Investigating the use of mTOR inhibitors in Tuberous Sclerosis Complex

A thesis submitted in requirement of University College London
For the degree of Doctor of Philosophy

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Abstract

Tuberous Sclerosis Complex (TSC) is a genetic disease caused by variants in the tumour suppressor genes *TSC1* and *TSC2*, located on chromosomes 9 and 16. (1, 2) Approximately 70% of cases are sporadic. The birth incidence has been estimated as 1 in 5,800 per year. The protein products of *TSC1* and *TSC2* (hamartin and tuberin) function together within the cell and have an inhibitory effect on the mammalian target of rapamycin (mTOR), a protein kinase that influences cell growth. Variant in either *TSC1* or *TSC2* leads to over-activation of the mTOR pathway and relatively uncontrolled cell growth. This, in turn, causes growth of benign tumours in various organs such as the brain, kidneys, skin, heart and lungs. (3-5)

Artificial inhibition of mTOR is a therapeutic option in patients with TSC. The mTOR inhibitor currently being used in TSC (everolimus) has significant side-effects. Metformin, which also inhibits mTOR and has a relatively benign side-effect profile, has never before been studied in TSC patients.

The aim of this project was firstly to investigate the impact of TSC on patients' lives and then to investigate the impact of mTOR inhibition via novel methods, and their effect on patients with TSC.

Initially I studied a large cohort of TSC patients to identify the morbidity of TSC and causes of early mortality. Subsequently I investigated the impact of TSC on quality of life. This was the first study to look at the impact of TSC on patients' physical, emotional, social and school functioning.

The final part of the thesis investigates the effect of mTOR inhibition, delivered in novel ways, on TSC related complications. Firstly, I studied the safety and effectiveness of using topical rapamycin in patients with facial angiofibromatosis. Secondly, I conducted a multi-centre double blind randomised placebo controlled trial of metformin in patients with TSC. Specifically, I investigated the safety and effectiveness of metformin on TSC-related lesions (renal angiomyolipomas, and cerebral subependymal giant cell astrocytomas), epilepsy, quality of life, and cognition.

Impact statement

Initially I studied a large cohort of TSC patients to identify the morbidity of TSC and causes of early mortality. The results of such a study may help clinicians and policy makers to decide where best to focus efforts and resources to reduce mortality in this patient group in the future. In addition, the results of this study will guide us on how to treat this condition and what organs we need to focus on.

The results of this study led on to instigating a Delphi process to obtain consensus on the management of TSC in the UK. This is the first UK guideline for the management of TSC, which has been published and circulated to all TSC clinics. This will unify the way TSC patients are treated in the UK.

Subsequently I investigated the impact of TSC on quality of life. This was the first study to look at the impact of TSC on patients' physical, emotional, social and school functioning. Results of such a study may help clinicians and policy makers decide where best to focus efforts and resources to improve the quality of life of patients with TSC. In addition, the results can be used to service plan, and to measure the impact (in terms of health gains) of clinical and social interventions.

The final part of the thesis investigates the effect of mTOR inhibition, delivered in novel ways, on TSC related complications. Firstly, I studied the safety and effectiveness of using topical rapamycin in patients with facial angiofibromatosis. We are hoping that reporting our study of sirolimus ointment will support family groups in streamlining funding for this treatment. This study highlights a need for a national study to determine optimal dosing

regimen of topical sirolimus for facial angiofibromas in TSC, to facilitate EMA licence application for this indication and to facilitate NHS funding for this treatment.

Secondly, I conducted a multi-centre double blind randomised placebo controlled trial of metformin in patients with TSC. Specifically, I investigated the safety and effectiveness of metformin on TSC-related lesions (renal angiomyolipomas, and cerebral subependymal giant cell astrocytomas), epilepsy, quality of life, and cognition. Metformin is a drug that potentially offers the benefit of mTOR inhibition without the side effect and cost profile of other mTOR inhibitors. Both everolimus and rapamycin are immunosuppressant drugs and cause significant side effects, however metformin has a very benign side effect profile. Treatment with everolimus or rapamycin is life-long, and costs thousands of pounds per year per patient. However metformin costs very little.

Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
AML	Angiomyolipoma
AMPK	AMP-activated protein Kinase
ASD	Autistic Spectrum Disorders
ATM	Ataxia Telangiectasia Mutated
BAD	Bcl-2-Associated Death
BRTC	Bristol Randomised Trials Collaboration unit
CRRT	Continuous Renal Replacement Therapy
DMD	Duchenne Muscular Dystrophy
ECG	Electrocardiogram
EEG	Electroencephalogram
EHCP	Education and Health Care Plan
eIF4B	eukaryotic Initiation Factor 4B
EIF4EBP1	Eukaryotic translation Initiation Factor 4E-Binding Protein-1
ER	Endoplasmic Reticulum
FASI	Facial Angiofibroma Severity Index
FDA	Food and Drug Administration
GA	General Anaesthetic
GFR	Glomerular Filtration Rate
HIF-1α	Hypoxia Inducible Factor - 1α
HRAS	V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG
HRCT	High-Resolution Computed Tomography

IBD	Inflammatory Bowel Disease
IFR	Individual Funding Request
IGF1	Insulin-like Growth Factor 1
IQR	Interquartile Range
LAM	Lymphangioleiomyomatosis
LD	Learning Disabilities
LOH	Loss Of Heterozygosity
MALA	Metformin Associated Lactic Acidosis
MHRA	Medicines and Healthcare products Regulatory Agency
MRI	Magnetic Resonance Imaging
mTOR	mammalian Target Of Rapamycin
NIHR	National Institute for Health and Research
NO	Nitric Oxide
OCT3	Organic Cation Transporter-3
PDCD4	Programmed Cell Death 4 protein
PDH	Pyruvate Dehydrogenase
PDK1	3-Phosphoinositide-Dependent protein Kinase-1
PGA	Physician's Global Assessment
PI3K	Phosphoinositide 3-Kinase
PIP3	Phosphatidylinositol (3,4,5)-triphosphate
PMAT	Plasma membrane Monoamine Transporters
PPARγ	Peroxisome Proliferator-Activated Receptor-γ
QoL	Quality of Life
RAP1A	RAS-RELATED PROTEIN 1A
RAP2	RAS-RELATED PROTEIN 2A

RfPB	Research for Patient Benefit programme
Rheb	Ras homologue enriched in brain
RHO	RAS HOMOLOG GENE FAMILY
ROS	Reactive Oxygen Species
RTK	Receptor Tyrosine Kinase
S6K1	RIBOSOMAL PROTEIN S6 KINASE
SEGA	Subependymal Giant Cell Astrocytoma
SEN	Sub-Ependymal Nodule
SNP	Single Nucleotide Polymorphism
SREBP1	Sterol Regulatory Element Binding Protein 1
SUDEP	Sudden Unexplained Death in Epilepsy
TAND	TSC Associated Neuropsychiatric Disorders
TOSCA	TuberOus SClerosis registry to increase disease Awareness
TSA	Tuberous Sclerosis Association
TSC	Tuberous Sclerosis Complex
VABS	Vineland Adaptive Behaviour Scale
VNS	Vagal Nerve Stimulators
WHO	World Health Organization
WPW	Wolff–Parkinson–White syndrome
YY1	factor Yin-Yang 1

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5.1 Abstract

Chapter one

1.1 Background

Tuberous Sclerosis Complex (TSC) is a genetic disease caused by variants in the tumour suppressor genes *TSC1* and *TSC2*, located on chromosomes 9 and 16. (1, 2) Approximately 70% of cases are sporadic. The birth incidence has been estimated as 1 in 5,800 per year. The protein products of *TSC1* and *TSC2* (hamartin and tuberin) function together within the cell and have an inhibitory effect on the mammalian target of rapamycin (mTOR), a protein kinase that influences cell growth and division through the regulation of protein formation. Variant in either *TSC1* or *TSC2* leads to over-activation of the mTOR pathway and relatively uncontrolled cell growth. This, in turn, causes growth of benign tumours (hamartomas) in various organs such as the brain, kidneys, skin, heart, lungs and bones, which are the clinical hallmarks of the disease. (3-5)

Artificial inhibition of mTOR is a therapeutic option in patients with TSC. The mTOR inhibitor currently being used in TSC (everolimus) has significant side-effects. Metformin, which also inhibits mTOR and has a relatively benign side-effect profile, has never before been studied in TSC patients.

The aim of this project was firstly to investigate the impact of tuberous sclerosis complex on patients' lives and then to investigate the impact of mTOR inhibition via novel methods and their effect on patients with TSC.

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1.2 Genetics of TSC

Tuberous Sclerosis Complex (TSC) is caused by variants in either *TSC1* or *TSC2* genes. They are suppressor genes and provide instruction for making the proteins hamartin and tuberin, respectively. (1, 2)

Two thirds of TSC cases are sporadic. These individuals have new variants, whilst the other one-third of cases are inherited in an autosomal dominant pattern. It is believed that *TSC1* variants appear to be more common in familial cases whilst *TSC2* gene variants occur more frequently in sporadic cases. (2)

The *TSC1* gene is located on the long arm of chromosome 9 at position 34.13 with base pairs of 132,891,348 to 132,945,269. The *TSC1* gene consists of 23 exons. The official symbol is TSC1 and the official full name is TSC complex subunit 1 provided by HGNC.

The other names for this gene are hamartin, KIAA0243, TSC1_HUMAN and tuberous sclerosis 1.(1, 6)

The *TSC2* gene is located on the short arm of chromosome 16 at position 13.3 with the base pairs of 2,047,804 to 2,088,720. The official symbol is TSC2 and the official full name is TSC complex subunit 2 provided by HGNC. (2) The other names for this gene are PP1R160, TSC2_HUMAN, tuberin and tuberous sclerosis 2.

The *TSC2* gene was originally given the name of TSC4. It was then found that there is no locus for tuberous sclerosis on chromosome 11 or chromosome 12, therefore, the TSC gene on chromosome 16 was designated TSC2.

The *TSC2* gene has 41 small exons spanning 45 kb of genomic DNA and encodes a 5.5-kb mRNA. (7) The *TSC2* gene is located next to the *PKD1* gene (poly cystic Kidney) in a tail-to-tail orientation. (8)

The protein product of this gene, tuberin, stimulates the intrinsic GTPase activity of the RAS-related protein RAP1A (RAS-RELATED PROTEIN 1A) rather than RAP2 (RAS-RELATED PROTEIN 2A), HRAS (V-HA-RAS HARVEY RAT SARCOMA VIRAL ONCOGENE HOMOLOG), Rac, or RHO (RAS HOMOLOG GENE FAMILY). (9) In addition, it is has been shown that tuberin stimulates RAB5 (RAS-ASSOCIATED PROTEIN) which is involved in the endocytic pathway. Variant in the *TSC2* gene may have an effect on the endocytic pathway which can lead to mis-sorting of internalized growth factor receptors or other signal-mediated membrane-bound molecules that would otherwise undergo lysosomal degradation. (10)

Hamartin and tuberin interact directly with each other and function in a complex. It has been shown that tuberin functions as a chaperone which prevents hamartin from self-aggregation and maintains the tuberin-hamartin complex in a soluble form. Any defect in the phosphorylation of tuberin can affect the formation of this complex.(11)

Manning et al discovered that amino acid residues ser939 and thr1462 of tuberin are PI3K (phosphoinositide 3-kinase)-regulated phosphorylation sites. They also found that a tuberin mutant lacking the PI3K-dependent phosphorylation sites has the ability to block growth factor-induced activation of S6K1 (RIBOSOMAL PROTEIN S6 KINASE) activity. (12)

It has been demonstrated that the TSC1-TSC2 complex inhibits the mammalian target of rapamycin (mTOR), leading to inhibition of ribosomal S6K1 and activation of EIF4EBP1 (eukaryotic translation initiation factor 4E-binding protein-1). (13)

Individuals with TSC are born with one mutated copy of the *TSC1* or *TSC2* gene in each cell. For some hamartomas to develop, a second variant involving the other copy of the TSC genes must occur. When the second variant occurs, the genes are no longer able to produce functional proteins. This in turns leads to formation of multiple hamartomas in various organs in the body.

Carbonara et al studied loss of heterozygosity (LOH) in both the TSC1 and TSC2 loci and seven tumour suppressor gene-containing regions, p53 (191170), NF1 (613113), NF2 (607379), BRCA1 (113705), APC (611731), VHL (608537), and MLM (155600), in 20 hamartomas from 18 tuberous sclerosis patients. Overall, 8 angiomyolipomas, 8 giant cell

astrocytomas, 1 cortical tuber, and 3 rhabdomyomas were analyzed. LOH at either TSC locus was found in a large fraction of the informative patients, both sporadic (7 of 14) and familial (1 of 4). A statistically significant number of LOH of TSC2 was observed in the sporadic group ($p<0.01$). It is possible that a large amount of loss of heterozygosity was seen in this cohort because of selection bias of the patient population. This study showed that none of the seven 7 tumour suppressor gene-containing regions had loss of heterozygosity. This implies that the loss of either *TSC* gene product may be sufficient to form hamartomatous lesions. They also showed that different markers in LOH in different lesions including SEGA and AML indicating that there might have been a different origin of the second hit variant.(14)

Different types of variants in *TSC1* and *TSC2* have been identified including large deletions, nonsense, missense, frameshift, and splicing errors. Large deletions and missense variants are most commonly seen in *TSC2* whilst the most commonly reported variant in *TSC1* gene is small truncating. *TSC2* gene variants are most commonly seen in the sporadic cases compared with the familial cases. (15, 16)

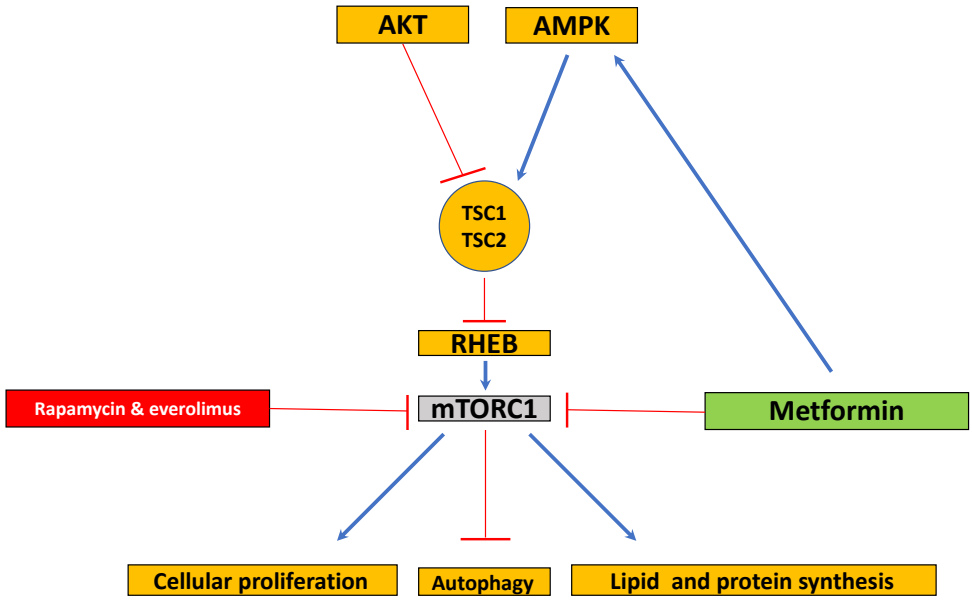
It is believed that patients with *TSC1* have milder disease compared with *TSC2*. (16-18) Joes et al found that learning disabilities were more common in patients with sporadic *TSC2* gene variant (59 [67%] of 88 cases) than sporadic cases carrying TSC1 variants (4 [31%] of 13 cases). Polycystic kidney disease has also been seen mostly in patients with *TSC2* with and without the involvement of the *PKD1* gene. (16)

Retinal hamartomas have also been mostly observed in patients with *TSC2* compared with *TSC1*. Aronow et al reported that retinal hamartomas are most commonly seen in patients

with giant cell astrocytoma, renal angiomyolipoma, cognitive impairment, and epilepsy than in those patients without these lesions.(19)

TSC has a high rate of new variants in which mosaicism is not uncommon similar to neurofibromatosis.(20) Some patients with TSC have no identifiable *TSC* gene variant. Jones et al looked for *TSC* gene variant in 150 patients. They found no variants in 1 of 19 familial cases and in 29 of 130 sporadic cases. Somatic mosaicism was frequently seen among the sporadic cases. Some cases had no identifiable variants, which might be because the analysis was not sensitive enough to detect low level mosaicism or not sensitive enough to detect non-somatic mosaicism.(16)

Figure 1.7.1: mTOR pathway



1.3 mTOR pathway

In order for cells to grow and divide, they require extracellular signals. Signal proteins such as the insulin growth factor family, stimulate cells to grow. These proteins bind to the receptor, tyrosine kinase (RTK), which then stimulates Phosphatidylinositol 3-kinase enzyme (PI3 Kinase) to produce Phosphatidylinositol (3,4,5)-triphosphate (PIP3). PIP3 then recruits two protein kinases such as protein-serine threonine kinase (Akt) and 3-Phosphoinositide-dependent protein kinase-1 (PDK1) (4).

Upon activation and phosphorylation of Akt, Hamartin and Tuberin are phosphorylated in the TSC complex. Hamartin and Tuberin are protein products of TSC1 and TSC2 respectively, which function together within the cell as a complex, and have an inhibitory effect on the mammalian target of rapamycin (mTOR). mTOR is present in two functionally different protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (21).

mTORC1 is localized to the lysosome and formed of mTOR, Raptor, mLST8, Deptor and PRAS40. PRAS40 is a substrate for Akt and mTOR. Upon phosphorylation of PRAS40, PRAS40 disconnects itself from mTOR1, thus alleviating the inhibitory effect on mTOR1 activity. mTORC2 is consists of mTOR, mLST8, Deptor, Rictor, mSIN1and Protor (22).

The two complexes mTORC1 and mTORC2 are molecularly different due to the presence of Raptor or Rictor proteins in complex 1 and 2 respectively. These proteins are essential as they function as a platform for the complex assembly and also help the complexes to bind to regulators and substrates (23).

The regulation between tuberous sclerosis complex (TSC) and mTORC1 is controlled by a small G protein called RHEB (Ras homologue enriched in brain). The GAP domain of TSC2 (Tuberin) within the complex stimulates the intrinsic GTPase activity of RHEB, and this leads to the conversion of RHEB-GTP into RHEB-GDP. TSC inhibits mTOR signalling through its GAP activity towards RHEB (24).

mTORC1 has two downstream targets, the ribosomal S6 kinases S6K1 and S6K2, and the eIF4E-binding proteins 4E-BP1 and 4E-BP2. Upon phosphorylation of 4E-BP1 by mTORC1, 4E-BP1 inhibits mRNA translation, whilst upon phosphorylation of S6K1 by mTORC1, S6K1 has a positive effect on translation initiation and elongation, which is achieved by modifying the activity of their downstream targets, such as ribosomal protein S6, eukaryotic initiation factor 4B (eIF4B), programmed cell death 4 protein (PDCD4), SKAR, CBP80 and eEF2K (23).

mTOR is a serine/threonine kinase which has an important role in regulating cellular processes such as growth, cell proliferation, cytoskeletal organization, transcription, protein synthesis, autophagy, apoptosis, lipid synthesis and mitochondrial metabolism. mTORC1 receives inputs from several major signals such as growth factors, genotoxic stress, energy status, oxygen, and amino acids. According to the environmental cues, these signals exert different effects on the TOR pathway. For example, growth factors inhibit TSC1-TSC2 complex and stimulate mTORC1. Other stimuli such as genotoxic stress, energy deficiency, and oxygen deprivation enhance TSC1-TSC2 activity and therefore inhibit mTORC1 (22).

Amino acids stimulate mTORC1 through the RagA-D family, which are small GTPases. mTORC1 stimulation leads to Rag translocation from the cytoplasm to lysosomal membrane. The GTPases are Rag A or B with either Rag C or D. When there is a lack of amino acids, Rag confirmation is inactive as RagA and RagB are GDP loaded, whilst in the presence of amino acids Rag is active, as RagA or RagB become GTP loaded. Upon activation of Rag, it relates to Raptor and this will relocate mTORC1 to the lysosomal membrane. This relocation will enable mTORC1 to relate to small GTPases, RHEB, on the surface of the lysosome (25).

Triglycerides are the most effective source of energy, simply because they are small in mass and produce a lot of energy. Studies have shown that mTORC1 also plays an essential role in the regulation of lipid synthesis, and mitochondrial metabolism and biogenesis. mTORC1 mediates lipid metabolism through lipogenic transcription factors, such as sterol regulatory element binding protein 1 (SREBP1), peroxisome proliferator-activated receptor-γ (PPARγ) and a phosphatidic acid phosphatase lipin-1 (26).

In addition to amino acids and growth factors, other stimuli, such as low ATP, can stimulate the mTOR pathway. mTORC1 is able to indirectly sense low ATP levels during nutrient deprivation through AMP-activated protein kinase (AMPK). Once the AMP and ATP ratio is high, AMPK phosphorylates TSC1/2, which will affect its GAP activity on RHEB. In addition, AMPK phosphorylates Raptor, which will also inhibit mTORC1 (25).

Other stressors, such as hypoxia, also have an effect on mTORC1. Hypoxia causes reduction in ATP and consequently, the activation of AMPK. The effect of hypoxia on mTORC1 is not only through AMPK, but it also can be through induction of expression of

REDD1/2 genes, which then block mTORC1. Furthermore, other stressors like DNA damage, can supress mTORC1, which is due to p53-dependent upregulation of AMPK. It has been shown that the two transcriptional targets of p53, Sestrin 1 and 2, activate AMPK during DNA damage which will lead to suppression of mTORC1 (27).

During hypoglycaemia and starvation, insulin levels drop, which increases the AMP:ATP ratio, which will then lead to inhibition of mTORC1. mTORC1 is also involved in mitochondrial biogenesis and oxidative metabolism. This is possibly achieved by modulating the interaction between PPARγ coactivator 1 (PGC1-α) and the transcription factor yin-yang 1 (YY1) (22). Studies have also shown that the mTOR pathway has a role in cytoskeletal organisation. CLIP-170/Restin is a member of the family of microtubule associated proteins, which are phosphorylated by mTORC1 (23). mTORC1 signalling is also believed to be involved in transcriptional regulation during hypoxic injury and inflammation. mTORC1 substrates such as HIF1α and STAT3 may play a role in this process(28).

The mTOR pathway is also known to be involved in the immune response which regulates the innate and adaptive immune responses. mTOR receives signals from T cell receptors, CD28, and interleukins. Dendritic cells, which are the most important antigen presenting cells, present antigens to T cells in order to activate them. Upon activation of the T cells, they initiate the adaptive immune response [42]. mTOR senses signals from the dendritic cells and helps the antigen recognition process. This will lead to activation, differentiation and haemostasis of T cells. mTOR is also able to regulate natural killer cell, neutrophil, and macrophage activity. In addition, it has been shown to have an important role in B cell differentiation and maturation (29).

The mTOR pathway plays an important role in other cellular processes such as autophagy. It is believed that mTORC1 inhibits autophagy. Autophagy is a catabolic process which involves cell degradation of unwanted or unnecessary cellular contents through lysosomal machinery. In order to maintain cellular energy levels during starvation, the degradation of dysfunctional cellular components is necessary to ensure cell survival(30) . When nutrients and energy are available, autophagy is inhibited by mTOR as it works as a sensor of intracellular amino acids and cell energy status. Starvation leads to reduction in intracellular amino acid levels which subsequently leads to reduction of mTOR activity, resulting in autophagy (31). mTOR also has been shown to have an effect on apoptosis. This pathway plays a crucial role in switching off and on the cell survival and death process. Depending on the environment that the cells go through, mTOR is able to promote cell death or survival. Ribosomal kinase S6K inactivates Bcl-2-associated death promoter (BAD) which is a pro-apoptotic protein. Inactivation of BAD will lead to inhibition of apoptosis. During cellular damage, the mTOR pathway can induce apoptosis by activating p53 and inhibiting the anti-apoptotic protein Bcl-2 (32)

1.4 Complications of TSC

1.4.1 Subependymal Giant Cell Astrocytoma (SEGA)

Approximately 95% of TSC patients have sub-ependymal nodules (SENs) (33). SENs are nodular lesions, located under the ependymal surface of the lateral ventricles or third ventricle, which form in foetal life. It is believed that SEGAs (Subependymal Giant Cell Astrocytomas) are derived from SENs (34). SEGAs develop in the tissues adjacent to the Foramen of Monro and cause clinical problems if they block cerebrospinal fluid pathways within the ventricular system, leading to obstructive hydrocephalus. SEGAs may also haemorrhage intermittently or secrete protein rich exudate, contributing to communicating hydrocephalus. They are one of the reasons for the excess mortality noted in TSC patients. The prevalence of SEGA ranges from 5%-20% in patients with TSC (35).

