who had infantile spasms and focal seizures had significantly larger
head circumferences than those who did not have seizures (Table 3). Additionally at 12 months, those who had infantile spasms, focal seizures, and at least one other type of seizure had significantly larger head circumferences than those who had just infantile spasms and those who just had focal seizures (Table 3). At 24 months, those who had infantile spasms and one other seizure type had significantly larger head circumferences than those who just had infantile spasms (Table 3). From this data, it appears that at any age, having more than one seizure type (regardless of how often seizures occur) causes a significant increase in head circumference z-score. Moreover, we know that infantile spasms are associated with worse neurodevelopmental outcomes (Chu-Shore et al., 2010) and from this data; we can see that in every significant difference noted above, the children had infantile spasms. Thus having infantile spasms and at least one other seizure type may be considered the most severe epilepsy, as represented by the greatest increase in head circumference.

**Brain measurements based on MRI data reveal similarities between TACERN participants and typically developing population**

In order to determine if there were obvious physiological differences that could account for the increase in head circumference and to determine if head circumference was an accurate measurement of brain size, we analyzed the MRIs of TACERN participants. Brain volumes calculated from MRI data showed a similar distribution of volumes across tissue types within the TACERN sample. Approximately 58% (of total brain volume) was cortical grey matter, 3% was subcortical grey matter, 22% was white matter, 3% was ventricular systems, and 14% was extra-cerebral spinal fluid (CSF) for all subjects in this cohort, with or without epilepsy. This pattern of volumes of tissue types
among our subjects is important to note, as the trend suggests that larger head circumferences are not because of individual volumetric differences of one tissue type in the brain, hydrocephalus, or any other acute abnormality, but rather an overall large brain. Moreover, in the volumetric analysis of these MRIs, tubers were included in the percentage of volume of tissue type they were found within; for example, if a tuber was in the white matter, it added to the total white matter volume. Thus, tubers do not appear to change the distribution of tissue volume in the brains of those with TSC. Any participant with an acute abnormality, such as hydrocephaly, was excluded from further analysis. Additionally, because volumetric measures were positively correlated to head circumference, we can conclude that this 1 standard deviation increase in head circumference is relative compared to body size (Figure 8).

To determine if the general trend of distribution of volume of each tissue type was similar to the typically developing population, we compared the percentage that each tissue type occupied between the two populations. The trend that the TACERN participants followed was similar to that of the typically developing population, therefore this suggests that large head size is not being caused by a structural difference induced by TSC (for example, the presence of cortical tubers). For example, the typical population that has a 52% of grey matter (Luders et al., 2002), while TACERN sample has 58% grey matter. We cannot say that there is no significant difference here, because Luders et al. (2002) did not report individual data points so we could not run two tailed t-tests between TACERN participants and the healthy controls. Therefore, while head circumference and consequently total brain volume was increased in the TACERN sample, it is not because any one tissue type is significantly increased.
Intracranial cavity (ICC), or total brain volume including everything within the skull, was positively correlated to head circumference based on all TACERN participants’ brain MRIs (Figure 8A). This is important, as it suggests that head circumference was an accurate measure of brain volume, as shown previously. White matter and subcortical and cortical grey matter volumes were also positively correlated with head circumference (Figure 8B, 9C, and 9D respectively). Positive correlations between the tissue volumes and head circumference is as expected, because the amount of tissue should be proportional to head size. As expected, males had overall larger brain volumes than females, as predicted by larger body size (World Health Organization, 2018). Lastly, larger head circumference was not due to increased CSF, as all individuals in this sample had normal CSF volumes as compared to the typically developing population (data not shown).

**Review and results of data analysis plan:**

1. TACERN infants’ head circumferences were about 1 standard deviation above the WHO’s healthy infants’ head circumferences.

2. The average head circumference z-score for all subjects, spanning each participants' first two years of life (individual average of each head circumference measurement) was 0.97.

3. TACERN infants’ head circumference z-score was proportional to epilepsy severity, based on the number of seizure types each infant has. With no seizures, TACERN infants had an average z-score of 0.57, with one seizure type the average was 0.881, and with two or more types of seizures, the average was 1.31
4. At 6 months of age, TACERN infants without epilepsy had an average z-score of 0.482, while those who did have seizures was 1.12. At 12 months of age, TACERN infants without epilepsy had an average z-score of 0.667, while those who did have seizures was 1.22. At 24 months of age, TACERN infants without epilepsy had an average z-score of 0.871, while those who did have seizures was 1.15.

