Neuroimaging in Paediatric Tuberous Sclerosis Complex

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NEUROIMAGING IN PAEDIATRIC TUBEROUS SCLEROSIS COMPLEX

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A thesis submitted in fulfilment of the requirements for the degree of Masters of Medicine by Research

School of Women's and Children's Health
Faculty of Medicine

2022

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Publication Details #1

Full Title:	The natural history of subependymal giant cell astrocytomas in tuberous sclerosis complex: a review
Authors:	Chan DL, Calder T, Lawson JA, Mowat D, Kennedy SE
Journal or Book Name:	Reviews in the neurosciences
Volume/Page Numbers:	29(3):295-301
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Status:	published
The Candidate's Contribution to the Work:	I performed the literature review, critically appraised literature, had input into structure of paper (in particular, suggested section on congenital SEGA), drafted and performed revisions of manuscript
Location of the work in the thesis and/or how the work is incorporated in the thesis:	This publication is located in Chapter 1.2 of my thesis. It represents the beginning of my wider literature review (Chapter 1) on the neuroradiological features of TSC which includes SEGA as well as cortical tubers, SEN and radial migration lines. As my paper was accepted in August 2017, I have update the literature review with new literature in the following chapter (Chapter 1.3).

Publication Details #2

Full Title:	Congenital subependymal giant cell astrocytoma in children with tuberous sclerosis complex: growth patterns and neurological outcome
Authors:	Chan DL, Kennedy SE, Sarkozy V, Chung C, Flanagan D, Mowat D, Farrar M, Lawson JA
Journal or Book Name:	Pediatric Research
Volume/Page Numbers:	89(6):1447–1451
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Status:	published
The Candidate's Contribution to the Work:	I co-designed the study with my supervisor Dr Lawson, collected data, measured SEGA on MRI, performed statistical analysis, drafted original manuscript and performed revisions of manuscript
Location of the work in the thesis and/or how the work is incorporated in the thesis:	The published manuscript of this work is located in Chapter 2 of my thesis. This study was conceptualised after I identified a paucity in literature regarding congenital SEGA during my literature review in Chapter 1. In particular, one study suggested rapid aggressive growth of congenital SEGA when compared to non-congenital SEGA, but no studies since had corroborated this finding. Chapter 2 details my attempt to address this through a retrospective case series of congenital SEGAs patients seen in our tertiary referral hospital over 10 years.

Candidate's Declaration

I confirm that where I have used a publication in lieu of a chapter, the listed publication(s) above meet(s) the requirements to be included in the thesis. I also declare that I have complied with the Thesis Examination Procedure.

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Firstly, to my amazing husband Jeff, thank you. I couldn't have done this without your support. To my darling daughter Hayley, you've given me a real reason to finish this. Thanks also to David, Mei Yong, Julia, Jennifer, Nathan and both Mums and Dads. Your practical support, cooking and words of wisdom have guided me along. And of course, enormous thanks to Clara, who is my family in many senses of the word.

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Soli Deo Gloria.

ABBREVIATIONS

TERM	DEFINITION
1.5T	1.5 Tesla
3T	3 Tesla
ABC	Adaptive behavior composite
ACT	Australian Capital Territory
ADC	Apparent diffusion coefficient
ADOS	Autism diagnostic observation scale
AED	Antiepileptic drugs
AML	Angiomyolipoma
ASD	Autism Spectrum Disorder
AUC	Area under curve
CARS	Childhood Autism Rating Scale
CBD	Cannabidiol
CI	Confidence interval
CR	Cardiac rhabdomyoma
CRF	Case report form
CSF	Cerebrospinal fluid
CT	Computed tomography
DD	Developmental disability
DICOM	Digital imaging and communications in medicine
DQ	Developmental quotient
DRE	Drug resistant epilepsy
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th edition
DTI	Diffusion tensor imaging
EEG	Electroencephalogram
EVE	Everolimus
EXIST-1	EXamining everolimus In a Study of Tuberous sclerosis complex-1
EMBASE	Excerpta Medica dataBASE
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
FLASH	Fast low angle single shot
FoV	Field of view
FS	Focal seizure
GARS	Gilliam Autism Rating Scales
GRE	Gradient echo
ICP	Intracranial pressure
ILAE	International league against epilepsy
IS	Infantile spasms
IQ	Intelligence quotient
IQR	Interquartile range
KD	Ketogenic diet
LAM	Lymphangioleiomyomatosis
MRI	Magnetic resonance imaging
MSEL	Mullens Scale of Early Learning

mTOR	mechanistic target of rapamycin
mTORC1	mechanistic target of rapamycin complex 1
NAWM	Normal appearing white matter
NMI	No mutation identified
n.s.	Not significant
NZ	New Zealand
OR	Odds ratio
PKD	Polycystic kidney disease
ROC	Receiver operating characteristic
RML	Radial migration line
SCH	Sydney Children's Hospital
SD	Standard deviation
SEGA	Subependymal giant cell astrocytoma
SEN	Subependymal nodule
SIR	Sirolimus
SVT	Supraventricular tachycardia
SWI	Susceptibility weighted imaging
T1WI	T1 weighted imaging
T2WI	T2 weighted imaging
TBC1D7	TBC1 Domain Family Member 7
TBP	Tuber brain proportion
TE	Echo time
TOSCA	TuberOus SClerosis registry to increase disease Awareness
TR	Relaxation time
TSC	Tuberous Sclerosis Complex
USA	United States of America
VIC	Victoria
Vineland-III	Vineland Adaptive Behavior Scales, 3rd Edition
WHO	World Health Organisation
WISC-III	Wechsler intelligence scale for children, 3rd Edition
WPPSI-III	Wechsler preschool and primary scale of intelligence, 3rd Edition

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PUBLICATIONS

Chapters 1.2 and Chapter 2 represent separate papers which have been published in peer-reviewed journals as follows:

Chapter 1.2: Chan DL, Calder T, Lawson JA, Mowat D, Kennedy SE. (2018). The natural history of subependymal giant cell astrocytomas in tuberous sclerosis complex: a review. Rev Neurosci, 29(3):295-301. doi: 10.1515/revneuro-2017-0027

Chapter 2: Chan DL, Kennedy SE, Sarkozy V, Chung C, Flanagan D, Mowat D, Farrar M, Lawson JA. (2021). Congenital subependymal giant cell astrocytoma in children with tuberous sclerosis complex: growth patterns and neurological outcome. Pediatr Res, 89(6):1447-1451. doi: 10.1038/s41390-020-1002-7. Epub 2020 Jun 9.

PRESENTATIONS

Chan DL, Flanagan D, Mowat D, Farrar M, Kennedy SE, Lawson JA. Congenital SEGA in children with tuberous sclerosis complex – natural history and neurological outcome. International TSC Research Conference, Washington 2017

Chan DL, Kennedy SE, Sarkozy V, Farrar M, Mowat D, Lawson JA. TSC neuroradiological markers, epilepsy and cognitive outcomes at 5 years of age. International TSC Research Conference, Tokyo 2018

Chan DL, Kennedy SE, Sarkozy V, Farrar M, Mowat D, Lawson JA. TSC neuroradiological markers, epilepsy and cognitive outcomes at 5 years of age. 46th Annual Tow Research Awards, Prince of Wales Hospital, Randwick, Sydney, 2018

AWARDS

Francis G. Byrnes and Vicky H. Whittemore Travel Award. Awarded by USA Tuberous Sclerosis Alliance. Washington, 2017.

ABSTRACT

This thesis explores novel aspects of the neuroradiological features of tuberous sclerosis complex (TSC), including the relationship between these features and subsequent epilepsy and developmental outcome in later childhood. The neuroradiological features of TSC include cortical tubers, subependymal nodules and subependymal giant cell astrocytoma (SEGA). Patients with TSC have high rates of refractory epilepsy, developmental delay and Autism Spectrum Disorder (ASD), but are affected to varying degrees leading to a wide phenotypic spectrum. Prior studies focussing on TSC neuroradiological markers have found some associations between lesion burden and severity of neurological dysfunction, but the literature remains inconclusive. Literature regarding congenital SEGA is limited but suggested they grew more aggressively than other SEGA.

The first study in this thesis addresses this literature gap through a case series of ten congenital SEGA at a single TSC referral centre. Using serial MRI, our study found median congenital SEGA growth rate of 1.1mm/yr in diameter or 150mm^3/yr volumetrically, which is lower than previous published reports. Congenital SEGA with volume >500mm^3 had significantly higher growth rates compared with smaller SEGA. Children with congenital SEGA tended to have more severe epilepsy, developmental disability and ASD compared to genotype-matched controls, suggesting that congenital SEGA may be a biomarker for poor neurological outcome, which is a novel finding.

The second study explores whether neuroradiological features of TSC on early MRI are biomarkers for neurological and developmental outcomes. Thirty-nine MRIs acquired between 18-36 months from children with TSC were scored blindly. We found children with drug resistant epilepsy (DRE) had more tubers and subependymal nodules (SEN) compared to children without DRE. More frontal tubers were found in children with impaired adaptive function and children with ASD. Therefore, tuber count and frontal tuber location on early MRI may contribute to predicting DRE, adaptive function and ASD at 5 years of age.

In conclusion, this thesis determines that neuroradiological markers have an important role in understanding TSC patients' neurological and developmental outcome at 5 years

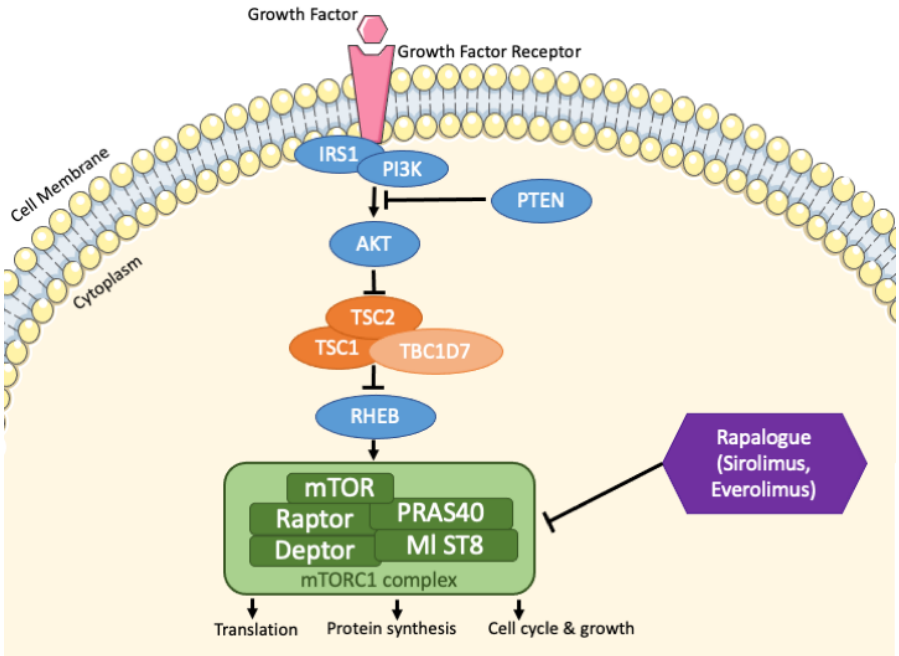
of age. Early identification of patients at risk for poor outcomes would open opportunities for early intervention and more aggressive epilepsy treatment with the aim of optimising neurological and developmental outcome.

CHAPTER 1 - Literature review: Neuroimaging markers of TSC

1.1 Introduction

Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder with an estimated incidence of 1 per 6000 livebirths, which is caused by a mutation in *TSC1* or *TSC2* gene [1]. Physiologically, the proteins encoded on these genes (hamartin and tuberin respectively) together with TBC1D7 form a tumour suppressing protein-complex known as "TSC complex", which downregulates mTORC1 function in the mechanistic (formerly mammalian) target of rapamycin (mTOR) pathway (Figure 1.1) [2]. Thus, mutations in either the *TSC1 or TSC2* gene cause a functionally defective TSC complex. The resultant overactivity of mTORC1 which underpins TSC disease, leads to cellular overgrowth, abnormal cellular differentiation and aberrant cellular migration (Figure 1.1). As mTOR is an essential cellular process in all cells, TSC is a multisystem disease.

Figure 1.1 (author's own). TSC1 and TSC2 form part of the mTOR pathway



Classically described as a neurocutaneous disorder, the dermatological signs of TSC include hypomelanotic macules, shagreen patches, facial angiofibromas, forehead plaques and ungual fibromas. Benign tumours can be found in multiple organs including but not limited to cardiac rhabdomyomas, pulmonary lymphangioleiomyomatosis (LAM), renal angiomyolipomas (AML) and retinal hamartomas. These clinical features are reflected in the TSC diagnostic criteria (Table 1.1) [3].

Table 1.1 Tuberous Sclerosis Complex diagnostic criteria

Major features	**Minor features**
Hypomelanotic macule (≥3, at least 5mm diameter)	"Confetti" skin lesions
Angiofibromas (≥3) or fibrous cephalic plaque	Dental enamel pits (≥3)
Ungula fibromas (≥2)	Intraoral fibromas (≥2)
Shagreen patch	Retinal achromic patch
Multiple retinal hamartomas	Multiple renal cysts
Multiple cortical tubers and/or RMLs	Nonrenal hamartomas
Subependymal nodules (SEN, ≥2)	Sclerotic bone lesions
Subependymal giant cell astrocytoma (SEGA)	
Cardiac rhabdomyoma	
Lymphangioleiomyomatosis (LAM)*	
Angiomyolipomas (AML, ≥2)*	

Definite TSC diagnosis: 2 major features, or 1 major with ≥2 minor features

Possible TSC diagnosis: Either 1 major feature or ≥2 minor features

Genetic diagnosis: A pathogenic variant in *TSC1* or *TSC2* is diagnostic for TSC (most TSC- causing variants are sequence variants that clearly prevent TSC1 or TSC2 protein production. Some variants compatible with protein production [e.g., Some missense changes] are well established as disease-causing; other variant types should be considered with caution).

***** A combination of the 2 major features LAM and AML without other features does not meet criteria for a definite diagnosis.

Adapted from: Northrup et al., 2021 [3]. *AML: Angiomyolipoma, LAM: Lymphangioleiomyomatosis, RML: Radial migration line, SEN: Subependymal Nodule, SEGA: Subependymal giant cell astrocytoma.*

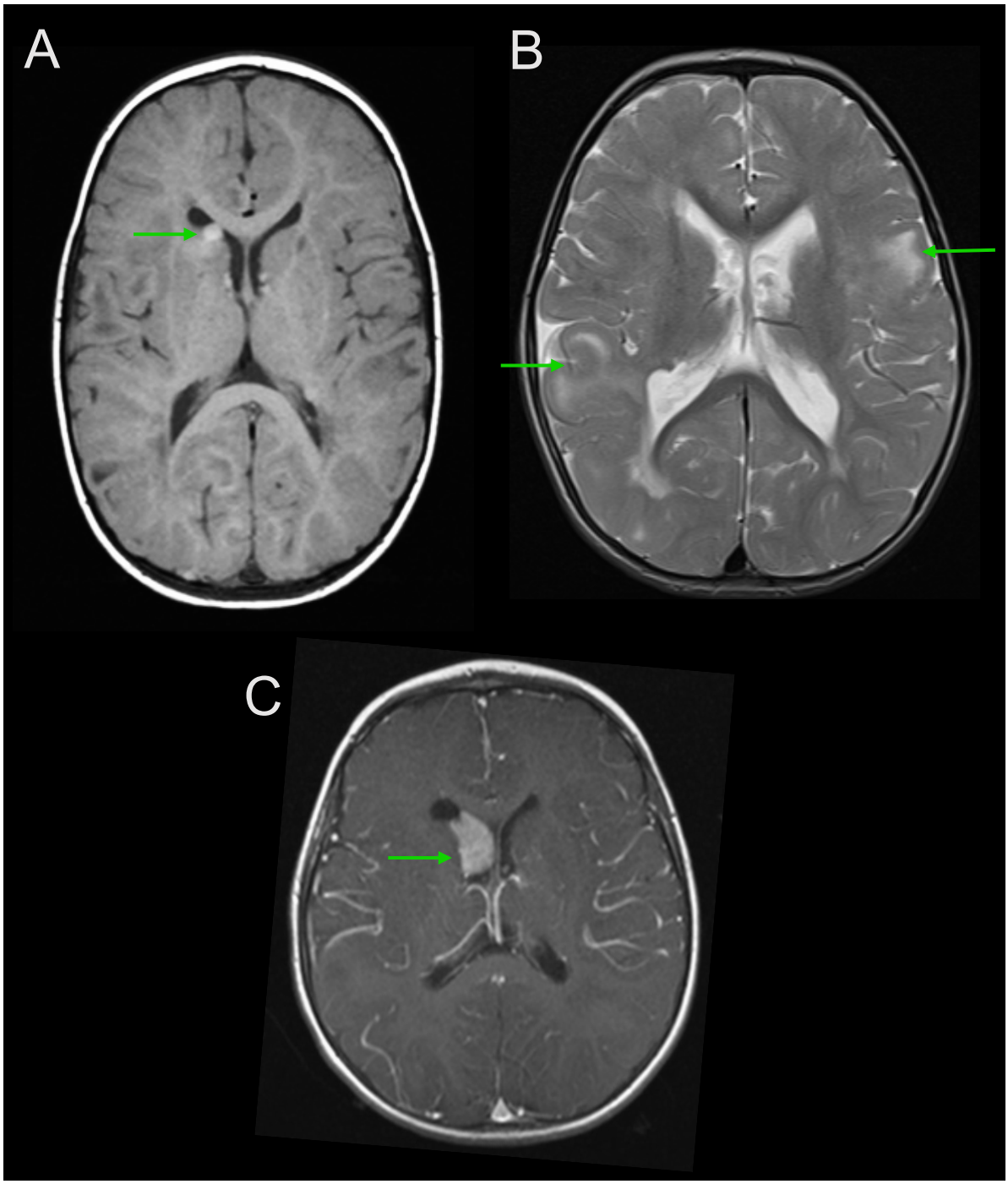
Undoubtedly, the biggest impact of TSC on affected children is felt through the disease's neurological manifestations, which can be divided into structural and functional (Table 1.2). Some structural brain changes are readily seen at birth on neuroimaging, and detection via ultrasound or foetal magnetic resonance imaging (MRI) can lead to an antenatal diagnosis. Cortical tubers, subependymal nodules (SEN) and subependymal giant cell astrocytoma (SEGA) together make up the classical triad of neuroradiological markers of TSC (Figure 1.2). Other TSC neuroradiological markers include radial migration lines (RML), cerebellar tubers, cortical malformation and white matter cysts. SEGA are particularly noteworthy, as they are a low-grade intraventricular tumour with potential to cause obstructive hydrocephalus by blockage of the foramen of Monro. Thus, serial MRI are recommended to monitor SEGA every 1-3 years up to the age of 25 [4].

Table 1.2 TSC features affecting structure and function of the brain

Structure	**Function**
SEGA	Epilepsy
SEN	
Cortical tubers	Development
RML	Intellectual disability
Cerebellar tubers	ASD
	Aggression
	Anxiety
Visible on MRI	Difficult to quantify
Present from birth	Emerge with age

ASD: Autism Spectrum Disorder, MRI: Magnetic Resonance Imaging, RML: Radial migration lines, SEN: Subependymal Nodule, SEGA: Subependymal giant cell astrocytoma.

Figure 1.2. Neuroradiological features of TSC on MRI



Magnetic Resonance Imaging (MRI) of three patients from Sydney Children's Hospital TSC clinic (A) T2 FLAIR axial image showing a subependymal nodule (SEN), (B) T2 axial image showing bilateral tubers, (C) T1 post-contrast axial image showing right sided SEGA with mild dilation of frontal horn.

TSC not only affects brain structure but also impacts neurological function. In contrast to MRI changes which are visible at birth, epilepsy, intellectual disability and autism spectrum disorder (ASD) emerge over time, with extremely variable neurodevelopmental outcome. Epilepsy affects approximately 80% of patients before 2 years of life and is often refractory, with earlier onset and infantile spasms associated with a poor cognitive outcome [5]. A wide phenotypic variability is seen in patients with TSC – whilst some patients are severely affected with drug-resistant epilepsy and profound developmental delay, others have normal cognition and only mild skin manifestations.

Biomarkers, short for 'biological markers', are defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.' [6] Currently, neuroimaging in TSC is limited to SEGA surveillance and epilepsy investigation. The objective of this thesis is to identify effective neuroimaging biomarkers in TSC, giving MRI studies a role in identification of patients at high risk of poor neurological outcome. As MRIs are widely available and provide quantifiable data, multiple possible candidate biomarkers exist for investigation. Reliable biomarkers of TSC would allow for personalisation of treatment through timely identification of candidates for aggressive epilepsy treatment, drug trials and early intervention.

While this thesis aims to focus on neuroimaging biomarkers, additional biomarkers have been used to be effective in prognosticating outcome in TSC. Serial EEG has been demonstrated to be a useful biomarker in predicting epilepsy onset in infants with TSC [7]. The adoption of EEG as a potential prognostic biomarker can be seen in the recent EPISTOP trial [8]. Even though EEG is increasingly used as a prognostic biomarker, there are remaining uncertainties and an unmet need for additional imaging biomarkers, with comparison to EEG.

The first chapter provides a comprehensive overview of current literature regarding neuroimaging markers of TSC. Appraisal of current knowledge will provide the rationale for this research thesis. This chapter begins with a literature review addressing SEGA (published in *Reviews in the Neurosciences* in 2018), followed by an update

incorporating newly published literature. Following this, other TSC neuroimaging markers are discussed, including cortical tubers, SEN, cerebellar tubers, and radial migration lines. The review also summaries the previous work in relation to TSC neuroimaging markers and their association with epilepsy, developmental disability and ASD.

1.2 SEGA

1.2.1 Link to Thesis

To begin my thesis, I undertook a wide review of the literature encompassing the neuroradiological markers of TSC. SEGA are of particular interest because of their growth and management implications, whereas other neuroradiological markers are generally static and do not require specific management. Chapter 1.2 was published in *Reviews in the Neurosciences* in 2018 entitled "The natural history of subependymal giant cell astrocytomas in tuberous sclerosis complex: a review" and represents the beginning of the literature review in this thesis. Preliminary literature search did not yield enough literature to support a systematic review. In future, further studies in this area would allow a systematic review to analyse a greater body of work.

1.2.2 Abstract

Tuberous sclerosis complex is an autosomal-dominant inherited condition with an incidence of approximately 1:6000 births, characterised by deregulated mTOR activity with multi-site hamartomas. Subependymal Giant Cell Astrocytoma (SEGA) are one such hamartoma, affecting up to 24% of patients with tuberous sclerosis complex. Their intraventricular location may lead to life-threatening obstructive hydrocephalus. Current management is hampered by a lack of understanding regarding the natural history, behaviour and growth patterns of SEGAs. We review current literature to summarise what is known about SEGA in the following areas: (1) Diagnostic Criteria (2) Prevalence (3) Origin (4) Imaging Characteristics (5) Growth rate (6) Genotype-phenotype correlation (7) Congenital SEGA (8) SEGA as a marker of severity of other TSC manifestations.

Keywords: Genotype; Growth; Neuroimaging; MRI; SEGA; TSC

1.2.3 Introduction

Tuberous Sclerosis Complex (TSC) is an autosomal dominant, inherited multisystem disorder characterised by benign tumours, with an estimated incidence of 1:6000 [9].

Mutations in the *TSC1* or *TSC2* tumour suppressor genes trigger a constitutive activation of the mechanistic target of rapamycin (mTOR) signaling pathway, with subsequent upregulation in cell growth and proliferation [10].

Cerebral manifestations of TSC include two related hamartomatous lesions - subependymal nodules (SEN) and subependymal giant cell astrocytomas (SEGA). Along with tubers and radial glial lines, these entities encompass the neuroradiological findings of tuberous sclerosis complex. SEN are seen in more than 80% of TSC affected individuals, whereas SEGA are reported to occur in 5-25%, typically arising within the first or second decades of life [11,12]. SEGA are slow-growing WHO grade 1 tumours composed of mixed glioneuronal cell lineage [13]. They are commonly located along the lateral ventricle wall, some being adjacent to the foramen of Monro. Due to this critical location, SEGA can contribute to morbidity and mortality by means of local mass effect causing obstructive hydrocephalus, or infrequently haemorrhage.

Detection of asymptomatic SEGA and monitoring of growth depend on active neuroimaging surveillance. Current consensus guidelines recommend that neuroimaging be performed every 1 to 3 years in patients with TSC, up to the age of 25 years [4], with the preferred modality being magnetic resonance imaging (MRI). More frequent imaging is advisable if SEGA are large, growing or symptomatic [14,15]. Large and symptomatic SEGAs have previously been managed by surgical resection, however randomised controlled trials have shown that medical management with mTOR inhibitors can cause reduction in size of SEGAs [16]. The mTOR inhibitor everolimus was approved by the FDA in 2010 for SEGAs associated with TSC that cannot be treated with surgery.

For clinicians involved in the management of TSC, current guidelines for surveillance and management pose several challenges. MRI is both a costly and limited resource which is not universally available, particularly in resource-poor countries. Young children and those who are uncooperative due to developmental disability or behavioural comorbidities require sedation or general anaesthesia to undergo MRI due to significant scan duration. Computed Tomography (CT) is an alternative to MRI but carries with it the risk of radiation exposure. Similarly, both surgical and medical

management of SEGA with mTOR inhibition carry risks. The duration of medical therapy in patients with SEGA has yet to be determined. With these complexities in mind, an improved understanding of the natural history of SEGA growth could assist in risk assessment and evaluating prognosis for individual patients.

To date, few studies have attempted to elucidate the natural history and growth of SEGA and their nodular precursors [17,18]. This report will review our current understanding of the natural history of SEGA and possible interrelationships between SEGA and other clinical manifestations of TSC.

1.2.4 Methods

A comprehensive review of the literature was conducted using Medline, EMBASE and the Cochrane Library, for relevant publications from 1985 through 2016. A series of searches was performed encompassing SEGA natural history, SEGA growth rates, SEGA treatment and TSC multisystem correlations. Major search terms included ‘Tuberous Sclerosis’, ‘subependymal giant cell astrocytoma OR astrocytoma’, and ‘growth OR growth rate OR size’. Additional searches were conducted to correlate clinical features, including renal angiomyolipomas, cognitive deficits, epilepsy, facial angiofibromas and cardiac rhabdomyomas. Studies were included if they were published in a peer-reviewed journal and available in English language. Reference lists of retrieved articles were manually consulted for additional relevant material. Author TC performed the initial literature review, which was updated and finalized by DLC. In total, 76 papers were retrieved, excluding one which was rejected due to publication being withdrawn.

1.2.5 Review

1.2.5.1 Diagnostic Criteria of SEGA

Cerebral tumours were noted as a feature of TSC by Dr Manuel R. Gomez in his seminal work "Tuberous Sclerosis" in 1960's and the term SEGA was originally coined by Russell et al. to distinguish it from other intracerebral tumour [19,20]. Since then, the definition of SEGA has been poorly standardised across the literature. A working definition was agreed upon by an invited expert panel at the International TSC Consensus Conference in 2012 as "a lesion at the caudothalamic groove with either a size of more than 1 cm in any direction or a subependymal lesion at any location that has shown serial growth on consecutive imaging regardless of size" [4]. In the same year, a paper resulting from the European TSC Consensus Meeting for SEGA and Epilepsy Management proposed a smaller size criteria for SEGA according to the following definition: "a tumour in a TSC patient that is usually characterized by a location near the foramen of Monro, >0.5cm in diameter, with any documented growth…" [21]. Whilst the definitions are similar, minor discrepancies suggest there is still no universally accepted SEGA definition to date. Most recent publications have partly conformed to the former definition [4], using a size criteria of at least 10mm diameter and evidence of growth. The recent EXamining everolimus In a Study of Tuberous sclerosis complex (EXIST-1) trial accepted as its inclusion criteria SEGA "with the longest diameter 1 cm or greater as assessed by multiphase MRI" along with evidence of lesional volume growth greater than 25%, worsening hydrocephalus or presence of new lesion when compared with previous scan [16]. Other studies have added to the diagnostic features of SEGA to include location in the region of the foramina of Munro, serial radiological growth and association with clinical symptoms of hydrocephalus [22,23].