1.4.2 Epilepsy

The brain is the organ most commonly affected in TSC. Most patients will have tubers, which are focal malformations of cortical development. It is anticipated that abnormal activity in the tubers, or the areas of brain immediately adjacent to them, lead to epileptogenesis, which causes epilepsy in approximately 80% of these patients (36). Significant numbers of these patients have refractory epilepsy. Epidemiological studies have shown that cognitive impairment is seen in just under half of patients with TSC. However, learning difficulties are frequently severe and are still one of the most distressing consequences of the condition. Many adults with TSC are unable to live independently and require state or family care. There are two explanations for cognitive disabilities in TSC; firstly, the presence of the cerebral tubers, and secondly, due to epilepsy (in particular epilepsy in early life such as infantile spasms). It is believed that the estimated IQ is bi-modally distributed in this population. 55.5% have normal IQ, 14% have mild to severe impairment and 30.5% have profound disability (IQ < 21) (35). In addition, a large number of individuals with TSC develop behavioural issues such as Autistic Spectrum Disorders (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) (37).

1.4.3 Angiomyolipomas (AML)

Renal involvement in this condition is also potentially serious and very common. Angiomyolipomas (AMLs) and cysts are the two characteristic types of renal lesions in tuberous sclerosis complex (TSC) [16]. Growth of AMLs in individuals with TSC is often first detected during childhood and they tend to increase in number and size with age (38). Approximately 80% of patients with TSC have AMLs and 30% have cysts (39). AML is the leading cause of death in individuals with TSC. The presenting features of AMLs are haematuria, pain, high blood pressure and renal failure. Haematuria can sometimes be life threatening (40). These patients are likely to require lifelong health-care follow-up.

1.4.4 Facial angiofibromas

Approximately 86% of patients with TSC have facial angiofibromas, which are benign tumours on the face. These lesions can have a huge psychological impact on a patient's self-esteem. In addition, they are known to cause recurrent bleeding, irritation, infection, facial scarring and disfigurement (41).

1.5 mTOR and TSC

mTOR inhibitors mimic the action of the TSC gene products, in inhibiting the action of mTOR and therefore regulating the PI3 kinase-mTOR-S6 Kinase intracellular growth pathway. mTOR inhibitors, such as rapamycin and everolimus, have been extensively studied in vitro, vivo and in human clinical trials on the appearance and evolution of TSC related tumourous lesions. Rapamycin effectively inhibits mTORC1 (42). Rapamycin is produced by Bacterium *Streptomyces hygroscopicus* which was first isolated from the soil on Easter Island (Rapa Nui) in 1965 (43). It was initially approved as an immuno-suppressant in 1999. This drug also has potent antifungal properties as well as anti-tumour activity (44).

1.5.1 mTOR inhibition in vitro

In vitro, studies on breast, nasopharyngeal and ovarian cancers have shown that rapamycin is an inhibitor of mTOR kinase activity and leads to reduction in cell growth and proliferation (45, 46). In addition, other studies have shown that mTOR inhibition in TSC renal AML cells was associated with a significant tumour response, including stimulation of apoptosis and reduction in cell proliferation and division (47).

1.5.2 mTOR inhibition in animal studies

These positive findings led scientists to invest in, and use, mTOR inhibitors in animal models of TSC, in the hope that this would lead to a reduction in tumour size, improving epilepsy and other related TSC issues.

Rapamycin was initially trialled in Eker rats which carry a germ line TSC2 variant. The effect of this drug was assessed on pituitary and renal tumours. It was noted that rapamycin improved their clinical state and prolonged their survival. It was also noted that the rats showed a significant decrease in the size of renal tumours. The effect was attributed to the down-regulation of ribosomal S6 kinase activity, causing reduction in cell size, and induction of apoptosis (48). Other animal studies have also shown that rapamycin is highly effective in the neuronal model of TSC. It was noted that rapamycin caused complete and sustained inhibition of the mTOR pathway, which led to a reduction in cell size and neurofilament abnormalities, and improvements in myelination. In addition, it improved survival in these animals(49).

mTOR inhibition by rapamycin has also been shown to improve learning deficits and epilepsy in TSC animal models (50). A brief treatment with rapamycin in adult mice with a heterozygous, inactivating variant in the TSC2 gene, which caused problems with learning and memory, rescued their behavioral deficits (50). Rapamycin treatment prevented the development of epilepsy in TSC1 (GFAP) CKO mice. For those animals that already had epilepsy, the drug was able to prevent seizures and prolong their lives (51).

1.5.3 mTOR inhibition in human studies

Bissler et al investigated rapamycin in TSC patients with renal AMLs and lymphangioleiomyomatosis. This study was a non-randomized, open-label trial to study whether rapamycin reduces the AML size in these patients. Rapamycin was given for 12 months. 18 patients completed a 24 month assessment. Kidney AML volumes were reduced to approximately 53% of the baseline value after 12 months of starting the drug. In addition, it led to significant improvements in pulmonary function in some patients with sporadic lymphangioleiomyomatosis. However, tumour regrowth to 86% of the baseline value was noted at 24 months and five patients had six serious adverse events while receiving rapamycin, including diarrhoea, pyelonephritis, stomatitis, and respiratory infections (52).

In a multicentre, phase 2 trial, Davies et al investigated the efficacy and safety of rapamycin in adults with TSC or sporadic lymphangioleiomyomatosis. 13 patients who had AMLs of more than 2 cm were treated with rapamycin for up to 1 year. Patients received daily oral doses of rapamycin to achieve initial trough blood levels of 3 to 6 ng/ml, with an increase to 6 to 10 ng/ml at 2 months. They reported that shrinkage of AMLs was seen in all patients, and the mean (±SD) reduction in the sum of the longest diameters at 12 months was 26.1±10.3%. However, no improvement in lung function was observed. They reported that all patients suffered from grade 1 or 2 adverse events such as mouth ulcers, hyperlipidemia, and peripheral oedema. The treatment had to be terminated in one patient due to side effects. In addition, the tumour size increased upon treatment cessation (53, 54).

Several other studies have demonstrated the efficacy of this drug in reducing the size of other TSC related solid tumours such as SEGA. Franz et al treated 4 TSC patients who had SEGA with rapamycin at standard immunosuppressive doses (serum levels 5–15ng/ml) from 2.5 to 20 months. It was reported that the treatment was well tolerated and all SEGAs showed regression. However, cessation of therapy resulted in tumour regrowth in one patient (55).

Everolimus, marketed by Novartis (Basel, Switzerland) under the trade name Afinitor, is the 40-O-(2-hydroxyethyl) derivative of rapamycin (sirolimus) and works similarly to rapamycin as an mTOR inhibitor.

Krueger et al investigated the efficacy and safety of everolimus in an open trial in 28 patients with TSC who had SEGA. Everolimus was given orally, at a dose of 3.0 mg/m^2, in order to attain a trough concentration of 5 to 15 ng/ml. The treatment was given for the median duration of 21.5 months. It was noted that everolimus was associated with a reduction of at least 30% of the volume of SEGA in 21 patients (75%) and at least 50% in 9 patients (32%). Single cases of grade 3 treatment-related sinusitis, pneumonia, viral bronchitis, tooth infection, stomatitis, and leukopenia were reported. It was also noted that the clinical and electrographic seizures were significantly reduced at 6 months (56). Facial angiofibromas had almost disappeared in 13/15 patients at 6 months of commencing the treatment. In addition, it was also reported that quality of life was improved in this group.

In a phase III international, multicentre, double-blind, randomized, placebo-controlled trial, Franz et al evaluated the efficacy and safety of everolimus in 117 patients, aged between 0.8-26.6 years with SEGA. Everolimus was associated with a significantly greater overall

SEGA response rate, compared with placebo (35% vs. 0%). The frequency of seizures was not different between the treatment and placebo group. It was demonstrated that everolimus was associated with greater partial skin lesion (facial angiofibromas) response (42% vs. 11%). In addition, AML response rates were 53% vs. 0%, compared with the placebo group (55).

Krueger et al have recently reported that everolimus was associated with a clinically relevant reduction in the overall frequency of clinical and subclinical seizures in individuals with TSC (56). There are other reports that demonstrate that mTOR inhibitors everolimus / rapamycin, are effective in the treatment of epilepsy associated TSC in in adults and children (57, 58). A recently published Phase III trial has shown that everolimus, when used as an adjunctive therapy, significantly reduced treatment-resistant focal seizures in individuals with TSC compared with placebo (59).

1.5.4 Side effects of mTOR inhibitors

The short-term side effects related to mTOR inhibitors (rapamycin and everolimus) are generally considered acceptable. The most common side effects are oral ulcers, acneiform rash, thrombocytopenia, hyperlipidemia, impaired wound healing, and immunosuppression [58]. Long term side effects are not well known. Reports from literature related to the use of rapamycin for prevention of kidney transplant rejection, suggested that rapamycin might be associated with impaired spermatogenesis and, as a consequence, may reduce male fertility. In addition, rapamycin crosses the blood–brain barrier and impairs memory in rats (60). Animal studies have also shown that the induction of autophagy by everolimus worsens tubular dysfunction during recovery from kidney injury (61). In addition to side effects, TSC related tumours do regrow on discontinuation of the drugs. Furthermore, there are significant cost implications to the use of these mTOR inhibitors. The use of everolimus costs approximately £20,000 per patient, per year.

1.6 Treatment options in Tuberous Sclerosis Complex

1.6.1 Treatment of Subependymal Giant Cell Astrocytomas (SEGA)

The management of asymptomatic SEGA lesions has been controversial because the natural history of these lesions is unclear. In the past surgical SEGA resection was performed only for those patients who either became symptomatic due to hydrocephalus or had a lesion with evidence of interval growth. (62) Surgical resection was the only treatment option in the past. Recently, medical therapy for SEGA with mTOR inhibitors has become a possibility. I conducted a study and looked at the indications for SEGA surgery and outcomes of patients who underwent surgical removal of SEGA lesions in our institution between 2000 and 2011. I collected information on age, sex, epilepsy history and cognitive status. I reviewed the indications for surgery, age at surgery, surgical approach, and the size and location of the lesions. I analysed mortality, completeness of tumour resection, intraoperative blood transfusion, shunt placements, and surgical complications. This paper was published in the European Journal of Paediatric Neurology. We concluded that surgery is a safe and effective treatment for SEGA. It remains the most appropriate treatment strategy for SEGAs that are amenable to surgery.

1.6.2 Treatment of epilepsy

Management of epilepsy has not been dissimilar to that of epilepsy due to other aetiologies. Anticonvulsant drugs and surgery have been the main treatment options. Infantile spasms are one of the epilepsy syndromes which is the most difficult to control with conventional antiepileptic medications. 10–20% of patients with infantile spasms have TSC. These patients are even more resistant to drug treatment than those without TSC.

Surgery plays a crucial role in the management of epilepsy in patients with TSC. In addition to epilepsy improvement, surgery can also improve IQ and cognitive behaviours over time. The first report on epilepsy surgery outcomes in TSC was by a group from Montreal Neurological Institute in 1966 (63). The outcome is thought to be better following surgery which involves the removal of a single tuber, or in patients with focal EEG abnormalities and focal seizures (64). The exact localisation of the epileptic foci can be difficult in this cohort as the majority of patients have more than one tuber. In addition, the neighbouring neuronal tissues can contribute to the generation of seizures. Non-invasive imaging techniques such as PET, SPECT and magnetoencephalography have been used in identifying the epileptic foci in individuals with TSC, and this has revolutionised this treatment strategy. In addition, the epileptogenic tubers are the ones which produce the largest volume of hypometabolism on FDG-PET scan (65). Other therapeutic surgical options such as Vagal Nerve Stimulators (VNS) have been used for medically retractable epilepsy in patients with TSC. Studies have shown that VNS is a safe and effective treatment option in patients with refractory epilepsy with TSC (66, 67). In addition to VNS and tuber resection, other surgical approaches such as corpus callosotomy and

hemispherectomy have also been used in managing epilepsy in patients with TSC (68)(62).

1.6.3 Treatment of angiomyolipmas (AML)

The best possible treatments for AML have logically focused on preserving renal tissue. The treatments have been no intervention, nephrectomy, or embolization. Total nephrectomy is not an ideal option, but may be indicated where a non-functioning kidney is causing uncontrolled hypertension (69). Partial nephrectomy can be done for these lesions; however it carries a significant risk of bleeding. Embolization is the most commonly used treatment for these lesions. It involves cutting the blood supply to the AMLs and thus reduces the risk of bleeding (70). However, this also carries significant side effects, such as post-embolization syndrome (71).

1.6.4 Treatment of facial angiofibromas

The best treatment options previously have been laser therapy and surgery. The laser technique essentially burns individual lesions reducing the size of the red lumps. However, the larger the area treated, the more painful this is and the longer the recovery. Approximately 50% of patients with TSC have learning disabilities and therefore they require general anaesthetic for laser treatment. Most of these patients require extensive or repeated laser sessions. In addition, laser therapy does not improve the background erythema and the rash gradually worsens again as new lesions form over the next 2-3 years. Surgery can also be used for single very large lesions but this leaves the rest of the rash unaffected. Some patients opt for laser therapy and ask for repeat treatments, but most feel it is too traumatic and ultimately futile.

Chapter 2

2. The impact of Tuberous Sclerosis Complex on patients

2.1 What are the causes of mortality in TSC?

Purpose

The purpose of this work was to identify the causes of death in patients with TSC. I was keen to know the causes of death in this cohort so that we could recommend the appropriate therapy and surveillance in order to reduce mortality and avoid life threatening complications in this cohort.

I investigated the burden of this disease by looking at the causes of mortality in this group of patients, which have rarely been studied, the one published account from the Mayo Clinic appeared in 1991. This study reported that the majority of patients died due to renal disease or SEGA lesions. I conducted a study to investigate the causes of death in a more contemporary population of patients to investigate whether causes of mortality in this patient group have changed over time or differ within a different healthcare system. (72) The results of such a study may help clinicians and policy makers to decide where best to focus efforts and resources to reduce mortality in this patient group in the future. In addition, the results of this study will guide us on how to treat this condition and what organs we need to focus on.

I traced all patients who had attended the Bath TS clinic from 1981 to 2015. The Bath TS clinic is a specialist supra-regional clinic and sees patients with TSC of all ages. I identified 284 patients from our database at the Bath TSC clinic with a definite diagnosis of TSC. I reviewed the medical records including medical notes, radiology images, and if applicable, death certificates and post-mortem reports to determine the age and cause of death in

those patients that had died. Patients were confirmed to be either alive or deceased through clinic records or through GP records if not actively attending clinic. If the death certificate or post-mortem details were not present in the medical records, they were obtained via the hospital bereavement services, primary care facilities, or requested from the General Register Office. Additionally, details regarding patient demographics and clinical details were reviewed.

2.1.1 Patients and methods

We investigated mortality in a large cohort of patients with TSC from a national/supra-regional referral clinic in the UK. We identified 284 patients who attended Bath TSC clinic between 1981 and 2015, and ascertained causes of death by reviewing medical records, death certificates, and post-mortem reports.

The Bath TSC clinic is a specialist supra-regional clinic; it sees patients with TSC of all ages. We reviewed a database of all patients who had attended the Bath TSC clinic from 1981 to 2015 inclusive. Of these patients, we identified 284 patients with a definite diagnosis of TSC, as defined by the International Tuberous Sclerosis Complex Consensus Group. (73) We reviewed the medical records, including medical notes, radiology images, and, where applicable, death certificates and post-mortem reports, in order to determine the age and cause of death. Patients were confirmed to be either alive or deceased through clinic records or through GP records if not actively attending clinic. If the death certificate or post-mortem details were not present in the medical records, they were obtained via the hospital bereavement services, primary care facilities, or requested from the General Register Office. Additionally, details regarding patient demographics and clinical details were reviewed. Ethical approval was not required but all patient details were

anonymized. This is a clinic cohort in which there was heterogeneity in terms of administered investigations. For example, not all patients were administered psychometric tests in a consistent fashion, but some tests were requested and administered when an initial clinical screen of abilities identified areas of concern. We divided the clinic cohort into two groups: those with and those without learning disabilities. Patients were given these diagnoses of learning disability based on their abilities to understand new or complex information or to learn new skills, or their ability to cope or live independently, and with such inabilities having started before adulthood. Although these categorizations were made on the basis of clinical assessment rather than according to any measurement tool, they broadly concur with the definitions of learning disability defined by the UK Department of Health.

The UK Department of Health defines learning disability as follows:

"Learning disability includes the presence of:

● A significantly reduced ability to understand new or complex information, to learn new skills (impaired intelligence), with;

● A reduced ability to cope independently (impaired social functioning);

● Which started before adulthood, with a lasting effect on development.

> Quotient, for example an IQ below 70, is not, of itself, a sufficient reason for deciding whether an individual should be provided with additional health and social care support." (74)

2.1.2 Results

Two hundred and eighty-four patients, with definite diagnoses of TSC, attended the Bath TS clinic from 1981 to 2015; all were included in this study. There were 149 (52%) patients with learning disabilities. The median age of patients with learning difficulties was 25 years (interquartile range [IQR] 15–36) and the median age of patients without learning difficulties was 28 years (IQR17–43). There was no gender difference in this cohort.

The median follow-up duration for all clinic patients was 8 years (IQR 3–17). Forty-one patients had a follow up duration of more than 20 years, 92 patients had a follow up duration of 10 to 20 years and 151 had a follow-up duration of less than 10 years. Eighteen patients (11 females, 7 males) in the clinic cohort died during this period. In two patients, the cause of death was considered not to be directly attributable to TSC. One patient died from ischaemic heart disease at age 80 years, and the other patient from pulmonary embolism, secondary to deep venous thrombosis of the leg at age 33 years. The pulmonary embolism was confirmed by computed tomography and there was no evidence of LAM. The age range of the 16 patients who died directly due to TSC was 17 to 60 years. Median age of death in this group was 33 years (IQR 26–46). Seventy one out of 284 patients in the clinic cohort were children aged 0 to 16 years. In the mortality group, 13 out of 16 patients who died of causes directly related to TSC had learning disabilities. Mortality was significantly more common in the patients with learning difficulties than the patients without learning difficulties (9% vs 2%; Fisher exact test, p=0.020; Table I). Death due to renal causes was seen in eight patients (6 females, 2 males). Three of these patients had chronic kidney failure and one of those three patients had polycystic kidney disease. Three died secondary to acute haemorrhage from renal angiomyolipomas. One of those three patients had had kidney haemorrhage requiring multiple embolizations; one had bilateral

multiple angiomyolipomas and diffusely abnormal kidneys. The remaining two patients died because of renal cell carcinoma. In our clinic cohort, four deaths were attributed to sudden unexplained death in epilepsy (SUDEP), one of which occurred in a case with no identified learning disabilities. SUDEP was defined according to the criteria published by Devinsky et al. (75) Age at death was between 18 years and 40 years. All four patients had developed epilepsy before the age of 16 years, and suffered from more than three generalized tonic–clonic seizures per year. Three patients were on antiepileptic monotherapy with carbamazepine, and one patient was on a combination of carbamazepine with clonazepam and also had a working vagal nerve stimulator in situ at the time of death. None of these patients were identified as being in status epilepticus at the time of death, but they were all assessed to have had an epileptic seizure immediately before death. Pulmonary lymphangioleiomyomatosis (LAM) was the stated cause of death in two patients, both of whom were female. One patient, aged 17 years, had developed heart failure due to LAM lesions in the lung, and the second patient, aged 19 years, died in her sleep secondary to massive pulmonary haemorrhage. One patient died at age 33 years due to a metastatic non-secreting neuroendocrine pancreatic tumour. One patient died aged 26 years because of SEGA. He was referred to our service in 1989, after neurosurgeons had already recommended a palliative approach because they assessed his SEGA to be inoperable at the time of diagnosis.

2.1.3 Discussion

This study, compared with the previously published report, provides important new insights into the causes of premature mortality in this patient group. (72) It confirmed renal causes as being the commonest cause of early death but it has also demonstrated that SUDEP is a significant and previously unreported risk in TSC. Patients with LD are significantly more likely to suffer premature death, and female patients are more at risk of death secondary to angiomyolipoma and LAM. In contrast with the previous report, SEGA was an uncommon cause of death. There were no deaths during childhood.

This was a retrospective study design and was based on a clinic population, which creates the main limitations of this study. Specialist clinics tend to ascertain more severe cases and we would anticipate this patient sample to have more medical issues and co-morbid factors than the overall population of people with TSC. The gender balance and prevalence of LD in our cohort, however, was similar to previously reported population based TS cohorts, suggesting that this clinic population was not grossly dissimilar from the TS population at large. (39) (76)

Another weakness of this study was that we did not have enough data to perform a survival analysis. Shepherd et al presented Kaplan Meier survival analysis.

A relative strength of this study, compared with Shepherd's study, is that it relied not only on death certificates, but also on other sources, such as post-mortem examination and medical notes. Studies have shown that death certificates can be completed inaccurately and can be misleading when relied upon as the sole source of mortality data. (77)

In our series, the median age of death was 33 years (IQR 26-46). None of the cases died in the paediatric age range (<16 years). Although we would expect TSC complications to become more prevalent with increasing age and possibly not to cause death in the paediatric age group, this finding contrasts with the earlier study, where 40% of the deaths were in children. (72) We believe that our study's finding of absent mortality in children and only single case of mortality secondary to SEGA reflects a period effect relating to increased awareness of this condition, improved surveillance within a specialist clinic population, and successful treatment within a specialist neurosurgical centre that has acquired significant experience in dealing with these mid-line tumours[8]. Although not reported from our clinic population, mortality in childhood in TSC may occur secondary to cardiac rhabdomyomas, which are TSC-related hamartomas that grow in foetal life and can cause a significant problem around the time of birth by such mechanisms as obstruction to blood flow or life-threatening arrhythmias. The cardiac rhabdomyomas tend to regress in size after the perinatal period and are unlikely to be a cause of death in cases ascertained through a TSC clinic, and this is a factor that would tend to bias downwards estimates of death in childhood in studies such as ours. (78)

In our clinic cohort, there was no significant gender imbalance (143 female and 141 male patients) but the mortality in females was twice that of males. Female patients died predominantly secondary to renal and lung complications. It is well known that pulmonary LAM occurs almost exclusively in women (79) and, therefore, it is not surprising that the two fatalities in this study secondary to LAM were females. One possible explanation for symptomatic LAM occurring almost exclusively in females is that LAM lesions are thought to express oestrogen and progesterone receptor proteins. (80) It is also noticeable that the two deaths associated with LAM occurred in women of child-bearing age, where there

is exposure to higher levels of circulating oestrogen than at pre-pubertal or post-menopausal ages. (81) In this study, six out of the eight patients who died from renal causes were female. One previous population-based study has shown angiomyolipomas to be slightly, but not significantly, more prevalent in women but the difference in prevalence is not so large as to explain the differential renal associated mortality between the genders. (38, 39) It is possible that the differential mortality is explained by the fact that angiomyolipomas also express oestrogen receptors, thus making them more likely to grow to a dangerous size in the female population, but it might also be a chance finding given that it is a relatively uncommon cause of death in a moderately-sized study population.

Thirteen of the patients who died had learning difficulties. The increased risk of early mortality in LD patients has not previously been reported. LD is a recognised feature of TSC and seen in approximately 50% of patients, (82) and in our cohort, 52% had LD. The increased risk of early mortality in the LD population of TSC patients is plausible for several reasons. Firstly, the LD population may be prone to having more hamartomas than non-LD patients. One study has previously reported that patients with LD have more TSC related renal hamartomas. (83) Secondly, patients with learning difficulties are at much higher risk of epilepsy than patients without learning difficulties, and therefore also at higher risk of SUDEP. (36) Finally, patients with LD are less likely to be able to communicate symptoms to their careers and their complications may not come to medical attention until they are more advanced. Based on these findings, patients with learning difficulties merit regular and close surveillance in order to minimise the risk of premature death.

Death due to renal causes was the commonest cause of death in this group. Two patients died due to renal cell carcinoma. These findings support the need for regular surveillance of this population for the development of potentially fatal renal lesions.

In our study four deaths were attributed to SUDEP. No SUDEP cases have previously been reported in TSC. Deaths due to status epilepticus have been reported in TSC. (84) The US Food and Drug Administration and Burroughs-Welcome developed criteria for SUDEP in 1993 which states that the death should not be the direct result of status epilepticus.

All patients had multiple risk factors for SUDEP. They were adults aged between 18 and 40 years at the time of death. SUDEP has been reported to be four times more likely in adults compared with children, and the peak age categories at time of death from this cause are between 20 to 40 years. (85) All four patients had developed epilepsy before the age of 16, suffered from multiple generalised tonic-clonic seizures per year and were on treatment with carbamazepine. Three of these patients had learning difficulties. Previous reports have linked all these factors to SUDEP. (86) It is also possible in TSC patients that residual cardiac rhabdomyomas may predispose patients to cardiac arrhythmia in the context of an epileptic seizure. Interestingly, two post-mortem reports of the SUDEP cases reported the presence of cardiac rhabdomyomas on post-mortem examination. These patients were being monitored in the hospital at the time of death and their ECG monitoring showed changes just seconds before their death. The ECG changes and the presence of rhabdomyomas suggest that the cause of death could be cardiac rather than cerebral.