5. The start of seizures increases head circumference at any age of seizure onset when TACERN infants had only one type of seizures, but not when TACERN infants had two or more types of seizures.

6. Using two tailed t-tests, it was determined that infantile spasms, more than focal seizures or other seizure types, increased head circumference the most.

7. Head circumference and brain volume were positively correlated, as determined by MRI data.

Discussion

Most TACERN participants have an increased head circumference, but not macrocephaly

When originally designing this project, we hypothesized the majority of the TACERN infants would meet clinical criteria for macrocephaly, as the literature intermittently reported that macrocephaly was a symptom of TSC and because of the presence of tubers and seizures in individuals with TSC; however this was not the case. As mentioned in the introduction, macrocephaly is defined as a head circumference that is two or more standard deviations above the mean, as reported by the WHO, (Lainhart et al., 2006), and the TACERN infants on average had an increase in head circumference z-score of one
standard deviation. Yet, we did have a rate of 19.8% infants who met criteria for macrocephaly, which is still elevated from the 3% rate in the typical population and a 15.7% rate in those with autism (Vignoli et al., 2015). This supported our hypothesis that the rate of macrocephaly is elevated in the TSC population. Most of our subjects had an increased head circumference but not increased enough to meet criteria for macrocephaly. Thus, we believe that an increased head circumference of approximately 1 standard deviation above the WHO mean, is a more accurate descriptor of head circumference differences in those with TSC. Additionally, it has been previously demonstrated that head circumference is an accurate predictor of brain volume, especially in young children; thus, differences in head circumference represents differences in brain volume (Bartholomeusz et al., 2002). Furthermore, since head circumference was positively correlated to brain volume in the TACERN sample, our analysis of head circumference is likely an accurate measurement of brain size. Thus, overgrowth of head circumference represents over growth in the brain.

**Epilepsy severity is proportional to the increase in head circumference in TACERN participants**

Through our analysis of epilepsy severity, we observed a number of trends that related epilepsy severity to head circumference. First, since we demonstrated that overall, TSC carries a risk of an increased head circumference, because we found that as a whole, the average head circumference z-score of the TACERN population was 0.97 (Figure 2A). The average head circumference z-score for TACERN participants without epilepsy was 0.57, while the average head circumference z-score for TACERN participants with epilepsy was 1.08; this difference was statistically significant (Figure 3). This supported
our hypothesis that those TACERN infants with epilepsy had a larger head circumference than those TACERN infants without epilepsy. We also determined that those with TSC without epilepsy increased z-score of head circumference over the course of their first two years, which suggests that even without epilepsy, TSC is associated with abnormal head growth as head circumference percentile is typically stable over the lifetime (Figure 6B).

The abnormal change in head circumference z-score in those without epilepsy could potentially be because the brains of those with TSC without epilepsy, still have abnormal cellular growth that leads to larger cells, cellular migration, and subtle microscopic differences and therefore they have overall larger brains. An example of these microscopic differences is that, some cells do not migrate properly, thus some portions of the brain are subtly structurally different from a healthy brain. For those who do not have tubers, there are likely still unhealthy cells within the brain. This is because those without tubers still have the mutation of one of the TSC genes that causes TSC and also cause the unhealthy cells, just likely at lower concentrations such that no visible tuber is formed. To confirm these hypotheses, in the future it would be helpful if more post-mortem studies were conducted to determine if these discrete cellular abnormalities are present in the brains of those with TSC without epilepsy and or without tubers, and to determine how exactly they change the brain. Crino et al. (2010) reviewed studies that have noted some microscopic differences, as mentioned above, in those TSC that were not visible on MRI, but were identified post mortem. This is important, because if those without tubers, still have an increased head circumference, potentially caused by these abnormal cells, then head circumference could be used as a diagnostic tool of TSC,
for individuals who do not have tubers which is a common way of diagnosing TSC. While each change is so minor, many such changes could constitute an increased head circumference.