1.2.5.2 Prevalence

The prevalence of SEGA in the recently published international cross-sectional study of a large cohort of TSC patients was 24.4% [24]. This figure is similar to the TSC Natural History Database, comprising 919 patients from 16 tertiary referral centres in USA and Belgium, which found 24% of TSC patients had SEGA [12]. Similarly, a retrospective

cross-sectional study of a large national referral centre for TSC in Netherlands with 214 TSC patients found prevalence of SEGA at 20% (22% in males, 18% in females) [25]. Single centre series which concentrate on SEGA surgery rates, find prevalence rates which are lower at 8-14% [22,26], but do not necessarily capture all asymptomatic SEGA or lesions not requiring excision.

SEGA primarily arise in the first two decades of life [27], although sporadic adult onset cases have been reported. Nabbout's series of 60 French children showed that the first MRI evidence of SEGA growth occurred between 1 and 9 years, with a mean age of 4 years. SEGA surgery occurs at a mean age of 9.7 years in Kotulska's study (2014), 10.5 years in Cuccia's study (2003) and 10.6 years in Goh's study (2004). This is in concordance with the EXIST-1 trial, where the mean age of patients enrolled was 9 years. The best evidence that de novo SEGA can continue to present in adulthood comes from Adriaensen who found new SEGA in 3 adult patients out of a group of 138 (2%), without radiological signs of SEGA 5 years previously. These 3 patients were aged 19, 23, and 41 years old, thus only 1 patient (0.7%) developed a SEGA beyond 25 years [28].

1.2.5.3 Origin from SEN

Serial neuroimaging studies have demonstrated a radiographic continuum between growing SEN and SEGA, suggesting nodules have the potential to gradually transform into SEGA [17,18]. Histologically, SEGA are indistinguishable from SEN. The first objective evidence that a SEN may transform into an obstructing SEGA was provided by a single patient case report by Fujiwara et al. (1989) using sequential CT data. Evidence for the evolution of SEN to SEGA was later corroborated by Nabbout et al. (1999) who retrospectively reviewed MRI imaging of 60 children with TSC and found 24 who had SEN localised near the foramen of Monro. Of these, 8 developed into SEGA during follow up of MRI between 1 and 9 years of age (mean 4 years).

It is possible that the biological mechanism by which SEN transform into SEGA involves a somatic event, a second TSC gene "hit", amounting to loss of heterogeneity and overactivity of the mTOR pathway. In 1997, Henske found allelic loss common in

renal and cardiac associated TSC hamartomas (56%), but rare in TSC brain lesions (4%) [29](Henske et al., 1997). However, a further study on SEGA pathogenesis found biallelic mutation of TSC1 or TSC2 in 5 of 6 SEGA [30] (Chan et al., 2004). An immunohistochemistry study of 9 SEGA from patients with TSC found loss of hamartin (the product of TSC1 gene) expression in all SEGA, and 6 also had no expression of tuberin (product of TSC2 gene) [31]. Most recently, Martin et al. found that "roughly two-thirds of hamartomas from TSC1/TSC2 patients harboured two TSC hits, including most RAs and SEN/SEGAs". Within the 18 SEGA and 2 SEN samples analysed, the second-hit mutation were almost exclusively copy-neutral loss of heterogeneity events, which occur during errors in mitotic recombination [32].

Nabbout et al (1999) suggested that risk factors for SENs to transform into SEGAs include nodule size above 5mm, incomplete calcification, and gadolinium enhancement. Menor, Marti-Bonmati, Mulas, Poyatos, and Cortina (1992) also reported that marked enhancement is a stronger indicator than size for transformation potential, although this finding has not been replicated to date.

1.2.5.4 Imaging Characteristics

Identifying SEGA represents a logistic and resource challenge in TSC management because of the characteristics of the target patient population. MRI is currently the recommended modality for screening SEGAs, although they can also be diagnosed via CT, antenatal and postnatal ultrasound. MRI shows well-circumscribed tumours as iso- or hypointense on T1 weighted images, and iso- or hyperintense lesions on T2 weighted images [33]. Their assessment is aided by contrast enhancement with gadolinium [34]. On CT, SEGA appear as a mass of uniform-density, often with partial calcification within the lesion. Regardless of modality, almost all SEGA are located in the region of the foramen of Monro, with a few cases reported to occur in the third ventricle. Associated findings may include ventricular dilatation and transependymal oedema secondary to CSF pathway obstruction.

1.2.5.5 Growth Rate

Studies evaluating serial SEGA growth are limited to a selection of small case series that predominantly focus on surgical management [22,35,36]. In addition, methods of measurement vary between maximal axial diameter, cross-sectional area or estimated volumetric values. These all report marked variability in tumour growth rates among their subjects (Table 1.3). Cuccia et al. (2003) conducted a retrospective analysis of all TSC patients who had SEGA excision between January 1988 and December 2000 at a single centre in Buenos Aires, Argentina (n=15). All tumours grew on serial CT or MRI imaging, and were observed for an average of 83 months prior to proceeding with surgery. The average growth rate was 3.4 mm/year in SEGA diameter (range 1-10mm). This study's strength was the extensive period of pre-operative sequential growth analysis, which ranged from 43 to 143 months (excluding one patient who needed immediate surgery), however the inclusion criteria pertained exclusively to surgical patients; 80% of whom presented with hydrocephalus. Hence the external validity of these results may be limited to the severe end of the SEGA spectrum. Small sample size further limits the robustness of this study.

Jiang et al (2011) demonstrated pre-operative growth of between 4.1 and 5.6 mm/year in a series of 2 patients. Park and colleagues observed SEGA growth in 4 patients (3 of whom had TSC) resulting in a mean growth rate of 2.53 mm/year [35,36]. Goh's paper was able to calculate SEGA growth rate in two patients for whom serial scans was available [26]. Interestingly they showed contrasting results, with one patient having no SEN visible on MRI 9 months prior to developing a 2 x 2 x 2cm SEGA with hydrocephalus. The other asymptomatic patient had a 1.8 x 1.4 x 1.7 cm SEGA which grew to 2.2 x 1.8 x 2.1cm in 5 years, with a resultant growth rate of 0.1cm/year. Krueger's phase 1-2 trial of everolimus was the only study identified that reported tumour volume growth before treatment (mean +0.54cm^3/year), in addition to volume reduction after therapy [15]. No SEGA regressed during the placebo arm of the EXIST-1 trial and 6 of 39 placebo patients had progression of SEGA. However, the exact pattern of growth over time, whether linear, exponential or sporadic, remains to be described in further detail.

Epidemiological trends seem to suggest younger patients have more aggressive SEGA growth. A case series of 59 SEGA patients found that growth rate was significantly higher in children compared to adults (75.6 vs 16.5% volume growth, p=0.03) [37]. In contrast, a similarly sized study of 43 patients with radiological evidence of SEGA found no association between patient age and SEGA size [25]. Torres et al. also observed SEGA growth to peak at puberty [27]. Histopathology of three SEGA developing in the first year of life were found to mimic malignant gliomas, with atypical histological pattern consistent with anaplastic features [38]. These features included necrotic foci, pseudopalisading, frequent mitotic figures and a high Ki67 index in the range of 15-20%, which together suggest that early SEGA within the first 12 months can belong in a more aggressive subset of SEGA.

The question of whether SEGA ever stop growing also remains. In Adriaensen's series looking at SEGA beyond the second decade of life, 10 of 43 patients (29%) showed a continued growth of SEGA over an average interval of 5 years. The remainder 33 patient's SEGA remained stable in size. The 10 SEGA which grew demonstrated an average size increase of 1mm/year [28].

Regrowth of SEGA after partial resection has been well described. Additionally, Kotulska described growth of contralateral SEGA in 15 patients (26%) 6 to 120 months after surgery but the growth rates were not quantified [39].

Table 1.3 Summary of literature regarding SEGA growth rate

Author	Year	Method	Number	Growth rate of diameter
Cuccia et al.	2003	Retrospective surgical patients	15	3.4 mm/yr
Adriaensen et al.	2014	Retrospective longitudinal study	10	1 mm/yr
Park et al.	2011	Prospective gamma knife surgery patients	4 (1 not TSC)	2.53 mm/yr
Kotulska et al.	2014	Congenital SEGA [TSC2 cohort]	4	16.2 mm/yr
Kotulska et al.	2014	Congenital SEGA [TSC2/PKD1 cohort]	3	65.2 mm/yr
Jiang et al.	2011	Retrospective surgical patients	2	4.1 mm/yr, 5.6 mm/yr
Goh et al.	2004	Retrospective chart review	2	1 mm/yr, >20 mm/yr

1.2.5.6 Genotype-phenotype correlations

Correlation between TSC2 gene mutation and a more severe TSC clinical phenotype has been extensively documented [12,40,41]. Kothare et al. (2014) also report that TSC2 mutation specifically confers an increased risk for more severe neurological manifestations. Patients with a known TSC2 mutation were 1.64 times more likely to develop SEGA than those with TSC1 mutation (p=0.01). A similar trend was reported by Dabora et al. (2001) in a retrospective, multicentre study, which remains the largest of its kind confirming severity of manifestations in relation to genotype. However, TSC mutation status was unknown in approximately one third of patients (297/919). Kotulska found that surgery for SEGA was on average performed at a younger age in patients with TSC2 mutation (6.8yr) when compared to patients with either TSC1 mutation (12.9yr) or no mutation identified (11.3yr).

1.2.5.7 Congenital SEGA

SEGA can rarely present as one of the earliest manifestations of TSC, with 28 reported cases of SEGA diagnosed antenatally or before 3 months of age [42,43]. With regards to congenital SEGA, patients with a contiguous TSC2/PKD1 deletion had SEGA which grew more rapidly (5.43 mm per month, n=3) compared with patients with a TSC2 sequence mutation (1.35 mm per month, n=4) [42]. Overall the growth rates of congenital SEGA have been reported as much higher than in the older TSC population.

1.2.5.8 SEGA as a marker of severity of other TSC manifestations

Few studies have investigated possible interrelationships between SEGA occurrence or growth and other manifestations of TSC. One study showed that patients with TSC who had infantile spasms have a higher chance of developing SEGA (OR 1.60, $p = 0.02$) compared with TSC patients without infantile spasms. This study also showed that patients with the TSC2 mutation were more likely to have SEGA (OR 1.67, $p = 0.01$) and infantile spasms (OR 1.64, $p = 0.01$) when compared with patients with TSC1 mutation, perhaps pointing to genotype being the common factor underlying this association. A retrospective review of clinical and radiological data from 27 cases of children with TSC concluded that there was no association between the progression of neuroradiological lesions (including SEGA, SEN and tubers) and neurological outcome [34]. Cuccia et al (2003) in their 12-year retrospective review noted that seizures and intellectual disability did not improve following SEGA surgical excision.

Menor et al. (1992) found that 80% of patients with SEGA had associated cardiac rhabdomyomas diagnosed as neonates. This observation has not been substantiated in recent literature and it may reflect the high prevalence of cardiac involvement in the neonate.

Like SEGA, renal angiomyolipoma (AML) exhibit variable growth rates, and are generally considered incapable of resolution without intervention [44]. Unlike SEGA however, increased growth has been observed in females compared to males, and renal AML have been linked with the development of pulmonary lymphangioleiomyomatosis in females, which rarely occurs before puberty. These findings may be explained by the

presence of estrogen and progesterone-receptor positive smooth muscle cells located within the AMLs [45]. To date, SEGA cells have not been shown to be hormone responsive and, other than a single series [27], the effect of puberty has not been observed in the natural history of SEGA.

1.2.6 Conclusion

SEGA are an important neurological manifestation of TSC due to their potential for growth and risk of complications, including life-threatening obstructive hydrocephalus. This comprehensive review revealed that the natural history and SEGA growth trajectory remains incompletely understood. The majority of studies have small sample sizes and are hampered by an inherent selection bias through inclusion of symptomatic or more severely affected patients. Additionally, there is a lack of standardised accepted definition of SEGA. It is suggested from these series that both the development and growth rate of SEGA is unpredictable, correlating only weakly with genotype or other clinical features.

The lack of large population data on the natural history of SEN and SEGA means there is no clear guidance for type and timing of intervention, whether medical and/ or surgical. This would support the current recommendations for SEGA imaging surveillance of all TSC patients from childhood until 25 years. However, the published growth rates of these tumours ranged widely from 0.4mm-5.6mm per year in diameter and no imaging features consistently predicted progression or growth rate. Therefore, there is no current evidence base to support guidelines on the frequency of neuroimaging after SEGA diagnosis.

Importantly, while the emergence of new tumours appears to slow after 20 years, there is clearly a risk for ongoing progression of existing SEGA and a small but important risk of de novo SEGA developing in adulthood.

Given widespread activation of the mTOR pathway is known to underpin this multisystem disorder, relationships between SEGA and the severity of clinical manifestations warrants further investigation. Addressing these issues may in turn

identify important predictive biomarkers for SEGA growth, and assist in better directing medical and surgical management.

Based on this review we suggest areas exist for improvement. First, a consensus definition of SEGA should be developed to assist with uniformity of new research. Second, there is a need for larger natural history studies, based on serial imaging and inclusive of other phenotypic and genotypic data. Perhaps TOSCA [24] or other similar cohorts may address this. Additionally, we found that the current understanding of SEGA evolution is predominantly two-dimensional; as such, there is significant potential for future volumetric MRI analyses to build on our knowledge of SEGA growth

1.3 Update to SEGA review

The above literature search was repeated prior to thesis submission to identify new literature regarding SEGA from February 2017 to November 2020 that was not included in the initial review. Eight relevant new studies were identified. The format of this update will follow the previous structure: (1) Diagnostic Criteria (2) SEGA prevalence (3) Origin from SEN (4) SEGA imaging (5) Growth rate (6) Genotype-Phenotype correlation (7) Congenital SEGA (8) SEGA as a marker of severity of other TSC manifestations. The chapter will additionally summarise knowledge in new areas: (9) Adult-onset SEGA (10) Clinical features (11) SEGA tissue within cortical tubers.

1.3.1 Update on SEGA Diagnostic Criteria

No new papers on SEGA definition. The 2012 Consensus SEGA definition remains the most current and accepted definition [4].

1.3.2 Update on SEGA prevalence

Since the initial literature review, data from a large multinational registry "TOSCA" (TuberOus SClerosis registry to increase disease Awareness) has been published. TOSCA is the largest TSC registry to date and included 170 sites over 31 countries. SEGA were reported in 25% of registry patients (544 of 2216) [46]. This prevalence is in line with previous reports by a joint registry between USA and Belgium (24%) and a national referral centre in Netherlands (20%) [12,25]. Unfortunately, TOSCA did not use the 2012 consensus SEGA definition because study recruitment commenced prior to the definition's publication. The paper acknowledges that 'no specific inclusion criteria was defined' for SEGA [46]. This methodology likely overestimates SEGA prevalence compared to the 2012 consensus SEGA definition [4], as it will include many SEN at the foramen of Monro not actively growing and not meeting size criteria. Furthermore, SEGA details were collected in this study using an electronic case report form (CRF) without centralised imaging review or standardised radiology protocol, which could lead to inaccuracies in SEGA measurement and diagnosis.

1.3.3 Update regarding SEGA origin from SEN

No new publications were identified regarding this.

1.3.4 Update on SEGA Imaging, including volumetric analysis

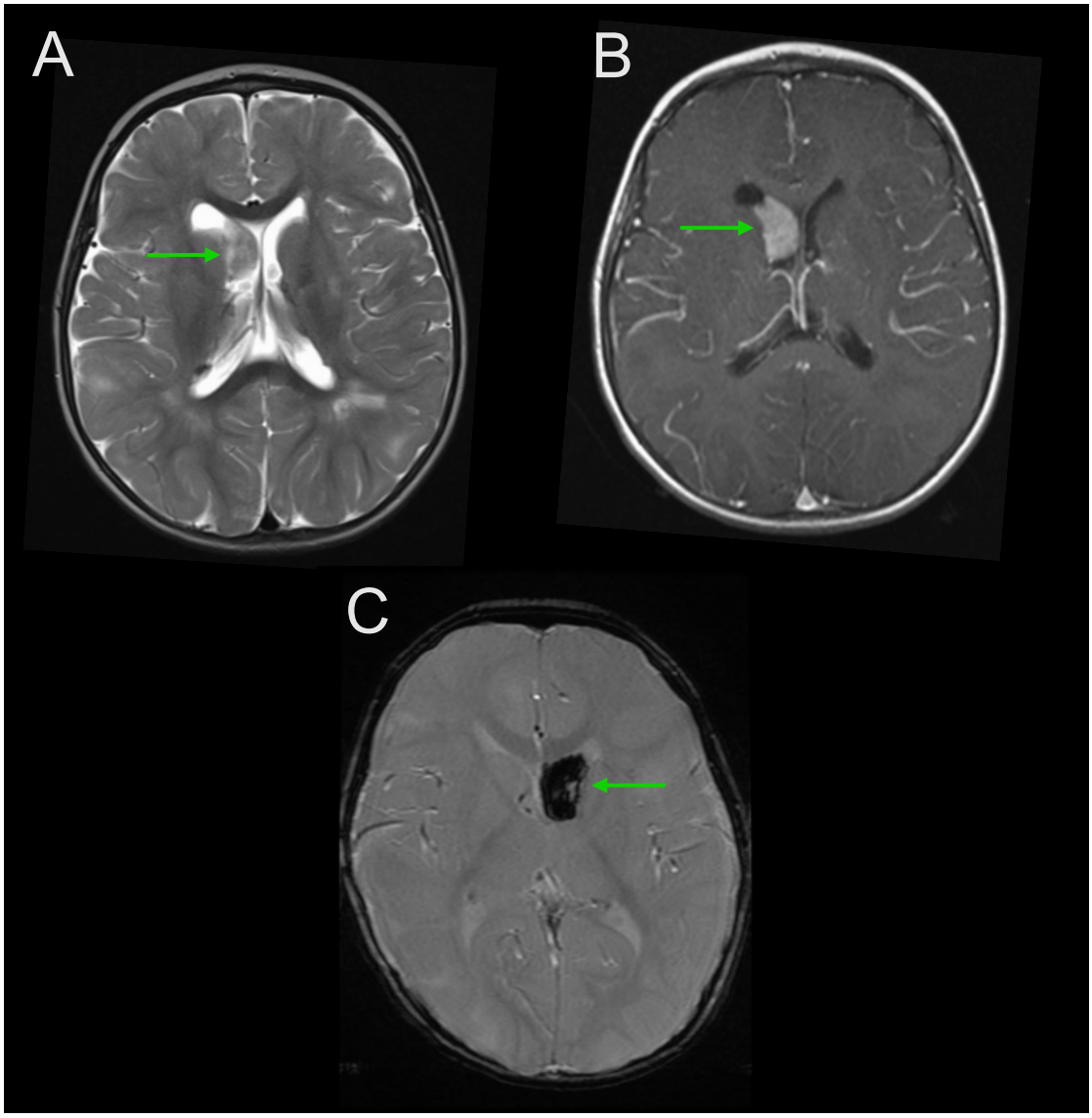
Mei et al. described SEGA radiological characteristics on MRI and CT imaging using a retrospective case series of 20 histologically confirmed SEGA [47]. The findings of this study are in line with Clarke's previous reports in 2006, that SEGA are usually iso- or hypointense on T1-weighted imaging (T1WI) and iso- or hyperintense on T2-weight imaging (T2WI) [33]. CT remains the preferred modality for detecting calcification within SEGA (Table 1.4), although calcification can be inferred from blooming artefact on gradient echo (GRE) or susceptibility weight imaging (SWI) sequences (Figure 1.3).

Table 1.4 Radiological characteristics of SEGA

CT (n=14)	**MRI (n=20)**
CT imaging: • Isodense (n=7) • Slightly hyperdense (n=7)	T1WI / T1 FLAIR: • Hypointense (n=10) • Isointense (n=5) • Hypointensity within isointensity (n=5) T2WI / T2 FLAIR: • Strongly hyperintense (n=13) • Isointense (n=1) • Hypointense (n=1) • Hyperintensity within hypointensity (n=5)
Marked heterogenous enhancement (n=14)	Moderate to strongly heterogenous enhancement with gadolinium (n=14)
Cystic degeneration (n=3) Amorphous calcification (n=14)	No cases had peritumoral oedema (n=0)

Adapted from: Mei et al., 2017 [47]

Figure 1.3 Examples of subependymal giant cell astrocytomas (SEGA) on MRI



Magnetic Resonance Imaging (MRI) of two patients from Sydney Children's Hospital TSC clinic (A & B) demonstrating a right frontal horn SEGA demonstrating (A) patchy hyperintensity on T2 axial sequence and (B) avid gadolinium enhancement on post-contrast T1 axial sequence. (C) Gradient echo (GRE) sequence showing blooming artefact of calcification in a left sided SEGA in another patient

Gadolinium contrast is routinely given in MRI of SEGA as an accepted method to increase diagnostic sensitivity [34]. However, in the last decade, there has been increasing concerns regarding long term effects of intracranial gadolinium deposition in deep brain nuclei after contrast-enhanced MRI imaging, even in the setting of normal renal function [48,49]. A recent study reported no statistical benefit in SEGA diagnosis by giving gadolinium enhancement compared to non-enhanced MRI [50]. Given the

SEGA consensus guidelines recommend routine neuroimaging surveillance every 1-3 yearly until at least 25 years [4], one could expect an antenatally diagnosed TSC patient to have between 8 to 25 MRIs before reaching 25 years. This paper challenges routine practice and suggests avoiding unnecessary cumulative gadolinium exposure in TSC patients. As the study included only 35 patients, it should be repeated on a larger scale to confirm findings prior to clinical implementation.

There have also been advances in volumetric analysis techniques for SEGA. Stawiski et al. made the first attempt at quantifying volumes of 30 SEGA using 4 different volumetric methodologies [51]. The standard planimetric method was compared to 3 open-source semi-automated methods of tumour segmentation (ITK-Snap, 3D Slicer and NIRFast). There was only moderate level of agreement between the 3 semi-automated segmentation methods, leading to conflicting results as to whether or not a SEGA had grown. Semi-automated volumetric analysis is a promising technology which requires further development and testing to ensure results are reproducible and reliable enough for clinical decision-making.

1.3.5 Update on SEGA Growth rate

None of the new publications calculated SEGA growth rate. The TOSCA investigators observed that childhood SEGA were more likely to exhibit growth over the follow-up period compared to adult-onset SEGA (22.7% vs 11.9%) [46]. This is in line with Tsai's finding that SEGA growth rate is higher in children compared to adults [37], and expert consensus recommendations for regular SEGA neuroimaging surveillance before the age of 25 [4].

1.3.6 Update on Genotype-Phenotype Correlation

The TOSCA registry found that SEGA were more likely to affect patients with a *TSC2* gene mutation compared to patients with a *TSC1* gene mutation (33.7 vs. 13.2%, $p < 0.0001$). [46]. Most patients with new adult-onset SEGA had a *TSC2* gene mutation (86%) [52]. This is in line with previous literature demonstrating *TSC2* mutations are associated with a more severe neurological phenotype [53].

1.3.7 Update on Congenital SEGA

One new case report of foetal SEGA was identified, with no other congenital SEGA cases reported. This *in utero* diagnosis was made at 22 weeks' gestation when a massive left intraventricular mass was seen on antenatal ultrasound. [54]. Postnatal MRI revealed a 4 × 4.5 × 7 cm vascular mass, which was hyperintense on T1WI and hypointense on T2WI. The reversal of usual MRI signal intensities is due to the neonatal unmyelinated brain. The patient was treated with 3 partial surgical resections and initiation of everolimus.

1.3.8 Update on utility of SEGA as a marker of severity of other TSC manifestations

No new articles were identified regarding this.

1.3.9 Adult-onset SEGA

Adult-onset SEGA are very rarely reported, with an incidence of 2.4% in the TOSCA study [52], where the oldest patient having new diagnosis of SEGA was 51 years old. None of these patients had history of previous SEGA. The incidence is in line with previous reported incidence of 2.2% in the Netherlands series [25]. Currently there are no recommendations for neuroimaging surveillance in patients over 25 years. This new data challenges this and suggests there should be a low threshold to MRI *TSC2* patients if there is any clinical suspicion.

1.3.10 Clinical features of SEGA

SEGA were previously believed to be asymptomatic until CSF flow obstruction, whereby the patient would experience symptoms of increased intracranial pressure (ICP). New evidence suggests that SEGA can present earlier with non-ICP symptoms including increased seizure frequency, behavioural disturbance and cognitive decline [46]. Patients with SEGA who present with increased seizure frequency have on average smaller tumour size than patients who present with headaches, vomiting or

visual disturbance (3cm vs 5cm) [47], although this paper did not appear to use the international diagnostic criteria for TSC diagnosis [14]. Furthermore, improvements in seizure frequency and learning disability have been previously documented following SEGA resection in patients with TSC [55].

1.3.11 SEGA tissue found within cortical tubers

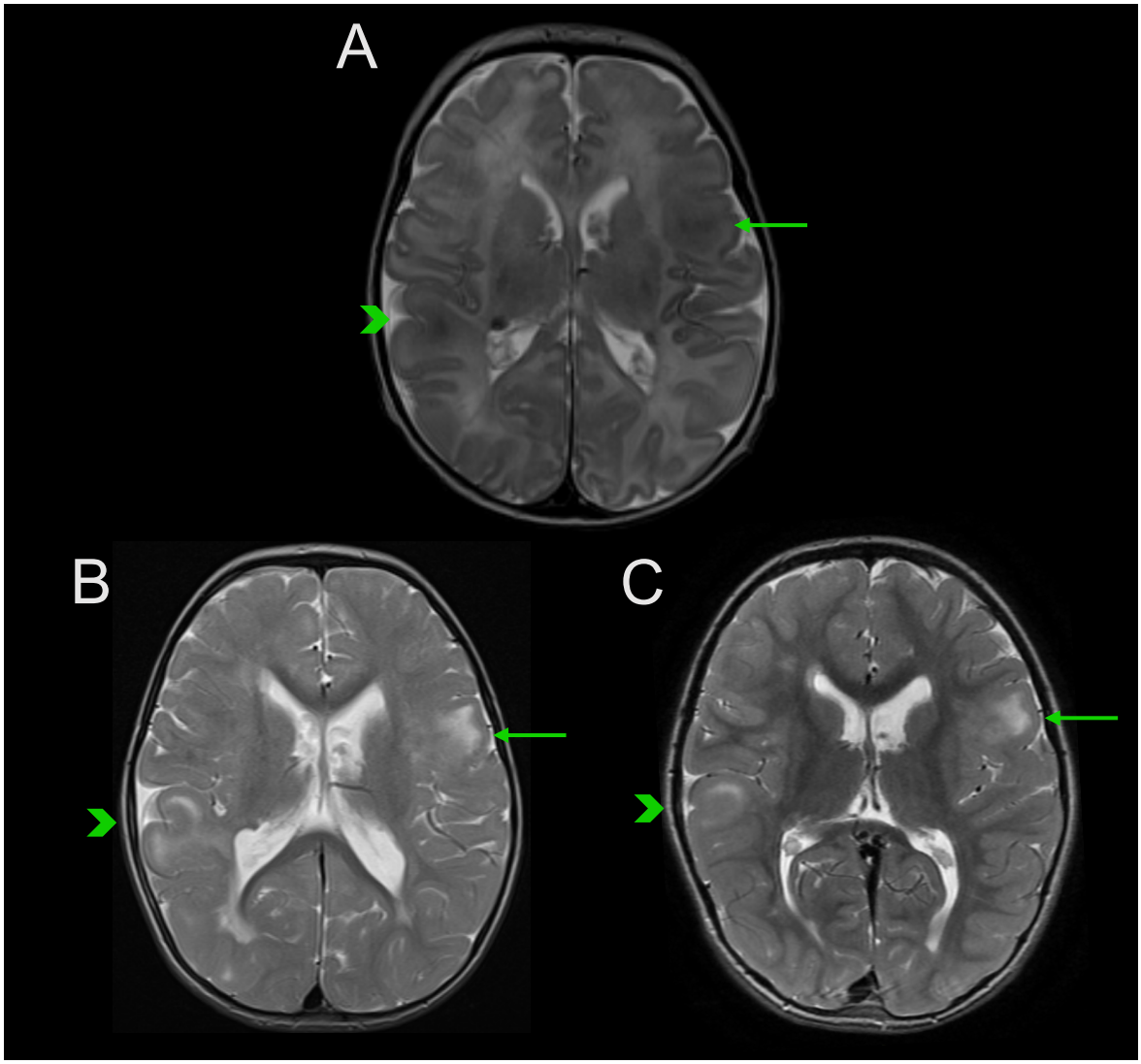
SEGA are usually located at the caudothalamic groove near the foramen of Monro, and this is acknowledged in the 2013 SEGA consensus definition [4]. SEGA have been rarely reported in the third ventricle or basal ganglia [56]. In 2017, SEGA tissue arising from within cortical tubers was reported for the first time by Roth's group [57]. Three of 75 patients who underwent cortical tuber resection for treatment-resistant epilepsy were found to have histology which contained SEGA tissue within the expected tuber histology. All three patients had early seizure onset (< 3 months) and refractory epilepsy. On CT, these cortical tubers with SEGA tissue appeared as calcified tubers. On MRI, two of the three tubers showed a T1WI-hyperintense/T2WI-hypointense core, with moderately strong gadolinium enhancement. Further studies are needed to understand the significance of this newly reported radiological finding, the rare histological finding of SEGA tissue within tubers, and clinical correlation with severity of early onset epilepsy in TSC.