One patient died due to a metastatic pancreatic tumour. Pancreatic tumours have been reported in individuals with Tuberous Sclerosis Complex and the mTOR pathway appears to be activated in pancreatic cancer cells. (87) We have recently seen two TSC patients with pancreatic tumours who are under investigation and treatment. It is incorrect to assume that all pancreatic lesions in individuals with TSC are benign and necessitate no treatment. All TSC patients should probably have intermittent abdominal MRI scans to check for pancreatic tumours as these tumours are not always easily picked up during kidney US scan surveillance.

Eight patients received mTOR inhibitors such as Rapamycin and Everolimus. Two patients received this treatment due to SEGA, one due to epilepsy, 4 due to angiomyolipomas and one due to pancreatic tumour. None of the patients who died in this cohort had ever received any mTOR inhibitors. mTOR inhibition has only recently become available for TSC patients in the UK and It is impossible to know if use of mTOR inhibitors would have had any impact on the mortality rate reported in this observational study.

In conclusion, this study has emphasised that renal disease is a major cause of mortality in TSC patients. We believe that lifelong surveillance of renal lesions, to enable appropriate management of potentially life-threatening complications, is warranted. The study has revealed also that SUDEP is a significant cause of mortality in these patients. Patients and carers should be warned about this risk. In our cohort, LD patients were at significantly greater risk of early mortality and this indicates the need for greater vigilance in these patients for the development of lesions, and indeed of symptoms that they may not be able to communicate clearly to their carers. Female patients are particularly vulnerable to pulmonary and renal disease. The mortality from pulmonary disease in

young women suggests that all post-pubertal female TSC patients should be screened for LAM and treated appropriately. Finally, pancreatic lesions, although rarely reported in TSC, are a reported cause of mortality and they should be looked for in TSC patients, not just by means of abdominal ultrasound, which is a relatively insensitive investigation, but also with intermittent abdominal magnetic resonance imaging.

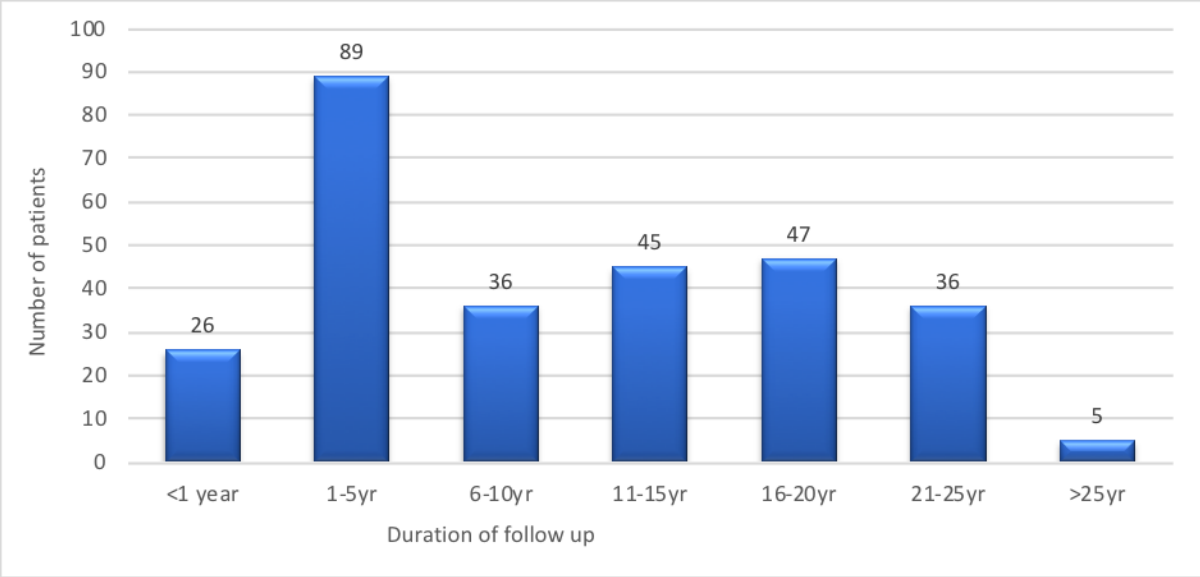


Figure 2.5.1: Shows all clinic patients and their follow up duration (from first to last clinic review).

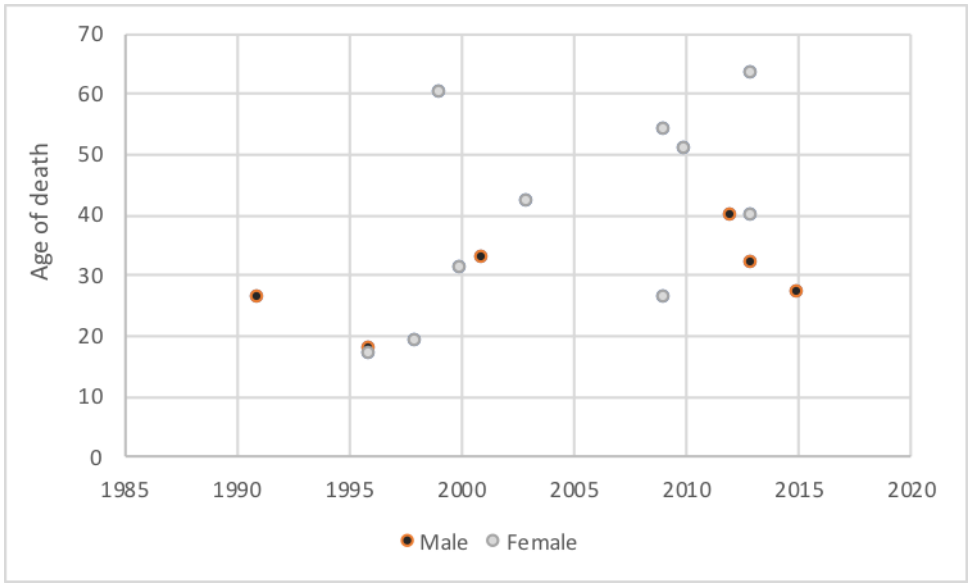


Figure 2.5.2: Shows the distribution of age, gender, and year of death of those patients who died from causes related to TSC.

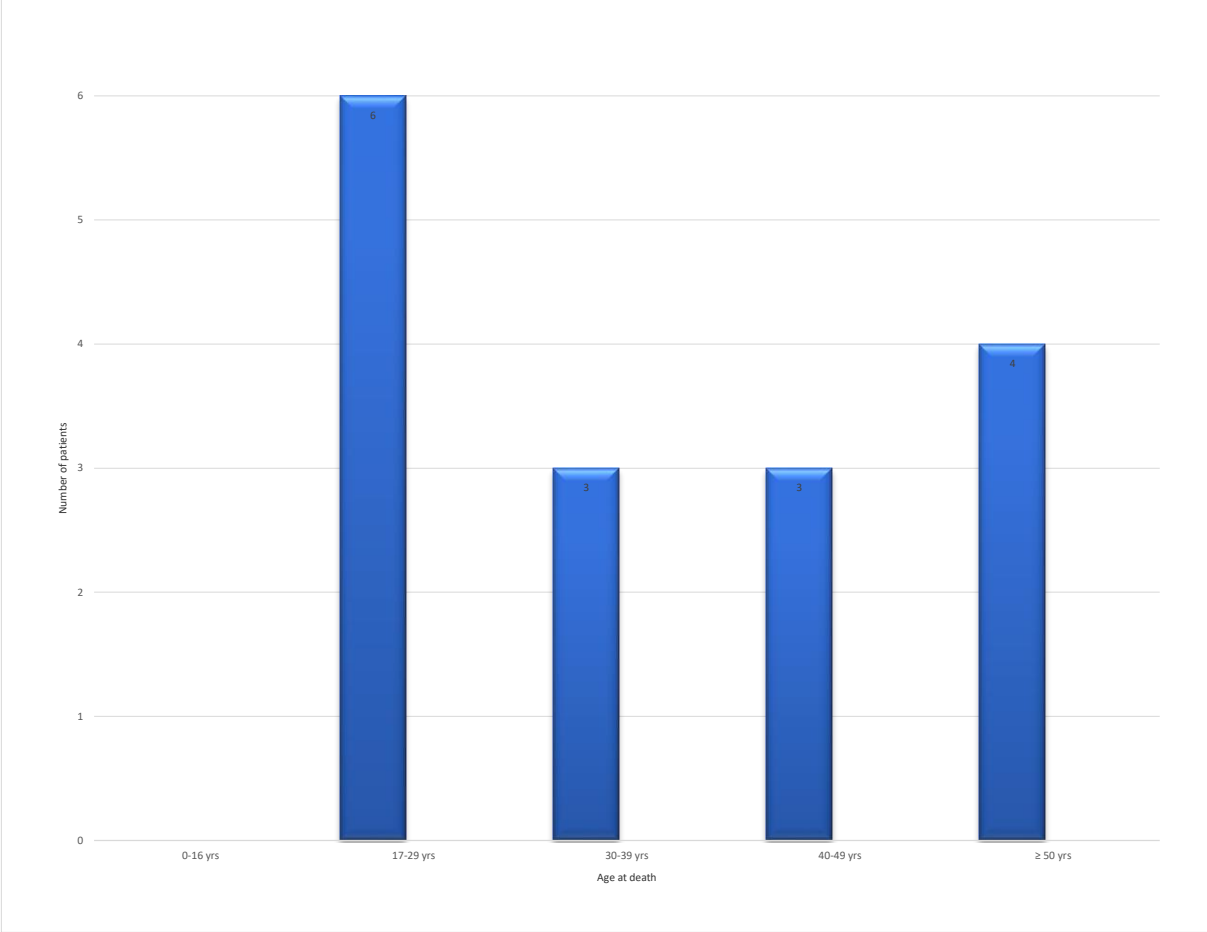


Figure 2.5.3: Distribution of age at death of those patients who died from causes related to TSC.

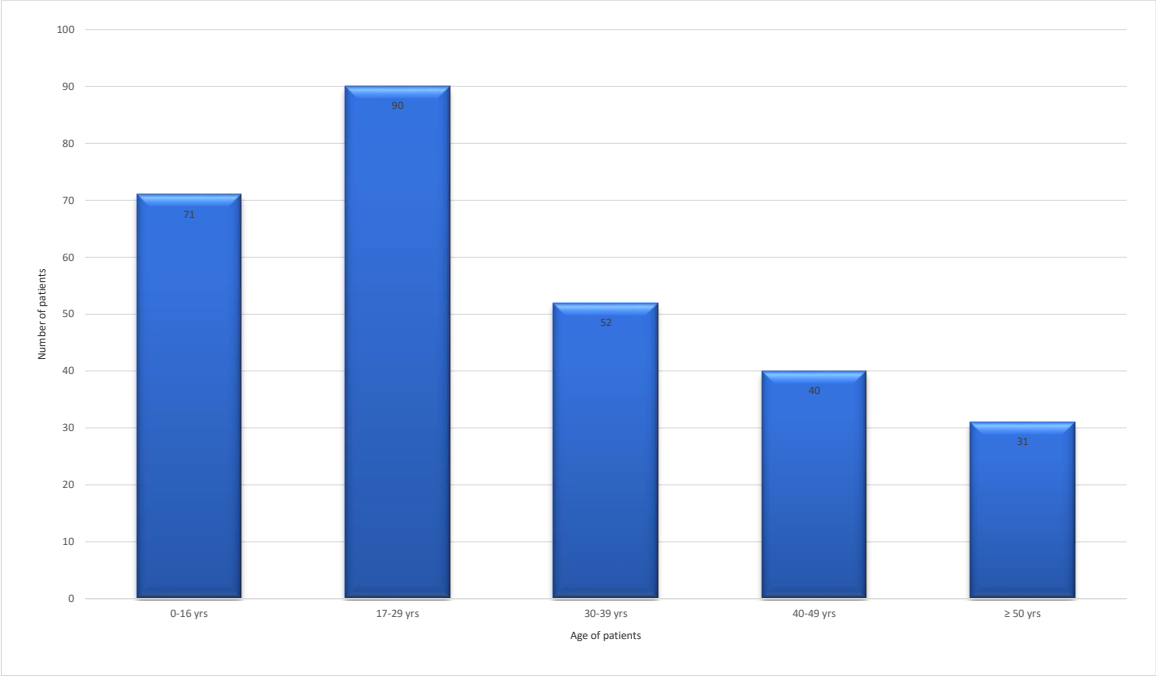


Figure 2.5.4: Distribution of age in 2015 of all patients at Bath clinic

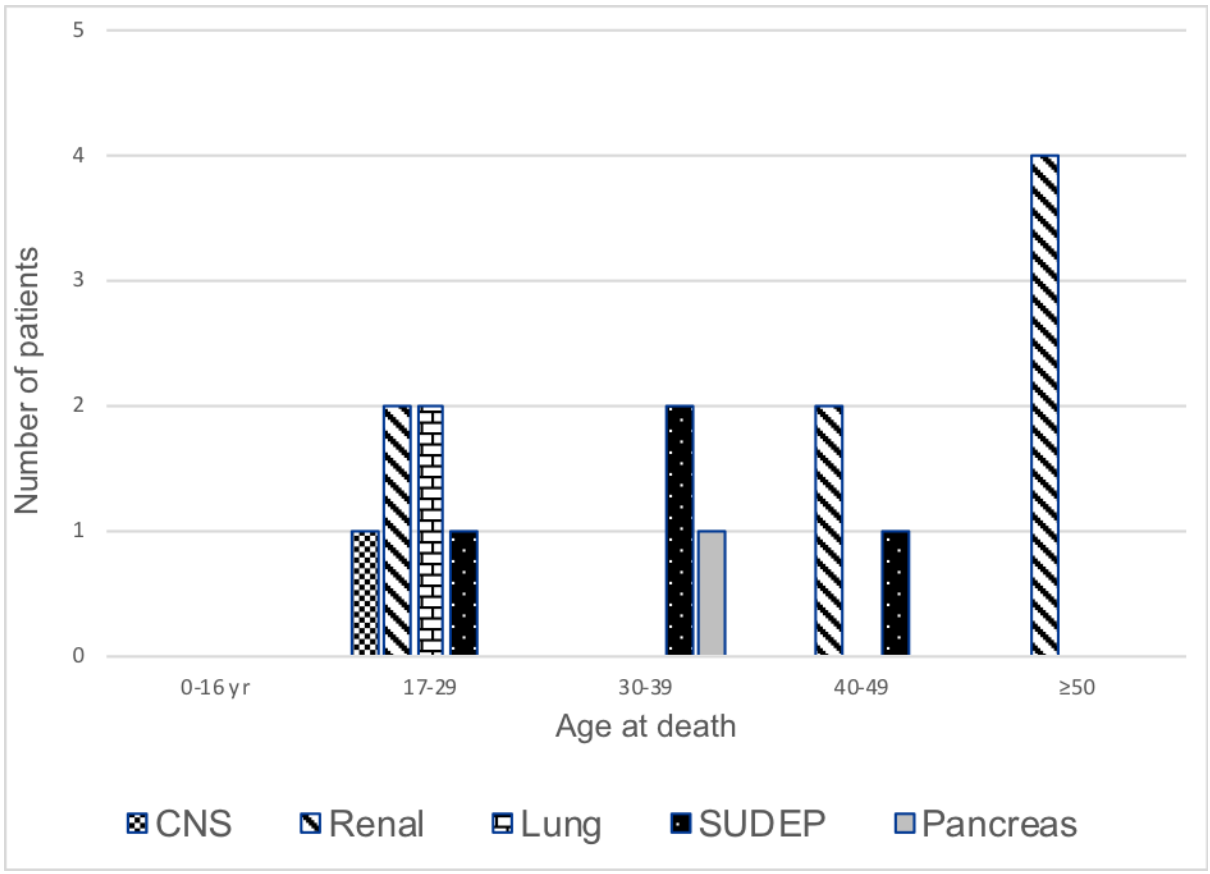


Figure 2.5.5: Causes and age of death

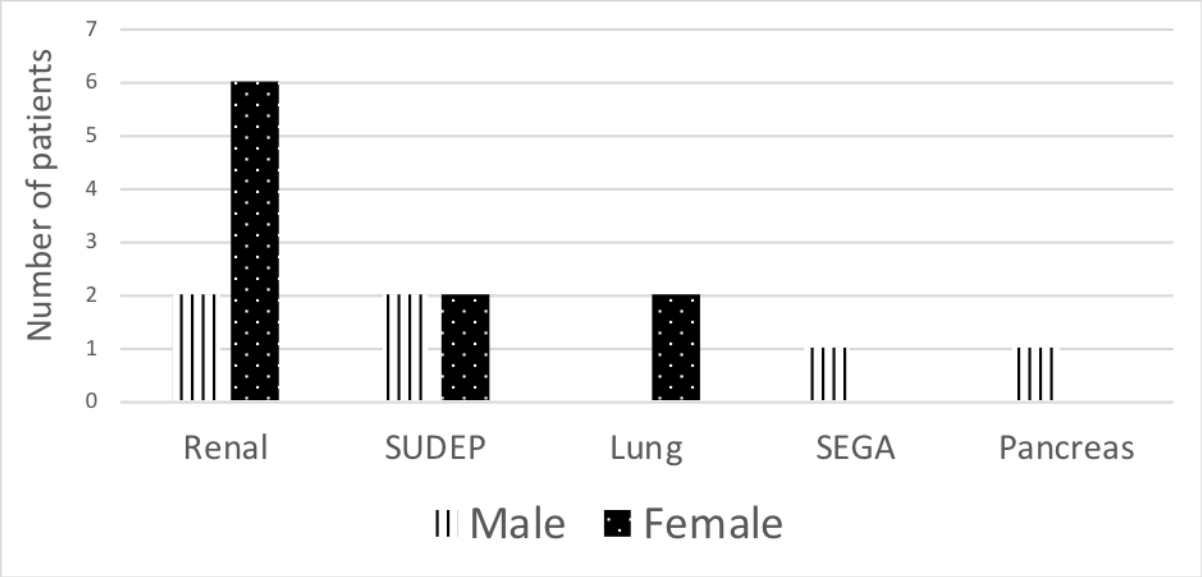


Figure 2.5.6: Causes of death and patient gender

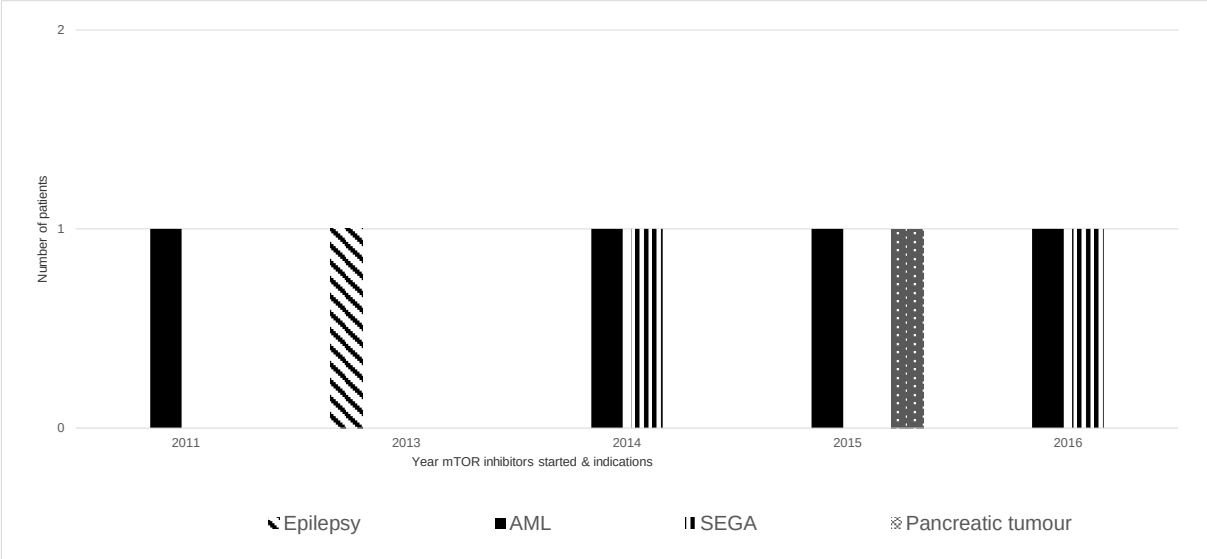


Figure 2.5.7: shows the number of patients who have been taking mTOR inhibitors, such as Everolimus or Rapamycin, the year of drug commencement, and indication. None of the patients who died in this cohort had ever received any mTOR inhibitors.

	Dead	Alive	Totals
Patients with LD	**13**	**135**	**148**
Patients without LD	**3**	**131**	**134**
Totals	**16**	**266**	**282**

Table 2.6.1: Comparison of mortality in learning difficulty and normal intellect patients. P = 0.02 (Fisher's exact test)

2.2 The UK guidelines for management and surveillance of Tuberous Sclerosis Complex

We have learnt from previous sections that complications of TSC can be life threatening, with significant impact on patients' quality of life.

The management of TSC has varied dependent on treating physician, local and national policies and funding. There were no current UK guidelines. We conducted a Delphi consensus process to reach agreement amongst UK experts on the management of patients with TSC. The Delphi process is a well established, commonly used and widely accepted approach to achieve consensus. It was first established by Dalkey et al.(88) The process has been used in other walks of life for example to gain consensus in programme planning, resource utilisation and policy making. In a Delphi process answers from expert respondents to a series of questions are collected in an iterative fashion until, where possible, consensus is achieved.

The Delphi process provides consensus guidance for the delivery of best clinical care. It is important that the subjects are selected carefully. A danger is that when questions address issues without an evidence base, some respondents may provide answers despite a lack of specific knowledge. It is crucial, therefore, that the respondents are experts in the field. It is generally believed that 15-20 subjects could be sufficient to take part in a Delphi process but the higher the number of the subjects and homogeneity of response, the better the outcome. There does not seem to be a universally agreed proportion for Delphi consensus. It has been suggested that this might depend on the sample numbers, aims of the research and resources available. Some papers have

suggested that consensus should be equated with 51% agreement amongst respondents; others recommend 60 or 70%.(89)

We conducted a Delphi consensus process to reach agreed guidance for the management of patients with TSC in the UK. We also reviewed the most recent international guidelines for TSC management (73).

We created a core committee to oversee the questions and analyse the data. A priori consensus was defined as 70% agreement among participants.

2.2.1 Participants

We invited 86 clinicians and researchers to complete the online survey. All the people surveyed were based in the UK. Clinicians were identified through the regional TSC clinics in the UK, Tuberous Sclerosis Association and British Paediatric Neurology Association while the researchers were identified through publications. A literature search was performed using MEDLINE, the Cochrane library and Google scholar. We invited all the first and last authors of papers on TSC published since January 2000 until January 2017 in the UK. Respondents were advised to complete only sections of the survey which were relevant to their expertise. As TSC is a multi-system disease, a cardiologist, for example, may not want to comment on the neurological management of TSC. Conversely a paediatrician who provides holistic care for his/her TSC patients may have relevant views on several aspects of the disease. This work has been completed with collaboration with the Tuberous Sclerosis Association (TSA). TSA is professional organisation providing support to families affected by TSC across the UK. Patients were not directly involved.

2.2.2 Materials

We drew up 55 questions in round one of our Delphi survey. In round two, 18 questions were asked to obtain consensus on the outstanding points that had been contentious in round one, or that needed clarification. The surveys were conducted over 6 months from June 2017-November 2017. A weekly electronic reminder was sent to the responders. The data were analysed by a core committee and subcommittee that consisted of UK experts in different fields within TSC. In addition, patient groups and the Tuberous Sclerosis Association were consulted.

Fifty-one experts (60%) responded to the survey. Two rounds were required to achieve consensus. The responders were neurologists, nephrologists, psychiatrists, psychologists, oncologists, general paediatricians, dermatologists, urologists, radiologists, clinical geneticists, neurosurgeons, respiratory physicians and neurodisability clinicians.

We reported the recommendations for the management of TSC based on the Delphi consensus results and expert opinions. For each aspect of TSC there was a committee to give expert opinion. Their opinion was based on their practice and latest evidence. The recommendations have been presented under two sections: Delphi consensus and expert opinion.

2.2.3 Round one questions

1. **What is your specialty?**
    a. Neurology
    b. Nephrology
    c. Psychiatry
    d. Oncology
    e. General Pediatrics
    f. Dermatology
    g. Urology
    h. Radiology
    i. Genetics
    j. Neurosurgery
    k. Respiratory
    l. Neurodisability
    m. Other (please specify)

2. **Do you think a genetic test should be offered?**
    a. Yes
    b. No
    c. Comments

3. **Do you think baseline brain imaging should be performed?**
    a. Yes
    b. No
    c. If yes, what modality, why?

4. **Do you think a baseline neuropsychiatric assessment should be performed?**
    a. Yes
    b. No
    c. Comments

5. **Do you think a baseline neuropsychology assessment should be performed?**
    a. Yes
    b. No

c. Comments

6. **Do you think a baseline standard EEG should be performed regardless of seizure activity?**
    a. Yes
    b. No
    c. Comments

7. **Do you think baseline kidney imaging should be performed?**
    a. Yes
    b. No
    c. If yes, what imaging modality, why?