Second, we determined that those with the most severe epilepsy, as characterized by having two or more types of seizures, had the largest head circumferences in the sample. This too supported our hypothesis that epilepsy severity would be correlated to head circumference. We determined that those with two or more types of seizures had an average head circumference z-score of 1.31, while those who had only one type of seizures had an average head circumference of 0.88 (Figure 4). At 6 months of age, those with two or more types of seizures had a significantly larger head circumference than those without epilepsy, but this difference was not statistically significant at 12 or 24 months (Figure 7). Because there was no statistical difference in those with and without epilepsy at 12 to 24 months, this suggests that 6 months may be a critical point in development for those with severe epilepsy, such that head growth rate begins to slow down, as head circumference was consistently high at 6, 12, and 24 months for those with epilepsy. Additionally, there were no statistically significant differences in head circumference at any age between those with 1 type of seizure and those without epilepsy (Figure 7).

Third, those with one type of seizure had a significant head circumference increase after their seizures began, but this was not seen in those with two or more types of seizures (Figure 6A). For infants with 1 seizure type, it appears that their heads grow at a similar rate to those without epilepsy until seizures start. For example, one participant with 1 seizure type had a consistent head circumference z-score of 0.66 before seizures
began, then after seizures began, head circumference z-score increased to 1.42. This participant represented the trend nicely, as there was a slight increase in head circumference, similar to those without epilepsy, before the seizures began. After seizures began, we observed a large increase in head circumference z-score, which may represent an anatomical change in the brain that could be causing the seizures. These trends are outlined below in Table 2.

**Table 2: Head Circumference z-score by Seizure Status**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Average head circumference z-score with epilepsy</th>
<th>Average head circumference z-score without epilepsy</th>
</tr>
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<tbody>
<tr>
<td>6</td>
<td>1.12</td>
<td>0.482</td>
</tr>
<tr>
<td>12</td>
<td>1.22</td>
<td>0.667</td>
</tr>
<tr>
<td>24</td>
<td>1.15</td>
<td>0.871</td>
</tr>
</tbody>
</table>

In every result of statistical analysis that was significantly different based on the type of seizures, infantile spasms were occurred in those who had larger head circumferences. This is important because we know that infantile spasms in particular are detrimental to infants’ development, leading to refractory epilepsy and epileptic encephalopathy (Chu-Shore *et al.*, 2010). This suggests that infantile spasms affect brain growth in a way that focal spasms or other seizure types do not, as there was only a significant post seizure onset z-score increase in those with infantile spasms alone, but not any other one seizure type. Another possibility is that infantile spasms typically have an earlier onset than focal seizures, so the change in head circumference may be greater from a younger age. If we can reduce the rate of refractory epilepsy, we also could be
reducing the amount of resulting cognitive impairments, and improving the outcomes of infants with TSC.

We conclude that increased head circumference could be used as a biomarker in TSC in one of three ways. First, having a head circumference that is slightly above average from a young age, could suggest that the infant may have TSC, if it has not already been diagnosed. A TSC diagnosis can then be confirmed with a genetic test. Second, if the increase in head circumference is slight, between half a standard deviation and 1 standard deviation above the mean, there is also a risk that this child may have mild epilepsy (1 seizure type). Third, for those who have an increased head circumference of about 1 standard deviation or above the mean, there is a risk of severe epilepsy (2 or more types of seizures). This biomarker could be further tested by performing regular electroencephalograms on all infants diagnosed with TSC in order to diagnose and track seizures, in addition to the yearly MRIs most individuals with TSC have to track tubers. If our conclusions were supported in the future, after additional research is conducted, only those who carry a risk of seizures would need to continue those diagnostic tests. Additionally, if the risk of severe epilepsy is supported then, perhaps these children can be proactively treated with an anti-epileptic in order to reduce the chance of refractory epilepsy as a preventative measure. As mentioned above, those with TSC have an approximate 50% chance of developing autism, and by having early onset severe epilepsy, individuals risk of developing increases, because the seizures damage healthy brain tissue. Thus, if we are able to control epilepsy, such that it does not damage healthy tissue, we could reduce the rate of autism in the TSC population. We could further this effort, by not only preventively treating epilepsy, but perhaps we could preventively treat
autism (i.e.: with applied behavioral analysis therapy) to further decrease the rate of autism in TSC.
References:
Bartholomeusz HH, Courchesne E, Karns CM. (2002). Relationship between head
circumference and brain volume in healthy normal toddlers, children, and adults.

challenges. Neuropsychiatric Disease and Treatment, Volume 14, 1-10.

from http://www.cdc.gov/ncbddd/autism/data.html

Chu-Shore, C. J., Major, P., Camposano, S., Muzykewicz, D., & Thiele, E. A.
Epilepsia, 51(7), 1236–1241.