1.4 Cortical Tubers

Of all the neuroradiological markers of TSC, cortical tubers have been the most widely studied. Tubers result from abnormal neuronal migration as a result of mTOR upregulation. They are visible from as early as 16 weeks' gestation, but foetal MRI is usually performed from 22 weeks onwards due to larger cerebral volume resulting in better diagnostic sensitivity [58]. The prevalence of tubers in TSC patients is approximately 80% amongst studies, with 82.2% of patients in the TOSCA registry having at least one cortical tuber [24]. Histologically, tubers are characterised by the disrupted cortical lamination, giant cells, dysmorphic neurons and astrogliosis [59]. Whilst tubers are usually considered static lesions, rarely they can undergo cyst-like change or haemorrhage. Some tubers have been implicated as an epileptic focus and are amenable to epilepsy surgery [60], whilst many other tubers are quiescent.

Radiologically, cortical tubers have the appearance of a swollen gyrus on MRI with hyperintense signal on T2WI with corresponding iso- or hypo-intensity on T1WI. These signal characteristics are only evident after approximately 18 months of age after myelination is complete. MRI in the newborn period show cortical tubers with the opposite signal pattern, with hypointensity on T2WI and hyperintensity on T1WI (Figure 1.4). Tubers can occasionally calcify or demonstrate gadolinium enhancement.

Figure 1.4 Changing MRI signal of tubers in first 2 years of life



Magnetic Resonance Imaging (MRI) of one patient from Sydney Children's Hospital TSC clinic. Serial axial T2 MRI sequences demonstrating a left frontal (arrow) and right parietal (arrowhead) tuber in patient with antenatally diagnosed TSC. (A) Tubers are T2 hypointense at Day 2 of life but evolve to become T2 hyperintense at (B) 26 months of life, which persists at (C) 5 years of life.

A subset of tubers demonstrates cyst-like characteristics in the tuber core, characterised by a central signal suppression on FLAIR suggesting a signal intensity similar to CSF and surrounded by a rim of FLAIR hyperintensity. They are named "cyst-like" and are not cystic tubers as the core lacks the epithelial lining seen in true cysts [61]. Cyst-like tubers do not show gadolinium enhancement [62]. Cyst-like tubers have an incidence of 24-46% in TSC patients [61–63], with higher rates in paediatric patients. Chu-Shore' cohort study of 173 patients found at least 1 cyst-like tubers in 57% of TSC patients

aged less than 18 years, compared with 25% of patients aged 18 or older [61]. Similarly, Jurkiewicz's smaller study of 73 patients found cyst-like tubers affect 82% of patients aged less than 7 years, compared with 18% of patients older than 7 years [62]. The reason for more cyst-like tubers in the younger patients is unclear but may be related to a more severe phenotype presenting earlier in young children, or a regression of the cyst-like structure in later life. Patients with *TSC2* mutation are more likely to have a cyst-like tuber compared with patients with *TSC1* mutation or patients with no mutation identified (56% vs 21% vs 33% respectively) [61].

Multifocal cyst-like tubers have been reported in a series of 8 children, with two in this group having progressive changes documented over time using serial MRIs [64]. These two patients presented with focal seizures at 3 and 9 months of age respectively and on repeat MRI 12 months later, both showed new multifocal cyst-like changes. Serial MRIs following this showed further evolution, with increase in size of existing cyst-like tubers and development of new cyst-like cavities in existing tubers [64]. Currently, it is unclear whether the majority of cyst-like tubers evolve over time or are radiologically stable. No new cases of cyst-like tuber evolution have been reported since this publication in 2009, suggesting this is a rare entity.

Manually Counting tubers

Counting tubers is a practical method of quantifying tuber burden which can be easily applied in the clinic setting as it does not require specialist techniques. The average number of tubers varies widely between individuals with TSC and even between studies. Patient selection bias may also contribute, where paediatric hospitals have more severe presentations of TSC associated with presentations at a young age. Goodman's 1997 metanalysis reported a median tuber count of between 5 and 10 using 5 studies [65]. A summary of median and mean tuber count is tabulated below (Table 1.5). A trend of increasing average tuber counts over time is noted, which may reflect advances in imaging resolution or methodology. Patients with *TSC2* mutation have on average more tubers than patients with *TSC1* mutation [53,66,67].

Table 1.5 Summary of mean and median tuber counts in studies

Study, year	n	Mean/Median tuber count	Range of tuber count
O'Callaghan 2004 [68]	41	Median 10	1-32, IQR 5-16
Doherty 2005 [66]	44	Mean 17	0-49
Jansen 2008 [69]	61	Mean 29	7-58
Kassiri 2011 [60]	24	Mean 10.9	1-28
Kaczorowska 2011 [70]	62	Mean 18.81	5-50
Jesmanas 2018 [71]	20	Mean 38.5	1-78

IQR: interquartile range

While quantifying tubers through tuber count is commonly used across studies, there are limitations to this methodology. One limitation of counting tubers is that this measure may underestimate the impact of large focal dysplasias made of several confluent tubers. Also, tubers spanning across multiple MRI slices may be double counted in error leading to inaccuracy. Conversely, small tubers may be missed due to generous slice thickness leading to wide inter-slice gap. For instance, Jesmanas et al. uses slice thickness of 5mm [71], whereas Jansen et al. uses slice thicknesses of 1.5mm without slice gap [69]. The studies by O'Callaghan, Doherty, Kassiri and Kaczorowska does not mention slice thickness at all [60,66,68,70]. This heterogeneity in study methodology may potentially impede comparison between different studies. Smaller tubers may also be inadequately visualised using low MRI magnet strength, inadequate MRI spatial resolution or CT imaging. Indeed, microtubers have been identified on 7T MRI imaging which were not evident on 1.5T MRI [72], although 7T MRI is limited to the research domain in Australia.

Tuber Brain Proportion (TBP)

Automatic methods of quantifying tubers have emerged in studies to reflect tuber load more accurately. These methods attempt to bypass the risk of human error and improve efficiency. In 2008 Jansen et al. introduced Tuber Brain Proportion (TBP), a ratio of total tuber volume divided by total brain volume excluding CSF [69]. Tubers were segmented using 3D FLAIR sequence using an automated tuber segmentation program.

In the study of 61 patients, mean TBP was 1.3% (0.2-5.1%). The study found TBP was a useful alternate marker for tuber load.

Classification of tubers

Studies have attempted to capture further detail regarding tubers from the MRI scan beyond total tuber count. Three methods of tuber classification have emerged which differentiate tuber types by their topographical location, size and radiological characteristics.

Tubers are most often categorised by their location in individual lobes (frontal, parietal, temporal, occipital) on either cerebral hemisphere. Studies have consistently reported that tubers are most commonly found in the frontal lobe [60,66,68] with up to 95% of patients having at least one frontal tuber in one study [68]. This may be in part due to the large contribution of the frontal lobe to the whole volume of the cerebral cortex [73].

Following reports of cyst-like tubers [61], Gallagher proposed a three-tuber-type classification system based on MRI signal characteristics in 2010 [74]. In this study, Type A tubers were defined as being isointense on T1WI and subtly hyperintense on T2WI and FLAIR. Type B tubers were hypointense on T1WI and homogenously hypointense on T2WI and FLAIR. Type C tubers have the same signal characteristics as a cyst-like tuber, that is, hypointense on T1WI, hypointense on T2WI and on FLAIR sequences have a hypointense core surrounded by a hyperintense rim. All patients in this study had a mix of 2 or 3 tuber types (29% and 71% respectively), and all patients had Type B tubers. Similar to Chu-Shore's paper regarding cyst-like tubers [61], Type C tubers were found to be significantly correlated with *TSC2* mutation [74].

Jesmanas et al. used the prior tuber classification system based on MRI signal characteristics and investigated the effect of tuber size in 20 patients [71]. Cystic tubers were the least prevalent tuber type. While patients had a median of 15.5 Type A tubers and 14 Type B tubers, 55% of patients did not have any Type C tubers (median 0). Type C tubers were the largest, with a median area of 129.36mm^2 compared with a median area of 36.5mm^2 for type A and 55.1mm^2 for type B tubers.

An alternative tuber classification system according to 3 sizes was proposed by Pascaul-Castroviejo et al. in 2013. Voluminous tubers were defined as those extending throughout at least 1 cerebral lobe. Medium-large tubers were defined as being greater than 3cm in diameter but less than an entire cerebral lobe, and medium-small tubers were those which were less than 3 cm in diameter [73]. The 3cm cut off was arbitrarily set by the authors. In their 45 TSC patients, 4 (9%) patients had voluminous tubers, 13 (29%) had predominantly medium-large tubers, and 28 (62%) had predominantly medium-small tubers. Forty-two patients (93%) had tubers of different sizes which fitted in more than one size classification, except three patients (7%) who had single medium-small sized tubers [73]. To date, no other paper has employed this classification system. One weakness of this system is that other authors may classify 'voluminous tubers' as malformations of cortical development.

Are tubers associated with epilepsy?

All tubers have epileptogenic potential as they are dysplastic tissue involving cortical grey matter. While some tubers have been implicated as the seizure onset-zone in focal epilepsy, many other tubers lie electrically inactive. Intracranial electrocorticography studies have demonstrated that the epileptogenic zone is within the tuber core and propagates outward [75], although some controversy exists with another paper suggesting that the epileptogenic zone can be situated in surrounding peri-tuberal tissue [76]. In Kassiri's cohort of 24 patients, 10 patients had intractable epilepsy and underwent surgical resection of the epileptogenic tuber. Follow up duration ranged from 1-6 years (average 2 years) over which time 9 patients had Engel class 1 outcome (seizure free), and one patient had Engel class 2 outcome (no seizures, only auras). While this case series constitutes class 3 level of evidence that removal of the appropriate tuber can lead to seizure freedom, it is highly dependent on correct localisation of the epileptogenic tuber [60].

There is reasonably strong evidence that increased total tuber count correlates with epilepsy severity in patients with TSC. The association between increased cortical tuber count and a more severe neurological phenotype has been postulated since the late 1980's [77,78]. An early metanalysis by Goodman in 1997 using 5 studies, pooled together 208 total TSC patients and found that patients with >7 tubers were 16 times

more likely to have poor seizure control (weighted OR 16.0; CI 5.1-46.5) compared with patients with <7 tubers [65]. This association between increased tuber count and worse epilepsy has been confirmed by more recent studies which primarily have univariate analyses [67,79].

Certain imaging characteristics have been associated with epilepsy severity. Patients who had at least one cyst-like tuber in Chu-Shore's study of 173 patients were more likely to have history of epilepsy (92% vs 76%, p=0.0038), drug resistant epilepsy (80% vs 54%, p=0.0007) and infantile spasms (57% vs 26%, p=0.00005) compared to patients without cyst-like cortical tuber [61]. In a smaller study of 20 patients, patients with predominantly Type C (cyst-like) tubers had higher rates of infantile spasms compared to patients with predominantly Type A tubers (83% vs 22%, p=0.02) [71].

Infantile spasms, an age-related seizure syndrome associated with TSC, has been associated with increased cortical tuber count. Goodman's metanalysis showed a strong association between increased cortical tuber count and history of infantile spasms (weighted OR 11.3, CI 3.75-37.08) [65]. In Doherty's series, all patients with >23 total tubers also had infantile spasms, and patients with infantile spasms had higher mean tuber count (per lobe and in all lobes combined) compared to patients without infantile spasms (for all lobes 23.8 vs 10.0, $p = 0.0001$) [66]. Kaczorowska similarly found a strong relationship between tuber count and presence of infantile spasms (Mann-Whitney test 2.209, $p = 0.027$) [70]. Wong's study suggested that parietally and occipitally located tubers may be associated with history of infantile spasms (p=0.009 and -.031 respectively) [80], but this result has yet to be replicated.

In summary, while there have been over 10 studies investigating tuber count and epilepsy severity, drug resistant epilepsy and infantile spasms, significant gaps remain in our understanding. These studies are limited by relatively small cohorts and contain primarily univariate analyses. Most focus on epilepsy frequency as the primary outcome measure, which can fluctuate over time and with changes in antiepileptic medication, rather than drug resistant epilepsy as a more robust outcome measure reflecting longitudinal seizure control. Multivariable analysis is necessary to tease apart the various contributions of tuber count, tuber location and epilepsy characteristic (such as

infantile spasms) to epilepsy outcome. Further studies are needed to address these literature gaps.

Are tubers associated with developmental delay and cognitive outcome?

It has been long postulated that increased total tuber count is associated with intellectual difficulty. Indeed, Goodman's metanalysis showed that patients with >7 tubers were 5 times more likely to have moderate-severe developmental delay (weighted OR 5.3; CI 2.3-11.9) compared with patients with less than or equal to 7 tubers [65]. Three studies have observed a significant negative linear correlation between tuber count and IQ score as a continuous variable [60,68,81]. Conversely, patients with no or few cortical tubers often have a normal developmental outcome. Zaroff's 2006 study included 7 patients with normal-range cognitive functioning (defined as Vineland Adaptive Behavior Composite score or Wechsler full scale IQ score of >=80). Three of these patients had no cortical tubers, while another 3 had unilateral tubers and one patient had bilateral cortical tubers [82]. In this small study, bilateral cortical tubers were significantly correlated to impaired cognitive functioning ($p=0.02$). No tuber location has been consistently implicated in developmental delay in TSC patients. Tubers in the frontal [60,83], temporal [83] and occipital lobes [68] have been each independently related to intellectual disability or developmental delay, but not replicated in subsequent studies. Whilst most studies are in agreement that increased tuber count increases risk of developmental delay in patients with TSC, a minority of studies have failed to show any association between tuber count and seizure control or developmental delay [66,80].

The relationship between tuber count and developmental delay is complex and difficult to tease apart due to childhood epilepsy being a significant confounding factor. In particular, infantile spasms and early seizure onset age are both independent risk factors for developmental delay [79,84] and there is significant interplay between factors. A 2011 cohort study including 62 patients by Kaczorowska et al. reported a significant correlation between right temporal lobe tuber count and impaired cognition (Kruskal-Wallis $H=9.135$, $p=0.01$), but concluded that cognitive outcome was more dependent on age of seizure onset due to stronger statistical association (Spearman rank correlation $R = -0.469$, $p<0.001$). In this study, presence of infantile spasms was also associated with cognition ($p=0.001$). Multivariate modelling was not performed as part of the analysis

so the various interactions between temporal tuber count, age of seizure onset, infantile spasms and cognition remain unknown. Several studies have used multivariate modelling to account for confounding factors with conflicting results, which will be summarised below.

O'Callaghan's 2004 study of 41 patients used a multivariate regression model and found a significant relationship between tuber count and IQ (regression coefficient =-1.2, $p<0.001$, $R^2 = 0.33$). Patients with history of infantile spasms had a lower mean IQ compared to patients without history of infantile spasms (70.7 vs 97.3, p=0.0005). Inclusion of infantile spasms into the regression model partially confounded the relationship but tuber count remained significantly associated with IQ (regression coefficient =-0.75, p=0.016, R^2 =0.47). While tubers accounted for 33% of variance in the IQ scores, addition of infantile spasms only slightly increased the accuracy of the regression model to account for 47% of variance in IQ scores. Current age and sex were the only other factors included in the analysis and were not found to correlate with IQ. Seizure onset age was not investigated in this study.

Raznahan's 2007 study of 48 TSC patients also found intellectual disability to be correlated with both infantile spasms and tuber count in univariate analysis (p=0.01 and p=0.04 respectively). However, on multivariate analysis by inclusion of temporal and frontal tuber counts, the relationship between infantile spasms and intellectual disability was rendered insignificant. Temporal tuber count and frontal tuber count was associated with intellectual disability on multivariate analysis (p=0.05 and p=0.06 respectively) [83].

Jansen et al. studied both tuber count and tuber brain proportion (TBP) as a marker of tuber burden in 61 patients. Patients scoring below average in cognitive testing had TBP ≥1%, whereas patients with above average results had TBP ≤1%. Patient's TBP negatively correlated with intelligence equivalent (regression coefficient -7.6, 95% CI -14.8 to 0.4), whereas tuber count was not related to cognitive outcomes. In final multivariate analysis, neither TBP nor tuber count were associated with cognitive outcome (only age of seizure onset remained associated). [69]

Finally, Bolton's study in 2015 included 125 patients with TSC. Higher tuber counts were associated with earlier age of seizure onset and seizure severity ($p<0.001$) on univariate analysis, but there was no direct association between tuber count and estimated IQ. Infantile spasms and status epilepticus were strongly associated with estimated IQ ($p=0.005$ and 0.004) in a multivariate analysis of variance which also included age of seizure onset ($p=0.9$). However, in the presence of epilepsy severity score none of these factors remained significantly associated to estimated IQ. A structural equation model was generated showing that *TSC2* gene mutation is associated with higher tuber count, which in turn is associated with epilepsy severity. The final step is that epilepsy severity is negatively correlated with intellectual outcome [67].

To summarise, over 10 studies have independently supported the hypothesis that higher tuber count is associated with intellectual disability or impaired cognitive outcome. However, there are multiple non-radiological determinants of cognitive outcome in patients with TSC, in particular, poor epilepsy control and infantile spasms. There is still significant uncertainty as the multivariate analyses of 4 studies have yielded conflicting results on whether tuber count is a significant biomarker of poor intellectual outcome in the presence of these other variables. While two studies found tuber counts to have a stronger association with intellectual disability than infantile spasms [68,83], another 2 studies reported early seizure onset age [85] and epilepsy severity [67] as the only significant predictors of intellectual disability. More research is needed to address this uncertainty in literature.

Are tubers associated with Autism Spectrum Disorder?

Previous studies investigating the relationship between tuber count and Autism Spectrum Disorder (ASD) have also had conflicting results. Samir's study found that 11 of 12 patients with ASD had more than >8 tubers (92%), compared with 1 of 14 patients without ASD (7.1%, $p=0.001$). However, two subsequent studies have found no relationship between total tuber count and ASD diagnosis. Weber's study of 29 patients with TSC found no correlation between Childhood Autism Rating Scale (CARS) score and total tuber count or number of lobes involved [86]. Similarly, Wong in 2006 reported no difference between tuber counts of 7 patients with ASD (12 ± 18.8) compared to 15 patients without ASD (12 ± 13.86) [80].

A few studies have suggested that temporal lobe location of tubers is associated with ASD [87,88]. Seri (1999) first reported all 7 ASD cases had temporal lobe tubers, whereas patients without ASD diagnosis had no temporal tubers. In Huang's study (2015), TSC patients with ASD had higher numbers of temporal tubers (6.67 ± 6.47 vs 3.12 ± 3.24, p=0.02) and insular tubers (1.17 ± 0 1.17 vs 0.23 ± 0.43, p=0.01) compared with patients without ASD. Most recently Numis et al. reported a trend for ASD patients to have their largest tuber burden in the left temporal lobe (p=0.09) even though this did not reach statistical significance [63]. In their logistic regression model, presence of left temporal interictal spikes increased the chance of ASD by more than 15 times (OR 15.1, CI 2.8 – 82.9, p=0.002). A recent metanalysis did not find any association between temporal tuber location and ASD, but could not perform metanalyses on other tuber locations or total tuber count due to limited existing studies [89].

Involvement of extra-temporal lobes have also been implicated in association with ASD. A retrospective study of 44 patients found occipital tuber count to be associated with pervasive developmental disorder (p=0.0074) [66]. In contrast, Samir's study reported that patients with ASD were more likely to have predominantly frontal tubers (75% vs 21.4%, p=0.04).

Of 86 TSC patients in Numis et al's study who had MRI available for analysis, 59% of 34 patients with ASD had cyst-like tubers compared to 30% of 52 TSC patients without ASD (p=0.015) [63]. Another study found higher prevalence of cyst-like tubers in ASD patients, but it did not reach statistical significance [88]. Using Gallagher's classification of tubers, patients with Type C tubers were more likely to have ASD (5/6, 83%) compared to patients with predominantly Type A tubers (3/9, 33%, p=0.057) [74].

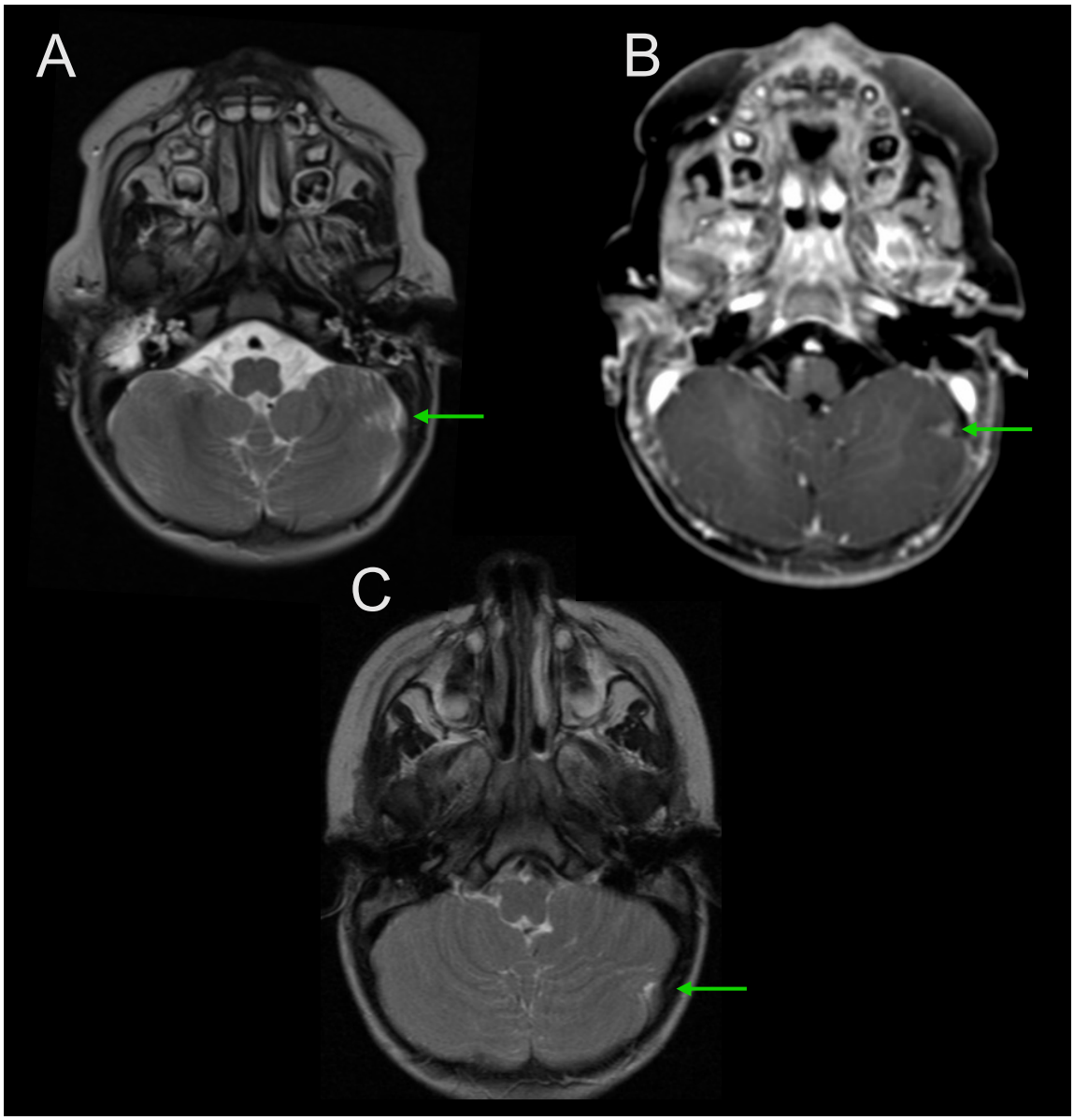
1.5 Cerebellar tubers and Cerebellar Atrophy

Cerebellar tubers are reported in 24-44% of patients with TSC [90,91]. These cerebellar lesions are not associated with clinical signs such as ataxia, nystagmus or intention tremor. Resected cerebellar tubers have shown similar histological features to supratentorial tubers, with aberrant neuronal migration and gliosis [92]. Specifically, thinning and gliosis are seen in the granular layer; crowding, gliosis, structural disorganisation and dystrophic changes in the Purkinje cell layer; and focal vacuolation of the molecular layer [92,93].

Radiologically, cerebellar tubers are predominantly wedge-shaped and located within either cerebellar hemisphere posteriorly [90,94]. The most common cerebellar lobes involved are situated posteriorly (crus I, crus II, VIIB) while midline structures such as the vermis and the flocculonodular lobe are usually spared [95]. “Retraction deformity” is a specific radiological feature described in 74.3-85.2% of cerebellar tubers, defined as a focal contour abnormality along the cerebellar margin associated with focal volume loss [90,93,95]. The MRI signal of cerebellar tubers is similar to cortical tubers with the majority showing hyperintensity on T2WI (Table 1.6). Gadolinium enhancement is reported in 30.1-51%, often in a classic striated “zebralike” pattern running along cerebellar folia, thought to be due to dysgenic cerebellar cortex disrupting the blood-brain barrier [90,93,96] (Figure 1.5). Calcification is also a major feature seen in 28.7-86.8% of cerebellar tubers and increases with age, seen as susceptibility artefact on gradient-echo or SWI sequences [90,93,95]. The average Apparent Diffusion Coefficient (ADC) value of cerebellar tubers is often higher than that of adjacent normal-appearing cerebellar parenchyma, proposed to be due to tuber gliosis and hypomyelination [93,94].

Almost all TSC patients with cerebellar tubers were found to have a *TSC2* mutation (31/34 in Toldo series, 41/42 in Boronat series, 8/8 in Jurkiewicz series), with other patients in the series having no mutation identified (NMI) [95,97,98]. Amongst children with *TSC2* genotype, almost half had cerebellar lesions (46%, 32/69) [99]. No patients with cerebellar lesion and *TSC1* mutation have been reported to date.

Figure 1.5 Examples of cerebellar tubers on MRI



Magnetic Resonance Imaging (MRI) of two patients from Sydney Children's Hospital TSC clinic, axial view. Left cerebellar tuber demonstrating (A) wedge shaped T2 hyperintensity and (B) T1 post-gadolinium enhancement. (C) MRI of different patient showing left cerebellar retraction deformity.

Table 1.6 Radiological characteristics of cerebellar tubers on MRI

MRI sequence (n=54)	**Vaughn et al.** [90] (n=54 tubers)	**Boronat et al.** [95] (n=106 tubers)
T1WI	• Hypointense 88.3% • Isointense 14.8% • Hyperintense 1.9%	• Hypointense 89.6% • Isointense 10.4%
T2WI	• Hyperintense 87% • Hypointense 13%	• Hyperintense 65% • Hyperintense with areas of hypointensity 21.7% • Hypointense 13.2%
T2 FLAIR	• Hyperintense 51.9% • Isointense 22.2% • Hypointense 25.9%	• Hyperintense 38.7% • Hyperintense with areas of hypointensity 17.9% • Isointense 15.1% • Hypointense 28.3%
Gadolinium enhancement	51% tubers (25/49) demonstrated enhancement on gadolinium administration	42% tubers (49/99) demonstrated enhancement on gadolinium administration
Calcification	53% tubers calcified – inferred by hypointensity on T2WI (and artefact on GRE when available)	86.8% hypointense on SWI or GRE

Based on: Vaughn et al. [90] *and Boronat et al.* [95]

Do cerebellar tubers evolve over time?

Unlike cortical tubers which are thought to be predominantly static, cerebellar tubers are relative inconspicuous at birth and become more prominent with time. Longitudinal series showed that 19.8 - 52.4% of cerebellar tubers changed imaging characteristics over time [90,93].