8. **Do you think baseline blood pressure should be checked at baseline?**
    a. Yes
    b. No
    c. Comments

9. **Do you think a baseline full blood count should be performed?**
    a. Yes
    b. No
    c. Comments

10. **Do you think a baseline liver function test should be performed?**
    a. Yes
    b. No
    c. Comments

11. **Do you think a baseline renal function test should be performed?**
    a. Yes
    b. No
    c. Comments

12. **Do you think baseline pulmonary function tests should be performed?**
    a. Yes
    b. No

c. Comments

13. **Do you think a baseline high-resolution chest computed tomography (HRCT) should be performed?**
    a. Yes
    b. No
    c. Comments

14. **At what age, should a baseline HRCT be performed?**

15. **Do you think a detailed skin examination should be performed at baseline?**
    a. Yes
    b. No
    c. Comments

16. **Do you think a detailed dental examination should be performed at baseline?**
    a. Yes
    b. No
    c. Comments

17. **Do you think a baseline echocardiogram should be performed?**
    a. Yes
    b. No
    c. Comments

18. **Do you think a baseline electrocardiogram should be performed?**
    a. Yes
    b. No
    c. Comments

19. **Do you think a detailed baseline ophthalmological examination should be performed?**

a. Yes
b. No
c. Comments

20. Any other tests or examinations which you think should be performed at baseline?

21. How do think SEGA (subependymal giant cell astrocytoma) lesions should be managed?

22. Should brain imaging be repeated in asymptomatic patients with SEGA lesions?
a. Yes
b. No
c. If yes, how often?

23. At what age, should routine imaging in asymptomatic patients with SEGA lesions be stopped?

24. Should brain imaging be repeated in asymptomatic patients without SEGA lesions?
a. Yes
b. No
c. If yes, how often?

25. At what age, should routine imaging in asymptomatic patients without SEGA lesions be stopped?

26. Do you think SEGA lesions which are actively growing and symptomatic should be treated?
a. Yes
b. No
c. If yes, what should be the first line treatment?

27. **Do you think SEGA lesions which are actively growing and asymptomatic should be treated?**
    a. Yes
    b. No
    c. If yes, what should be the first line treatment?

28. **Do you think assessments for associated neuropsychiatric conditions should be repeated?**
    a. Yes
    b. No
    c. If yes, how often?

29. **Do you think developmental status assessment should be repeated?**
    a. Yes
    b. No
    c. If yes, how often?

30. **Do you think children with infantile spams should be treated with anticonvulsant medications?**
    a. Yes
    b. No
    c. if yes, what should be the first and second line?

31. **Do you think multiple spikes on EEG in infants should be treated regardless of the seizure activity?**
    a. Yes
    b. No
    c. Comments

32. **Do you think multiple spikes on EEG in older children and adults should be treated regardless of the seizure activity?**
    a. Yes
    b. No
    c. Comments

33. Do you think kidney imaging should be repeated?

a. Yes
b. No
c. If yes, how often?

34. Do you think a renal function test should be repeated?

a. Yes
b. No
c. If yes, how often?

35. Do you think a full blood count should be repeated?

a. Yes
b. No
c. If yes, how often?

36. Do you think a liver function test should be repeated?

a. Yes
b. No
c. If yes, how often?

37. Do you think blood pressure measurement should be repeated?

a. Yes
b. No
c. If yes, how often?

38. How should growing kidney AMLs (angiomyolipomas) be managed?

39. Should kidney AMLs which are growing be treated?

a. Yes
b. No
c. If yes, what should be the first line?

40. **How should LAM (lymphangioliomyomatosis) lesions be managed?**

41. **Should HRCT be repeated in asymptomatic patients?**
    a. Yes
    b. No
    c. If yes, how often?

42. **Should HRCT be repeated in symptomatic patients?**
    a. Yes
    b. No
    c. If yes, how often?

43. **At what age, would you stop performing routine HRCT in asymptomatic patients?**

44. **Should pulmonary function tests be repeated?**
    a. Yes
    b. No
    c. If yes, how often?

45. **Should LAM which are growing be treated?**
    a. Yes
    b. No
    c. If yes, what should be the first line?

46. **Should skin examination be repeated?**
    a. Yes
    b. No
    c. If yes, how often?

47. **How should facial angiofibromas be managed?**

48. **Should facial angiofibromas be treated?**
    a. Yes

b. No
c. If yes, what should be the first line?

49. How should cardiac rhabdomyomas be managed?

50. Should echocardiogram be repeated?
a. Yes
b. No
c. If yes, how often?

51. Should electrocardiogram be repeated?
a. Yes
b. No
c. If yes, how often?

52. How should retinal hamartomas be managed?

53. Should detailed ophthalmic examination be repeated?
a. Yes
b. No
c. If yes, how often?

54. Should the liver and pancreas be imaged?
a. Yes
b. No
c. If yes, how often?

55. Any other tests or examinations which you think should be performed at baseline?

2.2.4 Round two questions

1. **Do you think a baseline assessment should be performed for: ADHD, ASD, sleep disorder and challenging behavior?**
    a. Yes
    b. No

2. **Do you think these assessments should be repeated?**
    a. Yes
    b. No
    c. If yes, how often?

3. **How often should brain imaging be repeated in asymptomatic patients with SEGA lesions?**
    a. Annually
    b. 1-3 years

4. **At what age should routine imaging in asymptomatic patients with SEGA lesions be stopped?**
    a. 25 years
    b. Should never stop
    c. Other (please specify)

5. **How often should brain imaging be repeated in asymptomatic patients without SEGA lesions?**
    a. Annually
    b. 1-3 years
    c. Others (please specify)

6. **At what age should routine imaging in asymptomatic patients without SEGA lesions be stopped?**
    a. 25 years
    b. Should never stop
    c. Other (please specify)

7. **Do you think all growing SEGAs should be discussed at an MDT meeting?**
    a. Yes
    b. No
    c. If yes, who should be present at MDT?
        i. TSC experts
        ii. Neurologist
        iii. Oncologist
        iv. Neurosurgeon
        v. Neuro-radiologist
        vi. Other (please specify)

8. **Do you think a baseline high-resolution chest computed tomography (HRCT) should be performed in females of child-bearing age?**
    a. Yes
    b. No

9. **Should we repeat HRCT (high-resolution chest computed tomography) scans in patients when it is difficult to establish if symptoms present especially in patients with learning disabilities.**
    a. Yes
    b. No
    c. If yes, how often?
        i. 5 yearly
        ii. 10 yearly
    d. When to stop?
        i. Never
        ii. Menopause

10. **Should skin examination be repeated? It may be useful to check on the progress of skin manifestations that cause morbidity such as facial angiofibroma and ungual fibroma.**
    a. Yes
    b. No

11. **How often should echocardiogram be repeated?**
    a. Until first sign of cardiac rhadomyoma regression
    b. Until complete regression

12. **What kind of ophthalmic examination should be repeated?**
    a. Fundoscopy by direct ophthalmoscopy
    b. Detailed ophthalmological review by an ophthalmologist
        i. How often?

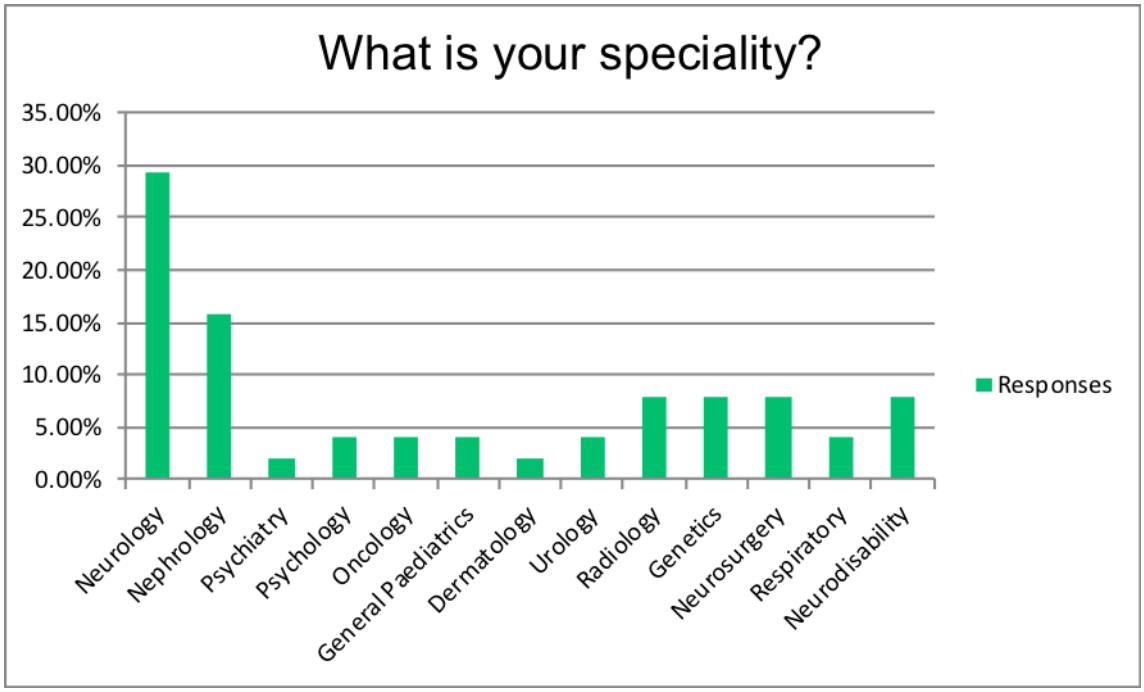
1. Annually
2. 2 yearly

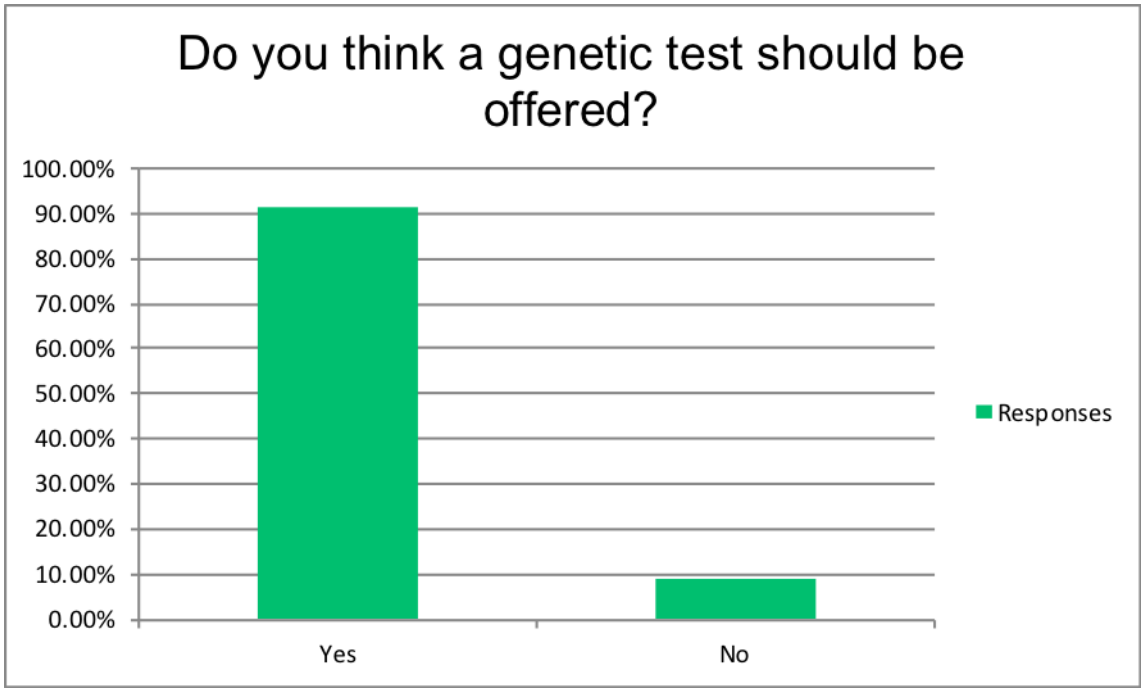
13. **Should the liver and pancreas be imaged? Studies have shown pancreatic and liver tumours can cause mortality in this cohort. The liver and pancreatic scans can be performed at the same time as the kidney scans.**
    a. Yes
    b. No

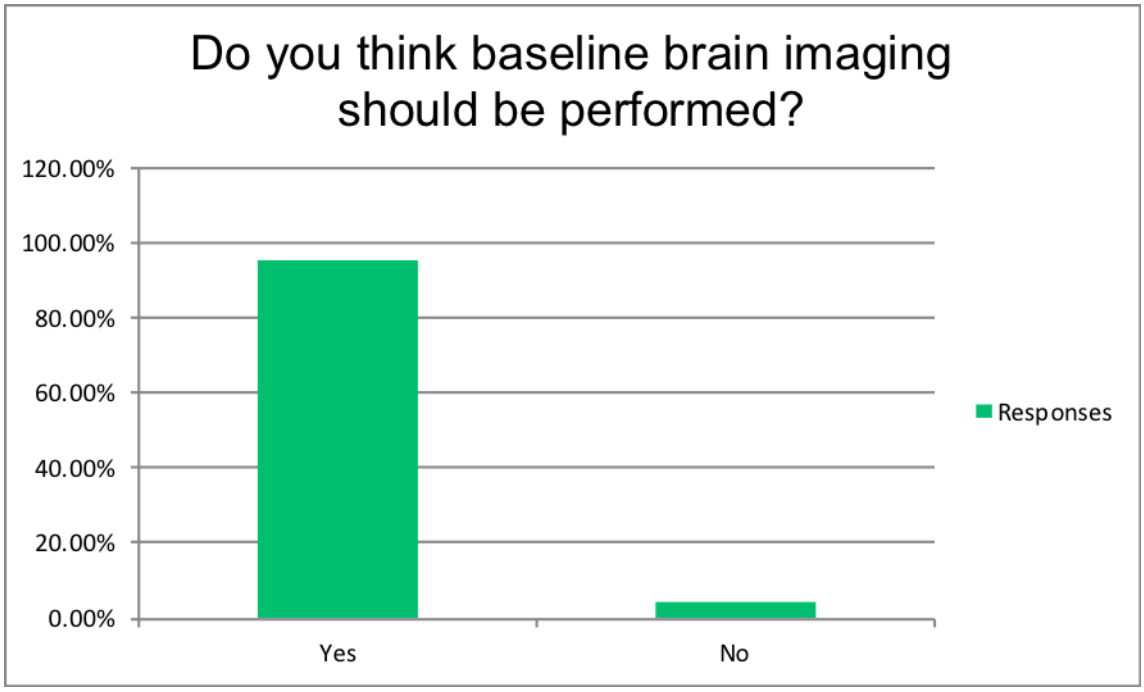
2.2.5 Results

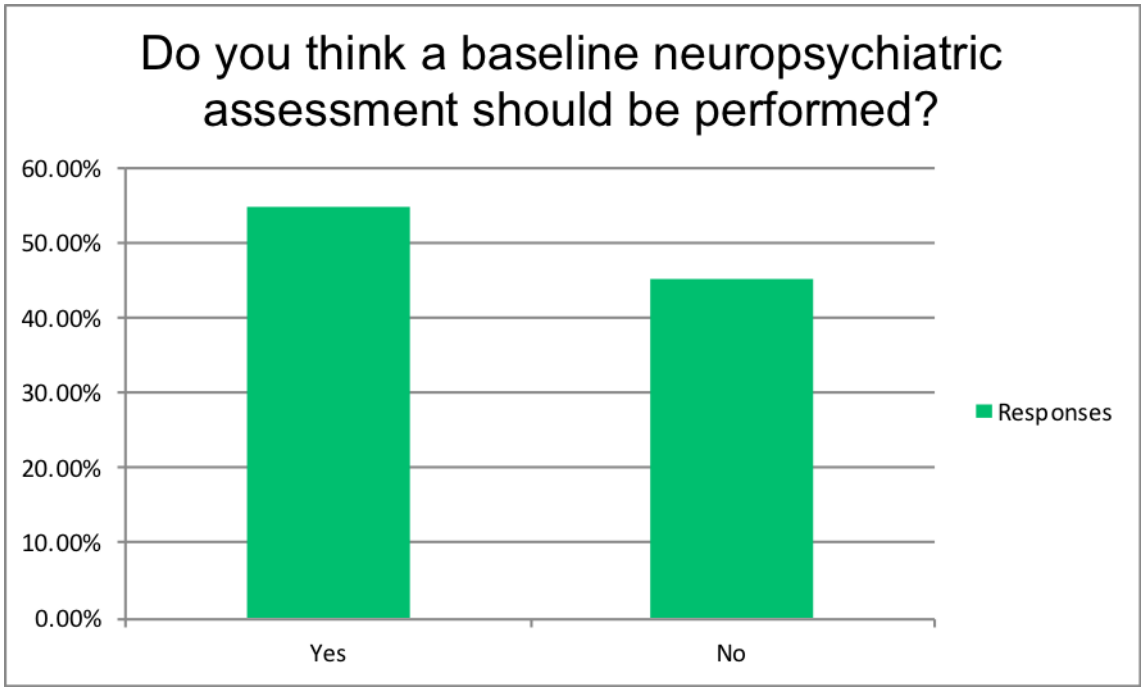
Fifty-one (60%) responded to the survey. Two rounds were required to achieve consensus. The responders were neurologists, nephrologists, psychiatrists, psychologists, oncologists, general paediatricians, dermatologists, urologists, radiologists, geneticists, neurosurgeons, pulmonologists and neurodisability clinicians.

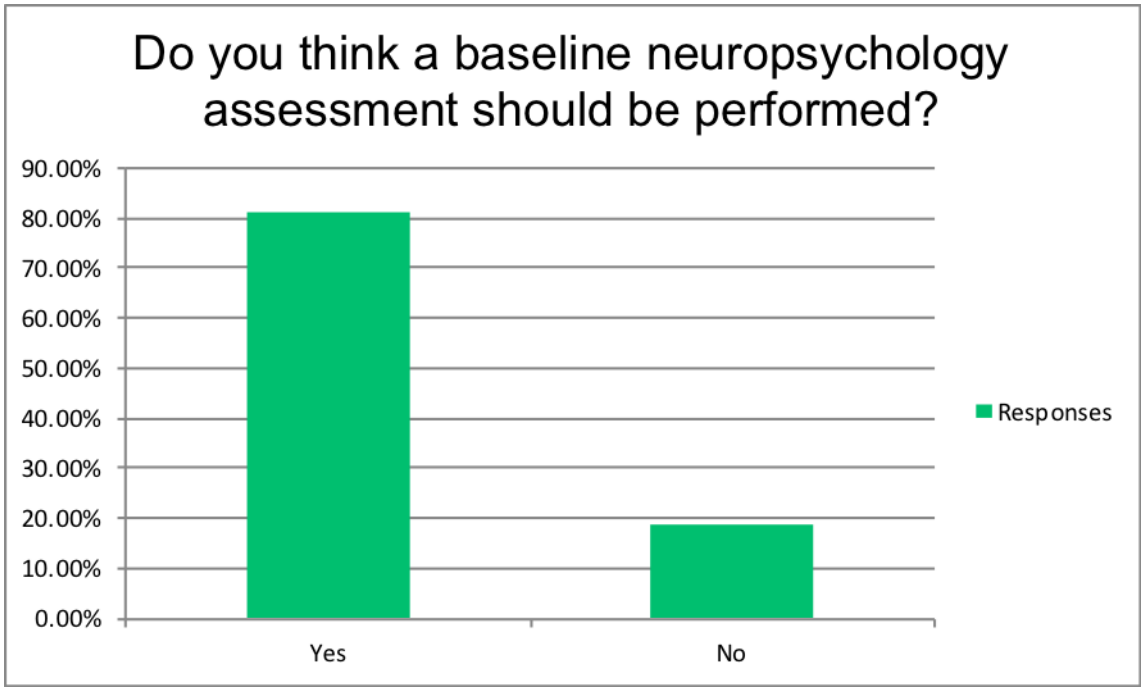
The results are presented here:

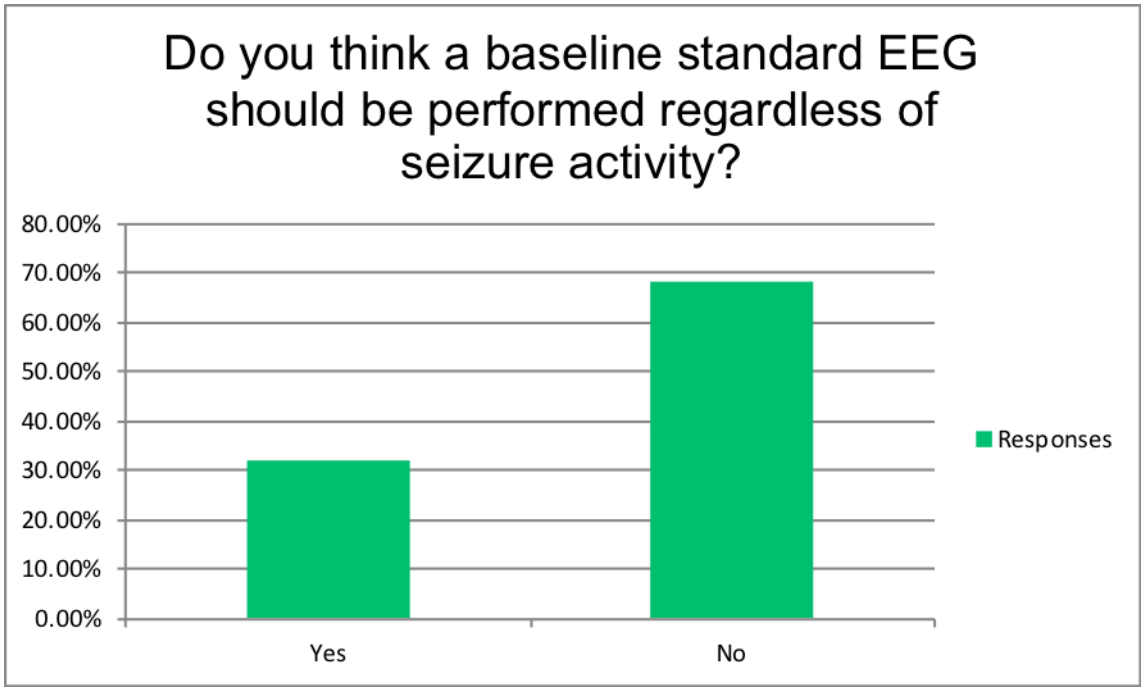

What is your speciality?
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
Neurology
Nephrology
Psychiatry
Psychology
Oncology
General Paediatrics
Dermatology
Urology
Radiology
Genetics
Neurosurgery
Respiratory
Neurodisability
Responses

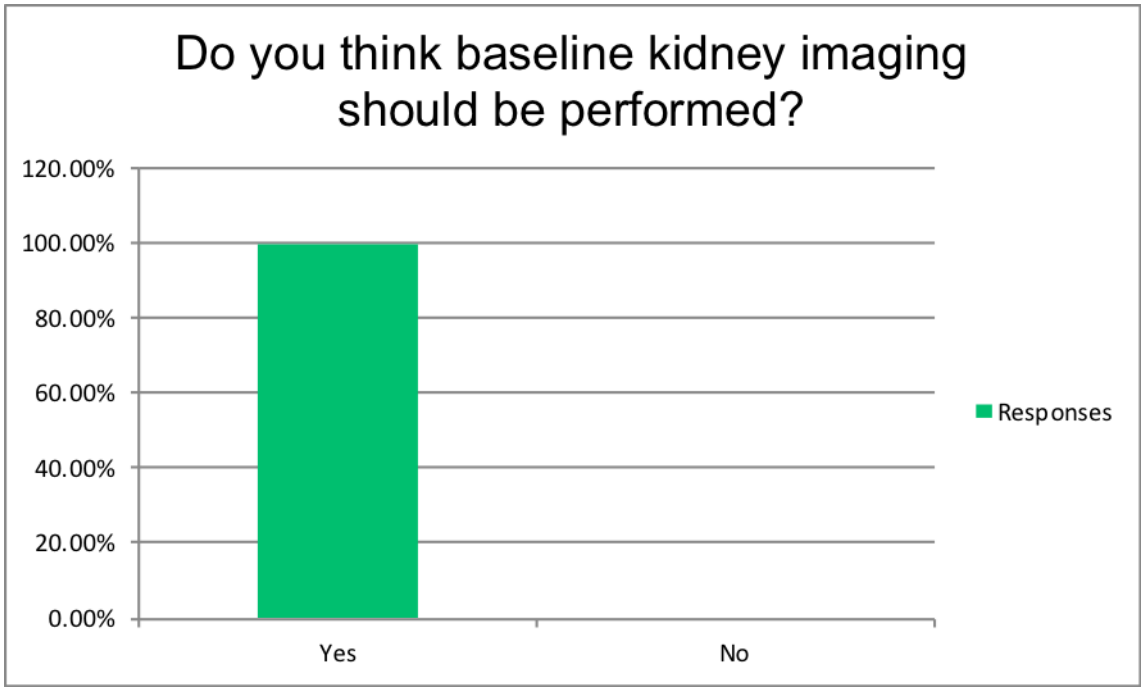

Do you think a genetic test should be offered?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