Kwiatkowski, D. J., Whittemore, V. H., & Thiele, E. A. Tuberous sclerosis
complex: Genes, clinical features, and therapeutics. Weinheim, Germany: Wiley-
Blackwell. p.176-179

Genotype/Phenotype Correlations in Tuberous Sclerosis Complex.

Sclerosis: A New Frontier in Targeted Treatment of Autism.
Neurotherapeutics, 12(3), 572-583.

Davis, P. E., Filip-Dhima, R., Sideridis, G., Peters, J. M., Au, K. S., Northrup, H., Bebin,
Tuberous Sclerosis Complex in Infants. Pediatrics, 140(6).


Vignoli, A., Briola, F. L., Peron, A., Turner, K., Vannicola, C., Saccani, M.,
complex: Searching for risk markers. *Orphanet Journal of Rare Diseases*

*Orphanet J Rare Dis, 10*(1).

from [http://www.who.int/childgrowth/standards/second_set/technical_report
_2/en/](http://www.who.int/childgrowth/standards/second_set/technical_report
_2/en/)

World Health Organization Head circumference-for-age. (2018). Available from

Figure 1. Average head circumference of TACERN participants and WHO reveals differences between typically developing children and children with TSC. By using the t-score formula and a two tailed t-distribution, significant differences at 6, 12, and 24 months were detected. Centimeters were used in this figure, because the WHO population would always represent a z-score of 0, thus centimeters makes the difference easier to visualize. *** Indicates p<0.001
Figure 2. Above average head circumference z-score of all TACERN participants is proportional to their height. **A.** The average head circumference z-score of all TACERN participants was 0.97, with no significant sex differences between TACERN participants (p=0.298, two tailed t-test, when $\alpha=0.05$). **B.** A positive correlation between head circumference and height (Pearson’s $r=0.867$; $p=2.96\times10^{-183}$, two tailed t-test, when $\alpha=0.05$), suggests that the larger head circumference was relative to body size. Average head circumference of TACERN participants was still above the expected average based on age and sex.
Figure 3. TACERN infants with epilepsy have a significantly larger head circumference z-scores than TACERN infants without epilepsy. Those who had any seizure type (red) had a significantly larger z-score than those who did not have seizures (blue) (p=0.0487, tailed t-test, when α =0.05). The average head circumference z-score for those with epilepsy was 1.08, while the average for those without epilepsy was 0.571. There was no significant difference in head circumference z-score between those TACERN participants without epilepsy compared to TACERN infants with any one type of seizure (i.e. infantile spasms). *Indicates p<0.05
Figure 4. Infants with two seizure types have a significantly larger head circumference z-score. As previously mentioned, individuals with epilepsy had larger head circumference z-scores, however when analyzed by the number of seizure types, only those with two or more seizure types had significantly larger head circumference z-scores than those with epilepsy. Those with no seizures had an average head circumference z-score of 0.571, one seizure type had a z-score of 0.881 and with two types of seizures, and the average z-score was 1.31. The average z-score of all participants with at least one type of seizure was 0.960. * Indicates p<0.05.
**Figure 5.** Analysis of head circumference z-score at 6, 12, and 24 months of all TACERN participants, by epilepsy status, reveals an increased head circumference that is consistent throughout infancy and larger for those with epilepsy. The closest measurement to each of the time points was used instead of average z-score across the first two years of life, as in Figure 2A; most infants were not seen at exactly 6, 12, or 24 months, therefore the closest visit to each age was used. At 6 months of age, head circumference z-score significantly differed between those who did and did not have seizures. The average head circumference z-score for those who did have seizures at 6 months was 1.12, the average for those who did not was 0.482. At 12 months of age, head circumference z-score was not significantly differed between those who did and did not have seizures (p=0.059, two tailed t-test, when α =0.05). The average head circumference z-score for those who did have seizures at 12 months was 1.22, the average for those who did not was 0.667. At 24 months of age, head circumference z-score was not significantly differed between those who did and did not have seizures (p=0.342, two tailed t-test, when α =0.05). The average head circumference z-score for those who did have seizures at 24 months was 1.15, the average for those who did not was 0.871. Although there was no statistically significant difference between those with and without epilepsy at 12 and 24 months, there are consistent increases in the mean z-score from 6 to 24 months. *Indicates p<0.05
Figure 6. Difference in pre and post seizure onset head circumference z-score for TACERN infants with one seizure type but not with two seizure types; and increase in z-score for TACERN infants without epilepsy overtime. A. For TACERN participants with one seizure type, their head circumference z-score was significantly different from pre to post seizure onset; this significant difference was conserved when tested with any participant who had any number of seizure types. However, for TACERN participants with two or more seizure types, there was no significant difference in pre and post seizure onset head circumference z-score ($p=0.229$, two tailed t-test, when $\alpha=0.05$). TACERN participants who do not have epilepsy had a significantly larger head circumference z-score from their first measurement to their last measurement. This suggests that even without epilepsy, those with TSC have larger heads than the typically developing population, and infants with TSC change percentile of head circumference, unlike the typically developing population. *Indicates $p<0.05$. ***Indicates $p<0.001$. 