The reported changes included increased size, calcification and enhancement. Reduction in size and enhancement have also been reported uncommonly [90]. The mean age of patients with changes in their cerebellar tubers was lower than the mean age of patients with static imaging of cerebellar tubers (2.8±2.8 yr vs 6.8±4.8 yr, $p<0.001$), suggesting that cerebellar tuber evolution tends to occur before 8 years of age and corresponds to a time of rapid brain growth and myelination [93]. In both series, there was no new development of cerebellar tuber over the follow up periods. Boronat found in a series of 58 adult and paediatric patients with TSC that the average age of patients with at least

one calcified cerebellar tuber was higher than patients without calcification (21.6yr vs 11.5yr) [95]. No cases of haemorrhage have been reported complicating cerebellar tuber [93,95].

How are cerebellar tubers different from cortical tubers?

Cortical tubers are usually confined to gyri whereas cerebellar tubers tend to be wedge-shaped and run parallel to folial architecture, causing disruption. The classic wedge shape (broad base towards the cortex and tip pointing towards the 4th ventricle) is thought to be related to cerebellar neuronal migration of the granular layer [94]. Cerebellar tubers are more likely to calcify, calcify at a younger age [99], and show gadolinium enhancement [94] compared to supratentorial tubers. Whilst cortical tubers have been reported to haemorrhage or undergo cystic change [61], neither have been reported with cerebellar tubers to date.

Are cerebellar tubers associated with other TSC neuroradiological features?

Cerebellar tubers are never an isolated radiological sign and are always accompanied by cortical tubers [73,91]. Patients with cerebellar tubers have more cortical tubers compared to patients without cerebellar tubers (17.9±8.9 vs 11.4±7.2, p=0.026) [91]. Toldo's study also agreed that cerebellar involvement is a powerful predictor of cortical tuber load [97]. Patients with cerebellar tubers are also more likely to have SEGA [90,100] and more SEN [97,100], but no differences in radial migration lines [97]. Global cerebellar atrophy has been reported to affect 4-13% of patients with TSC [34,90,91,94]. Global cerebellar atrophy is more prevalent in TSC patients with cerebellar tubers compared to patients without cerebellar tubers (17.1% vs 5.5%) [90].

Are cerebellar tubers associated with Developmental Delay or Impaired Adaptive Function?

Few studies have investigated cerebellar tubers as biomarkers for developmental delay and impaired adaptive behaviour. Weber et al. found that Vineland Adaptive Behavior Composite score (ABC score) was negatively correlated with cerebellar tuber count, that is, patients with more cerebellar tubers scored lower in adaptive behaviour [86]. However, a similar study by Eluvathingal found no association between presence of cerebellar tubers and Vineland ABC score or any of the Vineland subtests [96].

Due to the posterior fossa location of cerebellar tubers, they are thought not to be related to seizure pathogenesis. However, higher rates of epilepsy have been seen in patients with cerebellar tubers compared to those without (88% vs 68%, p=0.03), likely explained by the strong association between cerebellar tubers and higher cortical tuber count [97].

Lower cerebellar volumes have been associated with lower developmental quotient assessed by Mullens Scale of Early Learning (MSEL) in patients with *TSC2* gene mutation [101]. While this study did not assess presence of cerebellar tubers, it is of interest as cerebellar tubers are associated with cerebellar atrophy.

Are cerebellar tubers associated with Autism Spectrum Disorder?

Several studies have suggested an association between cerebellar tubers and autism. It has been argued that early cerebellar disruption contributes to later ASD [102]. Weber et al. found in 29 TSC patients that Childhood Autism Rating Scale (CARS) scores were positively correlated with number of cerebellar tubers, but not related to cortical tuber count or location. Similarly, another study found patients with cerebellar lesions (n=57) had higher overall autistic symptomology as assessed by the Gilliam Autism Rating Scale (GARS) compared to patients without cerebellar lesions (n=20), with right-sided cerebellar lesions associated with more severe impairments in social isolation and communicative and developmental skills [96]. In contrast, a recent detailed multivariate study found that cerebellar tubers were not the best predictor of ASD after adjusting for *TSC2* genotype and cortical tuber count [97].

1.6 SEN

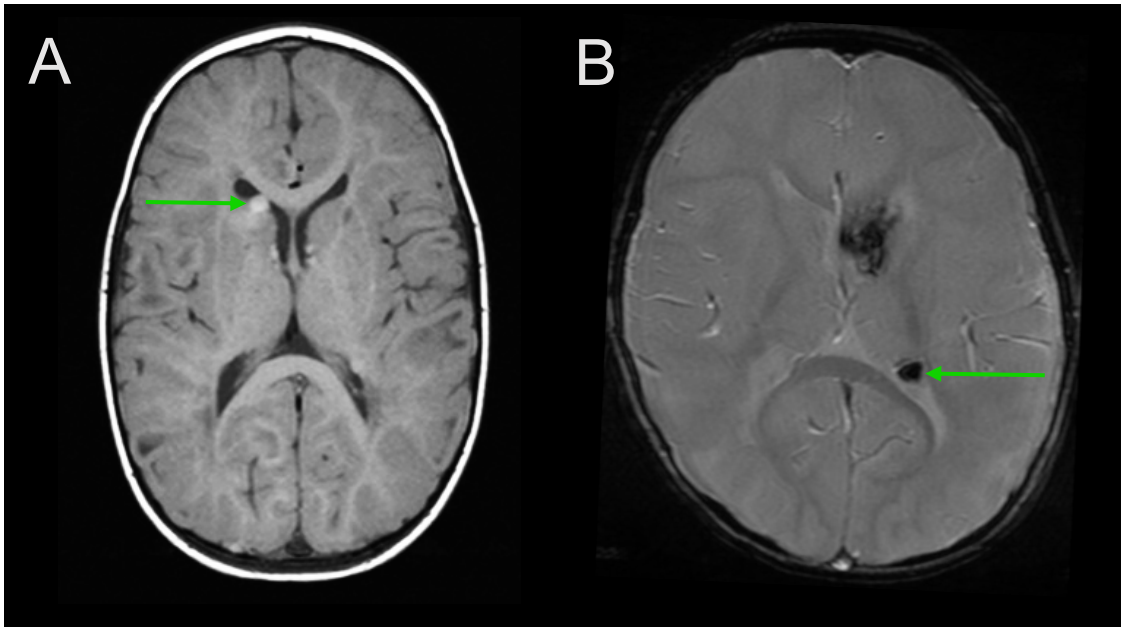
Subependymal nodules (SEN) are small round lesions arising from any part of the ependyma lining the ventricular system. They are usually <5mm, and by definition less than 1cm in diameter. SEN were found in 78.2% of TSC patients participating in the multinational TOSCA registry [24], with smaller studies reporting between 62-95% [23,53,103]. Patients usually have multiple SEN, with a median of 6 SEN in O'Callaghan's study of 39 patients (range 0-24, interquartile range 3-9) [23].

SEN are considered asymptomatic lesions which are not associated with epilepsy. Rarely in 5% of SEN, the lesion can undergo transformation into SEGA, especially if they are located near the caudothalamic groove. Because of this, SEN near the foramen of Monro warrant frequent MRI surveillance [4].

On MRI, SENs are isointense to grey matter on T1WI, with a rim of hyperintensity on T2WI. In some cases, the whole nodule can be T2WI hyperintense. SEN are present from birth but undergo progressive calcification over time, which changes their radiological characteristics. [104] Calcified SEN show blooming artefact on SWI sequence or phase shift on MRI (Figure 1.6) and appear hyperdense on CT. SEN can also show gadolinium enhancement due to their vascularity in up to 80% of cases [34]. Radiologically SEN can sometimes be confused with periventricular nodular heterotopia which occur in the same location, but do not calcify or enhance with gadolinium [105].

SEN count appears to be related to genotype, where patients with *TSC2* mutation have on average more SEN than patients with *TSC1* mutation (8 vs 6). SEN were more likely to be calcified in the TSC2 cohort compared to TSC1 cohort (p=0.015). Higher SEN numbers were seen where the *TSC2* mutation was predicted to cause absence of protein rather than a present TSC2 protein [53].

Figure 1.6 Examples of subependymal nodules (SEN) on MRI



Magnetic Resonance Imaging (MRI) of two patients from Sydney Children's Hospital TSC clinic. (A) Hyperintense SEN in right frontal horn of lateral ventricle on T2 axial sequence (B) Round blooming artefact consistent with calcification of SEN in left occipital horn of lateral ventricle.

Are SEN associated with other TSC neuroradiological features?

SEN count appears to be a marker for overall TSC disease burden in the central nervous system, with higher SEN count associated with higher cortical tuber count [106], cerebellar tubers and presence of SEGA [100]. These, particularly cortical tuber count, have been independently associated with poor epilepsy and developmental outcome (as previously discussed).

Are SEN associated with epilepsy and developmental delay?

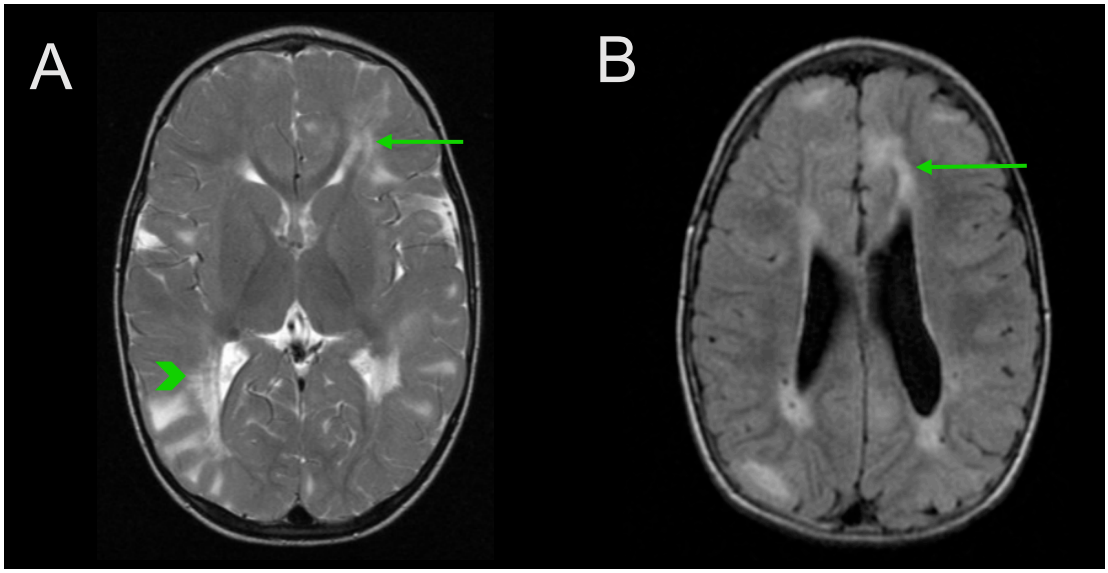
Few studies have found increased SEN number to be associated with poor seizure outcome [79], infantile spasms [107] and intellectual disability [107]. These two studies did not control for confounding factors such as tuber count. SEN were more frequently detected in TSC patients who underwent epilepsy surgery and had seizure recurrence (Engel class II-III-IV outcome, 100% vs 66.7%, p=0.009) [108]. It is not clear whether SEN count is independently associated with poor epilepsy and developmental outcome as studies to date have not accounted for the effect of tuber count.

1.7 Radial migration lines

Radial Migration Lines (RMLs) are curvilinear abnormalities extending from the ventricular system towards the cortical surface. Multiple RMLs are included as major criteria in the 2021 TSC clinical diagnostic criteria along with cortical tubers [3]. Approximately half of RMLs are associated with an overlying tuber, and those without this association tend to be smaller [53,109].

Radiologically, RMLs are characterised by T2WI hyperintensity relative to unaffected white matter and T1WI isointensity to grey matter [110]. RMLs are best evaluated on FLAIR on MRI, as the T2 bright CSF signal in the adjacent lateral ventricles which can be distracting, is attenuated in this sequence (Figure 1.7). Histologically, RMLs associated with tubers represent migration abnormalities, subcortical white matter disruption and hypomyelination [111].

Figure 1.7 Examples of radial migration lines (RML) on MRI



Magnetic Resonance Imaging (MRI) of two patients from Sydney Children's Hospital TSC clinic. (A) T2 axial sequence, showing RMLs not associated with tuber in left frontal area (arrow), and RMLs associated with overlying cortical tuber in right parieto-occipital area (arrowhead) (B) FLAIR axial sequence showing left frontal RML from another patient, with classic curvilinear appearance.

The frequency of RMLs in TSC has varied widely in literature, with most studies reporting a frequency of 15-20% [24,110], but studies from the Netherlands group reporting a frequency of 97-100% [53,109,112]. This discrepancy may be related to

differing definitions (i.e., whether RML associated with an overlying tuber is considered a separate entity or counted as a continuation of the tuber) and higher imaging resolution in recent years. One study of 30 MRIs found RMLs were the most frequent neuroradiological lesion with a mean of 47 RMLs per patient, compared with 27 tubers and 10 SEN per patient [109]. RMLs were found equally distributed between hemispheres and lobes. For such a common neuroradiological sign in TSC, there is an unexpected paucity of literature on RMLs. This likely reflects difficulties in quantifying these which involves a laborious process, compared with discrete easily countable lesions such as SEN or tubers.

RMLs are believed to be static lesions on serial MRIs. Given that immature myelination affects MRI white matter signal in the first 24 months of life, one would expect RMLs to become increasingly conspicuous on MR imaging after this age. One study reported a surprising trend of more RMLs in the younger age group and the paper's authors felt this was likely due to referral bias [109]. Future longitudinal study needs to be done to clarify this unexpected finding.

One study has reported higher RML count with *TSC2* mutations, particularly variants which lead to loss of TSC2 protein [53], but this was not statistically significant [53]. The study also identified two patients with RMLs but no tubers, both of whom had a *TSC1* mutation.

Few studies have correlated the features of RMLs with seizure, cognitive and behavioural outcome in TSC. Shepherd's 1995 study investigated presence of RMLs and found a higher proportion of TSC patients with mental impairment had migration lines on retrospective MRI review (13 of 41 patients, 32%), compared with TSC patients without mental impairment (3 of 34 patients, 9%) [113]. Additionally, RMLs were more common in the patients whose seizures started prior to 12 months of age (19/46, 41%) compared with patients whose seizures onset after 12 months of age (2/24, 8%). Looking at seizure subtypes, the patient group with infantile spasms were more likely to have RMLs (16/29, 55%) compared with patients who presented with other seizure types (5/41, 13%). This study did not investigate autism spectrum disorder (ASD) as an outcome measure.

While Shepherd's study dichotomised the presence or absence of RMLs, van Eeghen's 2013 study quantified RML frequency as an ordinal value through manual count. RML frequency was found to be strongly associated with age of seizure onset ($p=0.003$, $r=-0.60$), intelligence quotient ($p=0.01$, $r=0.48$), and rate of autistic features as measured by Social Communication Questionnaire (SCQ, $p=0.007$, $r=0.56$) [109]. However, the study also found a strong relationship between RMLs and other neuroradiological features such as tubers and SEN. Multivariate analysis was not done in this study to elucidate whether seizure onset was associated with presence RML or the other neuroradiological markers.

Mous' 2018 study address this problem by using multivariate regression analysis to investigate the association of tubers and RMLs with autism spectrum disorder (ASD) in 52 children with TSC [112]. RMLs were found in 98.1% of MRIs of the study group. On univariate analysis, both RML and tuber count were independently associated with ASD. However, after correcting for cognitive ability assessed through formal psychometric testing using WISC-III, WPPSI-III or non-verbal scales, only total tuber count remained significantly associated with ASD characteristics.

More recent studies have shifted focus to quantifying white matter connectivity through diffusion tensor imaging (DTI). This newer technology is not usually part of standard imaging protocols as it requires extra time for specialised sequences and post-acquisition analysis. It has been suggested that RMLs are responsible for white matter DTI abnormalities [109] and that underconnectivity in major white matter tracts such as the arcuate fasciculus contribute to development of ASD in TSC [114]. As the prevalence of ASD in the TSC population is as high as 50%, researchers are increasingly focussing on TSC patients to understand the pathophysiology of autism. More studies are needed to truly understand the clinical significance of decreased white matter connectivity on DTI and how this improves individual patient management.

1.8 Conclusions

Neuroradiological features of TSC on MRI hold potential to help further delineate and predict neurological and developmental outcomes of children with TSC. While most identified studies only investigate a single radiological variable, a small number of studies compare multiple neuroradiological biomarkers. Few studies have attempted to delineate the complex relationship between cortical tubers, epilepsy, intellectual disability and autism spectrum disorder by using multivariate analysis, but they have come to conflicting conclusions. Further research is required to address this. The present literature search has not shown any studies using MRI from a standardised timepoint in early childhood to predict later neurological and developmental outcomes. Few studies use drug resistant epilepsy as an outcome measure, and most past studies have used seizure frequency which can vary with changes to antiepileptic medication. There is also a data gap regarding congenital SEGA, and further studies are needed to confirm the reported aggressive growth rate compared to other SEGA.

These findings form the basis for this thesis' research question and the studies presented in Chapters 2 and 3. We hypothesise that neuroimaging markers in TSC are associated with severity of epilepsy, developmental delay and autism spectrum disorder. This thesis aims to:

1. Describe the incidence and growth rate of congenital SEGA
2. Investigate whether congenital SEGA can be used as a biomarker for poor epilepsy and developmental outcomes
3. Describe the incidence of other neuroimaging markers, including cortical tubers, cerebellar tubers, SEGA, and subependymal nodules
4. Investigate whether TSC neuroradiological changes on MRI performed between 18-36 months can be used as biomarkers for epilepsy and developmental outcomes at 5 years

CHAPTER 2. Congenital subependymal giant cell astrocytoma in children with tuberous sclerosis complex: growth patterns and neurological outcome

2.1 Link to thesis

In the previous chapter, I presented the literature review of SEGA and other neuroradiological makers in predicting neurodevelopmental outcomes for children with TSC. I identified a paucity of published data regarding congenital SEGA and their growth pattern over time, with one study suggesting rapid aggressive growth [42] but few other studies to corroborate this finding. It is important to understand the growth pattern of congenital SEGA as this determines the frequency of serial neuroimaging in this cohort. Frequent unnecessary MRI would expose children to risks associated with repeated general anaesthesia, whereas infrequent MRI may delay identification of SEGA growth and miss opportunities to treat medically with mTOR inhibitors. Furthermore, it is important to establish whether congenital SEGA is a marker for poor epilepsy and developmental outcomes. Here in Chapter 2, I investigate congenital SEGAs with a retrospective case series using patients seen in our tertiary referral hospital over 10 years. This study as published in *Pediatric Research* as follows and this published manuscript represents a chapter of this thesis.

2.2 Abstract

Background

Literature regarding congenital subependymal giant cell astrocytomas (SEGA) is limited, and suggests they are at risk of rapid growth and complications. We sought to characterize the growth patterns of congenital SEGA. The second part of the study was an exploratory analysis of congenital SEGA as a possible biomarker for poor neurological outcome.

Methods

This single-centre case series describes 10 patients with TSC who had SEGA diagnosed before 12 months. SEGA diameter and volumetric growth were analysed using serial MRIs. Neurological outcomes were compared to a genotype-matched group.

Results

All children with congenital SEGA had a *TSC2* mutation. Patients were followed for 1-8.7 years, during which median SEGA growth rate was 1.1mm/yr in diameter or 150mm^3/yr volumetrically. SEGA with volume >500mm^3 had a significantly higher growth rate compared with smaller SEGA (462mm^3/yr vs 42mm^3/yr, p=0.0095). Children with congenital SEGA had a high prevalence of severe epilepsy, developmental disability and autism spectrum disorder.

Conclusion

Congenital SEGA can follow a relatively benign course with a lower growth rate compared with published literature. Frequent neuroimaging surveillance is recommended for congenital SEGA with volumes exceeding 500 mm^3.

2.3 Background

Tuberous sclerosis is an autosomal dominant condition affecting 1:6000 livebirths.[115] Unregulated mTOR overactivation in affected individuals leads to hamartomas in multiple organs including the brain. In many patients, a causative mutation can be found in tumour suppressor genes *TSC1* or *TSC2*, although in approximately 15% of patients no mutation can be identified.[116] Subependymal giant cell astrocytoma (SEGA) are low-grade intraventricular tumours occurring in 5-14% of patients with tuberous sclerosis complex (TSC).[25] There is no universally agreed-upon definition of SEGA, but it is generally accepted as an intraventricular mass measuring greater than 10mm arising from the caudothalamic groove, or a subependymal lesion at any location which has shown serial growth on consecutive imaging.[4,117] Due to its location near the foramen of Monro, SEGA can block critical CSF pathways causing obstructive hydrocephalus. SEGA have a peak incidence in the first two decades of life [118], although there have been infrequent case reports of SEGA before 12 months of age.

We are only aware of 27 published cases of congenital SEGA (diagnosed before 12 months), most of which were associated with rapid SEGA growth and poor prognoses.[42,43,56,119–122] In the largest case series of 10 patients, Kotulska *et al.* reported a congenital SEGA growth rate of 2.78mm per month (33mm per year). [42] This is at least 6-fold higher than previously reported growth rates of non-congenital SEGAs (1-5mm per year).[25,36,123] Prior to the use of mTOR (mammalian target of rapamycin) inhibitors in SEGA management, standard treatment was limited to surgical resection, and/or ventriculoperitoneal shunting for hydrocephalus. Surgery is important to provide immediate relief of hydrocephalus in advanced cases, but have been associated with post-surgical neurological deficit and postoperative mortality in patients aged <3 years.[42] Due to the genetic mutation underlying TSC, SEGA can recur and thus surgical resection is not necessary curative. While mTOR inhibitors are increasingly being used without major acute adverse events in infants with TSC, there is a lack of long term safety data and no FDA approval for everolimus use in infants <1 year of age.[124]

The aim of this study is to characterize the incidence and growth patterns of congenital SEGA within cohort of children seen in a tertiary paediatric TSC management clinic. A

secondary aim was to explore whether congenital SEGA is a biomarker for poor neurological outcome.

2.4 Methods

This single-centre case series was derived from patients seen at the multidisciplinary Tuberous Sclerosis Clinic at Sydney Children's Hospital, a tertiary paediatric referral centre in New South Wales, Australia. The inclusion criteria were as follows:

- Patients seen in the TSC clinic aged less than 18 years between January 2006 and December 2016, with
- Clinical diagnosis of TSC using 2012 revised diagnostic criteria[14], and
- SEGA diagnosed before 12 months of age, and
- Two or more MRI accessible for growth analysis

Neuroimaging surveillance with MRI is routinely recommended every 1-3 years under the age of 25 according to consensus guidelines to screen for asymptomatic SEGA.[4] Following SEGA diagnosis, MRI is usually repeated within 12 months to establish a growth rate. Once the latter is known, frequency of repeat imaging is decided based on this. We defined SEGA as a lesion near the foramen of Monro measuring ≥10mm in maximal diameter in any plane. Congenital SEGA was defined as one which was diagnosed on MRI before 12 months of age. SEGA maximal diameter and volumetric growth from serial MRI were analysed using Analyze 11.0 software by two authors (DF, DC). SEGA volume was estimated by calculating the ellipsoid volume based on the measurements of the length of the major axis (a) and the lengths of the orthogonal semi axes (b & c) (Vol = 4/3 πabc). The presence of obstructive hydrocephalus secondary to SEGA location was also noted. Following initiation of mTOR inhibitor, patients were excluded from our growth analysis.

Neurological outcome data was collected through retrospective review of electronic medical record and clinic correspondence. Primary epilepsy outcomes were: presence of daily seizures, number of antiepileptic drugs trialled, trial of ketogenic diet (reflecting treatment resistant seizures). Developmental outcomes (only for age > 5 years) were:

diagnosis of severe developmental disability (DD) and autism spectrum disorder (ASD). Developmental diagnoses were based on expert clinical assessment by paediatric developmental specialist or paediatric neurologist. Neurological outcomes were compared to a group of genotype-matched (*TSC2*) children from our clinic, who were all diagnosed with TSC prior to 12 months of age (n=29) but who did not have SEGA. 26/29 were older than 5 years at the time of review so had developmental outcome data for comparison. Fisher's exact test, chi-squared, unpaired t-test and Mann-Whitney test was used for comparison of outcome measures between groups. Statistical analysis was performed using GraphPad Prism 7 for Mac OS X, GraphPad Software, La Jolla California USA, www.graphpad.com. This study received research ethics approval from the Sydney Children's Hospitals Network Human Research Ethics Committee (LNR/14/SCHN/445).

2.5 Results

2.5.1 Demographics

Of 109 paediatric patients seen in our clinic in the 10 years between January 2006 and December 2016, ten children had a diagnosis of SEGA prior to 12 months of age (9.2%). Four patients with congenital SEGA were male. Six patients were diagnosed with TSC after detection of cardiac rhabdomyomas – five antenatally, and one at six weeks of age (Table 2.1). The other four patients presented with focal seizures at 2 months, 5 months, and 9 months; and infantile spasms at 8 months of age. Following diagnosis of TSC, all patients had MRI identifying SEGA prior to 12 months of age. SEGA growth was monitored through serial MRI. In some cases, head ultrasound was used as long as the anterior fontanelle remained patent.

All patients underwent genetic testing, with TSC2 mutations identified in all individuals. One patient had a contiguous TSC2-PKD1 deletion. Eight families agreed to parental segregation genetic testing, which confirmed de novo mutations in these probands. The remaining parents who did not proceed to genetic testing did not have clinical manifestations of TSC, and the probands are presumed to have de novo mutations.

Table 2.1 Clinical characteristics of case series

Demographics					Maximal diameter analysis			Volumetric analysis		Neurological outcome			Treatment
No.	**Age at diagnosis**	**Gender**	**Gene**	**Presenting feature**	**Baseline SEGA max diameter (mm, age)**	**SEGA max diameter (mm, age)**	**Growth rate max diameter (mm/yr)**	**Baseline SEGA volume (mm^3)**	**Growth rate volumetric (mm^3/yr)**	**Seizure Frequency at last review**	**Severe Developmental Disability**	**ASD**	**mTOR treatment**
1	Antenatal	F	*TSC2*	CR	18mm at D2	21mm at 32 mo	1.15	1470	203.7	< daily	No	Yes	EVE for sz at 3.7yr
2	Antenatal	F	*TSC2*	CR	19mm at D5	22mm at 13 mo	2.90	2228	495.82	Daily	Yes	Yes	EVE for sz at 1.8yr
3	2 months	F	*TSC2*	FS	10mm at 2 mo	12mm at 53 mo	0.48	283	95.88	Daily	Yes	Yes	EVE for sz at 5.7yr
4	Antenatal	F	*TSC2*	CR, SVT	11mm at D2	11mm at 62 mo	0	276	8.98	Daily	No	No	-
5	5 months	F	*TSC2*	FS	16mm at 4 mo	16mm at 16 mo	1.11	1206	429.13	Daily	Yes	Yes	EVE for sz at 1.3yr
6	Antenatal	M	*TSC2*	CR	12mm at D1	13mm at 55 mo	0.1	387	26.52	Daily	Yes	Yes	SIR for sz at 5.4yr
7	9 months	F	*TSC2*	FS	17mm at 11 mo	31mm at 96 mo	2.22	1161	650.03	< daily	No	No	EVE for SEGA at 8yr
8	6 weeks	M	*TSC2*	CR	11mm at 2 mo	14mm at 85 mo	0.52	346	34.88	< daily	No	No	-
9	Antenatal	M	*TSC2*	CR	10mm at 2 mo	13mm at 14 mo	2.98	150	48.46	Daily	- *	- *	-
10	8 months	M	*TSC2+ PKD1*	IS	11mm at 9 mo	13mm at 22 mo	2.35	355	331.28	< daily	- *	- *	-

*CR: Cardiac rhabdomyoma; FS: focal seizure; SVT: Supraventricular tachycardia; IS: infantile spasm, ASD: Autistic Spectrum Disorder, D; days, mo: months, mTOR; mammalian Target Of Rapamycin, Avg: average, EVE: everolimus, SIR: sirolimus, *: Patient excluded from developmental analysis as <5 years of age*

2.5.2 SEGA growth

Median maximum SEGA diameter at baseline MRI scan was 12mm (range 10 - 19mm, Table 2.1). In 8 patients, the baseline scan was performed at or before 4 months of age. Patients had on average 4 MRIs over the radiological follow up period which ranged from 1 to 8.7 years (mean 3.6). Median SEGA growth rate was 1.1mm/yr using maximum diameter (range 0.0 - 3.0mm/yr). Using volumetric estimation, the median growth rate was 150mm^3/yr (range 9 – 650 mm^3/yr) (Figure 2.1). Most patients with SEGA volume <500mm^3 at baseline did not grow significantly. SEGA with volume > 500mm^3 had a higher median volumetric growth rate (462mm^3/yr vs 42mm^3/yr, p=0.0095). No patients in this study developed obstructive hydrocephalus or required neurosurgical intervention.