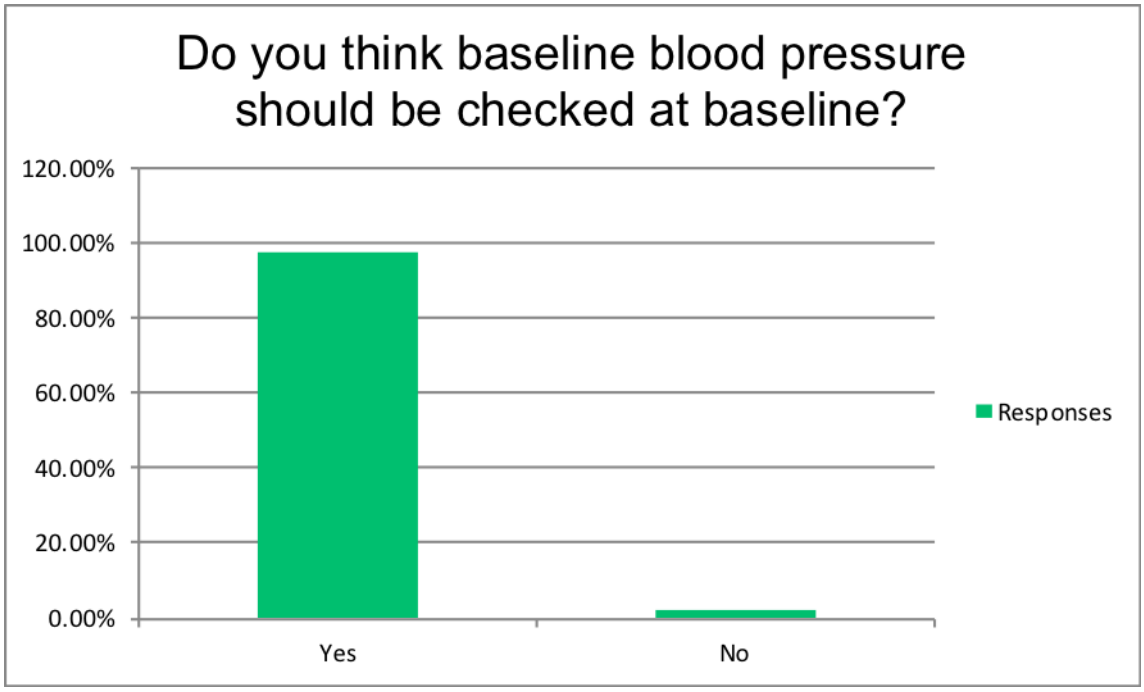

Do you think baseline brain imaging should be performed?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

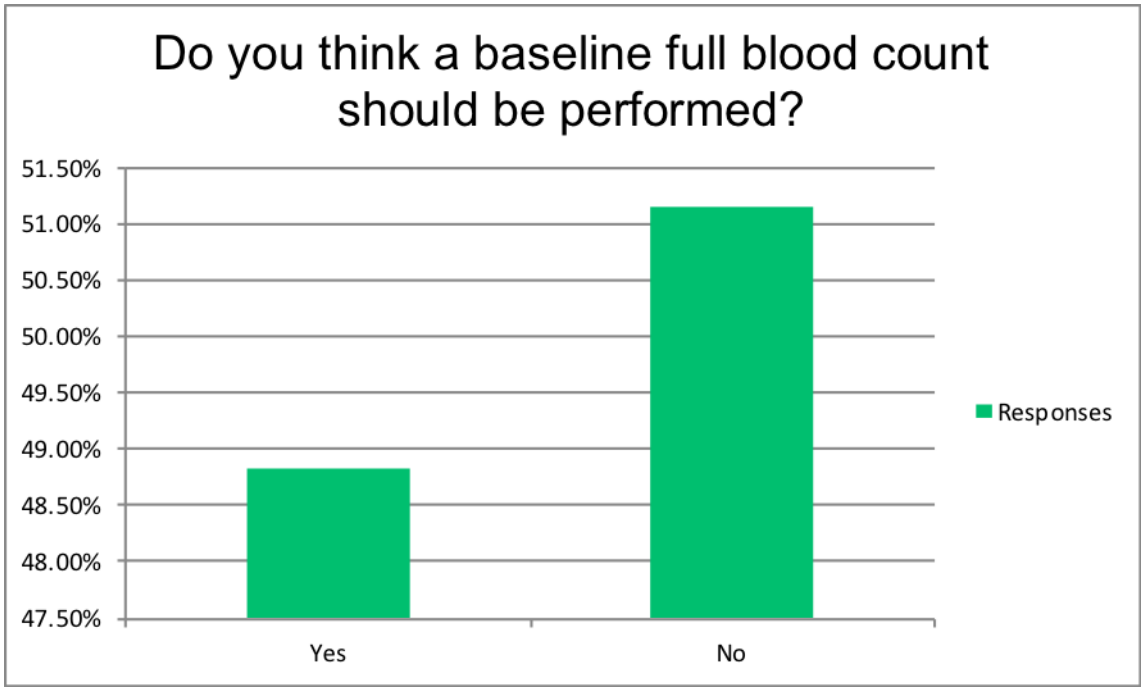

Do you think a baseline neuropsychiatric assessment should be performed?
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

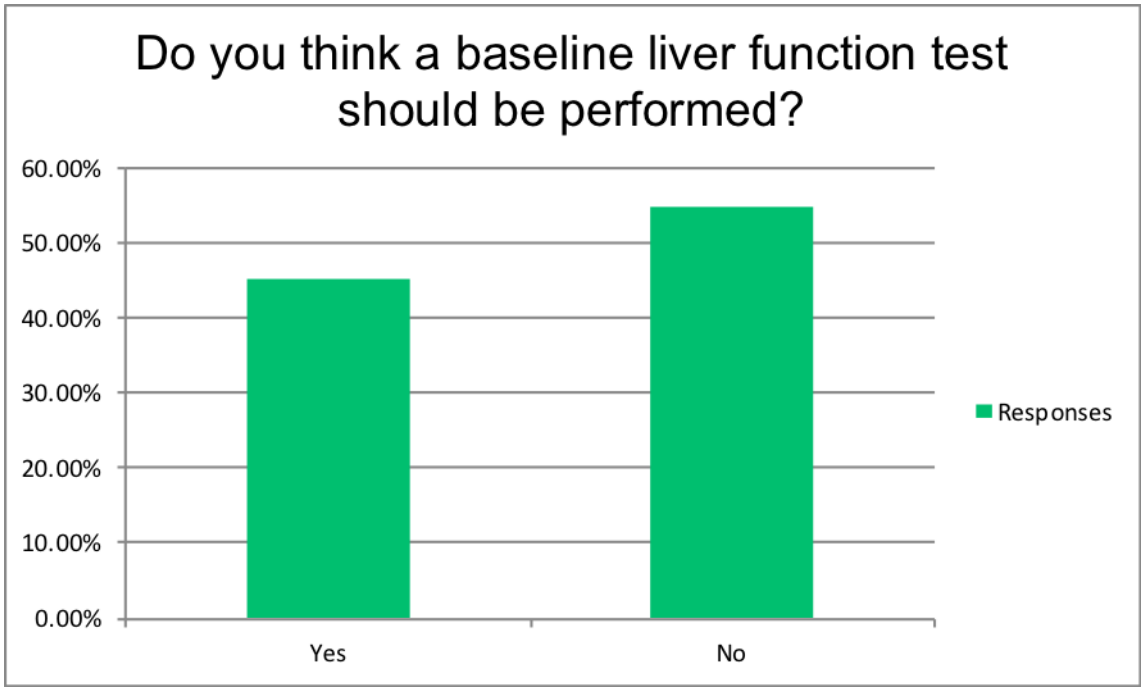

Do you think a baseline neuropsychology assessment should be performed?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

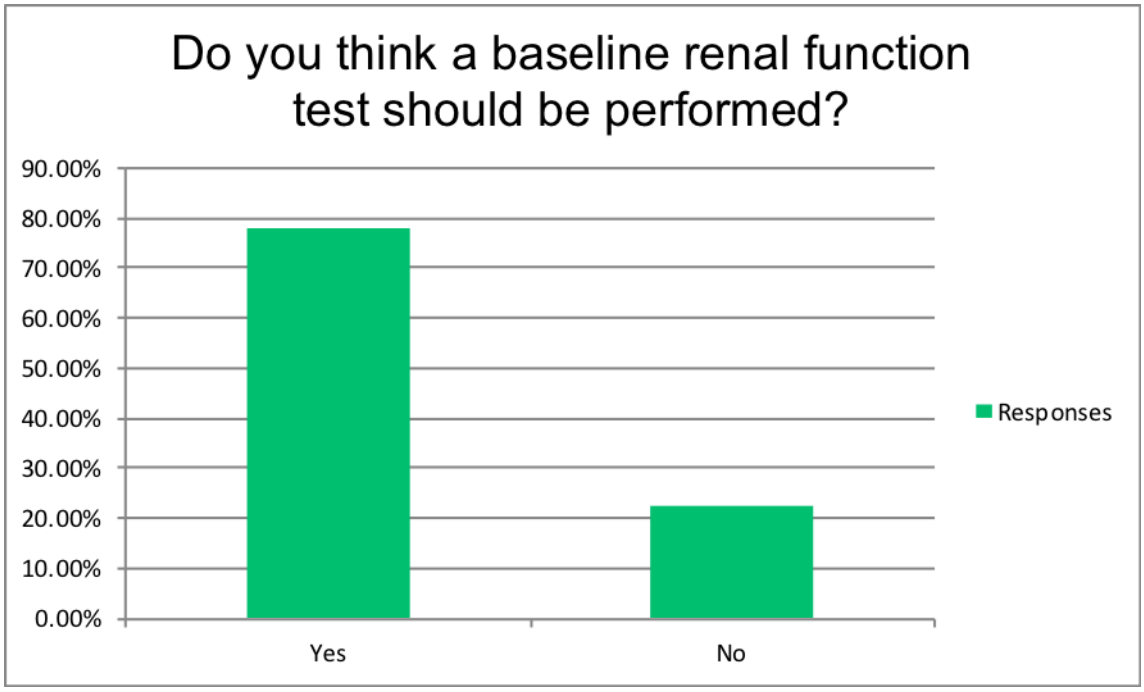

Do you think a baseline standard EEG should be performed regardless of seizure activity?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

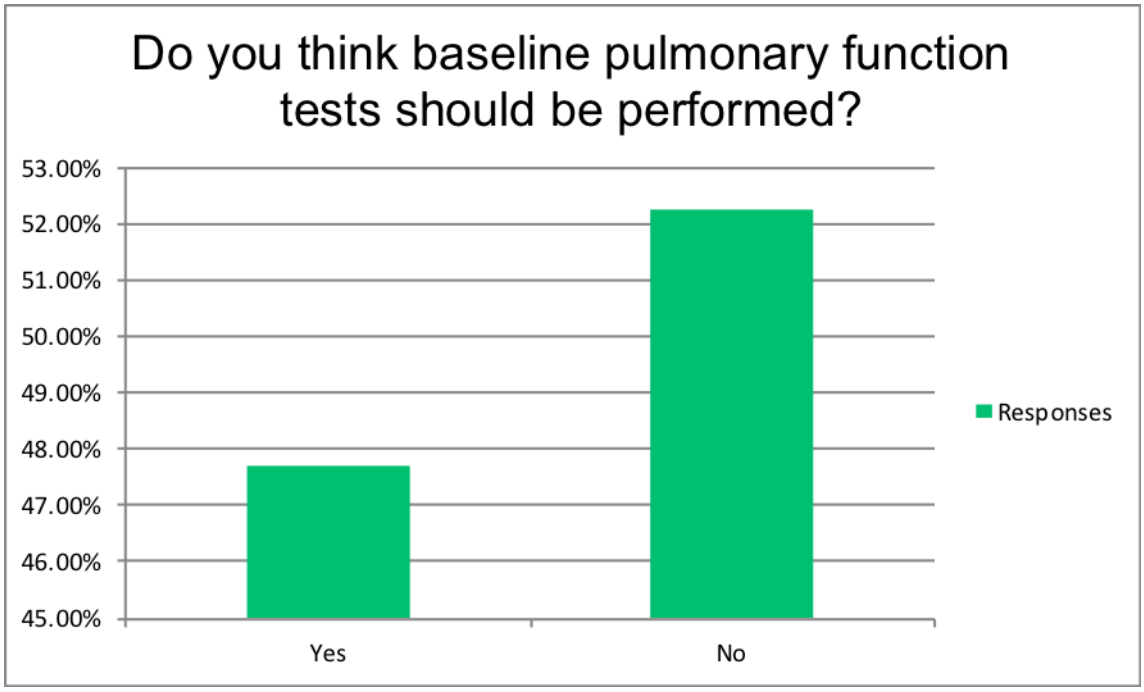

Do you think baseline kidney imaging should be performed?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

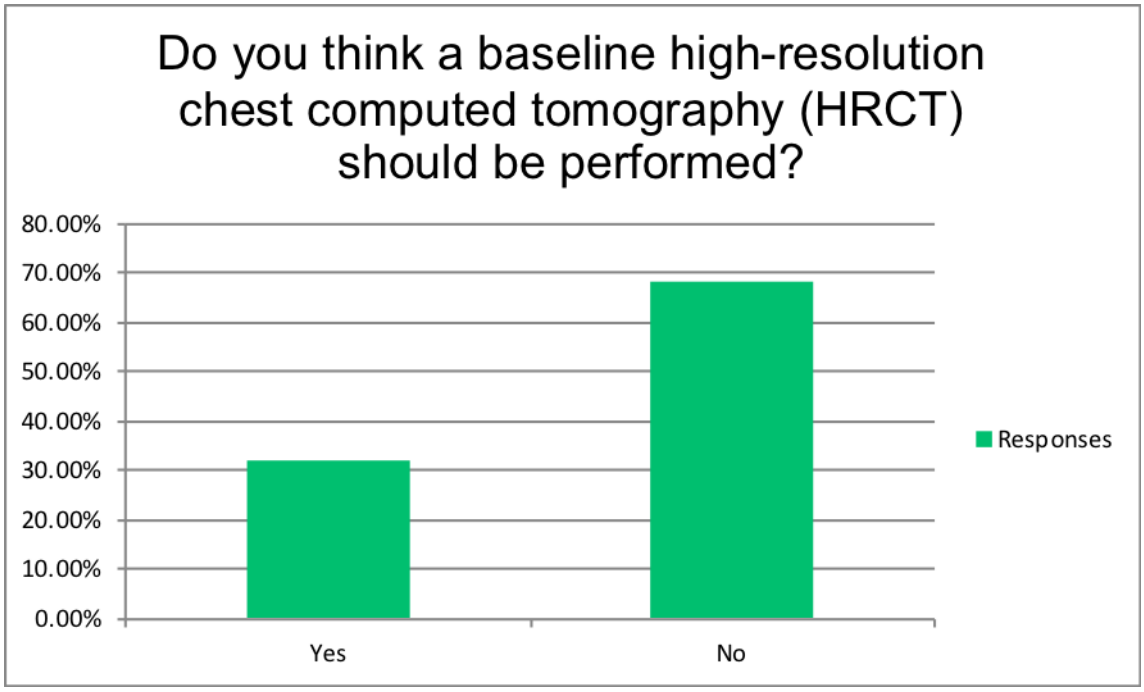

Do you think baseline blood pressure should be checked at baseline?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

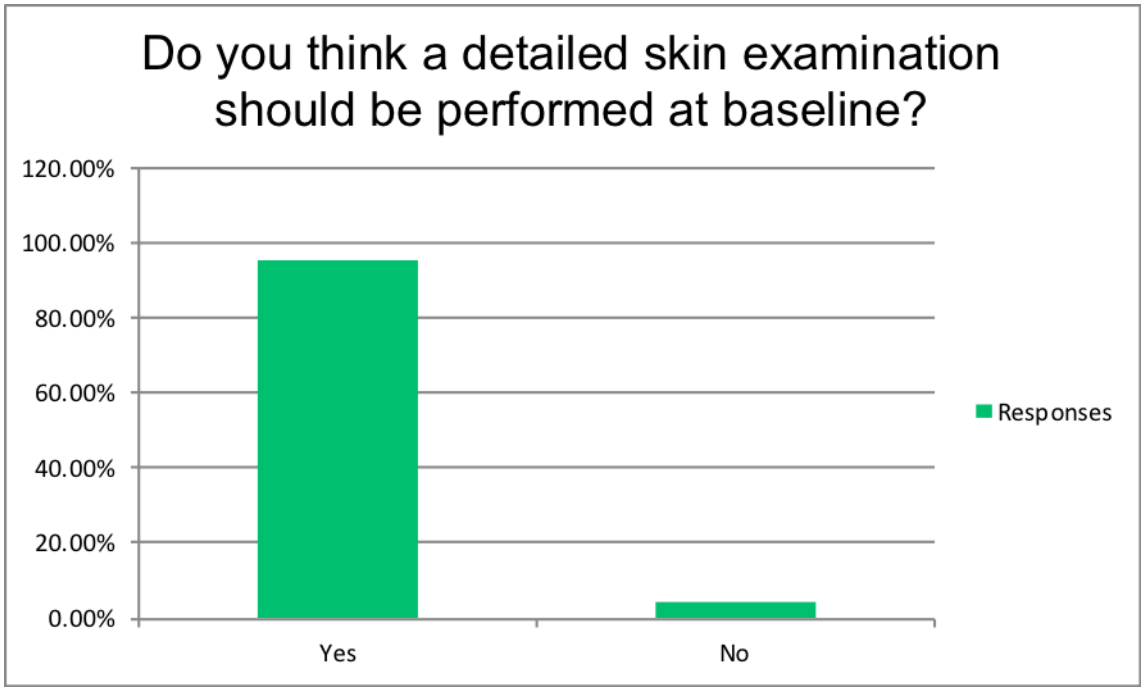

Do you think a baseline full blood count should be performed?
51.50%
51.00%
50.50%
50.00%
49.50%
49.00%
48.50%
48.00%
47.50%
Yes
No
Responses

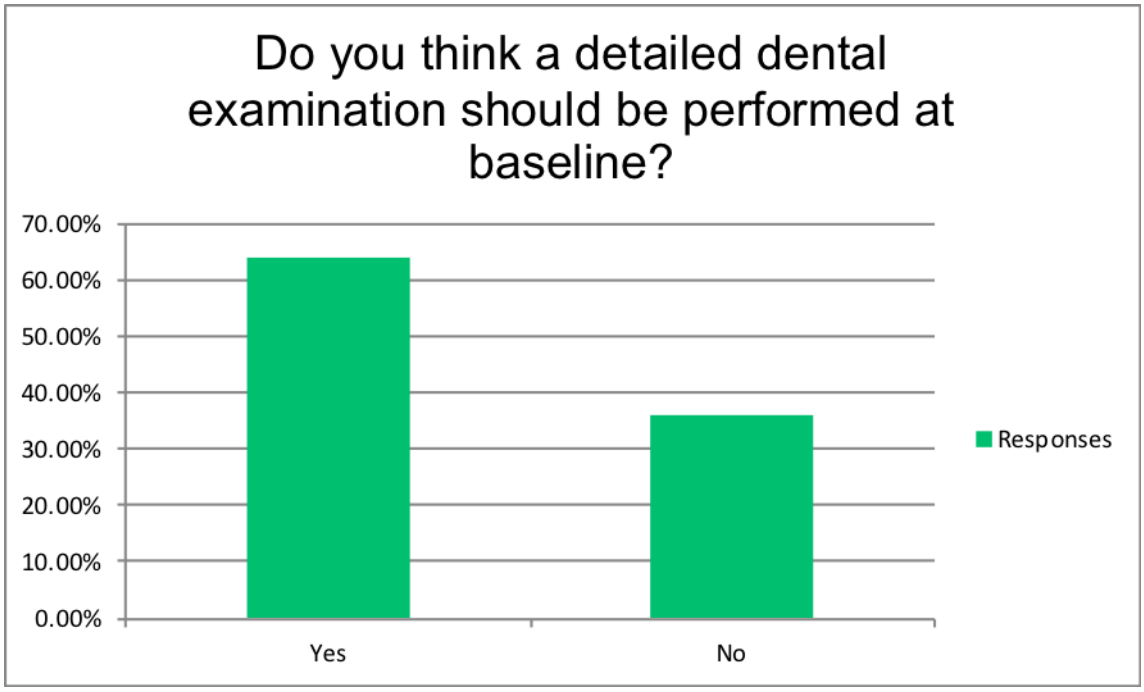

Do you think a baseline liver function test should be performed?
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

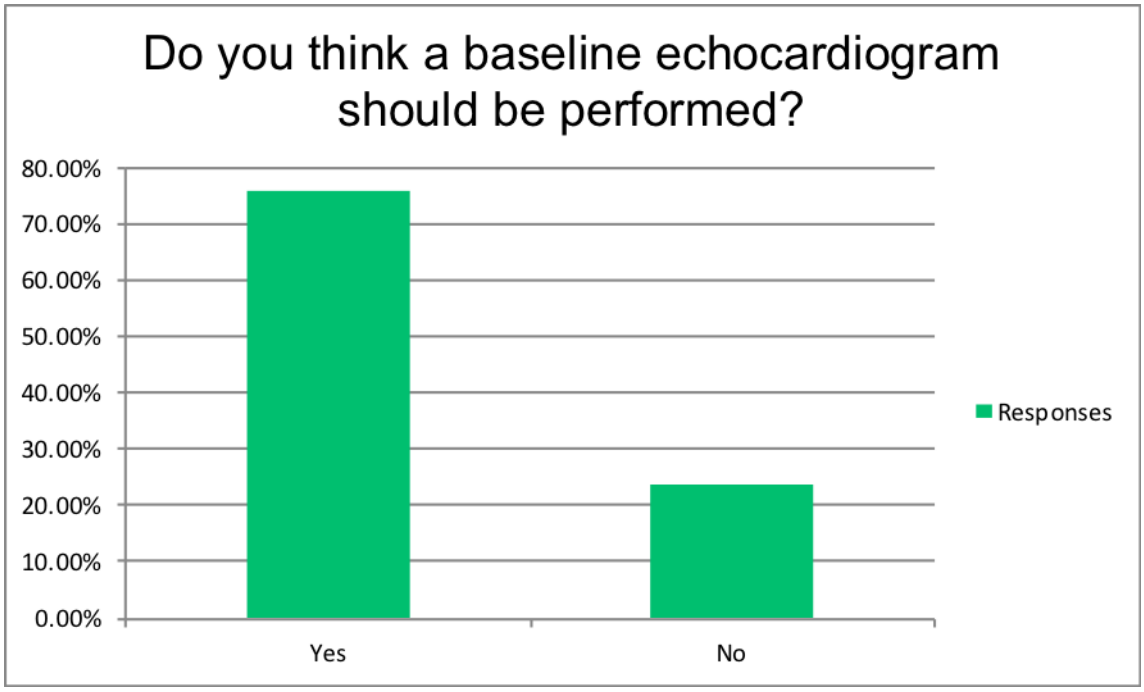

Do you think a baseline renal function test should be performed?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

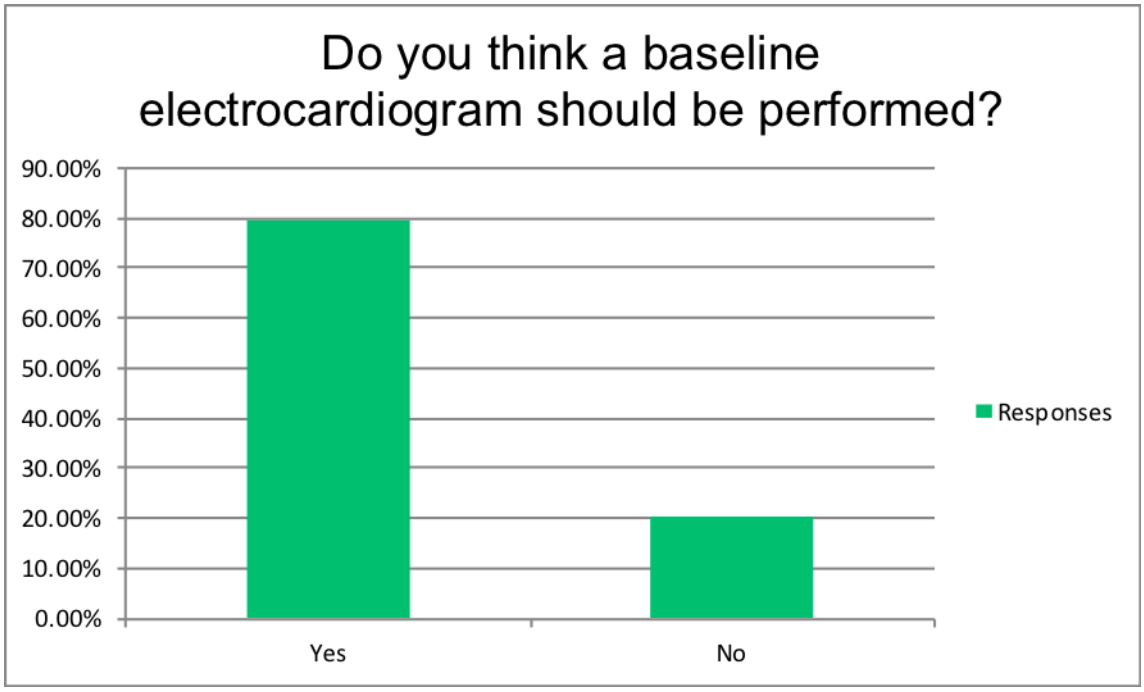

Do you think baseline pulmonary function tests should be performed?
53.00%
52.00%
51.00%
50.00%
49.00%
48.00%
47.00%
46.00%
45.00%
Yes
No
Responses