![Graph A: Average Head Circumference z-score](image1)

![Graph B: Average Head Circumference z-score](image2)
Figure 7. Difference in head circumference z-score between infants with two or more seizure types and infants without epilepsy is localized to 6 months of age. As seen in Figure 5, there were differences in head circumference between infants with and without epilepsy at 6 months of age, however that difference was driven by infants with 2 or more seizure types as shown here, this is the only significant difference. When analyzing the data in this manner, each subcategory had a fairly small sample size, thus other relationships may exist that were not present in our sample. * Indicates p<0.05
### Table 3: Two tailed t-tests of head circumference z-score by seizure status

<table>
<thead>
<tr>
<th>t-test</th>
<th>6 Months</th>
<th>12 Months</th>
<th>24 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS and FS vs no SZ</td>
<td>0.0078*</td>
<td>0.023*</td>
<td>0.492</td>
</tr>
<tr>
<td>IS and FS vs IS</td>
<td>0.0076*</td>
<td>0.174</td>
<td>0.806</td>
</tr>
<tr>
<td>IS vs all</td>
<td>0.720</td>
<td>0.024*</td>
<td>0.803</td>
</tr>
<tr>
<td>IS and OS vs no SZ</td>
<td>N/A</td>
<td>0.278</td>
<td>0.053</td>
</tr>
<tr>
<td>IS and OS vs IS</td>
<td>N/A</td>
<td>N/A</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

*Represents a significant difference
IS= infantile spasms, FS= focal spasms, SZ= seizures, All= IS, FS, and OS, OS=other seizure type; N/A represents too small of a sample size to run a two tailed t-test; Values represent p-values when $\alpha=0.05$ using a two tailed t-test
Figure 8. Brain volumes of all participants were correlated to average head circumference, suggesting head circumference is an accurate measure of brain size. This data includes all TACERN participants— with and without epilepsy. A. Total brain volume (ICC, intracranial cavity) was positively correlated with head circumference \((r=0.919, p=8.74\times10^{-131}, \text{two tailed t-test, when } \alpha =0.05)\). This suggests that larger head circumference was not due to measurement errors or hair volume. Additionally, head circumference is an effective measure of brain size. B. White matter (WM) volume was positively correlated and significantly related to head circumference \((r=0.884, p=6.91\times10^{-107}, \text{two tailed t-test, when } \alpha =0.05)\). C. Subcortical grey matter (GM) volume was positively correlated to head circumference \((r=-0.841, p=7.14\times10^{-87}, \text{two tailed t-test, when } \alpha =0.05)\). D. Cortical grey matter volume was also positively correlated to head circumference \((r=0.847, p=2.47\times10^{-89}, \text{two tailed t-test, when } \alpha =0.05)\). The linear fit of these graphs were similar but slightly better than a cubic fit, and a cubic relationship between volume \((\text{mm}^3)\) and head circumference \((\text{cm}^1)\) as previously demonstrated in the literature.