Six patients were started on mTOR inhibitor treatment at a median age of 4.5yr (range 1.3 - 8.0yr). The primary indication for this was refractory seizures (n = 5). One patient was commenced on mTOR inhibitor for rapid SEGA growth (patient 7), which was detected at 8 years of age (Figure 2.1). Everolimus was used in 5 patients, and sirolimus in one patient, due to drug availability in regional hospital.

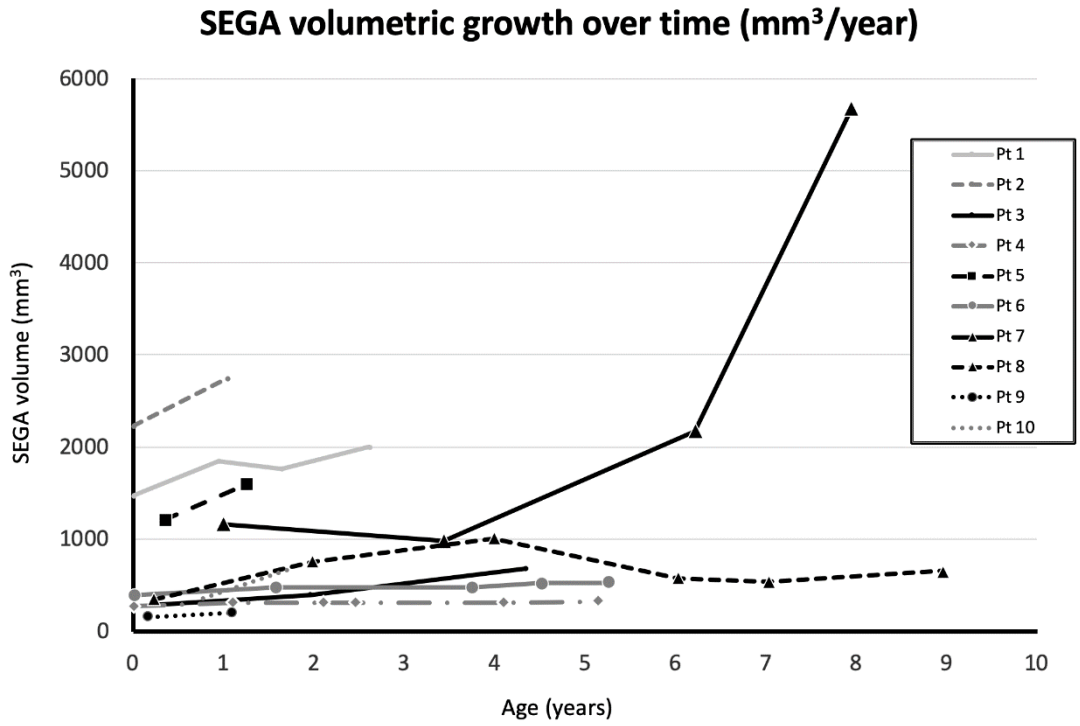


Figure 2.1 SEGA volumetric growth over time (mm^3/yr). This graph describes the SEGA volume (y-axis, mm^3) of included study patients over time (x-axis, age in years).

Table 2.2 Neurological outcomes of children with congenital SEGA compared with *TSC2* control group

	Congenital SEGA n=10	***TSC2* Control group n=29**	**Statistical testing**
Epilepsy outcome, n (%)			
Daily seizures	6 (60%)	10 (35%)	OR 2.9, 95% CI 0.7-10.4, p=0.3
Infantile spasm	6 (60%)	15 (52%)	OR 1.4, 95% CI 0.4-5.1, p=0.7
Trial of ketogenic diet	4 (40%)	5 (17%)	OR 3.2, 95% CI 0.8-13.8, p=0.2
Number of AEDs trialled*	5.5±0.9	4.3±0.4	t(37)=1.4, p=0.18
Age of seizure onset* (mo)	5.6±1.1	5.1±0.8	t(37)=0.3, p=0.7
	Congenital SEGA n=8**	***TSC2* Control group n=26****	**Statistical testing**
Developmental outcome, n (%)			
Severe DD	4 (50%)	7 (27%)	OR 2.7, 95% CI 0.6-11.5, p=0.4
ASD	5 (63%)	9 (35%)	OR 3.1, 95% CI 0.6-13.6, p=0.23

AED: Antiepileptic Drugs, ASD: Autism Spectrum Disorder, DD: Developmental disability, mo: months

** Results expressed as mean ± standard deviation*

*** Only patients >5 years included in developmental subgroup analysis*

2.5.3 Neurological outcome

The majority of children with congenital SEGAs had difficult to control epilepsy and while they tended to have higher scores for each measure for seizure severity, there was no statistical difference when compared to a group of 29 children with *TSC2* mutations who did not have congenital SEGA (Table 2.2). After 5 years of age, patient developmental outcome was evaluated by paediatric neurologist and/or developmental paediatrician (Table 2.1). Statistical analysis again did not yield any significant difference between the developmental outcomes of children with congenital SEGA compared to other children with *TSC2* mutations.

2.6 Discussion

In our cohort, patients with congenital SEGA show they can follow a relatively benign course, with lower growth rates compared with published literature. In these circumstances, we suggest that close monitoring is a safe and reasonable option. International consensus guidelines recommend routine MRI every 1-3 years in patients younger than 25 years who are asymptomatic, more regularly if a large or growing SEGA is present.[125] This guideline was adhered to in all cases in our series. We have shown that congenital SEGA with volume >500mm^3 at baseline are significantly more likely to demonstrate growth. In these cases, more frequent MRI's could be instituted. One patient required initiation of everolimus at 8 years of age for SEGA growth, highlighting the importance of ongoing surveillance imaging. Had mTOR inhibitor not been available or appropriate, neurosurgical options would have been explored.

The second part of this study was to explore whether congenital SEGA could be a potential biomarker for neurological outcome. Previous attempts to correlate neuroimaging features of TSC with outcome have focussed on exploring tuber count, tuber type, tuber location and tuber volumetric load.[61,65,66,68,69,74,126] We hypothesised that congenital SEGA could be a potential biomarker for worse epilepsy and poorer developmental prognosis. While the neurological outcomes in children with congenital SEGA tended to be poor, we did not find any statistical difference when compared to other children with *TSC2* mutation. Both small sample size and retrospective study design limited the interpretation of this exploratory study.

It is notable that 60% of our congenital SEGA patients were diagnosed after the discovery of cardiac rhabdomyomas, one at 6 weeks of age and the rest antenatally. Chung *et al.* showed using our clinic cohort that diagnosis of TSC prior to onset of seizures leads to improved epilepsy and developmental outcomes. [127] Cardiac rhabdomyomas may be more prevalent in children with *TSC2* mutations compared with *TSC1,* 54% vs 20% in one study.[128] Without cardiac rhabdomyomas, diagnosis of TSC may have been delayed until onset of seizures or symptoms of obstructive hydrocephalus. In our cohort, early SEGA diagnosis during which the lesion is still asymptomatic allowed for close surveillance and opportunities to use mTOR inhibitors rather than emergency neurosurgery.

To our knowledge, our study is the first to evaluate congenital SEGA volumetrically. Volumetric estimation is a more meaningful and sensitive measure of tumour growth compared to collecting linear data alone (e.g., maximal diameter). Indeed, patient 4's SEGA growth of 1mm in a non-maximal dimension was reflected in its volumetric growth rate of 8.98mm^3/yr averaged over 5 years, while the maximal diameter growth rate remained at 0mm/yr. We used the equation: volume = (4/3 $\pi \times$ abc), where a, b, and c are the radial width, length and depth respectively. This equation was also used in Tsai's SEGA growth study[37], and assumes tumours are perfectly ellipsoid. The a, b and c measurements, while reflecting all three dimensions, make the calculation susceptible to error. In an attempt to improve accuracy and reproducibility of volumetric analysis, there has been a recent shift towards semi-automated segmentation techniques. A recent study comparing three semi-automated segmentation methods against standard planimetric method suggested that the former had higher resolution to detect small changes of tumour volume.[51] However, the moderate level of agreement between the evaluated methods meant none of them demonstrated clear superiority. Interestingly, volumetric techniques have also been applied to quantify tuber load in TSC. Jansen created tuber segmentation maps to calculate tuber-brain-proportion (TBP), which were inversely related to age of seizure onset, intelligence and cognition but was not relevant on multivariate analysis.[69] Furthermore, cerebellar volume loss has been suggested as a marker for poor neurodevelopment in children with *TSC2* mutations. [129]

We acknowledge the limitations of our study. We had a small cohort, in the context of a rare complication (congenital SEGA) of an uncommon disease (TSC). Retrospective study design and tertiary center referrals may have led to bias towards a more severe phenotype in our case-mix. The strengths of our study lie in clinical follow up, availability of serial neuroimaging data and incorporation of tumour volumetric data.

Future studies in correlating SEGA to clinical outcomes would benefit from a larger cohort, multicenter recruitment and prospective study design. Standardising MRI scanning protocols with thin slices and volumetric sequences would allow for more accurate volumetric estimation and semi-automated volumetric methods. Indeed, a wider study correlating not only SEGA but multiple other TSC neuroimaging findings

with neurological outcome would be beneficial. This should include molecular analysis or genotype, and the cohort should be followed prospectively. We conclude that congenital SEGA, an important complication of TSC, should be monitored carefully with serial imaging and can follow a relatively benign course with a lower growth rate compared with published literature.

2.6.1 ADDENDUM to expand on volumetric analysis: To be read in conjunction with 4th paragraph of Chapter 2.6 (page 56 para 1)

Attention is turning to machine-guided automated image contouring utilising machine learning in areas such as radiation oncology. These semi-automated approaches reduce the effect of human error and are already showing merits in improving efficiency and increased precision. However, deep machine learning may be limited in clinical utility, as the algorithm learnt on one set of training images may not be necessarily generalisable to new data, particularly if the training set was small. This is currently not routinely applied to lesions in TSC but may be the future of volumetric analysis of SEGA and SEN, allowing deeper insights into lesion evolution over time.

2.6.2 ADDENDUM to expand on study limitations: To be read in conjunction with 5th paragraph of Chapter 2.6 Discussion (page 56 para 2)

Another limitation was that a single blinded rater assessed all the scans limiting assessment of reliability. A second rater was recruited when designing the study but was unable to complete rating of all the scans. A trained second rater was not available during the study period due to the impact of the COVID-19 pandemic. In the interests of preserving methodological reliability, the measures from the first reader were used for the study. Furthermore, all patients within this series had *TSC2* mutation, potentially limiting applicability to all children with TSC.

CHAPTER 3. Using TSC neuroradiological features on early MRI as biomarkers of epilepsy and developmental outcome at 5 years – a single-centre retrospective cohort study

3.1 Introduction

In my experience working with families with newly diagnosed TSC, the wide phenotypic spectrum of TSC is particularly difficult for parents to grasp and it is apparent they would benefit from having a more accurate picture of what difficulties their child might have in the years to come. Emerging evidence suggests that early identification of TSC patients at high risk for poor outcomes may allow for early interventions with vigabatrin (for those at high risk of epilepsy) [8] or targeted developmental stimulation and support (for those at high risk for developmental delay or Autism Spectrum Disorder) [130]. In Chapter 1, I reviewed the literature and found conflicting studies regarding the value of TSC neuroimaging markers as predictive factors for epilepsy and developmental outcome. In Chapter 2, I showed that congenital SEGA can follow a relatively benign course in growth but is associated with higher rates of severe epilepsy, Developmental Disability and Autism Spectrum Disorder. The above findings motivated the search for other neuroradiological markers for prognostication of outcome in children with TSC. Most previous studies have looked only at one neuroradiological factor, rather than multiple factors in the same cohort. Clarity regarding the relative contribution of different factors to neurodevelopmental outcome is critical to help clinicians to prognosticate and potentially select therapies and early intervention where appropriate. Therefore, in Chapter 3, I explore multiple neuroimaging markers within the same study (cortical tubers, SEN, SEGA, cerebellar tubers and RMLs). The aim of this study was to determine whether there is an association between early MRI neuroimaging features of TSC and developmental outcomes after 5 years of age. I hypothesised that patients with a higher tuber count would have worse epilepsy and a higher risk of poor developmental outcome.

3.2 Abstract

Background: Cortical tubers, subependymal giant cell astrocytomas (SEGA) and subependymal nodules (SEN) are the neuroradiological hallmarks of Tuberous Sclerosis Complex (TSC). Previous studies have suggested that neuroimaging-based biomarkers such as tuber type, location and burden may predict epilepsy and developmental outcomes in TSC.

Methods: This is a retrospective single-centre cohort study. Participants were included if they were seen between 2006-2018 in the multidisciplinary TSC clinic at Sydney Children's Hospital, had a confirmed diagnosis of TSC, and brain magnetic resonance imaging (MRI) performed between 18-36 months of age showed at least one TSC neuroradiological finding. Tubers (count and location), SEN, SEGA, cerebellar and RML involvement was quantified for each MRI scan. Epilepsy outcome at 5 years was assessed through retrospective chart review. Epilepsy status was categorised as poor seizure outcome (1 or more seizures a month) or favourable seizure outcome (less than one seizure a month). Drug resistant epilepsy was defined as having trialled more than 2 antiepileptic drugs. Developmental outcomes comprised of adaptive function, assessed using the Vineland-III comprehensive parent/caregiver form as a proxy measure of developmental outcome, and a diagnosis of autism spectrum disorder (ASD), which was ascertained through retrospective chart review. Neuroradiological markers were correlated between dichotomised epilepsy and developmental outcome groups. Fisher's exact test, unpaired t-test, odd ratios, Pearson correlation and binary logistic regression were performed as statistical analyses. Receiver operating characteristic (ROC) curves were used to investigate the diagnostic accuracy of radiological variables.

Results: 39 children were included. The most common neuroimaging finding were cortical tubers (100%) and SEN (100%), followed by cerebellar tubers (44%) and SEGA (26%). Patients had a median of 20.5 cortical tubers (IQR 29 - 33). Tubers were most commonly located frontally (47%). There was no difference between tuber counts of patients who had 1 or more seizure a month (poor seizure outcome) compared with patients who had <1 seizure a month (favourable seizure outcome). However, children with drug resistant epilepsy (DRE) had higher total tuber count (median 31.5 vs 9.5, p=0.0007) and SEN count (median 9.0 vs 7.0, p=0.004) compared to children without

DRE. Children with impaired adaptive function had more total tubers (33.0 vs 22.0, p=0.026) and frontal tubers (15.0 vs 11.0, p=0.023) than children with age-appropriate adaptive function. Children with coexisting ASD had more total tubers (32.0 vs 25.5, p=0.049) and frontal tubers (14.5 vs 11.0, p=0.013) than children without ASD. Multivariable logistic regression showed DRE is associated with higher cortical tuber count independent of early onset of seizures. However, tuber count was not a significant predictor of adaptive function or ASD at 5 years in the presence of poor seizure outcome. ROC curve results are described for a novel combined Tuber+SEN score, which improved AUC compared to using tuber count or SEN count alone. Using a cut-off of > 31 SEN+Tuber correlated with a likelihood ratio of 4.4 for developing DRE (LR 4.4, sensitivity 73%, specificity 83%).

Conclusion: Neuroradiological findings on early MRI did not fully predict poor seizure outcome (1 or more seizures a month) but may be useful as predictors of drug resistant epilepsy, impaired adaptive function and ASD at 5 years. Poor seizure outcome is a significant risk factor for both developmental delay and ASD.

3.3 Background

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem overgrowth disorder. Mutations in tumour suppressor genes *TSC1* or *TSC2* result in loss of mTOR regulation [116], leading to hamartomatous growths in multiple organ systems including the central nervous system. Structurally in the brain, TSC is expressed as cortical tubers, subependymal giant cell astrocytomas (SEGA) and subependymal nodules (SEN) which can be visualised and quantified using neuroimaging techniques such as magnetic resonance imaging (MRI). Patients with TSC are at high risk of neurodevelopmental comorbidities, with a large multinational registry reporting epilepsy in 83.5%, intellectual disability in 54.9% and Autism Spectrum Disorder (ASD) in 20.7% [24]. Children are affected to varying degrees, leading to a wide spectrum of disease phenotype.

Previous attempts to identify potential neuroimaging-based biomarkers have primarily focussed on tuber count and location, however there is still to date no consensus regarding whether the involvement of a particular hemisphere or lobe is predictive of outcomes. Studies have found increased tuber count to correlate with increased severity of epilepsy [65,66]. Increased tuber count has also been associated with lower cognitive scores, especially tubers in the right frontal [60], right parietal [60] and right temporal lobes [70]. Moreover, tubers in the temporal lobe [70,81], frontal lobe [79] and occipital lobe [23,66] have all been separately suggested to increase risk of ASD. The number of cyst-like tubers has also been suggested as a potential marker for ASD but not thoroughly investigated to date [63,88]. Interactions between neurodevelopmental outcomes are complex and entangled, with earlier onset of epilepsy and infantile spasms associated with developmental delay and ASD [70,131]. Despite multiple publications, no neuroimaging biomarker has yet been widely accepted into clinical practice.

We hypothesise that early MRI changes such as cortical tuber count and location may be useful biomarkers of epilepsy and developmental outcomes in children with TSC. This study is unique to previous publications in its use of MRIs from a standardised timepoint between 18-36 months, and use of number of antiepileptic drugs at 5 years as a surrogate marker of epilepsy severity. Our study aims to describe the incidence of

these neuroimaging markers in our cohort and to correlate these with the patients' epilepsy severity, presence of impaired adaptive function and presence of Autism Spectrum Disorder at 5 years of age.

3.4 Methods

3.4.1 Study Design and setting

This is a retrospective single-centre cohort study at Sydney Children's Hospital (SCH), a tertiary paediatric referral hospital in New South Wales (NSW), Australia. The SCH multidisciplinary TSC clinic was founded in 2006 and is one of only two specialist TSC clinics nationally. Our clinic is the major referral centre for children with TSC in NSW and captures approximately 80% of the NSW TSC population, as well as receiving several interstate referrals [127]. The monthly clinic is staffed by a multidisciplinary team including paediatric neurologists, developmental paediatrician, geneticists, paediatric nephrologist and dermatologist. Most patients are followed at our institution's TSC clinic on an annual basis.

3.4.2 Inclusion and exclusion criteria

Children with TSC who attended the SCH multidisciplinary TSC clinic between 2006-2018 were reviewed for study inclusion.

The inclusion criteria were as follows:

1. Patients seen in the TSC clinic aged <18 years between 2006-2018, with
2. definite diagnosis of TSC using the updated diagnostic criteria [14], and
3. MRI performed between 18-36 months of age which could be retrieved for analysis, and
4. this index MRI had at least one radiological marker of TSC, defined as either
    a. cortical tuber, or
    b. subependymal nodule (SEN), or
    c. subependymal giant cell astrocytoma (SEGA)

Patients were excluded for coexisting neurological diagnoses which were unrelated to TSC and could independently confer a poor prognosis (i.e., neonatal ventriculitis, ischaemic stroke).

3.4.3 Neuroimaging- acquisition, deidentification and storage

Patients at the SCH multidisciplinary clinic will receive an MRI brain at time diagnosis, and every 1-3 years until the age of 25 years for SEGA surveillance as recommended by the expert consensus guidelines [4]. As such, most patients diagnosed in infancy will have an MRI between 18 and 36 months of age. In this study, we chose to restrict analysis of the index MRI to 18-36 months period as the study aimed to identify biomarkers from early imaging. We excluded MRI performed before 18 months of age as tubers have variable signal change due to incomplete myelination. Where a patient had multiple MRIs performed between 18 months and 3 years of age and more than one was able to be retrieved, the earlier MRI was used for analysis.

MRI were collated from multiple hospitals on CD from which raw DICOM files were extracted and collated into a password-protected drive on a secure hospital network. Imaging files were assigned a 3-digit study identification number (TSC###) and anonymised using DicomBrowser (version 1.8.0b5), developed by the Neuroinformatics Research Group, © 2006-2015 Washington University (http//rsb.info.nih.gov/ij/). All patient identifiers within DICOM fields were removed, including patient first name, last name, date of birth, age, sex, address, referring doctor and study site. This allowed for unbiased analysis and scoring of the anonymised MRI scans. MRI examinations which were performed at Prince of Wales Hospital (13 of 39 scans) were performed on Siemens Avento 1.5T. The MRI parameters for these scans are shown below in Table 3.1. The other 26 scans were performed at other hospitals (Table 3.4) using a variety of MRI scanner models and different acquisition protocols.

Table 3.1 Imaging protocol at Prince of Wales Hospital for TSC neuroimaging

Sequence	Acquisition Plane	TR (ms)	TE (ms)	Matrix	FoV (mm x mm)	Slice Thickness (mm)
T1	Sagittal	465	13	192*256	230*230	5
T2	Axial	4240	107	235*384	192*220	7
T2 FLAIR[a]	Axial	8000	114	205*320	176*220	5
Epi_Diffusion	Axial	3700	109	192*192	220*220	5
Epi ADC	Axial	3700	109	192*192	220*220	5
T1	Axial	700	8.7	448*512	192*220	7
Mag	Axial	49	40	177*256	201*230	2
Pha	Axial	49	40	177*256	201*230	2
mIP	Axial	49	40	177*256	201*230	16
SWI	Axial	49	40	177*256	201*230	2
FLAIR[a]	Coronal	8000	105	184*256	175*220	5
T1	Coronal	700	8.7	512*512	220*220	5
T2	Coronal	6540	111	314*512	192*220	5
T1 gadolinium	Coronal	405	17	240*320	206*220	5
T1 gadolinium	Axial	405	17	240*320	206*220	5
T1 gadolinium	Sagittal	701	17	240*320	230*230	5

ADC: Apparent diffusion coefficient, FLAIR: Fluid-attenuated inversion recovery, FoV: Field of view, SWI: Susceptibility weighted imaging, TE: Echo time, TR: Relaxation time

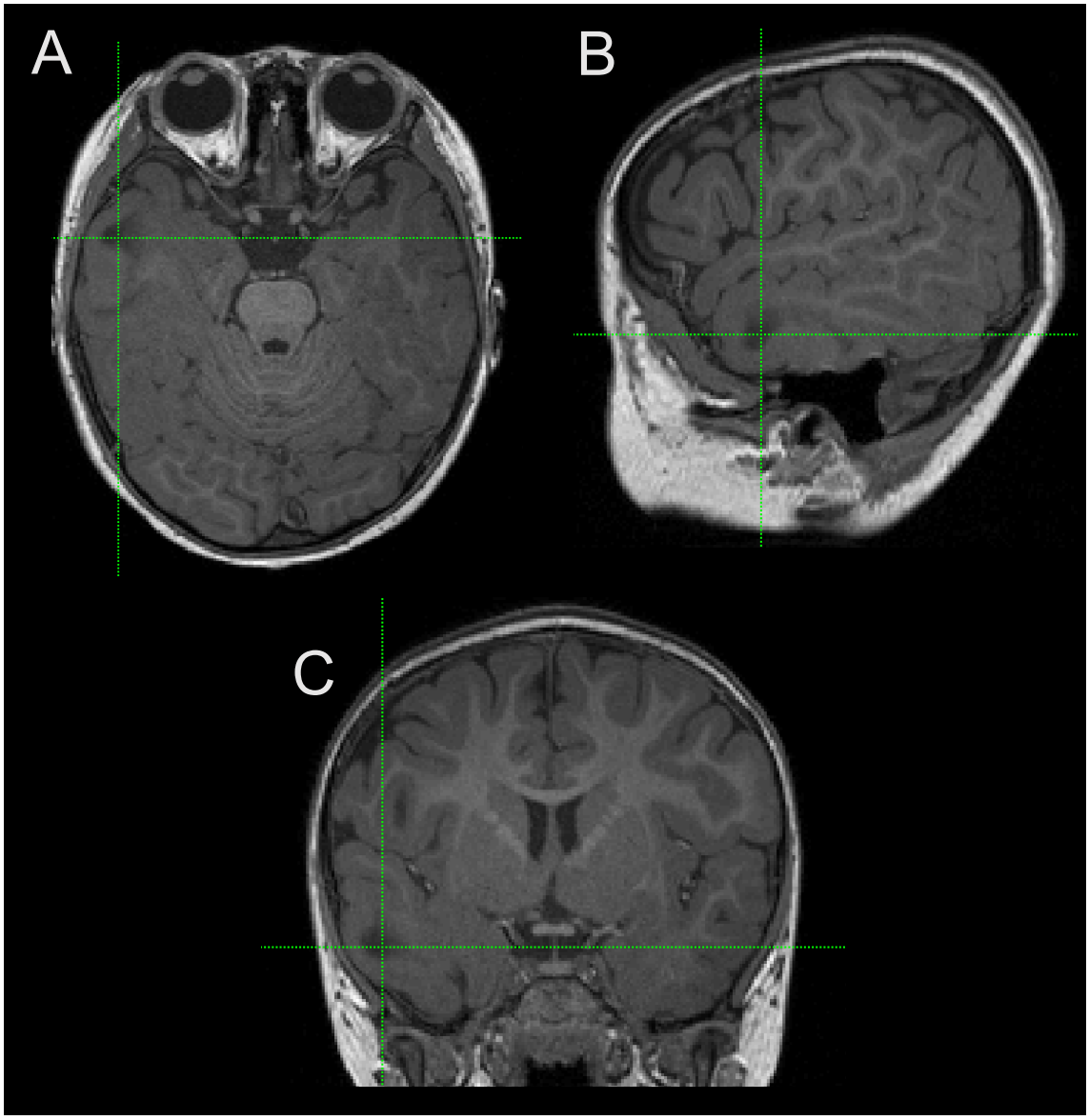
[a] Inversion time 2500

3.4.4 Neuroimaging analysis

Following anonymisation, MRIs were independently reviewed by the candidate as a paediatri neurologist with 5 years additional expertise in TSC. MRIs were viewed using RadiAnt DICOM viewer software by Medixant (Version 2020.1. Mar 9, 2020. URL: https://www.radiantviewer.com.) This software was chosen for its ease of use when interrogating a tuber in three planes across multiple sequences simultaneously, compact installation and compatibility between different devices. The candidate scored each MRI according to their neuroimaging characteristics over a two-week period in May 2018. Independent scoring from a second rater was planned but unable to be completed due to illness. While we spent time to identify and collaborate with another trained independent rater, this did not eventuate due to lack of funding and the later impact of

COVID-19. Multi-planar views aided precise localisation of tubers and 3D T1-weighted sequences acquired with isotropic millimetric voxel resolution (ie, 1 × 1 × 1 mm^3, with no interslice gap) were used where available (Figure 3.1). The imaging sequences were read in the following order: T1, T2, FLAIR, diffusion, T1 with contrast.

Figure 3.1 Right temporal tuber on MRI in multiplanar view



Magnetic Resonance Imaging (MRI) from an anonymised study patient demonstrating a right temporal lobe tuber using 3D T1-weighted sequence acquired with isotropic millimetric voxel resolution (1 × 1 × 1 mm^3, with no interslice gap). Multi-planar view allow simultaneous visualisation of tuber in (A) axial, (B) sagittal and (C) coronal planes.

To ensure consistency, a standardised scoring proforma was developed based on literature review and underwent multiple revisions during initial study design. The final proforma was used to score all included scans and is attached below (Figure 3.2). The following radiological definitions were agreed upon by the candidate and supervisor based on published literature. A cortical tuber was defined as having the appearance of a swollen gyrus with hyperintense signal on T2WI and corresponding hypointensity on T1WI. SEN was defined as a small round lesion arising from the subependymal lining measuring <1cm in any diameter, with isointense or hyperintense signal on T2WI, with possible calcification. SEGA was defined as a lesion near the foramen of Monro measuring >1cm in maximal diameter when measured in any plane [4]. Signal intensity within SEGA can be variable, and while SEGA often show gadolinium enhancement, this was not required for diagnosis. A cerebellar tuber was defined as a wedge-shaped T2 hyperintensity seen in the cerebellar hemispheres, often with a retraction deformity. Radial migration lines (RML) were defined as curvilinear or band-shaped lesions traversing deep white matter and hyperintense to normal appearing white matter (NAWM). This was categorised as to whether >50% of white matter volume was affected (RML>50%), estimated through visual analysis using FLAIR axial sequences.