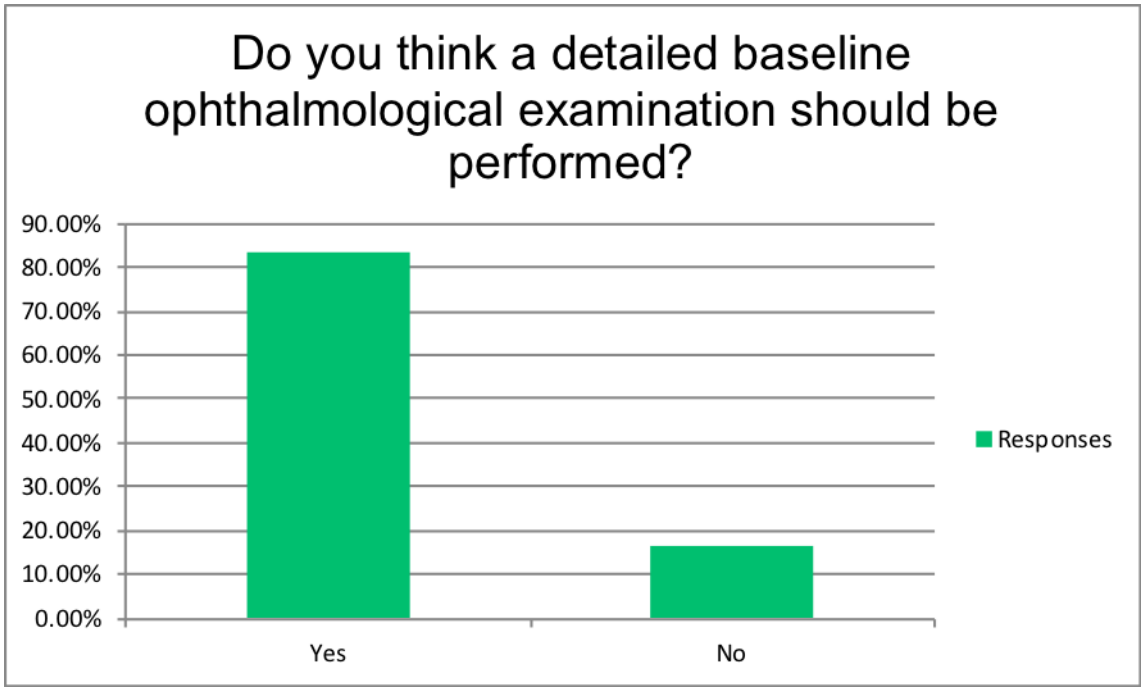

Do you think a baseline high-resolution chest computed tomography (HRCT) should be performed?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

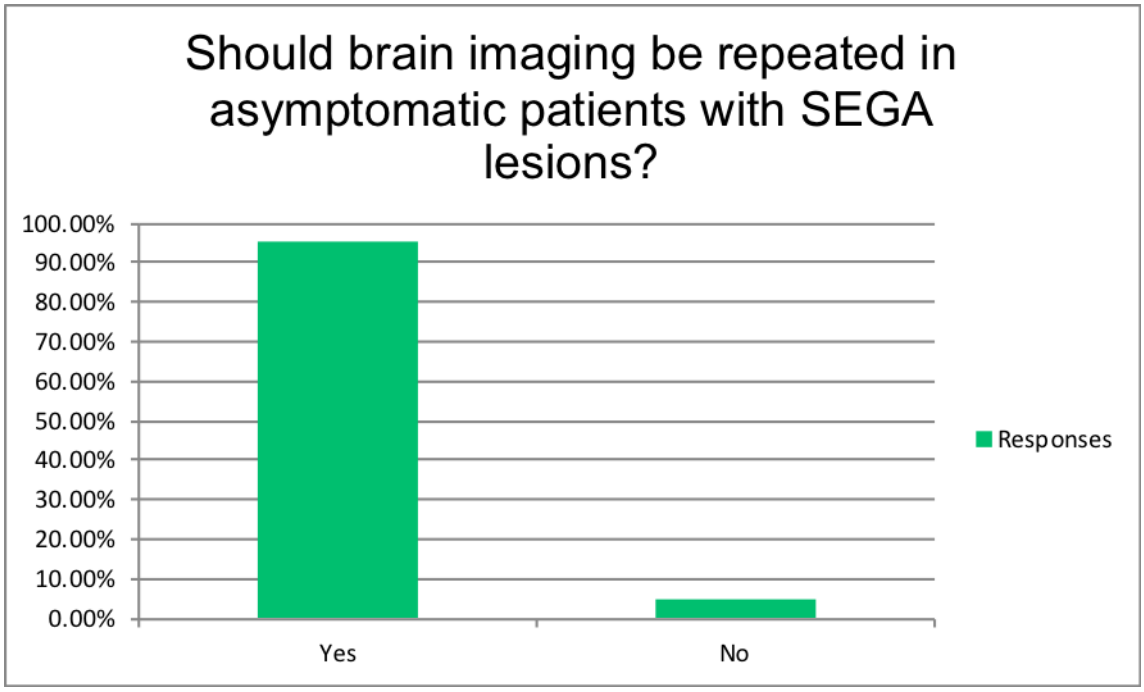

Do you think a detailed skin examination should be performed at baseline?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

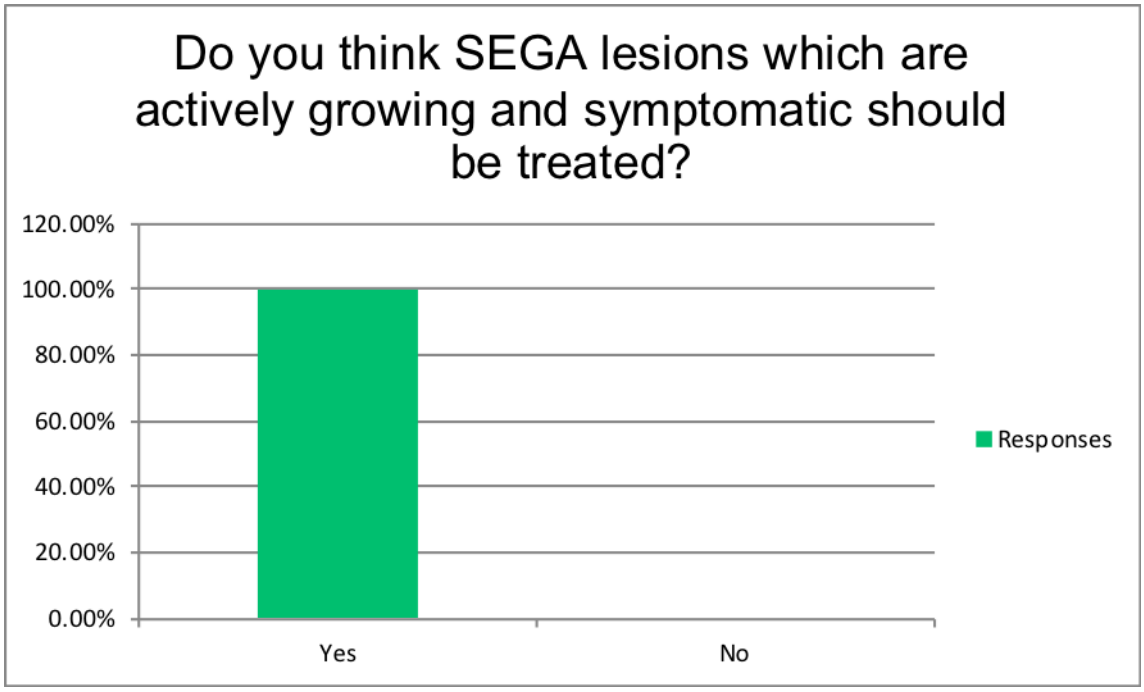

Do you think a detailed dental examination should be performed at baseline?
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

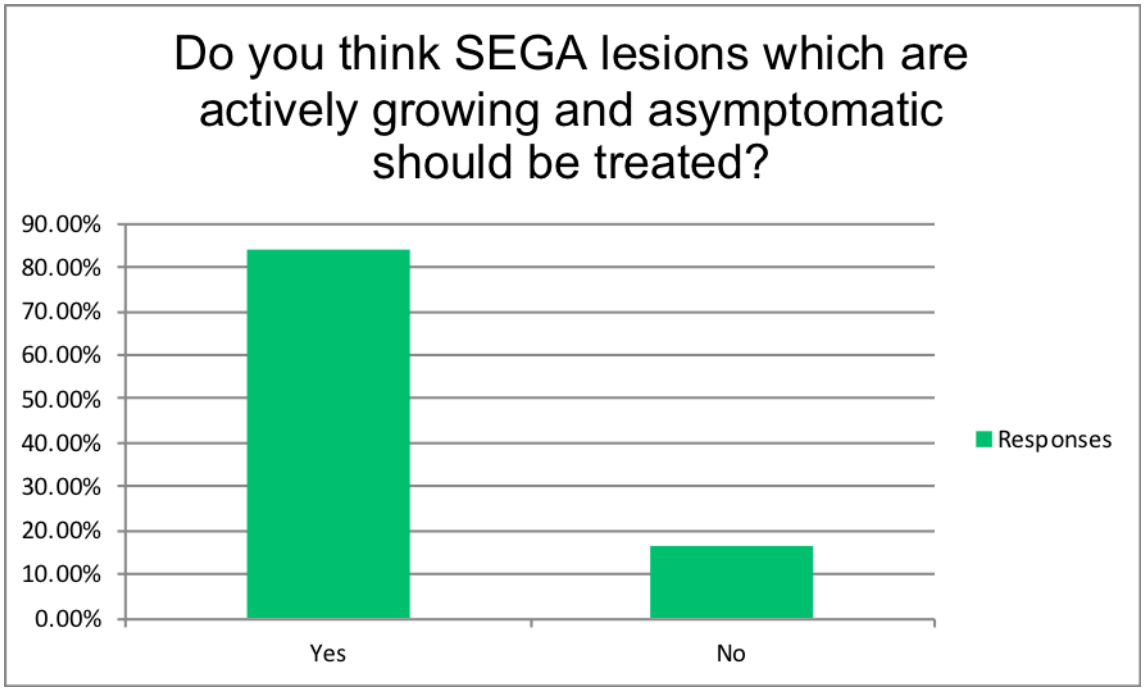

Do you think a baseline echocardiogram should be performed?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

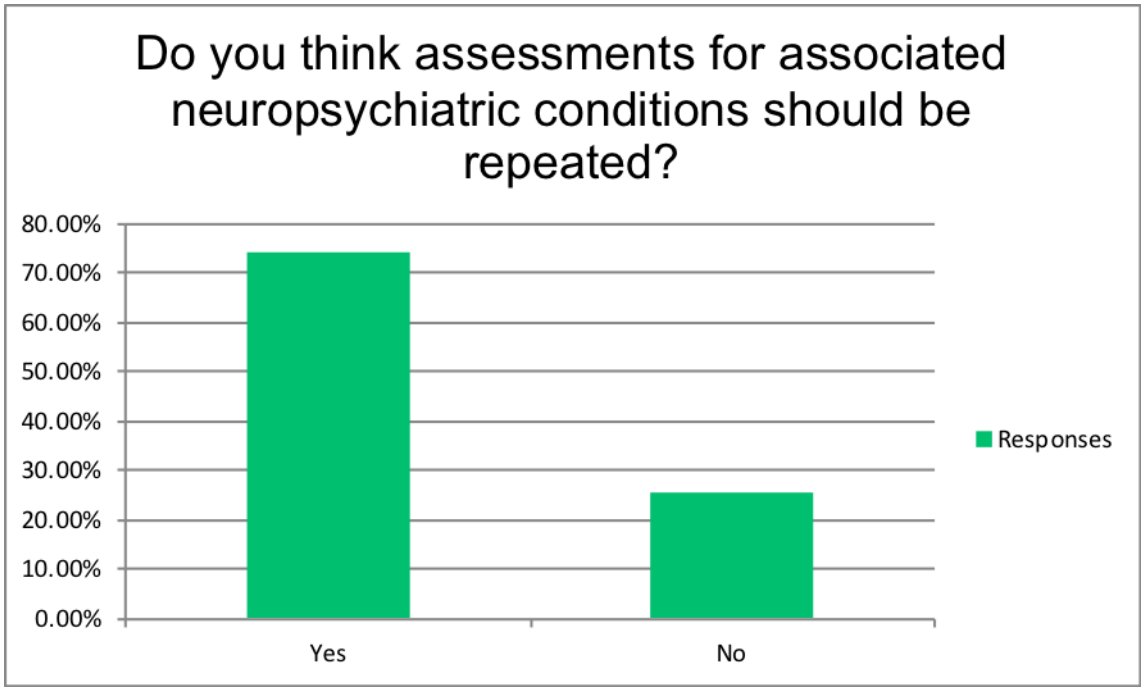

Do you think a baseline electrocardiogram should be performed?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

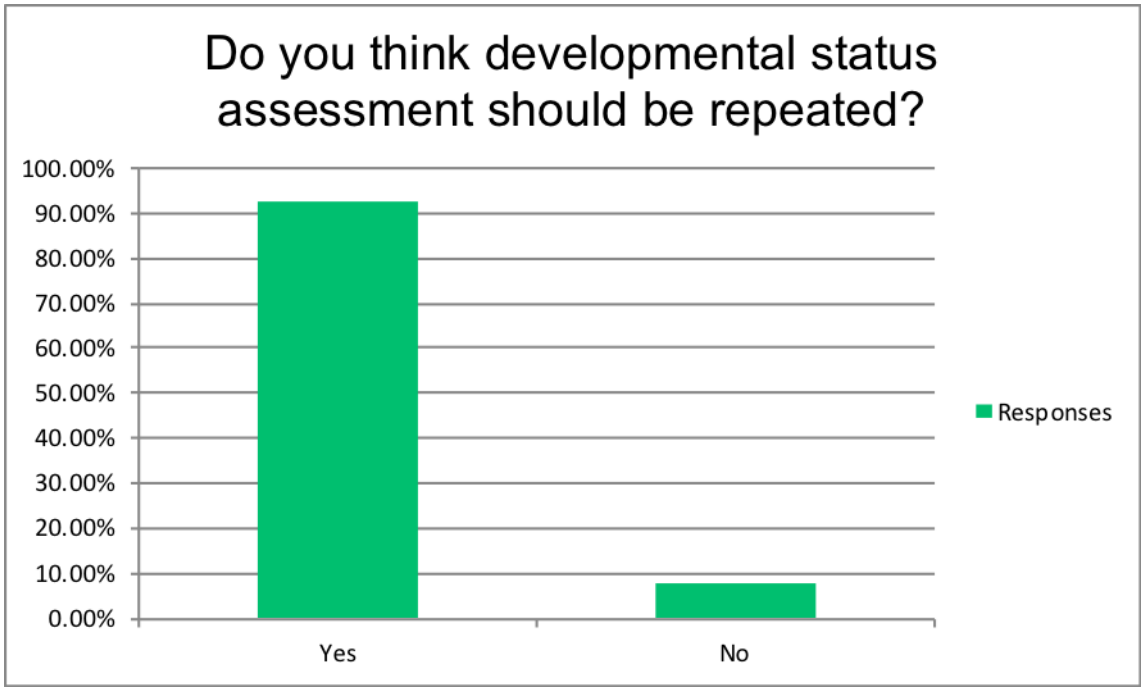

Do you think a detailed baseline ophthalmological examination should be performed?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses


Should brain imaging be repeated in asymptomatic patients with SEGA lesions?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

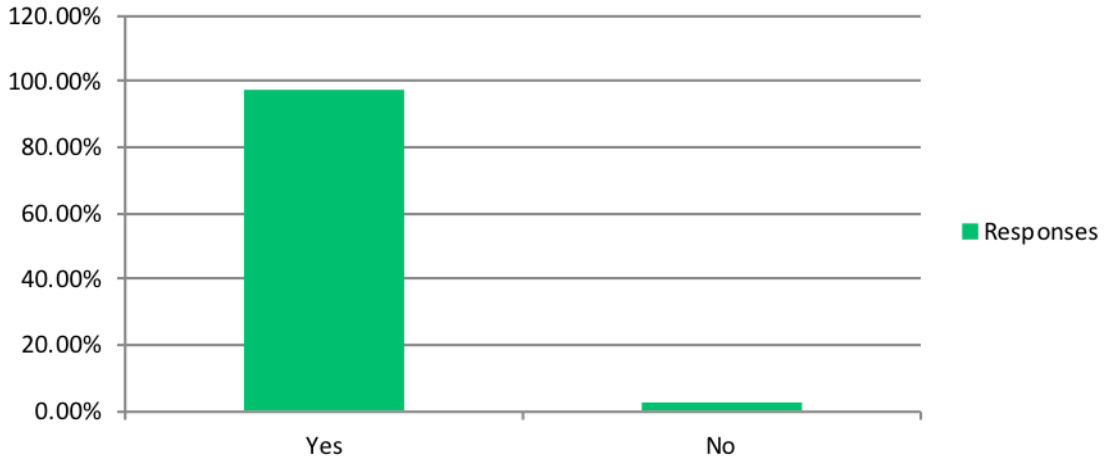

Do you think SEGA lesions which are actively growing and symptomatic should be treated?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

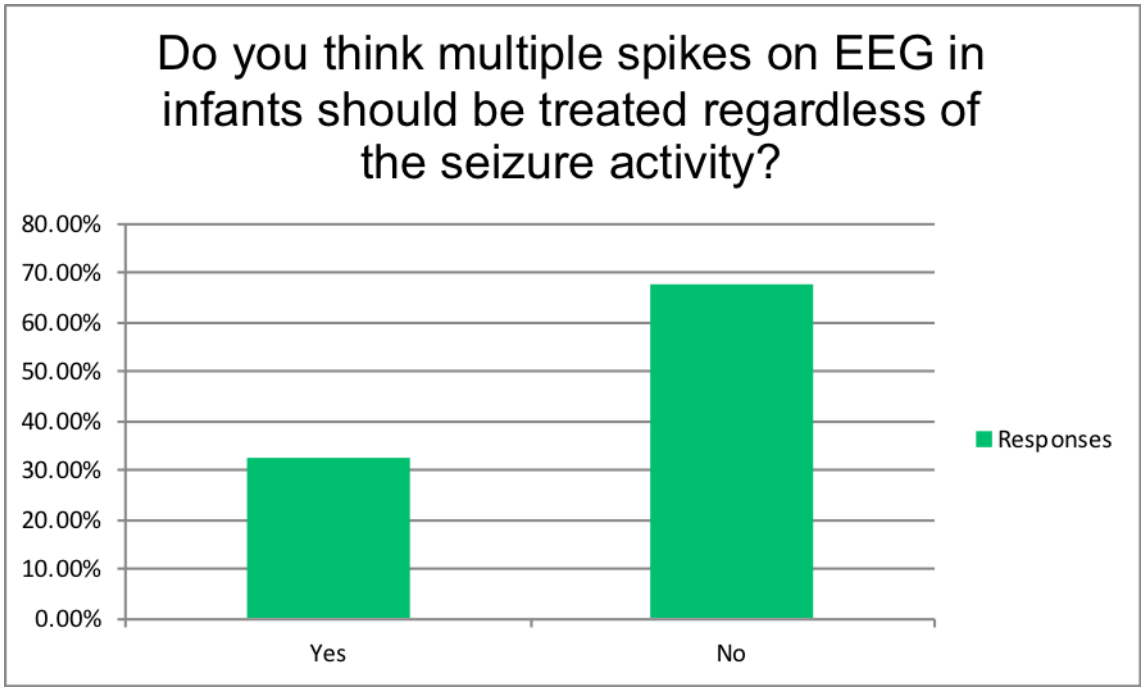

Do you think SEGA lesions which are actively growing and asymptomatic should be treated?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

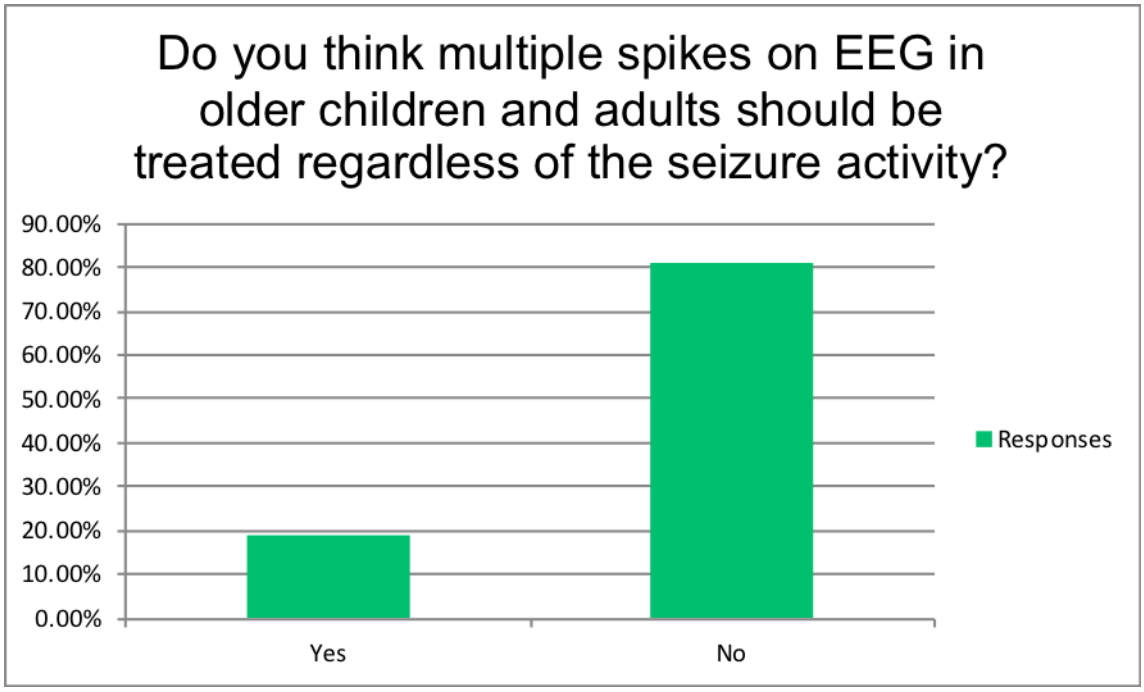

Do you think assessments for associated neuropsychiatric conditions should be repeated?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

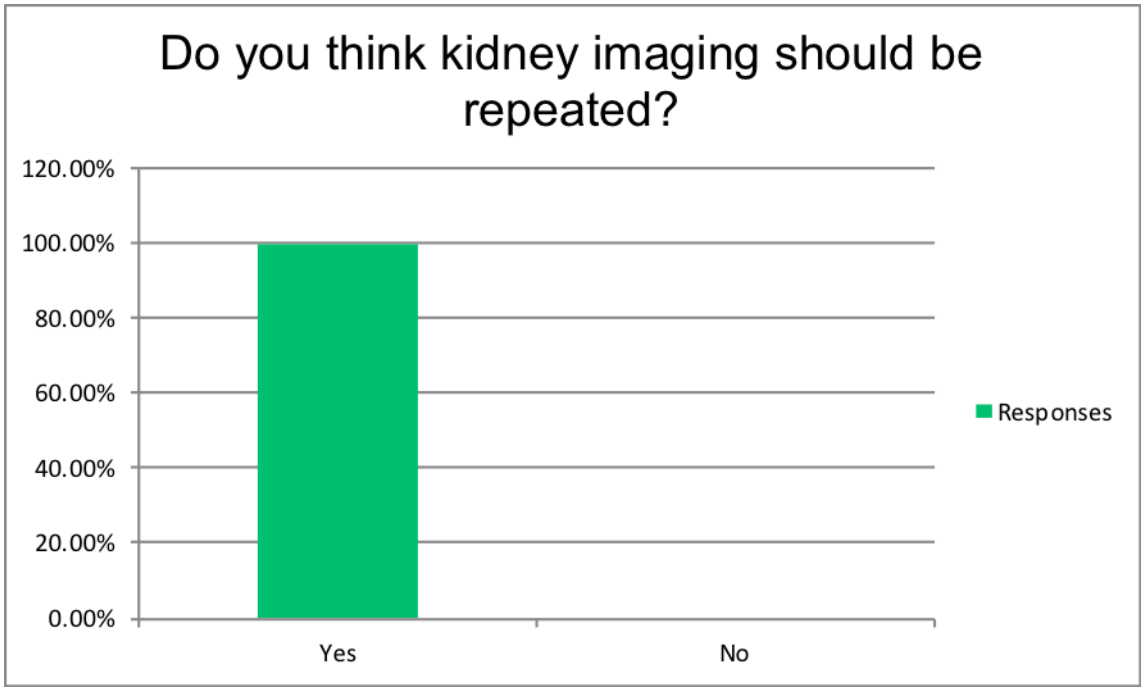

Do you think developmental status assessment should be repeated?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

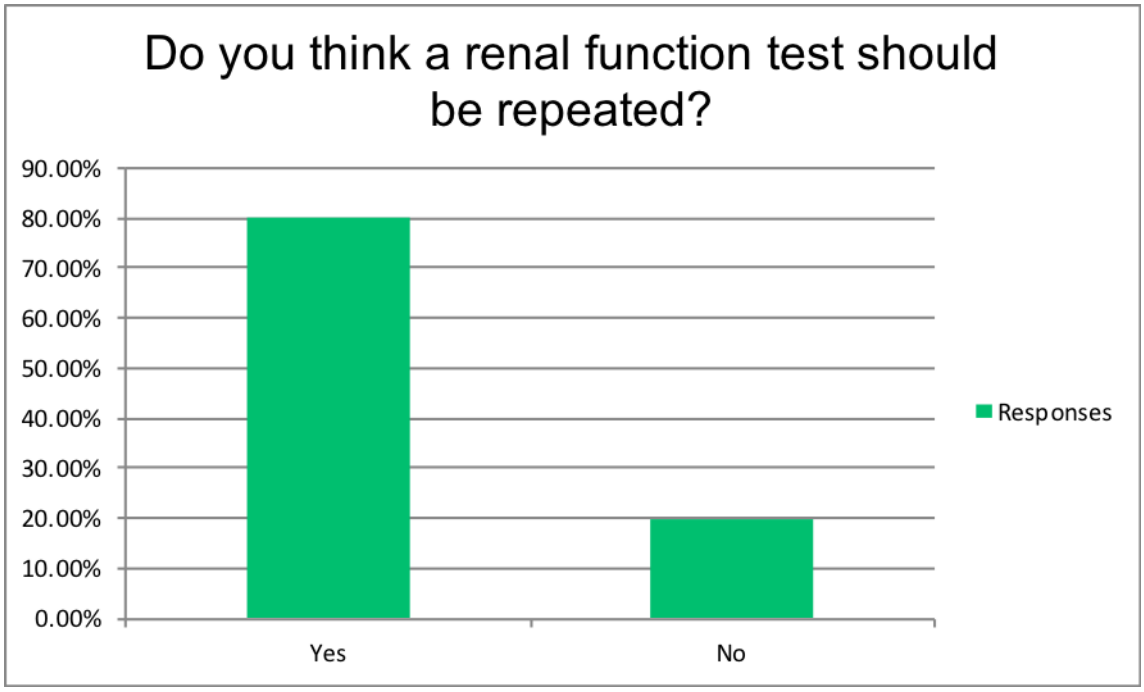
Do you think children with infantile spasms should be treated with anticonvulsant medications?