MRI ANALYSIS FORM V8 - FINAL

Reviewer:
☐ JL ☐ DC

Date of Review: _ _ / _ _ / _ _ _ _

Patient ID: _ _ _

Date of Scan: _ _ / _ _ / _ _ _ _

MRI location: _____
Tesla Strength:
☐ 1.5 ☐ 3

Sequences:
☐ T2 ☐ FLAIR. ☐ Diffusion
☐ T1+C ☐ SWI/T2GRE ☐ FLASH

☐ Sagittal ☐ Axial ☐ Coronal

Cortical Tuber Count

Areas of cortical gray matter distortion with hypo- or isointense subcortical signal intensity in T1W images and increased signal on T2/FLAIR sequences[1]. Check axial and coronal planes. If unmyelinated then use T1.

	Frontal	Parietal	Temporal	Occipital
R				
L				

SEN count

Lesions originating from ventricle wall and protruding into lumen, with variable signal intensity[2]

	SEN count
R	
L	

SEGA count

SENS >=1cm maximum diameter in any axis, located at foramen of Munro OR Radiological evidence of previous SEGA resection

	SEGA count
R	
L	

☐ Lesion near FoM to monitor for SEGA study

Cerebellar involvement

Wedge shaped, T2 hyperintensity is suggestive of cerebellar tuber[3]

	Tuber	Atrophy	Other
R			
Vermis			
L			

RML (Radial Migration lines)

Curvilinear or band-shaped lesions traversing the deep WM and hyperintense to the NAWM (normal appearing white matter) on FLAIR or T2[2]

☐ <50% affected ☐ >50% affected

Other comments:

1 Distinctive MRI features of the epileptogenic zone in children with tuberous sclerosis. Jahodava et al, European Journal of Radiology 2013
2 The Neuroanatomical phenotype of TSC: focus on radial migration lines. Van Eeghen et al, Neuroradiology. 2013
3 Centre of epileptogenic tubers generate and propogate seizures in tuberous sclerosis. Kannan et al, Brain 2016

Figure 3.2 Final proforma for MRI scoring

3.4.5 Epilepsy outcomes – seizure frequency and DRE

Epilepsy outcomes of study participants were ascertained through retrospective chart review, using the first clinic visit letter after the patient reached 5 years old. Primary outcomes measures were seizure frequency (dichotomised as one or more seizures a month, or seizures less than monthly) and presence of drug resistant epilepsy (DRE). The International League Against Epilepsy (ILAE) definition of drug resistant epilepsy (DRE) was used, that is, the "failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [132].

Basic demographic data was also collected during chart review: sex, genotype, age at MRI scan, age at last follow up. Surrogate measures of epilepsy severity were also collected, including age of seizure onset, history of infantile spasms, number of antiepileptic drugs (AEDs) at time of clinic review, total number of AEDs trialled to date, use of ketogenic diet (KD), mTOR inhibitor, cannabidiol and epilepsy surgery.

3.4.6 Developmental outcomes – adaptive function and ASD diagnosis

Intellectual disability is defined by the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) as concurrent impairments in intellectual function and adaptive function [133]. Assessment of intellectual function requires standardised psychometric IQ testing which has limitations in our patient population due to a high portion of moderate-severe intellectual disability, and only reflects performance on the day of testing. In contrast, adaptive function is assessed through parent/carer interview, who report the patient's usual functional abilities in their usual environment.

The Vineland Adaptive Behavior Scales, 3rd Edition (Vineland-III) is a validated interview-based assessment scale for patients aged 0 to 90 years to assess adaptive skills and behaviour. Adaptive function is assessed across 3 domains: Communication, Daily Living Skills, Socialization. Motor Skills and Maladaptive Behaviour are included in the interview as optional domains. Scores from the three primary domains are combined into the Adaptive Behavior Composite score (ABC score) which is a global

score standardised to age norms. The normative mean for the ABC score is 100 with a normative standard deviation of 15. The lowest possible ABC score is 20 and highest possible score is 160. ABC score <70 (2 standard deviations below the mean) is indicative of impaired adaptive function, whereas ABC score ≥ 70 is indicative of average-range adaptive function.

The Vineland-III assessment is part of routine clinical care for all patients of the Sydney Children's Hospital multidisciplinary TSC clinic. Parents and caregivers are asked to complete this questionnaire annually prior to review with the developmental paediatrician at their annual TSC clinic visit. The online parent-caregiver form is used with automatic assessment scoring and report generation through Q-global, a web-based platform for test administration by Pearson Clinical group. The Vineland-III reports are stored as part of the patient's medical record.

Formal diagnosis of Autism Spectrum Disorder (ASD) by 5 years of age was ascertained through retrospective chart review, as documented by the TSC clinic developmental paediatrician.

3.4.7 Statistical analysis

Statistical analyses were performed using GraphPad Prism (version 9.1.2 (225) for Mac OS X, GraphPad Software, La Jolla California USA, www.graphpad.com) and IBM SPSS (version 26.0 for Macintosh, IBM Corp, Armonk, NY: IBM Corp). Statistical significance was defined as a p-value of <0.05. Demographic features of the cohort and the incidence of neuroimaging findings were reported descriptively.

This was an exploratory study looking at the potential for TSC neuroimaging markers to predict epilepsy and developmental outcomes of children at 5 years of age. The sample size was necessarily small due to Tuberous Sclerosis Complex being a rare disease. As we had limited numbers in the cohort, patients were dichotomised into 2 groups according to the outcome measures (Table 3.2). Seizure outcome at 5 years, DRE and ASD diagnosis outcome measures were naturally dichotomous upon data collection. Adaptive function was initially categorised into average range (ABC score ≥70, n=12),

mild impairment (ABC score 50-69, n=10), moderate impairment (ABC score 35-49, n=3), severely impaired adaptive function (ABC score <35, n=4). These groups were later aggregated into a dichotomous variable as small numbers in individual groupings were insufficient for meaningful analysis.

Table 3.2 Study outcome measures

Outcome measures	Dichotomised outcome group
Epilepsy outcome 1: Seizure outcome at 5 years	"Favourable seizure outcome" (seizure free or seizures < monthly) vs. "Poor seizure outcome" (one or more seizures a month)
Epilepsy outcome 2: DRE	"No DRE" (2 or less AEDs trialled by 5 years) vs. "DRE" (more than 2 AEDs trialled by 5 years)
Developmental outcome 1: Adaptive function	"Average-range adaptive function" (Vineland-III ABC score >70) vs. "Impaired adaptive function" (Vineland-III ABC score <70)
Developmental outcome 2: ASD diagnosis	"No ASD diagnosis" vs "ASD diagnosis"

ABC score: Adaptive Behavior Composite score, ASD: Autism Spectrum Disorder, DRE: Drug Resistant Epilepsy

For each dichotomised outcome group (Table 3.2), the presence and amount of TSC neuroimaging markers were measured (i.e., total tuber count, SEN count, presence of SEGA, presence of cerebellar tuber, whether RMLs affected >50% of white matter). Cortical tuber count was further analysed within each cerebral lobe – frontal, parietal, temporal, occipital. Continuous variables were compared between two groups using unpaired T-test and expressed as median and interquartile range. Categorical variables were compared across groups using Fisher's exact test and expressed as a whole number prevalence (%). Odds ratios were calculated between outcome groups. Pearson correlation coefficient was used to assess relationship between two continuous variables. Receiver operating characteristic (ROC) curves were used to investigate the diagnostic accuracy of tuber count for adaptive function and ASD outcomes, and tuber

count and SEN count for DRE outcome. Binary regression analysis of each outcome measure was performed, using covariates which had previously demonstrated a significant relationship on univariate testing.

3.4.8 Ethics

This study received research ethics approval from the Sydney Children's Hospitals Network Human Research Ethics Committee (LNR14/ SCHN445).

3.5 Results

3.5.1 Description of study cohort

The process of searching and selecting participants for inclusion in this study is presented in Figure 3.3. Review of the Sydney Children's Hospital TSC patient database between 2006-2018 identified 125 patients, of whom 39 were eligible for inclusion (Figure 3.3).

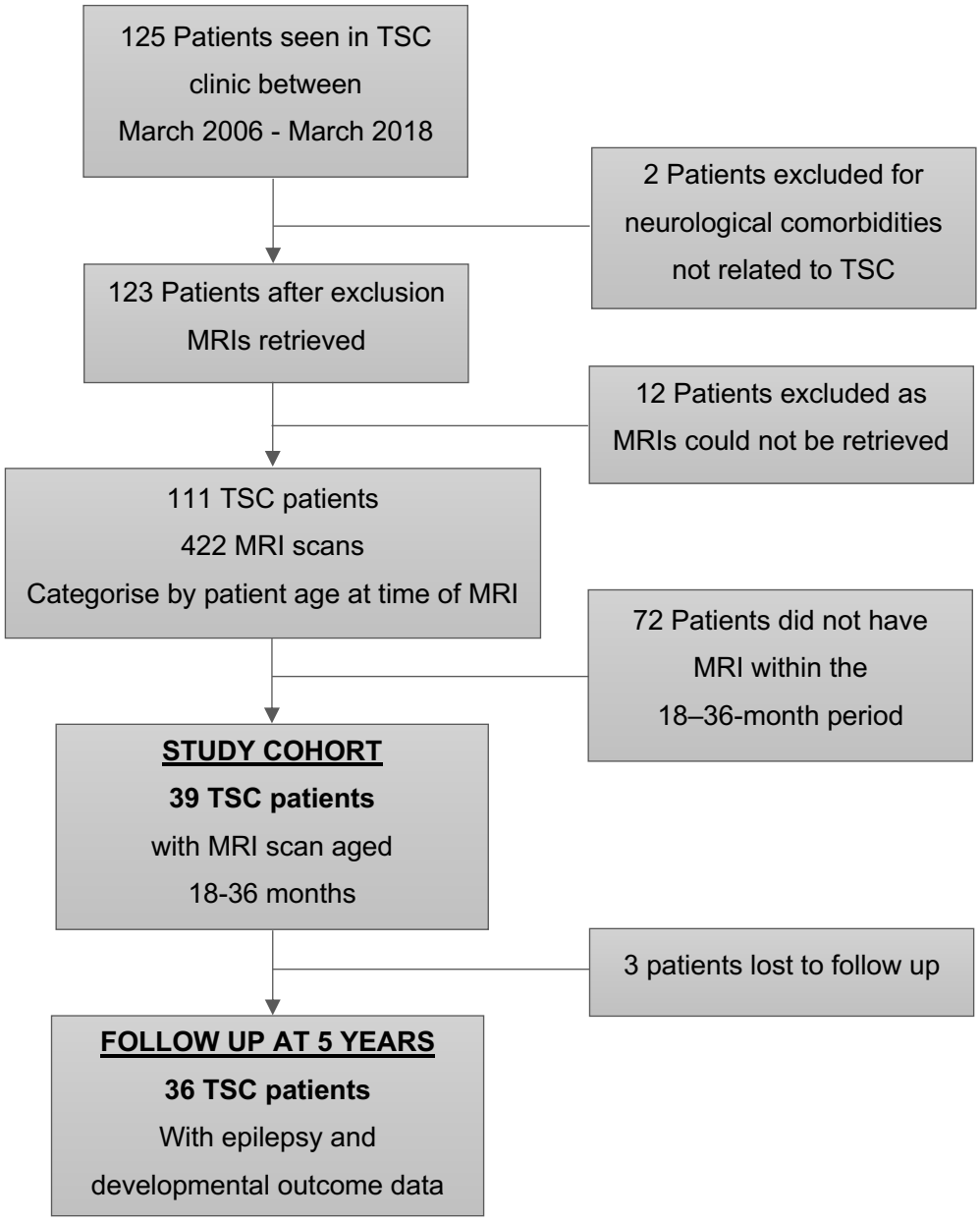


Figure 3.3. Study flow diagram

MRIs were analysed from 39 patients (16 male, 23 female). The median age at MRI was 2.1 years. Of 31 patients who had TSC gene molecular testing, 25 had mutation in *TSC2*, 2 had a mutation in *TSC1* and 4 had no mutation identified (NMI). Median patient age at last clinic follow up is 8.2 years (Table 3.3).

Table 3.3 Demographics of study cohort (n=39)

Characteristics	**Baseline Data**	
Gender, n (%)		
Male	16	(41)
Female	23	(59)
Patients with molecular testing, n (%)	31	(79)
Genotype testing, n (% of tested patients)		
TSC1	2	(6)
TSC2	25	(81)
NMI	4	(13)
Median age at MRI, yr (IQR)	2.1	(1.8-2.3)
Median age at last clinic follow up, yr (IQR)	8.2	(5.2-10.8)

NMI: no mutation identified, SD: standard deviation, yr: years

3.5.2 Quality, consistency and location of imaging data

MRIs of study patients were retrospectively retrieved from 9 hospitals across NSW, Victoria and New Zealand, with collation of MRI occurring from June 2017 to June 2018. A minority of MRIs occurred outside of NSW with patients moving to NSW in later childhood. All MRIs occurred under general anaesthesia in tertiary hospitals within Australia and New Zealand; because of patient's young age they are unable to lie still for scan acquisition. MRI protocols varied between patients due to the retrospective study design and were specified by the radiologist at the local site where imaging was undertaken. The majority of scans had T1-weighted, T2-weighted and fluid attenuated inversion recovery (FLAIR) sequences as a minimum dataset. In one study the T2-

weighted sequence was not performed, and in another the FLAIR sequence was omitted. Gadolinium contrast was given in 36 of 39 cases. Twenty-two scans (56%) included either susceptibility weight imaging (SWI), gradient echo (GRE) or fast low angle single shot (FLASH) sequences, which aided detection of calcification within SEN, SEGA and cortical tubers.

Thirty-one (79%) of 39 MRI studies were performed on 1.5 Tesla MRI machines, 8 studies were performed on a higher field 3 Tesla MRI machine (3T MRI). A third of the studies were performed at our institution's campus at Prince of Wales Hospital, another third at the Children's Hospital at Westmead, and the rest were performed at a variety of other sites (Table 3.4). The most frequently used MRI model was the Siemens Magnetom Avanto 1.5T, with 16 (41%) of scans performed on this scanner.

Table 3.4 Location of MRI brain imaging (n=39)

Imaging Site	MRI Scanner	Number of MRIs
Prince of Wales Hospital	Siemens Magnetom Avanto 1.5T	13
Children's Hospital at Westmead	Philips Intera 1.5T	10
Children's Hospital at Westmead	Siemens Magnetom Verio 3T	3
John Hunter Hospital	Siemens Magnetom Vision 1.5T	1
John Hunter Hospital	Siemens Magnetom Verio 3T	2
Liverpool Hospital	Siemens Magnetom Skya 3T	1
St George Hospital	GE Signa 3T	2
The Wollongong Hospital	Philips Achieva 1.5T	1
Canberra Hospital, ACT	Siemens Magnetom Avanto 1.5T	2
Royal Children's Hospital, VIC	Siemens Magnetom Avanto 1.5T	1
Royal Children's Hospital, VIC	Siemens Magnetom Aera 1.5T	1
Wellington Hospital, NZ	Philips Achieva 1.5T	2
Total		39

ACT: Australian Capital Territory, VIC: Victoria, NZ: New Zealand, 1.5T: 1.5 Tesla, 3T: 3 Tesla

3.5.3 Prevalence of TSC neuroradiological findings

All patients had evidence of cortical tubers on their early MRI. A total of 1011 tubers were counted over 39 MRI scans, with a median of 20.5 (IQR 29.0-33.0) cortical tubers per patient (range 2-46). Approximately half of all tubers were located in the frontal lobe (47%), followed by temporal (21%), occipital (17%) and parietal lobes (15%). There was no difference in tuber distribution between hemispheres (Figure 3.4).

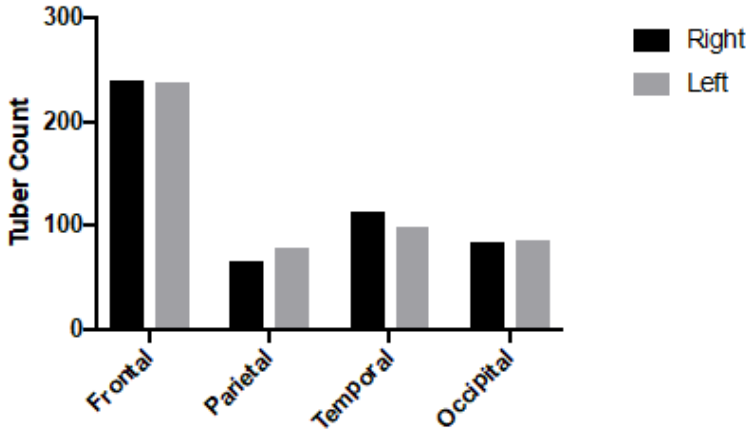


Figure 3.4 Distribution of all tubers by cortical lobe

All patients had subependymal nodules (SEN) with a median of 9.0 (IQR 7.5-11.0) per child. SEGA were seen in 10 (26%) of patients (6 right-sided, 4 left-sided). None of the patients in this series had bilateral or multiple SEGA. Cerebellar tubers were seen in 44% of patients (n=17). The prevalence of neuroradiological findings in this cohort are summarised in Table 3.5.

Table 3.5 Prevalence of neuroradiological findings (n=39)

TSC neuroradiological finding	Prevalence, n (%)
Cortical tuber	39 (100%)
SEN	39 (100%)
SEGA	10 (26%)
Cerebellar tuber	17 (44%)
RML>50%	19 (49%)

RML>50%: greater than 50% of white matter affected with RMLs, SEGA: subependymal giant cell astrocytoma, SEN: subependymal nodule

A weak correlation was found between cortical tuber count and SEN count (Figure 3.5). No significant difference was found in the median tuber counts of patients with SEGA compared to patients without SEGA (31.0, IQR 23.5-39.5 vs 29.0, IQR 17.5-33.0, p = 0.15), or in median tuber counts of patients with cerebellar tubers compared to patients without (33.0, IQR 16.5-36.0 vs 25.5, IQR 19.8-32.3, p = 0.28). Patients with over 50% of white matter affected by RML had a higher median tuber count compared to patients with less than 50% white matter affected by RML (33.0, IQR 29.0-37.0 vs 21.5, IQR 8.8-30.5, p = 0.0006).

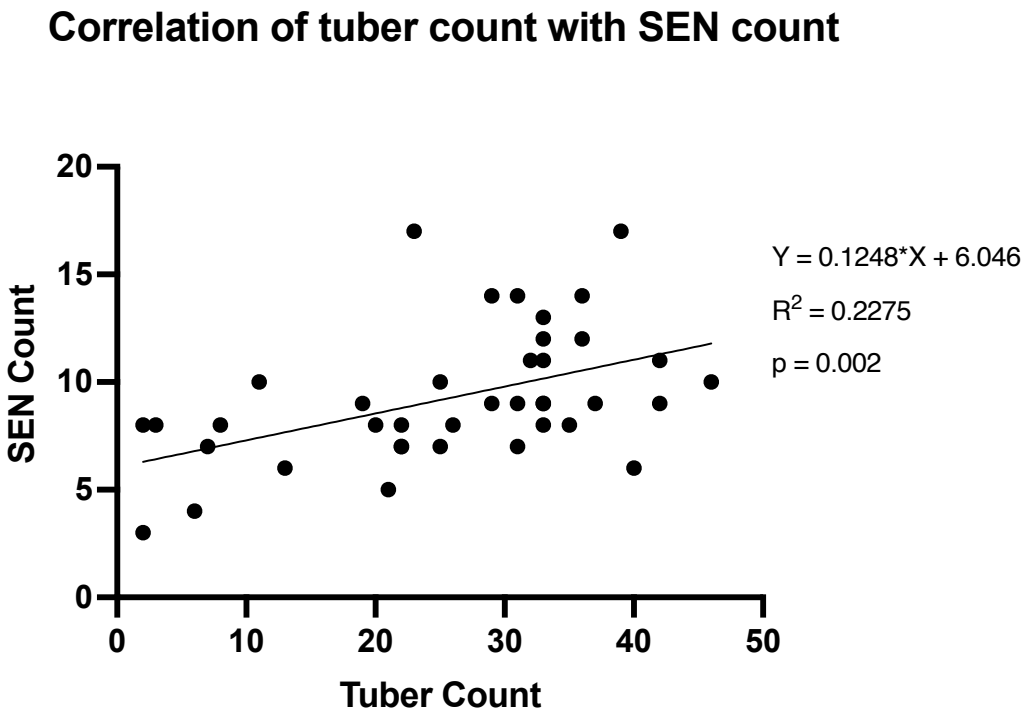


Figure 3.5. Linear regression of tuber count and SEN count

3.5.4 Clinical characteristics of cohort at 5 years

Five-year outcome data were not available for 3 patients who were lost to follow up, leaving 36 patients for outcome analysis. All 36 patients had epilepsy, with seizure onset ranging between 1 and 18 months of life (median 5.0, IQR 3.0-8.0), reflecting a severely affected cohort. Twenty-one patients (54%) had infantile spasms. Thirty patients (83%) had met the criteria for drug resistant epilepsy by 5 years, that is, trialled more than 2 antiepileptic medications. The current daily number of different antiepileptic medications ranged from 0 to 5 (median 3.0, IQR 1.8-4.0). The number of antiepileptic drugs trialled by 5 years ranged from 1 to 13 (median 5.5, IQR 3.0-7.0). At 5 years of age, one patient was seizure free without antiepileptics, 15 patients had less than monthly seizures, 5 patients had at least one seizure a month and 15 patients had at least one seizure a week.

Patients were divided into two groups determined by seizure frequency at 5 years for further analysis. The “poor seizure outcome” group had 20 patients who had one or more seizures per month. The “favourable seizure outcome” group comprised of 16 patients who had seizures less than monthly. Patients in the poor seizure outcome group had earlier age of seizure onset compared to the favourable seizure outcome group (Table 3.6). Children with poor seizure outcome were also currently on more daily AEDs, had a higher number of medication trials in the previous 5 years and were more likely to have DRE. Both groups had a similar proportion of infantile spasms. Patients with poor seizure outcome also had higher rates of accessing complex therapies such as ketogenic diet, cannabidiol (CBD), mTOR inhibitor and epilepsy surgery but this did not reach significance (Table 3.6). The main indication for starting mTOR inhibitor in the poor seizure outcome group was for refractory seizures (12/13 seizures, 1/13 SEGA), while the main indication for starting an mTOR inhibitor in the favourable seizure outcome group was SEGA (4/5 SEGA, 1/5 seizures).

Table 3.6 Seizure characteristics and genotype of study cohort (n=36)

	Favourable seizure outcome group (n=16)	**Poor seizure outcome group (n=20)**	**Significance testing**
Median age of seizure onset, mo (IQR)	7.5 (4.3-12.0)	3.8 (2.0-5.8)	$p = .007$
Presenting with infantile spasms	9 (56%)	12 (60%)	$p > .999$
Median number of daily AEDs (IQR)	1.5 (1.0-2.0)	4.0 (3.0-4.0)	$p < .0001$
Median number of AED trials (IQR)	3.0 (2.0-5.0)	6.0 (5.3-8.0)	$p = .0002$
DRE	10 (63%)	20 (100%)	$p = .004$
Ketogenic diet	2 (13%)	6 (30%)	$p = .257$
CBD	2 (13%)	7 (35%)	$p = .245$
mTOR inhibitor	5 (31%)	13 (65%)	$p = .533$
Epilepsy surgery	0 (0%)	4 (20%)	$p = .113$
Underwent genetic testing	n=11	n=16	
TSC1 mutation	0 (0%)	1 (6%)	
TSC2 mutation	9 (82%)	13 (81%)	
NMI	2 (18%)	2 (13%)	

AED: Antiepileptic drugs, CBD: Cannabidiol, DRE: Drug Resistant Epilepsy, IQR: Interquartile Range; mo: months, mTOR: mammalian targets of rapamycin

Twenty nine of 36 (81%) Vineland-III assessments were completed at a median age of 10.6 years (IQR 8.3 - 13.4). Seven patients did not have Vineland-III results as part of their medical record for analysis. The mean Adaptive Behavior Composite (ABC) score was 64.9 ± 25.0, consistent with a high rate of impaired adaptive functional in our cohort. Seventeen patients (59%) had ABC scores falling below two standard deviations of the mean (ABC score <70), indicative of impaired adaptive function. Twelve patients (41%) had a Vineland-III ABC score within two standard deviations of the mean, indicative of average-range adaptive function. Nineteen of 36 (52%) children were diagnosed with ASD by 5 years of age.

3.5.5 Clinico-radiological correlations

Epilepsy outcome 1: Seizure outcome at 5 years

No neuroradiological markers were found to be associated with seizure outcome at 5 years of age. There was no difference between cortical tuber counts of the poor seizure outcome group (patients who had one or more seizures a month) compared with the favourable seizure outcome group (patients who had <monthly seizures) (Table 3.7). On analysis of tubers by each individual lobe, no differences were found in frontal/parietal/temporal/occipital tuber counts between the poor and favourable seizure outcomes groups. Similar rates of SEN were observed between the two groups. No difference was observed between groups with regards to presence of SEGA, cerebellar tubers or RML involvement (Table 3.7).

Table 3.7 MRI features of patients by seizure outcome group at 5 years (n=36)

	Favourable seizure outcome group (n=16)	**Poor seizure outcome group (n=20)**	**Significance testing**
Median cortical tuber count (IQR)	28.5 (15.0–34.5)	30.0 (19.3–35.3)	$p = .307$
Frontal tuber count	12.5 (7.25-15.8)	13.0 (10.0-18.0)	$p = .182$
Parietal tuber count	4.0 (2.0-5.0)	4.0 (2.0-4.0)	$p = .494$
Temporal tuber count	6.0 (3.0-8.0)	6.5 (3.0-7.8)	$p = .347$
Occipital tuber count	4.0 (1.0-7.0)	4.0 (2.3-6.0)	$p = .408$
Median SEN count (IQR)	8.0 (6.0-11.8)	9.0 (8.0-10.8)	$p = .157$
Presence of SEGA	5 (31%)	5 (25%)	$p = .723$
Presence of cerebellar tubers	5 (31%)	10 (50%)	$p = .320$
RML>50%	9 (56%)	10 (50%)	$p = .749$

IQR: Interquartile Range, NMI: no mutation identified, RML>50%: greater than 50% of white matter affected with RMLs, SEGA: subependymal giant cell astrocytoma, SEN: subependymal nodule

Epilepsy outcome 2: Drug Resistant Epilepsy (DRE)

Thirty (83%) of children met definition for DRE at 5 years of age. Patients with DRE had higher tuber counts and SEN counts compared to patients without DRE (Table 3.8). Tuber counts in the frontal, parietal and temporal lobes were significantly higher in the patients with DRE compared to patients without DRE. There were no differences in presence of SEGA or cerebellar tubers between the two groups (Table 3.8). While more than half of the DRE group (56%) had more than 50% of white matter volume affected by RMLs, none of the patients without DRE had this level of RML involvement (0%, $p = 0.02$).

Table 3.8 MRI features of patients by DRE at 5 years (n=36)

	No DRE (n=6)	**DRE (n=30)**	**Significance testing**
Median cortical tuber count (IQR)	9.5 (2.0-23.0)	31.5 (21.8-36.0)	$p = .0007$
Frontal tuber count	4.5 (0.8-11.0)	13.5 (11.0-17.0)	$p = .001$
Parietal tuber count	1.5 (0.8-4.3)	4.0 (2.0-5.0)	$p = .038$
Temporal tuber count	2.0 (0.0-3.3)	7.0 (4.0-8.3)	$p = .0002$
Occipital tuber count	1.5 (0.8-4.8)	4.5 (2.8-7.0)	$p = .0568$
Median SEN count (IQR)	7.0 (3.8-8.0)	9.0 (8.0-11.3)	$p = .004$
Presence of SEGA	3 (50%)	7 (23%)	$p = .317$
Presence of cerebellar tubers	1 (17%)	14 (47%)	$p = .367$
RML>50%	0 (0%)	17 (56%)	$p = .020$

DRE: drug resistant epilepsy, IQR: Interquartile Range, RML>50%: greater than 50% of white matter affected with RMLs, SEGA: subependymal giant cell astrocytoma, SEN: subependymal nodule

Cortical tuber count was found to be weakly correlated to number of antiepileptic drugs (AEDs) trialled by 5 years (Figure 3.6, $p = 0.02$).

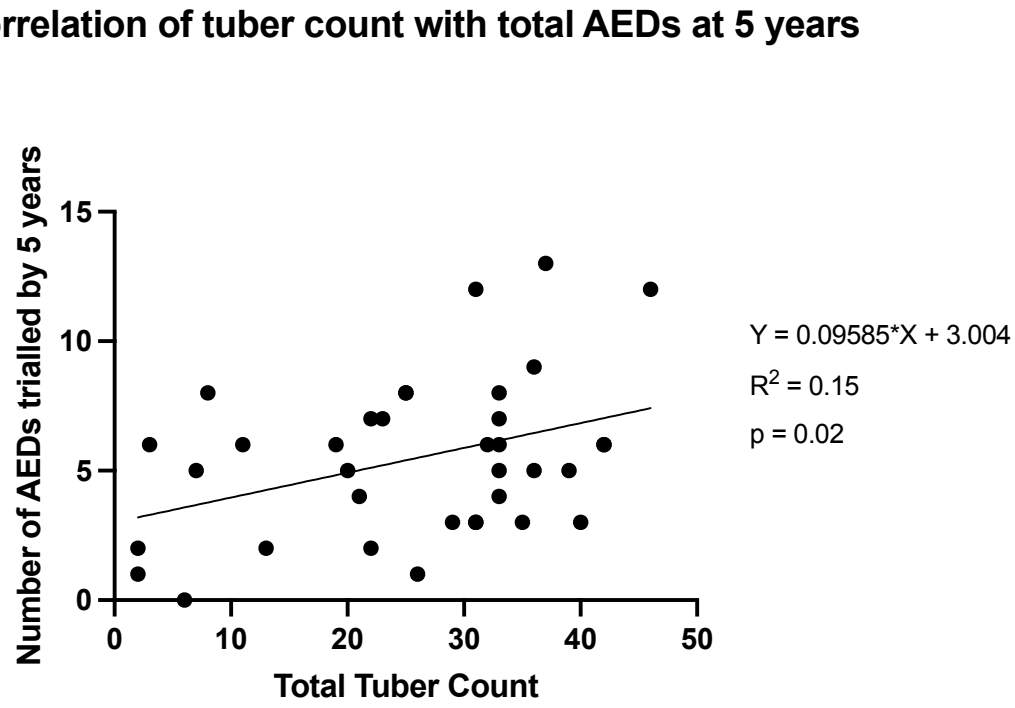


Figure 3.6 Linear regression of tuber count and number of AEDs trialled by 5 years

Post-hoc receiver operator characteristic (ROC) curve analysis was used to explore the predictive value of tuber and SEN counts regarding DRE. Tuber count performed slightly better as a predictor of DRE than SEN count (Figure 3.7). A combined score of SEN+Tuber was highly predictive of DRE (Figure 3.7) The p-values, cutoff points optimized for sensitivity and specificity, 95% CIs, sensitivities, and specificities obtained for each of tuber count, SEN count and tuber + SEN count are shown in Tables 3.9, 3.10 and 3.11. A tuber count of 23 or more, a SEN count of 7 or more and a combined count of more than 31 had the best specificity balanced by sensitivity to predict DRE.

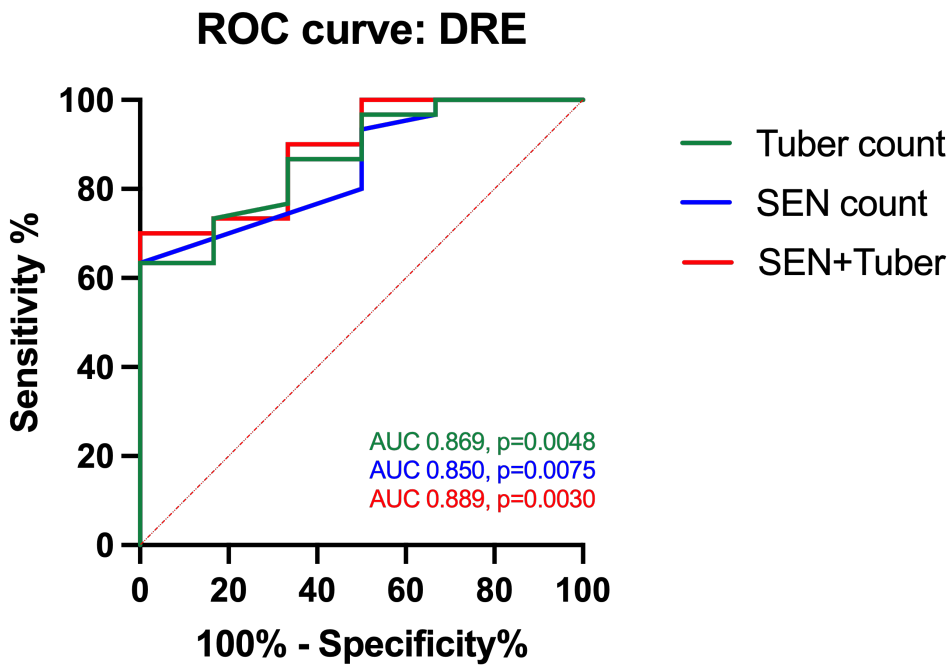


Figure 3.7 ROC curve of DRE using Tuber count, SEN count and combined SEN+Tuber score

Table 3.9 Data for ROC curve of Tuber Count

Tuber count	Sensitivity	95% CI	Specificity	95% CI	Likelihood ratio
> 2.500	100.0	88.65% to 100.0%	33.33	5.923% to 70.00%	1.500
> 4.500	96.67	83.33% to 99.83%	33.33	5.923% to 70.00%	1.450
> 6.500	96.67	83.33% to 99.83%	50.00	18.76% to 81.24%	1.933
> 7.500	93.33	78.68% to 98.82%	50.00	18.76% to 81.24%	1.867
> 9.500	90.00	74.38% to 96.54%	50.00	18.76% to 81.24%	1.800
> 12.00	86.67	70.32% to 94.69%	50.00	18.76% to 81.24%	1.733
> 16.00	86.67	70.32% to 94.69%	66.67	30.00% to 94.08%	2.600
> 19.50	83.33	66.44% to 92.66%	66.67	30.00% to 94.08%	2.500
> 20.50	80.00	62.69% to 90.49%	66.67	30.00% to 94.08%	2.400
> 21.50	76.67	59.07% to 88.21%	66.67	30.00% to 94.08%	2.300
> 22.50	73.33	55.55% to 85.82%	83.33	43.65% to 99.15%	4.400
> 24.00	70.00	52.12% to 83.34%	83.33	43.65% to 99.15%	4.200
> 25.50	63.33	45.51% to 78.13%	83.33	43.65% to 99.15%	3.800
> 27.50	63.33	45.51% to 78.13%	100.0	60.97% to 100.0%	
> 30.00	60.00	42.32% to 75.41%	100.0	60.97% to 100.0%	
> 31.50	50.00	33.15% to 66.85%	100.0	60.97% to 100.0%	
> 32.50	46.67	30.23% to 63.86%	100.0	60.97% to 100.0%	
> 34.00	30.00	16.66% to 47.88%	100.0	60.97% to 100.0%	
> 35.50	26.67	14.18% to 44.45%	100.0	60.97% to 100.0%	
> 36.50	20.00	9.505% to 37.31%	100.0	60.97% to 100.0%	
> 38.00	16.67	7.337% to 33.56%	100.0	60.97% to 100.0%	
> 39.50	13.33	5.310% to 29.68%	100.0	60.97% to 100.0%	
> 41.00	10.00	3.460% to 25.62%	100.0	60.97% to 100.0%	
> 44.00	3.333	0.1710% to 16.67%	100.0	60.97% to 100.0%	

Table 3.10 Data for ROC curve of SEN Count

SEN count	Sensitivity	95% CI	Specificity	95% CI	Likelihood ratio
> 3.500	100.0	88.65% to 100.0%	16.67	0.8549% to 56.35%	1.200
> 4.500	100.0	88.65% to 100.0%	33.33	5.923% to 70.00%	1.500
> 5.500	96.67	83.33% to 99.83%	33.33	5.923% to 70.00%	1.450
> 6.500	93.33	78.68% to 98.82%	50.00	18.76% to 81.24%	1.867
> 7.500	80.00	62.69% to 90.49%	50.00	18.76% to 81.24%	1.600
> 8.500	63.33	45.51% to 78.13%	100.0	60.97% to 100.0%	
> 9.500	43.33	27.38% to 60.80%	100.0	60.97% to 100.0%	
> 10.50	33.33	19.23% to 51.22%	100.0	60.97% to 100.0%	
> 11.50	23.33	11.79% to 40.93%	100.0	60.97% to 100.0%	
> 12.50	16.67	7.337% to 33.56%	100.0	60.97% to 100.0%	
> 13.50	13.33	5.310% to 29.68%	100.0	60.97% to 100.0%	
> 15.50	6.667	1.185% to 21.32%	100.0	60.97% to 100.0%	

Table 3.11 Data for ROC curve of SEN+Tuber Count

SEN+Tuber count	Sensitivity	95% CI	Specificity	95% CI	Likelihood ratio
> 7.500	100.0	88.65% to 100.0%	16.67	0.8549% to 56.35%	1.200
> 10.50	100.0	88.65% to 100.0%	50.00	18.76% to 81.24%	2.000
> 12.50	96.67	83.33% to 99.83%	50.00	18.76% to 81.24%	1.933
> 15.00	93.33	78.68% to 98.82%	50.00	18.76% to 81.24%	1.867
> 17.50	90.00	74.38% to 96.54%	50.00	18.76% to 81.24%	1.800
> 20.00	90.00	74.38% to 96.54%	66.67	30.00% to 94.08%	2.700
> 23.50	86.67	70.32% to 94.69%	66.67	30.00% to 94.08%	2.600
> 27.00	83.33	66.44% to 92.66%	66.67	30.00% to 94.08%	2.500
> 28.50	76.67	59.07% to 88.21%	66.67	30.00% to 94.08%	2.300
> 29.50	73.33	55.55% to 85.82%	66.67	30.00% to 94.08%	2.200
> 31.00	73.33	55.55% to 85.82%	83.33	43.65% to 99.15%	4.400
> 33.00	70.00	52.12% to 83.34%	83.33	43.65% to 99.15%	4.200
> 34.50	70.00	52.12% to 83.34%	100.0	60.97% to 100.0%	
> 36.50	66.67	48.78% to 80.77%	100.0	60.97% to 100.0%	
> 39.00	60.00	42.32% to 75.41%	100.0	60.97% to 100.0%	
> 40.50	53.33	36.14% to 69.77%	100.0	60.97% to 100.0%	
> 41.50	50.00	33.15% to 66.85%	100.0	60.97% to 100.0%	
> 42.50	46.67	30.23% to 63.86%	100.0	60.97% to 100.0%	
> 43.50	40.00	24.59% to 57.68%	100.0	60.97% to 100.0%	
> 44.50	36.67	21.87% to 54.49%	100.0	60.97% to 100.0%	
> 45.50	30.00	16.66% to 47.88%	100.0	60.97% to 100.0%	
> 47.00	20.00	9.505% to 37.31%	100.0	60.97% to 100.0%	
> 49.00	16.67	7.337% to 33.56%	100.0	60.97% to 100.0%	
> 50.50	13.33	5.310% to 29.68%	100.0	60.97% to 100.0%	
> 52.00	10.00	3.460% to 25.62%	100.0	60.97% to 100.0%	
> 54.50	6.667	1.185% to 21.32%	100.0	60.97% to 100.0%	

Developmental outcome 1: Impaired adaptive function

Children with impaired adaptive function had significantly more tubers than children with average-range adaptive function as assessed by Vineland-III (Table 3.12). Frontal tuber count was also significantly higher in patients with impaired adaptive function, compared to patients with average-range adaptive function. On sub-analysis, patients with impaired adaptive function had higher numbers of right frontal tubers (median 8.0 IQR 6.0-10.0 vs median 5.5 IQR 2.5-6.8, $p = .02$) but no significant difference in left frontal tuber count (median 7.0 IQR 4.0-9.0 vs median 6.0 IQR 3.3-7.0, $p = .08$). There were no differences when comparing tuber counts of parietal, temporal or occipital lobes between groups. There was also no difference in SEN count, presence of SEGA or cerebellar tubers between groups (Table 3.12).

Table 3.12 MRI features of patients by adaptive function level (n=29)

	Average-range adaptive function (n=12)	**Impaired adaptive function (n=17)**	**Significance testing**
Median cortical tuber count (IQR)	22.0 (14.5-31.8)	33.0 (24.0-36.5)	$p = .026$
Frontal tuber count	11.0 (7.0-13.8)	15.0 (11.0-18.0)	$p = .023$
Parietal tuber count	4.0 (2.3-5.0)	4.0 (2.0-5.5)	$p = .290$
Temporal tuber count	4.5 (3.0-6.0)	7.0 (3.5-8.5)	$p = .096$
Occipital tuber count	4.0 (1.0-6.3)	5.0 (3.5-6.5)	$p = .077$
Median SEN count (IQR)	8.5 (7.3-10.8)	9.0 (7.5-11.5)	$p = .311$
Presence of SEGA	4 (33%)	3 (18%)	$p = .402$
Presence of cerebellar tuber	3 (25%)	9 (53%)	$p = .251$
RML>50%	3 (25%)	9 (53%)	$p = .251$

IQR: Interquartile Range, RML>50%: greater than 50% of white matter affected with RMLs, SEGA: subependymal giant cell astrocytoma, SEN: subependymal nodule

A moderate negative correlation was found between cortical tuber count and Vineland-III ABC score (Figure 3.8).

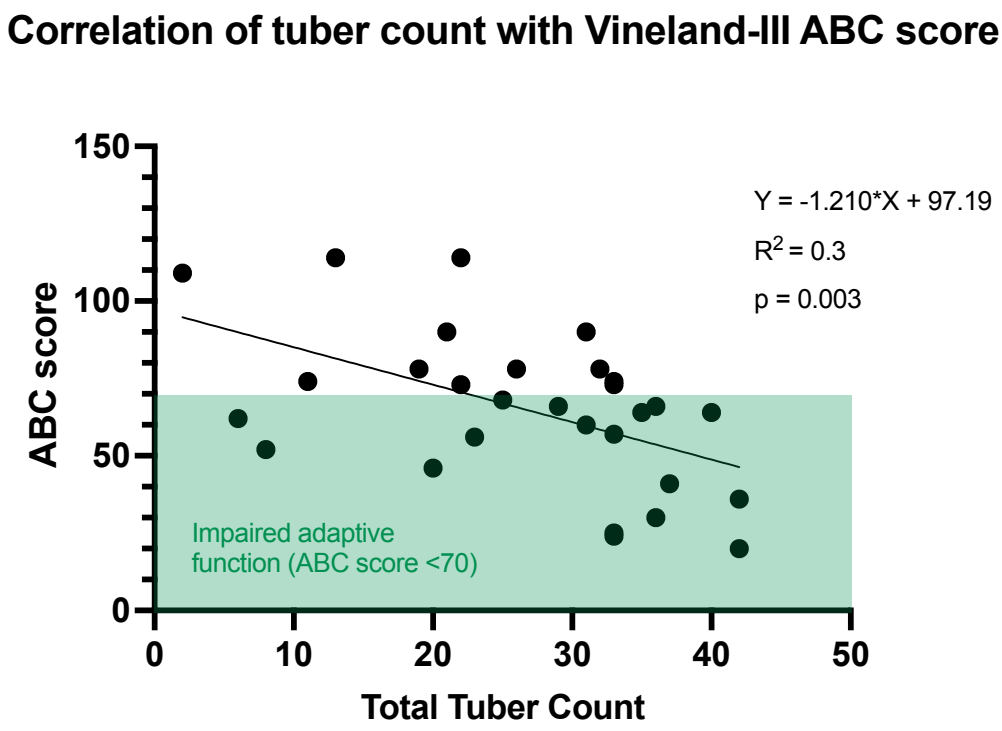


Figure 3.8 Linear regression of total tuber count and Vineland-III Adaptive Behavior Composite score

Post-hoc ROC curve analysis was used to explore the predictive value of tuber count for adaptive function. The curve demonstrated AUC of 0.753 (Figure 3.9) and the tuber cut off values, p-values, sensitivity, specificity, 95% CI and likelihood ratios are listed in Table 3.13. We found a good balance at a tuber count cut-off of greater than 22.5, which yielded a sensitivity of 82% and specificity of 58% for impaired adaptive function.

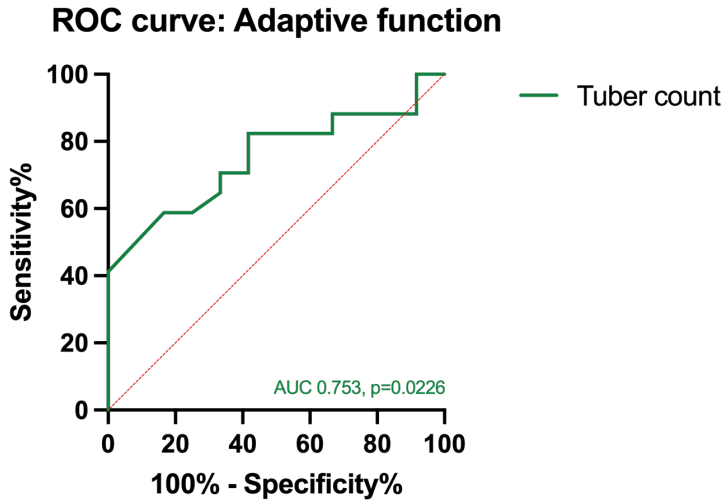


Figure 3.9 ROC curve of Adaptive function using Tuber count

Table 3.13 Data for ROC curve of Tuber Count for adaptive function

Tuber Count	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 4.000	100.0	81.57% to 100.0%	8.333	0.4274% to 35.39%	1.091
> 7.000	94.12	73.02% to 99.70%	8.333	0.4274% to 35.39%	1.027
> 9.500	88.24	65.66% to 97.91%	8.333	0.4274% to 35.39%	0.9626
> 12.00	88.24	65.66% to 97.91%	16.67	2.961% to 44.80%	1.059
> 16.00	88.24	65.66% to 97.91%	25.00	8.894% to 53.23%	1.176
> 19.50	88.24	65.66% to 97.91%	33.33	13.81% to 60.94%	1.324
> 20.50	82.35	58.97% to 93.81%	33.33	13.81% to 60.94%	1.235
> 21.50	82.35	58.97% to 93.81%	41.67	19.33% to 68.05%	1.412
> 22.50	82.35	58.97% to 93.81%	58.33	31.95% to 80.67%	1.976
> 24.00	76.47	52.74% to 90.44%	58.33	31.95% to 80.67%	1.835
> 25.50	70.59	46.87% to 86.72%	58.33	31.95% to 80.67%	1.694
> 27.50	70.59	46.87% to 86.72%	66.67	39.06% to 86.19%	2.118
> 30.00	64.71	41.30% to 82.69%	66.67	39.06% to 86.19%	1.941
> 31.50	58.82	36.01% to 78.39%	75.00	46.77% to 91.11%	2.353
> 32.50	58.82	36.01% to 78.39%	83.33	55.20% to 97.04%	3.529
> 34.00	41.18	21.61% to 63.99%	100.0	75.75% to 100.0%	
> 35.50	35.29	17.31% to 58.70%	100.0	75.75% to 100.0%	
> 36.50	23.53	9.555% to 47.26%	100.0	75.75% to 100.0%	
> 38.50	17.65	6.191% to 41.03%	100.0	75.75% to 100.0%	
> 41.00	11.76	2.090% to 34.34%	100.0	75.75% to 100.0%	

Developmental outcome 2: Autism Spectrum Disorder (ASD)

Children with a diagnosis of ASD had more total tubers and frontal tubers compared to children without ASD (Table 3.14). There was no difference found the between tuber counts of parietal, temporal or occipital lobes between groups. There was also no difference in SEN count, presence of SEGA or presence of cerebellar tubers between groups (Table 3.14).

Table 3.14 MRI features of patients by ASD diagnosis (n=36)

	No ASD (n=20)	**ASD diagnosis (n=16)**	**Significance testing**
Median cortical tuber count (IQR)	25.5 (9.3-33.0)	32.0 (20.5-39.3)	$p = .049$
Frontal tuber count	11.0 (3.8-15)	14.5 (11.0-18.8)	$p = .013$
Parietal tuber count	3.5 (1.3-5.0)	4.0 (2.3-5.8)	$p = .130$
Temporal tuber count	6.0 (3.0-8.0)	6.5 (3.0-7.8)	$p = .351$
Occipital tuber count	4.0 (1.0-6.8)	4.0 (2.5-6.8)	$p = .194$
Median SEN count (IQR)	8.0 (7.0-11.8)	9.0 (8.3-10.8)	$p = .321$
Presence of SEGA	4 (20%)	6 (38%)	$p = .285$
Presence of Cerebellar tuber	8 (40%)	7 (44%)	$p > .999$
RML>50%	8 (40%)	9 (56%)	$p = .503$

IQR: Interquartile Range, RML>50%: greater than 50% of white matter affected with RMLs, SEGA: subependymal giant cell astrocytoma, SEN: subependymal nodule

Post-hoc ROC curve analysis was used to explore the predictive value of tuber count for ASD diagnosis. The curve demonstrated AUC of 0.655 (Figure 3.10) and the tuber cut off values, p-values, sensitivity, specificity, 95% CI and likelihood ratios are listed in Table 3.15. A tuber count cut-off >27.5 yielded a sensitivity of 62.5% and specificity of 55% for ASD outcome.

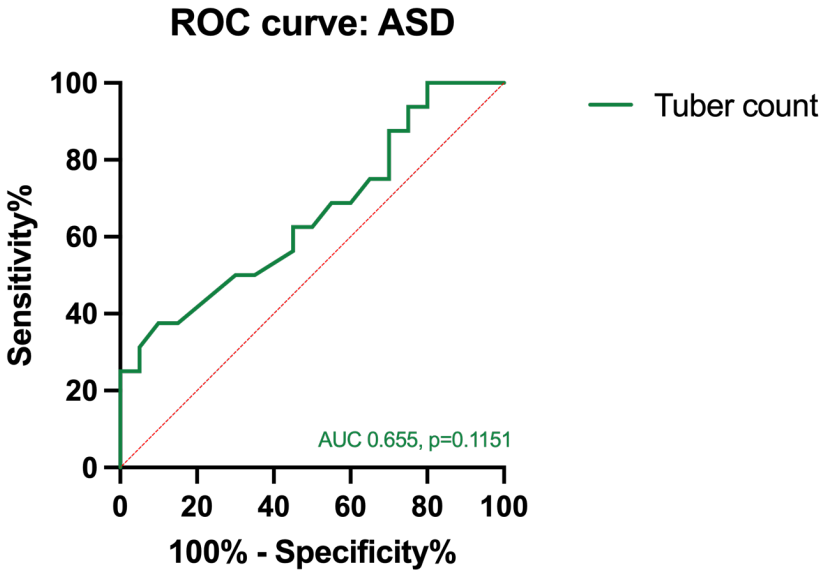


Figure 3.10. ROC curve of ASD outcome using Tuber count

Table 3.15 Data for ROC curve of Tuber Count for ASD

Tuber count	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
> 2.500	100.0	80.64% to 100.0%	10.00	1.777% to 30.10%	1.111
> 4.500	100.0	80.64% to 100.0%	15.00	5.237% to 36.04%	1.176
> 6.500	100.0	80.64% to 100.0%	20.00	8.066% to 41.60%	1.250
> 7.500	93.75	71.67% to 99.68%	20.00	8.066% to 41.60%	1.172
> 9.500	93.75	71.67% to 99.68%	25.00	11.19% to 46.87%	1.250
> 12.00	87.50	63.98% to 97.78%	25.00	11.19% to 46.87%	1.167
> 16.00	87.50	63.98% to 97.78%	30.00	14.55% to 51.90%	1.250
> 19.50	81.25	56.99% to 93.41%	30.00	14.55% to 51.90%	1.161
> 20.50	75.00	50.50% to 89.82%	30.00	14.55% to 51.90%	1.071
> 21.50	75.00	50.50% to 89.82%	35.00	18.12% to 56.71%	1.154
> 22.50	68.75	44.40% to 85.84%	40.00	21.88% to 61.34%	1.146
> 24.00	68.75	44.40% to 85.84%	45.00	25.82% to 65.79%	1.250
> 25.50	62.50	38.64% to 81.52%	50.00	29.93% to 70.07%	1.250
> 27.50	62.50	38.64% to 81.52%	55.00	34.21% to 74.18%	1.389
> 30.00	56.25	33.18% to 76.90%	55.00	34.21% to 74.18%	1.250
> 31.50	50.00	28.00% to 72.00%	65.00	43.29% to 81.88%	1.429
> 32.50	50.00	28.00% to 72.00%	70.00	48.10% to 85.45%	1.667
> 34.00	37.50	18.48% to 61.36%	85.00	63.96% to 94.76%	2.500
> 35.50	37.50	18.48% to 61.36%	90.00	69.90% to 98.22%	3.750
> 36.50	31.25	14.16% to 55.60%	95.00	76.39% to 99.74%	6.250
> 38.00	25.00	10.18% to 49.50%	95.00	76.39% to 99.74%	5.000
> 39.50	25.00	10.18% to 49.50%	100.0	83.89% to 100.0%	
> 41.00	18.75	6.592% to 43.01%	100.0	83.89% to 100.0%	
> 44.00	6.250	0.3206% to 28.33%	100.0	83.89% to 100.0%	

3.5.6 Multivariable analysis using logistic regression

Children who had drug resistant epilepsy (DRE) at 5 years had higher total tuber and SEN count on MRI (Table 3.8), as well as having earlier age of seizure onset (median 4.0 months IQR 2.8-8.0 vs median 9.5 months IQR 6.8-9.5). We performed a logistic regression using DRE as the outcome and covariates of total tuber count and seizure onset age. In this binomial regression model, total tuber count was significantly associated with DRE (B = 0.123, S.E. = 0.057, p = .02, OR = 1.142, CI 1.021 - 1.276), while seizure onset age was not significant (B = -0.258, S.E. = 0.135, p = .056, CI 0.593 - 1.006). We did not include tuber count in individual lobes, SEN count or RML into the model design due to significant collinearity between covariables.

Children with impaired adaptive functioning as assessed by Vineland-III were more likely to experience 1 or more seizure per month (76.5% vs 16.7%, OR = 16.25, CI 2.6 - 86.7, p = .0025) as well as having higher mean total and frontal tuber counts (Table 3.12). We performed regression analysis using impaired adaptive function as an outcome and the variables of cortical tuber count and poor seizure outcome. We did not include frontal tuber count in the model due to its strong relationship with total tuber count/significant collinearity. Having more than monthly seizures was the strongest predictor of impaired adaptive function, inferring 22-fold increased odds (B = 3.110, S.E. = 1.151, p = .007, OR = 22.415, CI 2.350 - 213.771). Total tuber count was not a predictor of impaired adaptive function in the presence of poor seizure outcome (B = 0.101, S.E. = 0.058, p = .084).

Children diagnosed with ASD were likely to experience 1 or more seizure a month (87.5% vs 30%, OR 16.33, CI 2.7-81.7, p = .001) as well as having more total tubers and frontal tubers (Table 3.14). Regression analysis of ASD was performed, where covariates were total tuber count and poor seizure outcome. Again, we did not include frontal tuber count in the model due to its strong relationship with total tuber count. Having more than monthly seizures was significantly associated with ASD, inferring 19-fold increased odds (B = 2.965, S.E. = 0.967, p = .002, OR = 19.389, CI 2.915 -

128.987). Total tuber count did not have a significant relationship with ASD in presence of poor seizure outcome in the modelling (B = 0.64, S.E. = 0.039, p = .104).

3.6 Discussion

3.6.1 Study Overview

In this study, we describe the incidence of neuroimaging features of TSC in a single-centre cohort and explore the relationships between TSC neuroimaging biomarkers and epilepsy and developmental outcomes at 5 years of age. Our current study supports the hypothesis that neuroradiological findings of TSC on early MRI scans may be useful in predicting drug resistant epilepsy, adaptive function and Autism Spectrum Disorder at 5 years of age. The major finding was that the number of cortical tubers present on early MRI was associated with increased risk of having drug resistant epilepsy, impaired adaptive functioning and ASD at 5 years of age (Figure 3.11). These associations were also present between number of frontal tubers and each of these three functional outcomes. Through multivariable logistic regression, we showed total tuber count to be significantly associated with drug resistant epilepsy even in the presence of early seizure onset age, however the odds ratio was modest (1.14). However, having one or more seizures per month significantly increased the likelihood of impaired adaptive functioning and ASD at 5 years of age by 22-fold and 19-fold respectively, rather than any neuroimaging finding. This suggests that the relationship between structural changes and outcomes is complex with important interplay between epilepsy and cognitive and behavioural outcomes (Figure 3.11). Despite this complexity, tuber count is a useful biomarker for predicting outcomes.