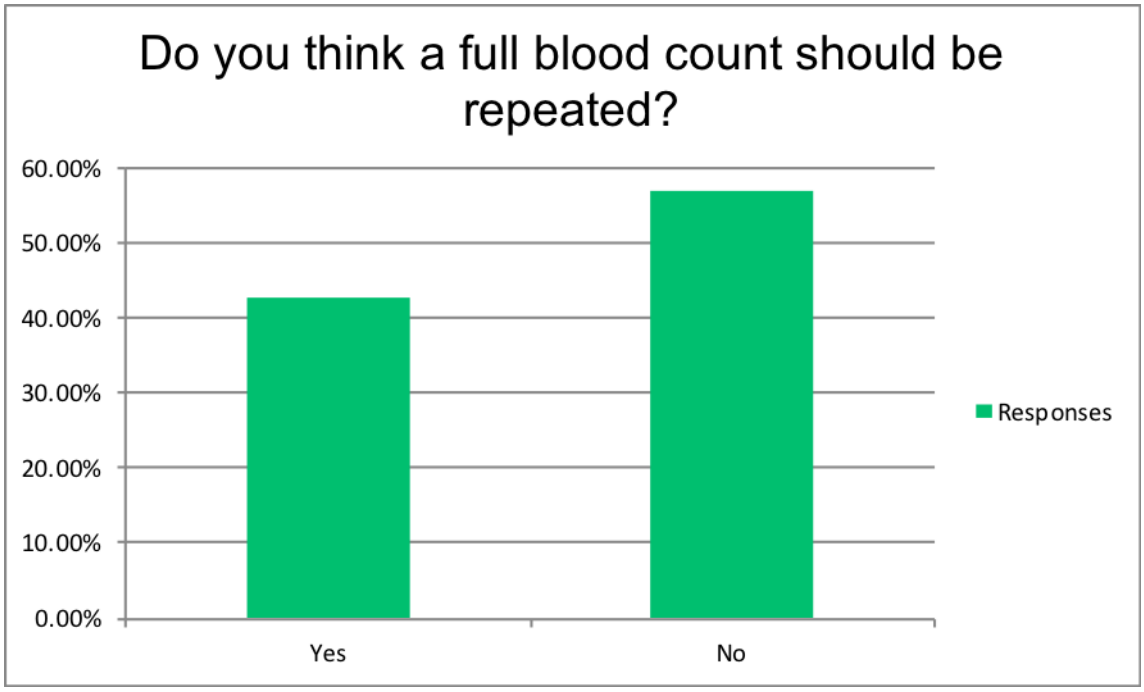


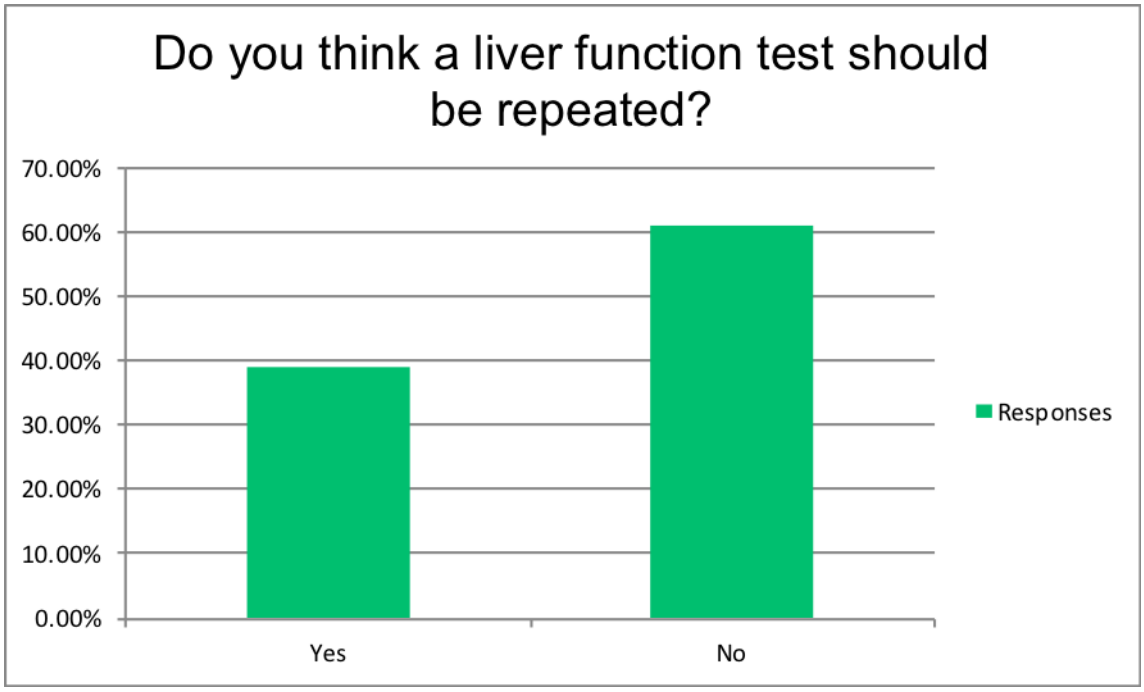

Do you think multiple spikes on EEG in infants should be treated regardless of the seizure activity?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

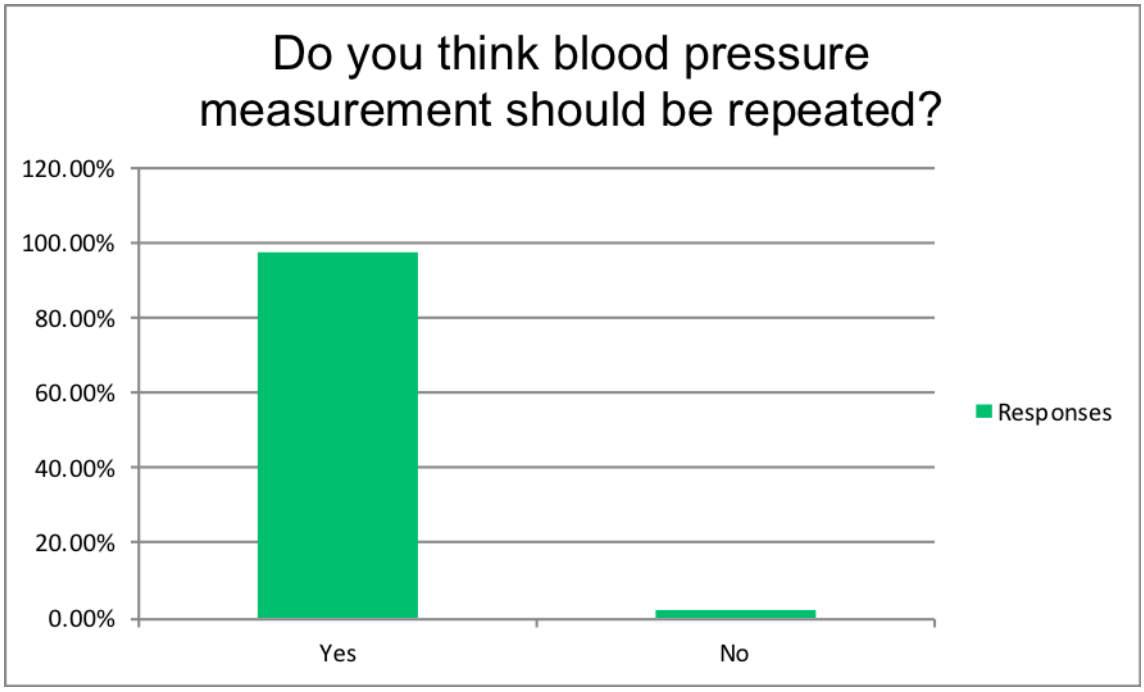

Do you think multiple spikes on EEG in older children and adults should be treated regardless of the seizure activity?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

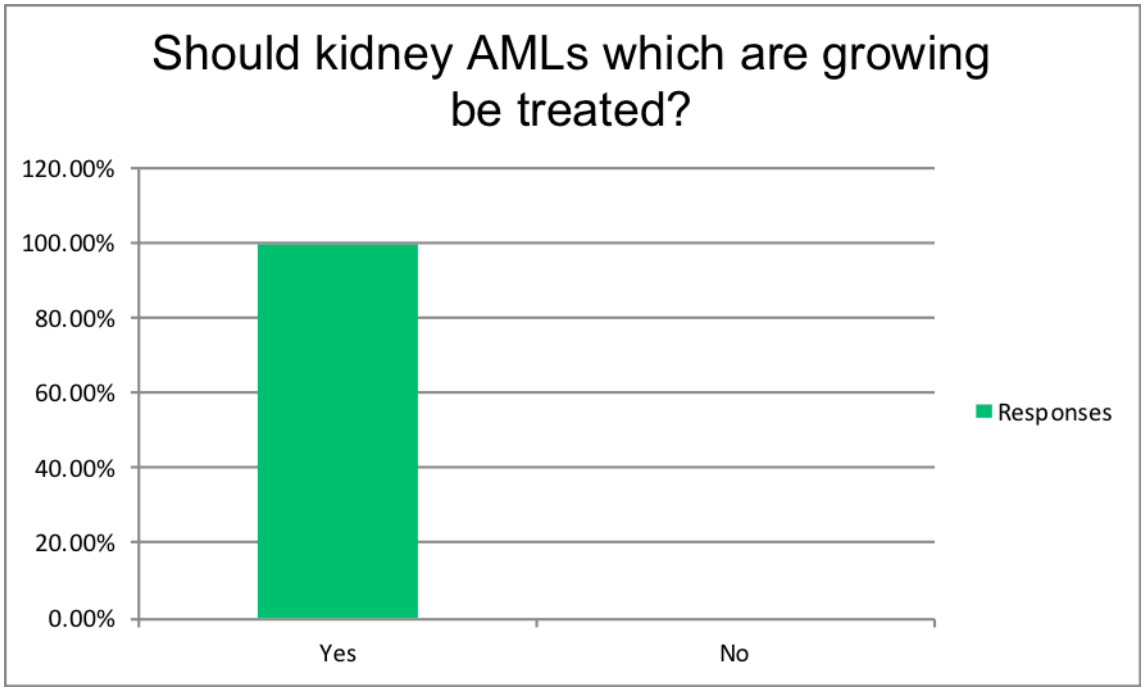

Do you think kidney imaging should be repeated?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

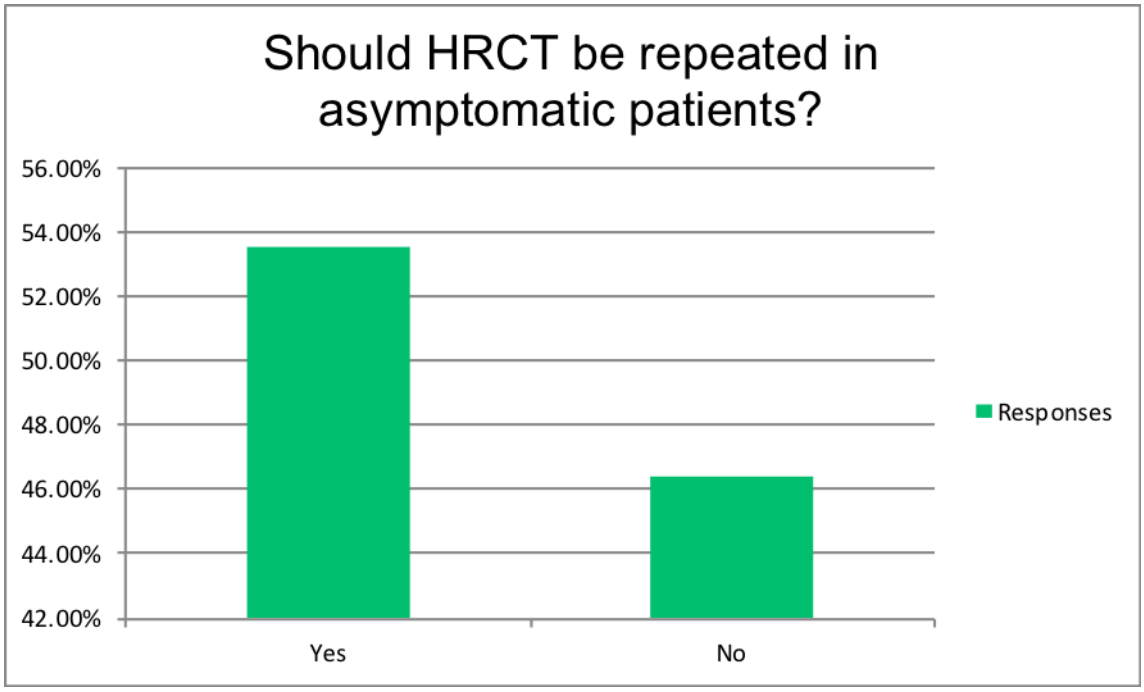

Do you think a renal function test should be repeated?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

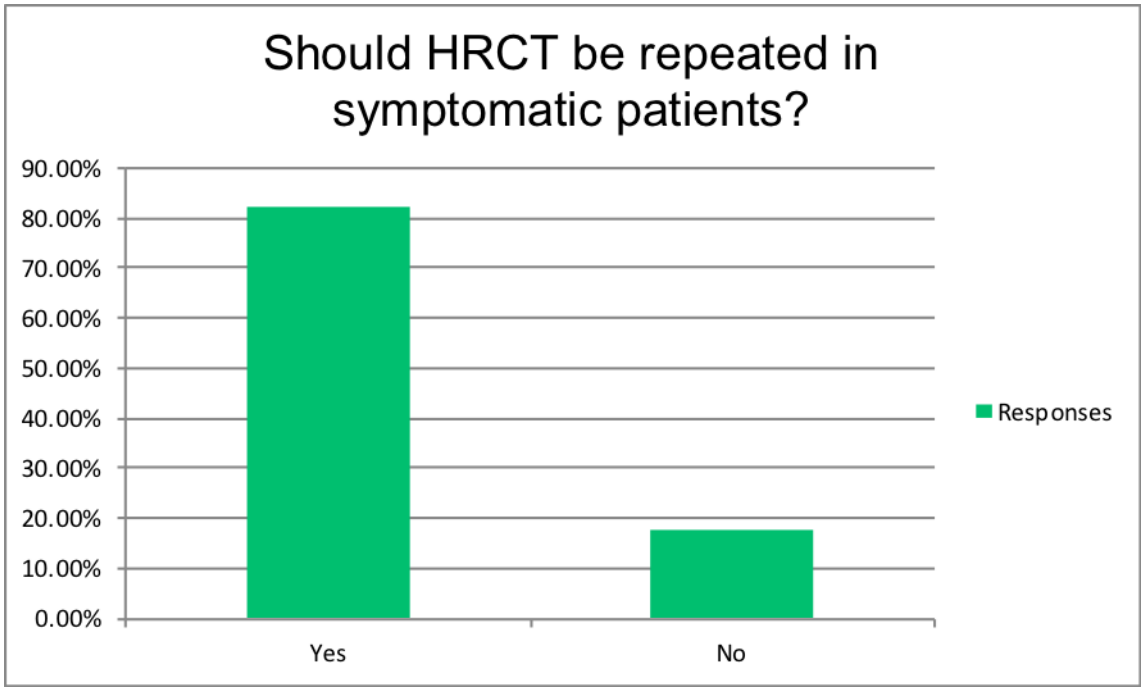

Do you think a full blood count should be repeated?
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

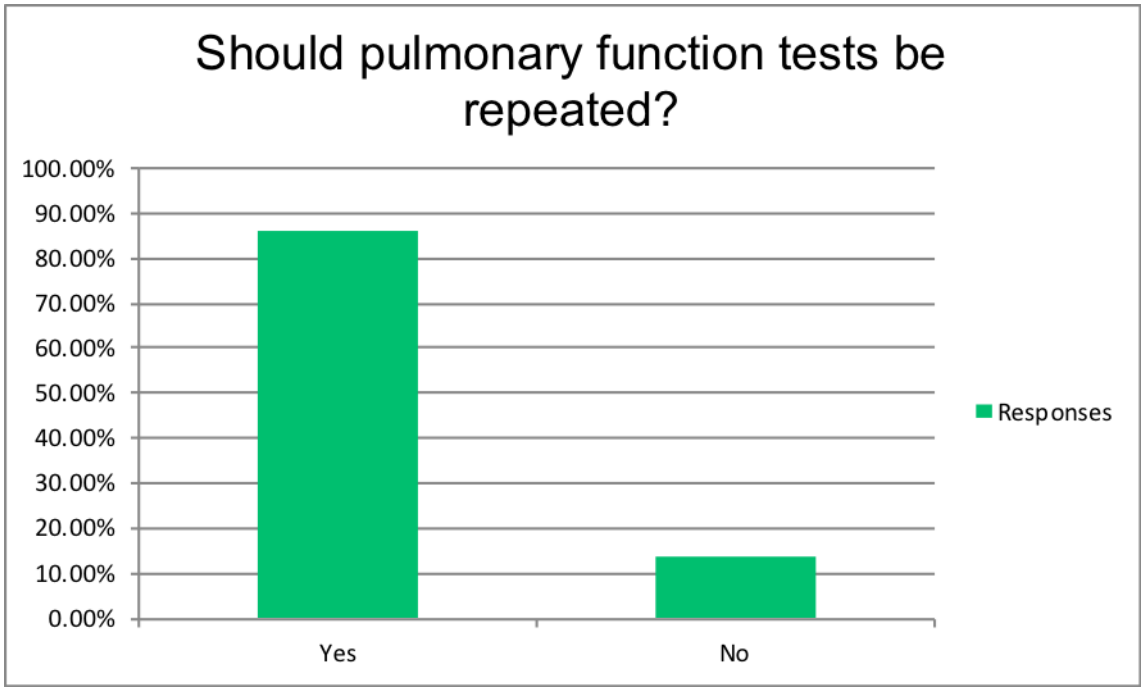

Do you think a liver function test should be repeated?
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

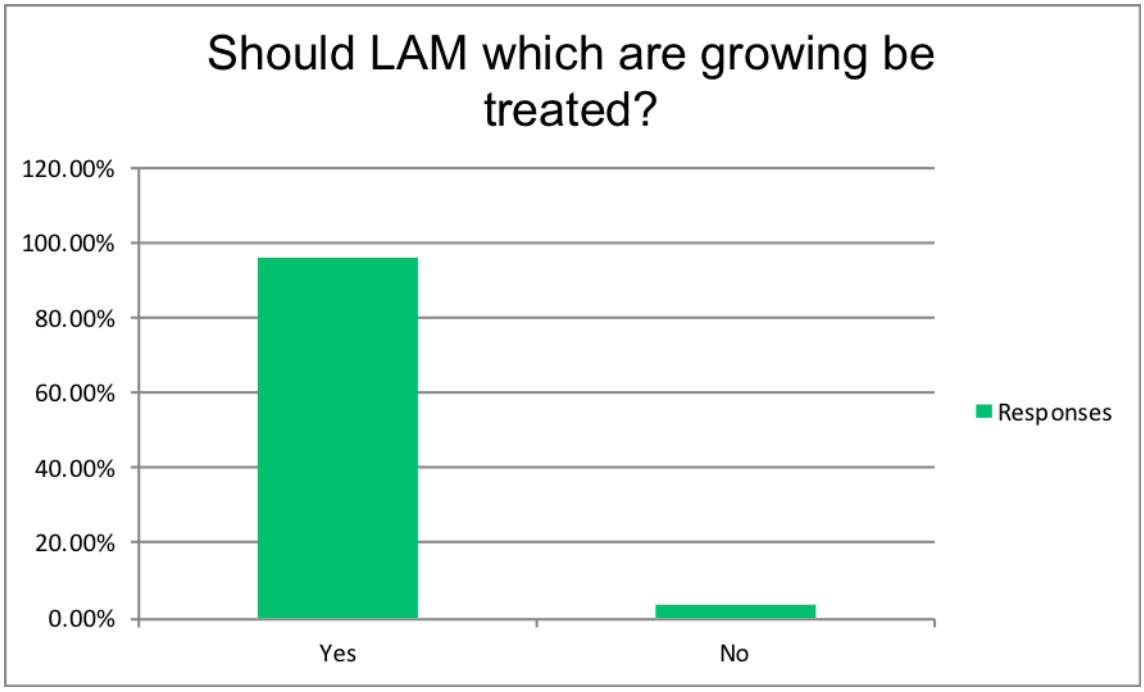

Do you think blood pressure measurement should be repeated?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

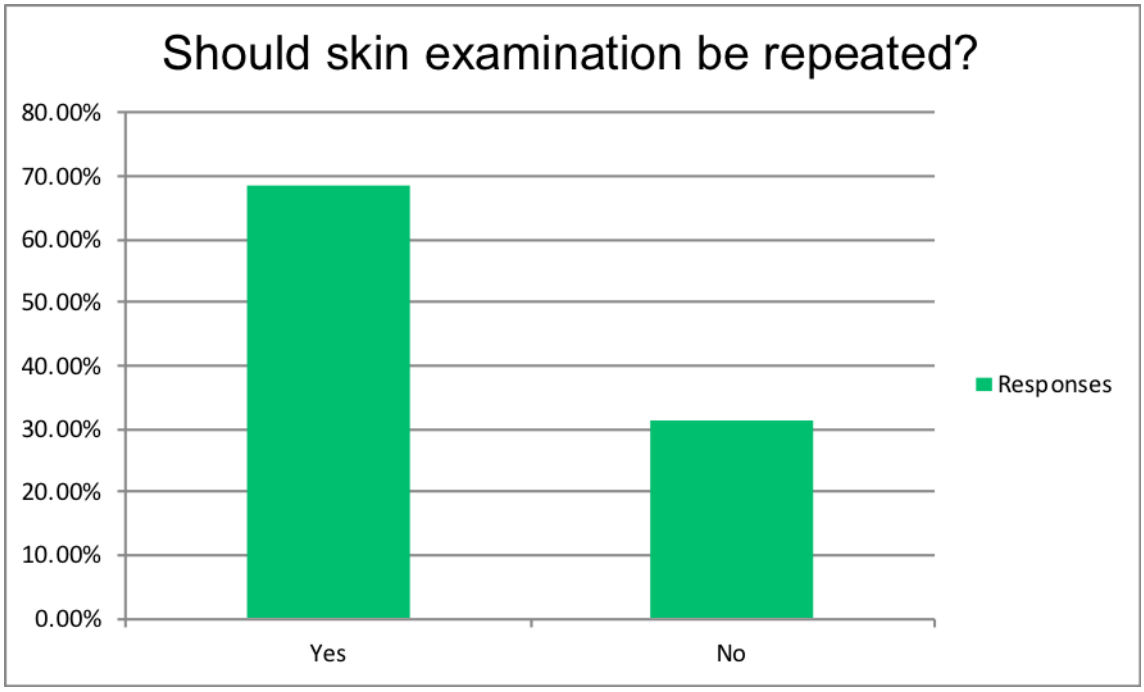

Should kidney AMLs which are growing be treated?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