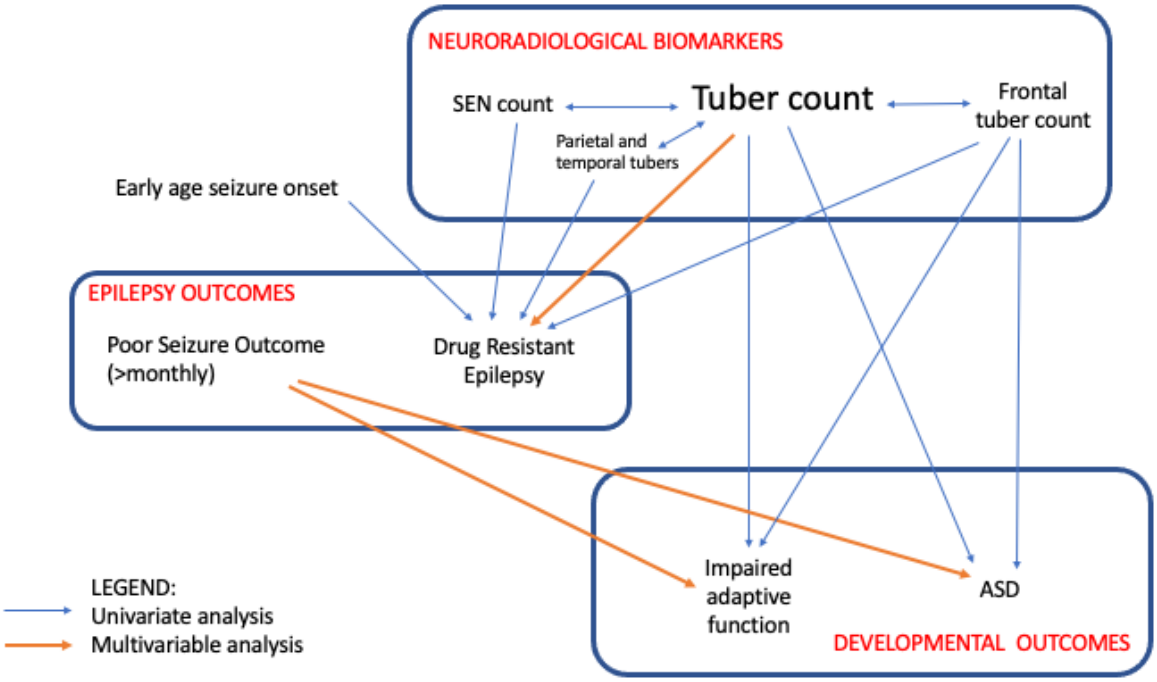


Figure 3.11. Diagram summarising the significant relationships in this study between TSC neuroradiological biomarkers, epilepsy outcomes and developmental outcomes

In our current study, patients who had drug resistant epilepsy had significantly higher tuber count compared to patients without DRE (median 31.5 vs 9.5, p=0.0007). Using our ROC analysis to address Goodman's meta-analysis, our patients who had more than 7.5 tubers were 1.9 times more likely to have DRE, but this had low specificity (LR 1.867, sensitivity 93%, specificity 50%). Using a cut off >26 tubers improved the likelihood ratio and specificity of testing with a trade off in sensitivity (LR 3.8, sensitivity 63%, specificity 83%). A combined SEN+Tuber score improved the AUC compared to using SEN or Tuber count alone. Using a cut-off >31 for SEN+Tuber count more than quadrupled the likelihood of developing DRE (LR 4.4 sensitivity 73%, specificity 83%). The correlation between tuber count and number of AEDs trialled was weak and DRE was present in 2 out of the 5 children who had less than 8 tubers.

We explored whether tuber location was a stronger correlate of poor seizure control and while each of frontal, parietal and temporal tuber count was associated with DRE, none had a stronger correlation with number of AEDs trialled. It should be noted that the prevalence of DRE by 5 years of age was very high within our cohort (83%) and likely

affected our analysis. A smaller proportion of children had unfavourable epilepsy outcome as defined by the presence of one or more seizures per month (55%) and we did not find a statistically significant difference in tuber count or other neuroradiological features between these children and those that had less frequent seizures. This may be due to the limitations of seizure outcome variable only reflecting the patient's current epilepsy control at the time of data collection, rather than whether their epilepsy was difficult to control through the years.

The number of SENs was also higher in children who had drug resistant epilepsy, and SEN count was weakly correlated with tuber count. We propose that the presence of SEN is a marker for the degree to which normal brain development was interrupted by TSC, rather than directly contributing to epileptogenesis. Similarly, children with DRE had more RML involvement and RML was also correlated with tuber count. On a multivariable analysis including tuber count and seizure onset age as covariates, total tuber count came out significantly correlated with DRE while seizure onset age was rendered insignificant. While tuber count was significantly correlated, the odds ratio of 1.14 reflects only a modest effect on DRE. The addition of either SEN or RML to the model rendered results of the statistical model insignificant due to significant collinearity between the neuroradiological variables. Taken together, these results suggest that while tuber count, SEN count and RML on early MRI may be associated with DRE, the association is not strong and the overall prevalence of DRE is high. Perhaps the most practical use would be to identify patients who are likely to have a more favourable outcome. The two patients with the lowest number of tubers observed in this study (2 tubers only) did not have drug resistant epilepsy at follow up ages of 9.7 and 14.5 years. Future studies of larger cohorts could explore whether there is a lower threshold for tuber numbers, below which monitoring may be able to be altered and families could be reassured of a favourable neurocognitive prognosis for their TSC affected child.

As well as epilepsy outcomes, our study also investigated the correlation of neuroradiological markers to adaptive behaviour and autism spectrum disorder. We chose the 5-year time point as it is the age of school entry and determines educational supports in Australia. We found TSC patients with impaired adaptive function had on

average more tubers than patients with average-range adaptive function. Goodman's meta-analysis similarly showed that patients with >7 tubers were 5 times more likely to have moderate-severe developmental delay (weighted OR 5.3; CI 2.3-11.9). While we found a tuber count of 7 had high sensitivity for impaired adaptive function, as with DRE, we found a very low specificity in our cohort (8.3%). Our findings are concordant with previous reports of negative correlations between tuber count and intellectual ability, but other studies used various psychometric scales to assess this, including Wechsler intelligence scales [60,68], Raven's matrices [68] or Mullen's scale of Infant Development [67].

TSC-associated intellectual disability has been associated with tuber counts in the frontal [60,83], temporal [83] and occipital lobes [68] in separate studies. Similar to our findings, Kassiri also reported a negative correlation between IQ and right frontal tuber counts (right frontal, $P < 0.001$, $R^2 = -0.30$) [60]. They described a cohort of 24 patients of whom 19 (79%) scored below 2 standard deviations of the mean on either Wechsler scales or Vineland Adaptive Behaviour Scales. The mean tuber count of their cohort was 10.9. In contrast, our study had a slightly larger cohort (n=36), used neuroimaging at a standardised time point (18-36 months) and assessed patients using one behavioural scale (Vineland-III) rather than a mixed methodology. We observed a higher mean tuber count (26) and a lower rate of impaired adaptive function (59%). Our MRI data are likely of higher resolution as some of the imaging data from Kassiri et al. were over 2 decades old, allowing small individual tubers to be accurately delineated. It is not surprising that right frontal tubers are associated with poorer developmental outcomes, as the dysplastic tuberous tissue could disrupt critical right frontal lobe circuitry associated with higher executive function. This is supported through resting state functional MRI studies from 115 children showing that right frontoparietal connectivity is associated with higher non-verbal intelligence. [134] However, the correlation between tuber count and Vineland-III ABC score was only moderate on linear regression and one of the 2 children with less than 8 tubers had impaired adaptive function while 5 out of 16 (31%) of children with more than 25 tubers had adaptive function scores within the normal range. Our ROC curve showed that patients with >28 tubers were 2.1 times more likely to develop impaired adaptive function by 5 years, demonstrating a sensitivity of 71% and specificity of 67%.

Logistic regression of adaptive function outcome found that tuber count was not significant in the presence of poor seizure outcome (having one or more seizures per month). Having greater than monthly seizures increased the odds of impaired adaptive function by 22-fold. Previous studies have also shown infantile spasms and age of seizure onset are both risk factor for intellectual disability [68,83,135], but our data did not show any association which is likely an effect of small numbers. Jansen's study of 61 TSC patients also identified an association between tuber count and intellectual outcome, but on multivariable analysis the only independent determinant of cognition index was age of seizure onset (infantile spasms, *TSC2* genotype, and tuber brain proportion was also included) [69]. Similar to our study results, Bolton's 2015 prospective study of 125 paediatric TSC patients found epilepsy severity score was the strongest predictor of intellectual impairment, even in the presence of other epilepsy associated variables such as age of seizure onset, history of infantile spasms and status epilepticus. While there was no direct association between tuber burden and intellectual disability in Bolton's study, latent variable and structural equation modelling supported a causal link between tuber load and epilepsy severity, and in turn epilepsy severity and estimated intellectual ability (p=<0.001) [67].

Autism is highly prevalent and affects up to 60% of children with TSC. The 16 children in our study who had ASD (44%) had significantly more total tubers (29.6 ± 11.4 vs 22.7 ± 12.6, p=0.049) and frontal tubers than children without ASD (14.8 ± 6.5 vs. 10.2 ± 5.6, p=0.012). Samir's study of 30 patients did not use mean tuber count per lobe, but instead took a more global approach by stratifying patients according to which lobe had the most tubers. Twelve patients in their study had ASD (40%), of whom 75% had tubers predominantly in the frontal lobe, compared to 21.4% of patients without ASD (p=0.04). A larger study using 52 patients investigated the associations between tuber count, IQ and autism spectrum disorder using Autism Diagnostic Observation Scale (ADOS) total score and subdomains (social affect, restricted and repetitive behaviours). [112] This study reported a mean tuber count (27.5 ± 20.2, range 0-81), IQ/DQ <70 in 65.4% and ASD in 48.1% of patients, which were similar to our study findings. Tuber counts in each separate lobe were initially all associated with ADOS total severity score in their study, but after correcting for cognitive function, a significant association

between frontal tuber count and restrictive and repetitive behaviours emerged. We did not have ADOS data to replicate these subtests in our study. However, we acknowledge that this effect may be swayed by the fact that the majority of the tubers are frontally located in their study (58%), as well as our study (47%). The frontal dominance of tubers been previously reported [60,66,68] and is not surprising as the frontal lobes encompasses the largest volume of the cerebral cortex.

In our study, multivariable analysis showed poor seizure outcome (having one or more seizures per month) was a significant contributor to ASD with an odds ratio of 17. The relationship between total tuber count and ASD was no longer significant in the presence of poor seizure outcome. It is well established that epilepsy is a significant risk factor for ASD in the TSC cohort. A recently published meta-analysis investigating factors associated with ASD in TSC patients pooled together 42 studies with a total of 3542 children [89] and found positive associations between ASD and epilepsy, infantile spasms, and seizure onset in infancy. However, no associations between ASD and total tuber count could be concluded due to limited number of studies and mixed methodology between studies. Meta-analysis was only possible for temporal tuber location, where no association was found, but not frontal tuber location. We suggest larger cohort studies into frontal tuber location and standardised methodology in future studies to address this.

We did not find any associations between the presence of SEGA, cerebellar tubers, RML involvement and neurobehavioural outcomes. This contrasts with previous studies, which have suggested that cerebellar tuber count and RML count are associated with increased risks of intellectual disability and ASD. Weber et al's study of 29 TSC patients reported that patients with higher cerebellar tuber count had lower Vineland ABC scores and higher Childhood Autism Rating Scale scores [86], although a larger study of 112 patients reported that cerebellar tubers were not the best predictor for ASD after adjusting for cortical tuber count and *TSC2* genotype [97]. RMLs have been associated with intelligence quotient and rate of autistic features [109], but Mous' study found RML count was not significantly associated with ASD after accounting for total tuber count and cognitive ability [112].

Taking a broader approach, the results of this study confirm the complex interactions between the MRI, epilepsy and developmental variables in children with TSC. Figure 3.11 illustrates graphically the multiple interactions between these variables in this study but is not exhaustive, we have not included factors such as RML in the figure for clarity. The main finding from this study is that increased tuber count is associated with DRE, and in turn, poor seizure outcome is a strong risk factor for both developmental delay and ASD. However, there are many additional interrelationships in Figure 3.11 which remain unclear, in particular the relative contributions, if any, of SEN and RML to developmental outcome. The results of this study ultimately reflect the complex nature and incompletely understood pathogenesis in TSC at present.

3.6.2 Strengths

The large size of our multidisciplinary tertiary TSC clinic allowed accrual of reasonable numbers of patients with a rare disease for this study. In analysing MRI performed between the small window of 18-36 months, we were able to avoid the signal changes brought upon by the normal myelination process of infancy, but the scans were still early enough to be used for prognostication for later school-aged outcomes. The use of data from Vineland-III assessments, an internationally accepted validated assessment tool, for 80% of our cohort strengthened the developmental outcome variable for this study. However, as the Vineland-III ABC score was based solely on parental report, there can be a tendency to overestimate patient ability, with resultant underdiagnosis of lower adaptive level in the study cohort. Finally, we developed proforma to collect TSC neuroradiological markers which standardised data collection and can be used for subsequent TSC neuroradiological studies.

As seizure frequency fluctuates over time in response to normal waxing-waning patterns and changes in pharmacotherapies, and are also dependent on seizure type, we propose that the cumulative number of AEDs trialled may be more helpful in reflecting the degree of epilepsy severity in each patient. Of course, this assumes that the choices of AED by practitioners are appropriate to seizure type. Given our patients were seen through a single clinic, the pattern of AED prescribing is likely to be consistent between

patients. To the author's knowledge, this is the first study to use number of AEDs trialled as a surrogate marker for seizure control in the context of imaging biomarkers.

3.6.3 Limitations

We acknowledge some limitations to the current study. Our retrospective study design may lead to data inaccuracies due to gaps in documentation and bias, although efforts were made to reduce bias such as through anonymisation of imaging data. Whilst retrospective imaging data in our study did not allow for a standardised imaging protocol, two thirds of MRIs were performed within the two hospitals within the Sydney Children's Hospital network. Other hospitals would have likely used different protocols.

We found that SEN could be missed on patients with low disease burden with thick MRI slices, and suggest routine T2 or FLAIR 3D millimetric acquisition would increase sensitivity of imaging to detect SEN. Slice thickness of 5mm may decrease spatial resolution and sensitivity to detect subtle radiological changes. Higher MRI field strength can also demonstrate additional tubers and white matter abnormalities which are not evident on lower field scans [72], although 7T MRI is not readily available and limited to the research arena currently. We acknowledge that through selecting early MRIs of TSC patients between 18-36 months we may have inadvertently introduced selection bias, skewing our cohort towards a more severe phenotype. A single blinded rater assessed all the scans limiting assessment of reliability. A second rater was planned when designing the study but was unable to continue due to illness. A trained second rater was not available during the study period due to the impact of the COVID-19 pandemic. Manual counting of tubers and estimation of RML burden is laborious and potentially introduces inaccuracies, although there is no universally accepted method of lesion quantification currently in TSC clinical practice and it is inexpensive and easily accessible at multiple sites compared to machine learning.

Our patient cohort has a more severe phenotype than that of published literature, as 100% of our patients had epilepsy, 83% had drug resistant epilepsy and median seizure onset age at 4 months. Use of validated tools in the diagnosis of ASD such as the

Autism Diagnosis Observation Schedule (ADOS) or the Childhood Autism Rating Scale (CARS) would have allowed for quantification of autism symptoms and subtest analysis.

Moreover, while it would have been pertinent to consider whether *TSC1* or *TSC2* genotype contributed epilepsy and developmental outcomes, it was not attempted due to the paucity of *TSC1* patients in this cohort. While we did not collect detailed genotype data, we recognise that some *TSC2* mutations, such as those affecting codon 905 have been linked with a mild phenotype [136].

We acknowledge that multiple statistical comparisons were made in this study, which may increase the chance of false positive results. Dichotomised outcome variables may ignore subtle variations in continuous variables such as the Vineland ABC score, and may potentially fail to detect small but significant differences between datasets as a result. Regression analysis using multiple variables was limited due to small sample size and significant collinearity between dependent variables, particularly the neuroradiological markers. Therefore, the possible biomarkers suggested in this study should be validated by a larger prospective study.

3.6.4 Clinical implications

We have shown that patients with higher tuber counts have modestly increased odds of drug resistant epilepsy, and patients with 1 or more seizure a month have strongly increased odds of developing impaired adaptive function and ASD at 5 years. From ROC curve analysis, a combined tuber+SEN score of >31 has a sensitivity of 73.3% and specificity 66.7% for DRE. Whilst our results are preliminary, exploratory and cannot yet be directly translated into clinical practice, we suggest that patients with a combined tuber+SEN score of >31 may benefit from more frequent clinical review, EEG monitoring and aggressive treatment of epilepsy. Early detection may allow for early intervention to optimise outcome in high risk TSC patients. Epilepsy should be aggressively treated in TSC patients, as our results suggest that increased seizure burden is associated with impaired adaptive function and ASD diagnosis.

3.6.5 Future directions

In view of this study's findings, further efforts in exploring neuroradiological biomarkers in predicting outcome in TSC patients would benefit from a prospective study design. Neuroimaging should be performed using a standardised MRI protocol at 18 months and analysed by two blinded reviewers using the proforma, with contemporaneous collection of follow up outcome data at predetermined time points. Other radiological variables such as tuber volume, tuber-brain proportion, tuber type, cyst-like tubers may be fruitful avenues for further investigation. Harnessing machine learning to distinguish RML vs NAWM using diffusion-weighted MRI tractography is a future avenue for more precise quantification of RML burden. Addition of EEG as a neurophysiological biomarker may allow for a more comprehensive understanding of the varying contributions of early epilepsy and neuroimaging towards poor developmental outcome. Further consideration and exploration of the various contributions of TSC genotype will be important in future imaging studies. International registries such as TOSCA, or establishment of a national TSC registry, would allow for larger numbers to be recruited. Future studies may benefit from radiomics and machine learning to further define the association between neuroradiological features, epilepsy, and developmental outcome of children with TSC.

3.7 Conclusion

In summary, this study shows that neuroradiological findings on early MRI may be useful as predictors of drug resistant epilepsy at 5 years. Using a combined tuber+SEN score has improved AUC for DRE compared to tuber count or SEN count alone, improving sensitivity and specificity. Adaptive function and ASD at 5 years of age were both associated with increased total and frontal tuber counts, and total tuber count showed moderate negative correlation with Vineland-III ABC score on linear regression. However, these relationships became insignificant in the presence of epilepsy outcome on multivariable analysis. Developmental outcomes were better predicted by poor seizure outcome group (having one or more seizures per month) than neuroradiological features, which conferred an increased odds of 22-fold and 19-fold of

developing impaired adaptive function and ASD respectively. Further studies and prospective trials investigating early MRI imaging as prognostic biomarkers in TSC are warranted.

CHAPTER 4. Discussion and conclusion

This master's thesis arose out of a clinical need to find neuroradiological biomarkers for patients affected with tuberous sclerosis complex (TSC) and their families. TSC is a common neurogenetic condition affecting 1:6000 newborns and imparts a significant risk of chronic neurological complications such as drug resistant epilepsy, developmental delay, and autism spectrum disorder. The developmental trajectory can be drastically altered in those affected. On the other hand, a minority of children with TSC have normal-range intellectual abilities. Currently, there are no effective radiological biomarkers to distinguish which children might benefit from closer clinical monitoring, pre-symptomatic antiepileptic drug trials or early intervention programs. Prognostication and risk stratification are important for patients with TSC as it helps both clinicians and parents plan for the future, and potentially optimise outcomes.

The overall aims of my thesis were to:

1. Describe the incidence of congenital SEGA
2. Investigate whether congenital SEGA can be used as a biomarker for poor epilepsy and developmental outcomes
3. Describe the incidence of other neuroimaging markers, including cortical tubers, cerebellar tubers, SEGA, and subependymal nodules
4. Investigate whether TSC neuroradiological changes on early MRI can be used as biomarkers for epilepsy and developmental outcomes at 5 years

4.1 Overview of thesis findings

This thesis began in Chapter 1 with a literature review summarising knowledge regarding SEGA and other TSC neuroimaging biomarkers. I reviewed the current understanding regarding the natural history of SEGA (published in *Reviews of the Neurosciences*) and identified a number of limitations including a lack of standardised definition of SEGA, a need for larger natural history studies and a lack of volumetric

data. Moreover, there was a paucity of literature around congenital SEGA with relatively few published cases. Interestingly, the growth rates of congenital SEGA were reported to be significantly higher than later onset SEGA.

In Chapter 2, I designed a retrospective case series to describe congenital SEGA growth rate using our TSC cohort at Sydney Children's Hospital to address this literature gap. This case series of 10 patients represents a significant contribution to literature, as to my knowledge there were only 28 published reports of congenital SEGA at the time of the study (published in *Pediatric Research).* Our 10 patients with congenital SEGA demonstrated a median growth rate of 1.1 mm/yr in maximal diameter or 150 mm^3/yr volumetrically. This is lower than the previous congenital growth rate reported [42], and more in line with growth rate of non-congenital SEGA. Whilst the cohort was relatively small, this study further informs parents of newborns with newly diagnosed TSC and concomitant congenital SEGA. I found that congenital SEGA with volumes >500mm^3 at baseline scan had significantly higher growth rates compared to smaller SEGA. Clinical vigilance is always required in patient care, however, patients with SEGA <500 mm^3 may potentially be able to have MRI monitoring less frequently than the high-risk group. This would relieve the pressure on limited medical imaging resources in tertiary hospitals and decrease cumulative general anaesthesia risk in patients already at high risk of developmental difficulties. It is important to acknowledge that all patients within this series had *TSC2* mutation, potentially limiting applicability to all children with TSC. The patients with congenital SEGA had a numerically higher prevalence of severe epilepsy, developmental delay and autism spectrum disorder compared to genotyped-matched controls but this was not statistically significant, possibly due to small numbers. The question remained as to whether MRI neuroimaging features could be used as effective biomarkers to prognosticate future outcomes in children with TSC.

To address this, in Chapter 3 I investigate a cohort of 39 patients who had MRI at a relatively early age, in an attempt to identify imaging biomarkers which would predict for adverse neurological and developmental outcomes at 5 years of age. This is the first study to use early MRI of patients scanned between 18-36 months to investigate TSC neuroradiological markers. The main findings were that patients with drug resistant epilepsy had more cortical tubers, SEN and RML. Patients with higher total and frontal

tuber counts were also at increased risk of low adaptive function and Autism Spectrum Disorder by 5 years. Cortical tuber count was mildly correlated with number of antiepileptic drug (AED) trials by 5 years (a surrogate marker for epilepsy severity) and also inversely correlated with Vineland-III ABC score. Multivariable regression analysis showed that cortical tuber count was associated with DRE even in the presence of early age of seizure onset. However, the relationships between cortical tuber count and the developmental outcomes (impaired adaptive function and ASD) were made redundant when epilepsy severity was taken into account. These study findings suggest that despite previous inconclusive studies in the field, MRI biomarkers on imaging performed between 18-36 months should not be ignored in the management of children with TSC. Whilst our results are preliminary and hypothesis generating, these candidate biomarkers should be further explored and clarified in larger multicentre prospective trials.

4.2 Limitations

Overall, the studies in this thesis were done to investigate neuroimaging features of TSC using MRI. A weakness of both studies was the retrospective study design, leading to non-homogenous imaging protocols, appreciating that imaging protocols differ between institutions and change over time with research and development. The statistical power was limited by small numbers, but overall, the numbers were reasonable considering TSC is a rare disease, and congenital SEGA is a rare complication of a rare disease. In our second study, we lost 3 patients to follow up and had 5-year outcome data for 36 patients out of the original 39. The majority of families had completed the Vineland-III questionnaire as part of their routine TSC clinic care (80%), reflecting a good responder rate from families who are actively engaged in their children's healthcare.

4.3 Future Directions

In this thesis I have shown that MRI markers in TSC are independent factors which can help predict clinical outcome. The markers used in the study in this thesis are macroscopic in nature, allowing for practical use in a clinical setting. Going forward, more studies are needed to confirm the effectiveness of these radiological biomarkers in

predicting poor seizure and developmental outcome in patients with TSC. It is important for any future studies looking at epilepsy and developmental outcome to consider comparison of the prognostic value of MRI alone verses EEG alone verses the combination of EEG and MRI, to determine whether MRI gives any incremental prognostic power compared to EEG alone. Multicentre trials are needed to harness a larger patient population in this rare disease. A larger patient recruitment may also allow for subgroup analysis of MRI within the 18-36 months category to determine the optimal timepoint for neuroimaging. Use of MRI prior to 18 months can be considered in the future if novel imaging techniques allow tubers to be reliably visualised despite incomplete myelination. We chose to focus on macroscopic MRI changes such as tuber count and tuber location because they can be practically applied in the clinic setting and found them to be of value. Future studies can concurrently explore these biomarkers along with others such as tuber type, radial migration lines, cortical cysts and volumetric tuber analysis. Furthermore, microscopic MRI changes such as diffusion tensor imaging (DTI), magnetic resonance spectroscopy and other measures can be used to interrogate white matter integrity and other microstructural disruptions caused by TSC. As no involvement with neuroradiology colleagues was undertaken in this thesis work, future formal collaborations would allow more detailed analysis and assessment of inter-rater reliability.

Collaboration is vital in doing research in a rare disease cohort. Registry trials, although not interventional in nature, enable the sharing of important patient data for hypothesis generating and observational research. The establishment of such registries will further forge collaborations between TSC centres, which will in turn aid trial conduct, planning and patient accrual. In today's environment where funding is scarce, there exists mechanism to reduce costs where possible such as open-source data such as REDCap and telemedicine reviews. EPISTOP included select Australian patients from Queensland along with a primarily European cohort, attesting to the success of multinational collaboration in a rare disease. Setting up an Australian TSC research collaboration would be the next step in order to allow pooling of imaging data to investigate further research directions outlined in this thesis.

Advances in radiological equipment and techniques could be potentially fruitful in the discovery of new biomarkers, such as refinements in acquisition techniques, stronger head coils, magnet strength, post-processing with MRI physicists and use of functional imaging such as fMRI. Given identification of individual tubers is painstaking and requires specialist expertise, future research may benefit from application of machine learning techniques. Big data radiomics is an evolving field which shows promise, but we acknowledge there will likely be practical challenges in training machines to identify tubers, accurately delineate lesion margins and minimise false positives.

Epilepsy management has changed in the last decade with a move towards preventative treatment to optimise epilepsy and developmental outcomes for young children with TSC. The EPISTOP trial screened TSC patients prior to seizure onset using EEG and randomised patients to preventative treatment vs conventional treatment. Preventative treatment comprised of starting vigabatrin when epileptiform activity was detected on EEG (prior to clinical or electrical seizure onset). Infants in the preventative treatment arm had significantly longer time to seizure onset (364 days vs 124 days) and on 24-month follow had reduced risk of clinical seizures (OR = 0.21), drug resistant epilepsy (OR = 0.23) and infantile spasms (OR = 0) [8]. Further early preventative trials are needed to confirm the positive effects of using prophylactic vigabatrin, as well as using precision therapy mTOR inhibitors, in pre-seizure children diagnosed with TSC.

Whilst this is an active area of research, currently there are relatively few applicable biomarkers to identify patients at high risk of poor neurodevelopmental outcome other than EEG. We have shown that whilst neuroimaging markers are independent contributory factors to outcome, there are other cofactors which are complex and interlinked. We propose that a big data approach with machine learning combining neuroimaging biomarkers together with neurophysiology data (i.e., EEG), clinical phenotype and genotype could help us further understand and predict children at risk for poor TSC neurological and developmental outcomes. A more aggressive treatment approach and novel therapeutics could be offered to children identified to be at highest risk of poor outcome. Thus, further confirmation of TSC radiological biomarkers will assist future trials with a view to improving the neurodevelopmental trajectory and natural history of TSC disease for all children.

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