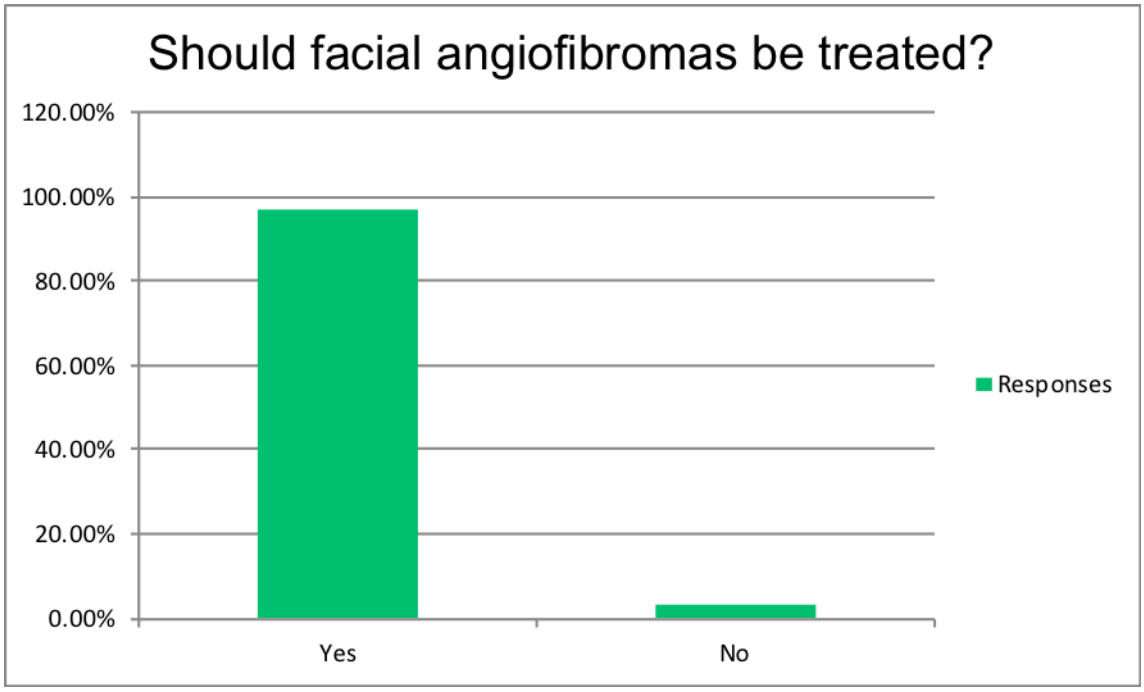

Should HRCT be repeated in asymptomatic patients?
56.00%
54.00%
52.00%
50.00%
48.00%
46.00%
44.00%
42.00%
Yes
No
Responses

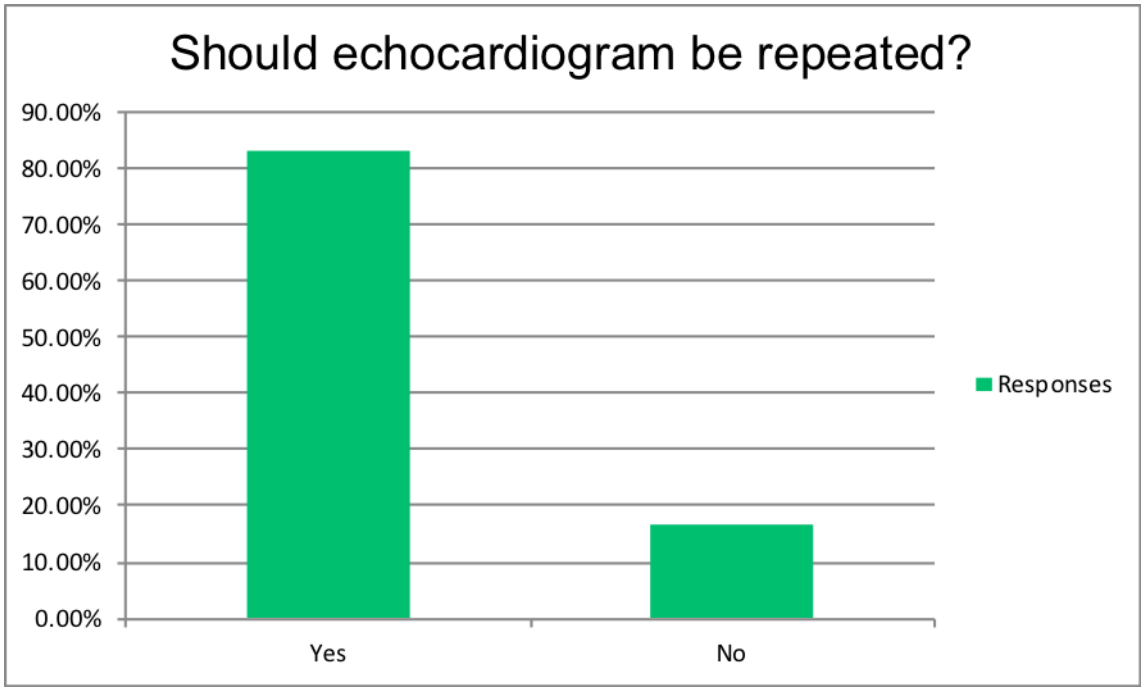

Should HRCT be repeated in symptomatic patients?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

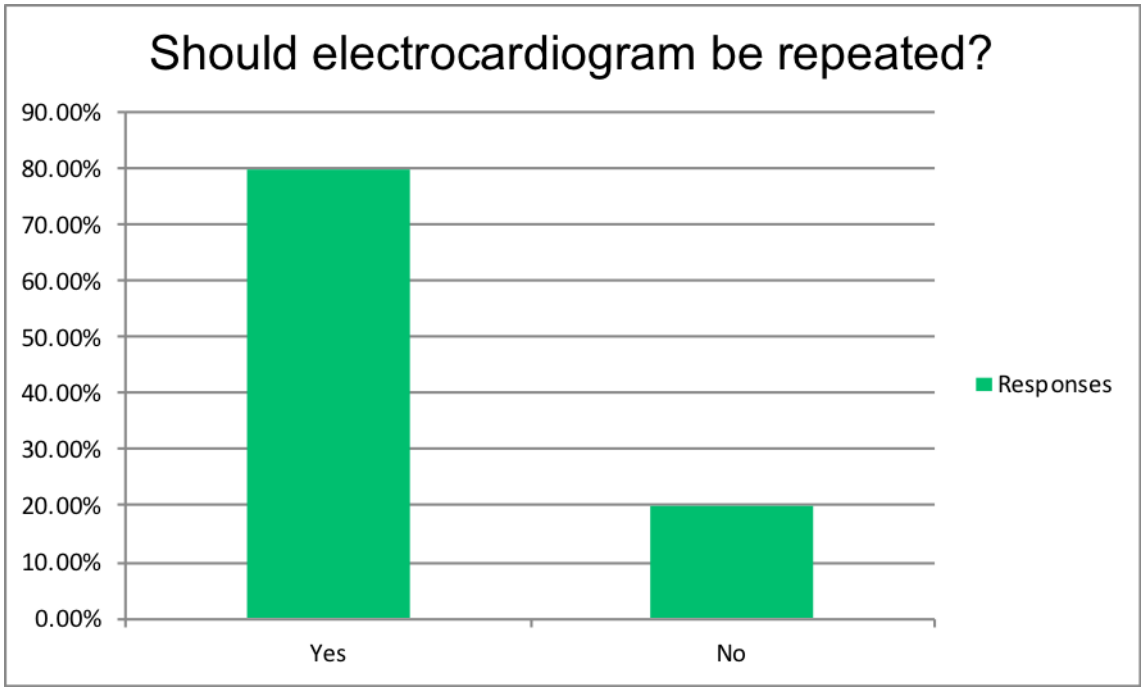

Should pulmonary function tests be repeated?
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

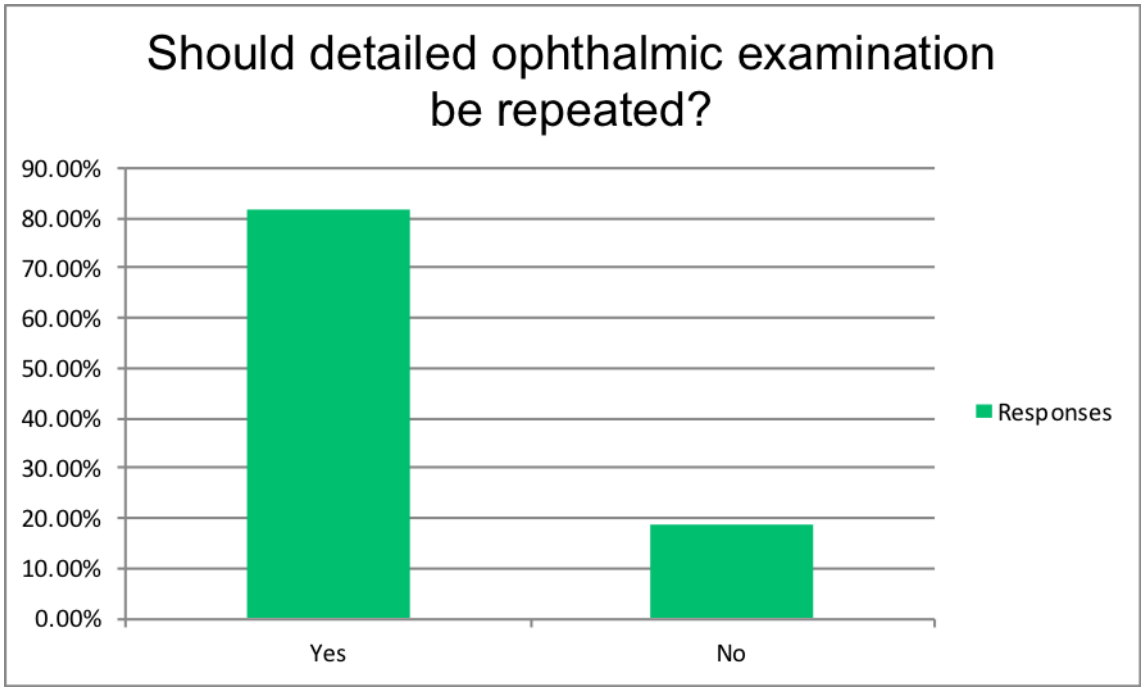

Should LAM which are growing be treated?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses

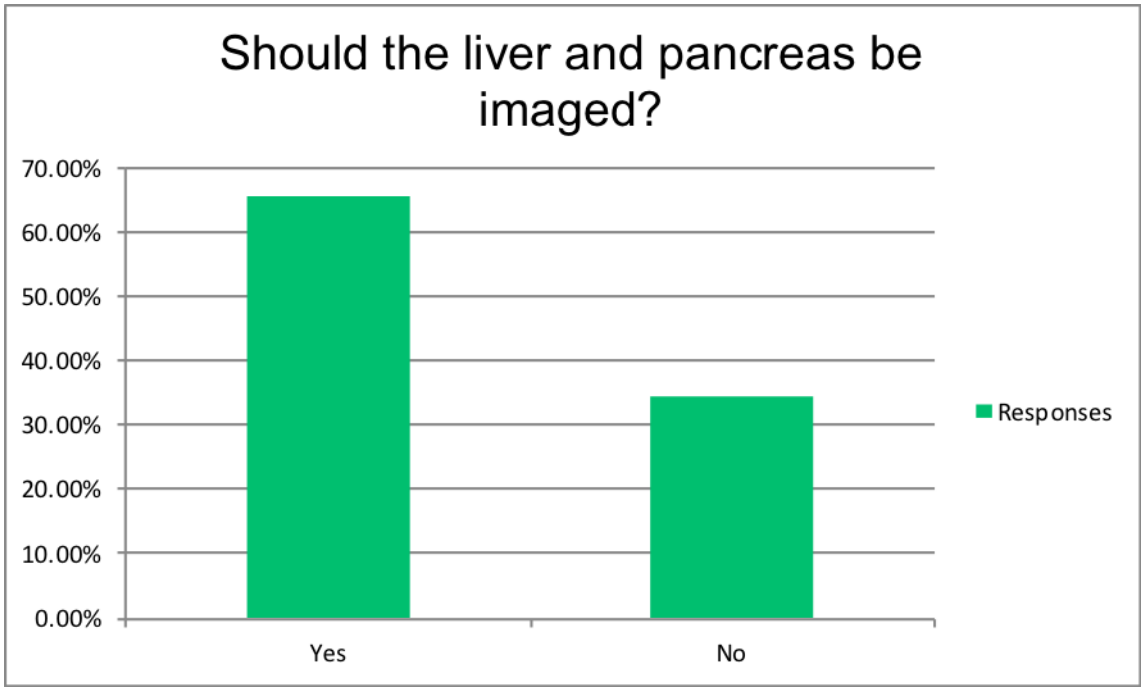

Should skin examination be repeated?
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses


Should facial angiofibromas be treated?
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Yes
No
Responses


Should echocardiogram be repeated?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses


Should electrocardiogram be repeated?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses


Should detailed ophthalmic examination be repeated?
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses


Should the liver and pancreas be imaged?
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Yes
No
Responses

2.2.6 Recommendations:

Genetics – recommendations and results of consensus

Delphi consensus

There was consensus to offer a genetic test at baseline in those with definite or probable TSC. Testing may clarify the diagnosis of TSC in cases that do not fulfil clinical criteria for a definite clinical diagnosis and is a prerequisite for the application of genetic technologies in family planning.(90, 91)

Expert opinion

All patients should have a three-generation family history obtained to determine if additional family members are affected or at risk of TSC. Genetic testing and counselling should be offered to individuals with TSC when they reach reproductive age. First-degree relatives of affected individuals should be offered clinical assessment and where possible genetic testing, in families in which a variant has been identified in the index case.

Epilepsy– recommendations and results of consensus

Delphi consensus

There is debate as to whether a baseline standard EEG (electroencephalogram) should be performed in all individuals with TSC regardless of seizure activity. There was no consensus on this in this survey. 68% of the responders suggested no baseline standard EEG when there is no suspicion of seizure activity.

There was no consensus on the need for treatment of interictal epileptiform abnormalities on EEG in infants, children and adults with TSC without clinical seizure activity. There are some suggestions that treating multiple spikes on EEG before onset of clinical seizures may reduce the risk of later clinical seizures, modify later phases of epileptogenesis, and reduce the risk of both drug-resistance and neurodevelopmental delay associated with epilepsy.(92) However, currently this has not yet been corroborated with prospective randomised trials.

Expert opinion

A standard EEG in individuals with suspected seizure activity should be performed. Perform 24-hr video EEG if changes in sleep, behaviour, or cognitive or neurological function are not explained by a standard EEG.

Parents should be taught to recognise epileptic spasms and focal seizures in infants, and EEG should be repeated urgently if there is suspicion of seizures. Paediatric neurologists should be involved in their care. Families and patients should be counselled about Sudden Unexpected Death in Epilepsy (SUDEP).(93)

Vigabatrin is the recommended first-line therapy for epileptic spasms in infancy. Hormonal therapies (oral prednisolone or ACTH) should be used if treatment with vigabatrin is unsuccessful.(94) Anticonvulsant therapy of other seizure types in TSC should generally follow the principles used in other epilepsies. Everolimus should be offered, if possible, to individuals with treatment resistant focal seizures. Epilepsy surgery should be considered for medically refractory TSC patients, but special consideration should be given to children at younger ages experiencing neurological regression. Epilepsy surgery must be performed at designated epilepsy surgery centres in the UK.

Sub-Ependymal Giant Cell Astrocytoma (SEGA)

SEGA- recommendations and results of consensus

Delphi consensus

There was consensus that patients with newly diagnosed or suspected TSC should have magnetic resonance imaging (MRI) of the brain to assess for the presence of SEGA.

There was consensus to perform MRI of the brain every 1-3 years in asymptomatic TSC patients without SEGA younger than 25 years of age, to monitor for growth or new occurrence. It is believed that the majority of these lesions stop growing in third decade of life.(62) However, there are some case reports of SEGA growth after the age of 25.(62) There was consensus to stop performing routine brain MRI scan at the age of 25 if there is no evidence of SEGA lesions.

There was consensus that MRI scan should be repeated in asymptomatic patients with SEGA. 56% of the responders suggested that the scan should be repeated 1-3 yearly. There was no consensus as to when to stop routine brain MRI scans in individuals with SEGA lesions. However, most responders suggested that this should be judged case by case, depending on their learning disabilities, communication abilities, and the SEGA interval growth.

There was consensus that symptomatic and asymptomatic SEGA lesions, which are actively growing and likely to enlarge beyond 1cm, should be treated.

There was consensus that the first line treatment for growing SEGA lesions should be surgery. There was consensus that growing SEGA lesions should be discussed at an MDT meeting that should include neurosurgeons, neurologists (paediatric or adult as appropriate), neuroradiologists and clinicians expert in the management of TSC (if not already covered by clinicians already listed).

Expert opinion

Patients with large or growing SEGA, or with SEGA causing ventricular enlargement who remain asymptomatic, should undergo MRI scans more frequently, and the patients and their families should be educated regarding the potential for new symptoms.

Surgical resection should be performed in a specialised centre with expertise in resecting intraventricular lesions.(62) In determining the best treatment option, discussion of the complication risks, adverse effects, costs, length of treatment, and potential impact on TSC-associated comorbidities should be included in the decision-making process. Patients, carers and parents should be part of this decision making process.

The NHS England commissioning policy states that the inclusion criteria for treatment with everolimus are as follows:

“Patient presents with SEGA lesion(s) and has at least one lesion of baseline longest diameter 1cm as assessed by multiphase MRI and is considered not amenable to

surgery as assessed by a properly constituted MDT (as defined in the Governance Arrangements).

Specifically, MDT decides that:

- SEGA is too difficult to remove surgically; OR
- SEGA needs reduction in size prior to surgery; OR
- SEGA lesion(s) are multiple or infiltrative; OR
- Surgery has been performed and there is residual SEGA (i.e. it was not possible to completely excise) that needs treating.

AND

The patient presents with:

- significant growth in target SEGA lesion(s) (as decided by properly constituted MDT since patient's last annual MRI); OR
- unequivocal worsening of non-target lesions of SEGA; OR
- the appearance of new lesion(s) of baseline longest diameter 1cm; OR
- symptoms of new or worsening hydrocephalus (but where urgent surgery is not required); OR
- patient presents for the first time with lesion(s) of baseline longest diameter 1cm (accounting for patients not cared for in a surveillance programme); OR
- partially excised SEGA lesion(s) known to be growing before surgery.

Exclusion criteria:

Any patient presenting with raised intracranial pressure (a surgical solution would be necessary as it would not be possible to wait for mTOR inhibition to take effect).”

Neurodevelopmental and Neuropsychiatric disorders

Recommendations and results of consensus

Delphi Consensus

There was consensus that a baseline assessment for neuropsychiatric/neurodevelopmental conditions including autism spectrum disorder and attention deficit hyperactivity disorder, or marked behavioural disturbances should be performed.

There was consensus to use the TSC Associated Neuropsychiatric Disorders (TAND) check list annually to check if there is any evidence of neuropsychiatric/neurodevelopmental disorders. In depth assessment should be undertaken when indicated. Treatment should follow the NICE guidelines.

There was consensus that developmental status should be formally evaluated at key developmental time points and periods of transition, which are infancy (0-3 years), preschool (3-6 years), middle school (6-11 years), adolescence (12-18 years), and as clinically indicated. This is to identify specific educational/cognitive disorders and intellectual disabilities. It informs treatment and care planning and choice of educational provision. Consideration should be given to the need for support for special educational needs and an assessment for an Education and Health Care Plan (EHCP).

Expert opinion

Marked changes in behaviour/cognitive status (either sudden or insidious), should prompt investigation for possible medical complications of TSC (e.g. SEGA; seizures; non-convulsive status; metabolic disturbances; adverse side effects of medications,

etc.).

TSC clinics should have established links and care pathways with developmental paediatric, educational and CAMHS specialist services to help ensure an integrated, responsive and timely multidisciplinary approach, which includes consultation and liaison.

Kidney

Angiomyolipoma (AML)

AML – recommendations and results of consensus

Delphi consensus

Consensus was reached on the management of kidney AMLs. For newly diagnosed or suspected patients with TSC, an MRI of the abdomen should be performed to assess for the presence of AML and renal cysts, regardless of age. MRI is the optimal imaging modality as some lesions, such as fat poor AMLs, can be over looked on ultrasound scans. CT is an alternative to MRI but the cumulative dose of ionising radiation needs to be considered. Both the brain and kidney MRI scans should ideally be combined and be done at the same time. MRI of the abdomen may also reveal aortic aneurysms or extrarenal hamartomas of the liver, pancreas, and other abdominal organs that can also occur in individuals with TSC.(93)

Patients should be screened for secondary hypertension by measuring blood pressure. At the time of diagnosis renal function (i.e. glomerular filtration rate) should be evaluated.(95, 96)

There was consensus that all patients with TSC should have regular kidney imaging. 68% of the responders suggested that kidney imaging should be repeated annually and 28% suggested that the kidney imaging should be repeated every 2 years.

There was consensus that blood pressure measurement and kidney function tests should be repeated annually.

There was consensus that growing AMLs measuring ≥3cm in diameter should be treated with mTOR inhibitors.

Expert opinion

For existing patients, if general anaesthetic (GA) is not required, they should have annual MRI of the abdomen to assess for the progression of AML and renal cystic disease throughout their lifetime. If GA is required, and on MRI the anatomy and pathology are judged to be easy to interpret by ultrasound scan, then the next surveillance scan or two could be ultrasound. Patients with Vagal Nerve Stimulator may not be able to have MRI scan. CT scan can be performed for those patients.

Everolimus is licensed for the treatment of growing AMLs that are > 3cm and not acutely bleeding in adults. It is funded for use in the NHS for adults and children (for whom it is off license) because the results from Exist-1 show it is highly effective in children.(97, 98) Selective embolization or kidney-sparing resection are possible second-line therapies for asymptomatic AMLs.

Embolization followed by corticosteroids is first-line therapy for AML presenting with acute haemorrhage. Nephrectomy should be avoided, if possible.

Lung

Lymphangioleiomyomatosis (LAM)

LAM – recommendations and results of consensus

Delphi consensus

There was consensus in this Delphi process to perform a baseline high-resolution chest computed tomography (HRCT) in females of child-bearing age. There was consensus to repeat HRCT in patients with LAM. The frequency of the scans should depend on patient's disease progression. There was consensus that individuals with LAM detected on HRCT should have annual pulmonary function testing and 6-minute walk test where practicable, and these should be repeated more frequently in those have progressive disease.

There was consensus to offer HRCT every 5-10 years to women without LAM on their baseline scan, or if symptoms of LAM develop. This should be performed until the menopause. There was no consensus on performing routine baseline pulmonary function testing or 6-minute walk test in patients who are newly diagnosed, or have suspected TSC.

There was consensus that mTOR inhibitors should be used to treat individuals with LAM if there is evidence of progressive LAM lesions.

Expert opinion

Assessment of those with LAM who are unable to perform pulmonary function testing, including those with learning difficulties, may be more difficult. Carers should be aware of the significance of increasing dyspnoea and the symptoms of pneumothorax. Clinical assessment should include a discussion of LAM symptoms, including exertional dyspnoea and pneumothorax with the individual and their carers. Six-minute walk testing or informal assessment of hypoxaemia during exertion may still be possible and may identify progressive lung disease. HRCT can be performed as described above and if there are otherwise unexplained worsening of respiratory symptoms.

Adult males should also undergo testing if they are symptomatic. Adolescent and adult females should be offered counselling on oestrogen use. All patients should be offered counselling regarding smoking risk. There is some evidence that oestrogen-containing contraceptives can exacerbate pulmonary LAM.(80, 81)

mTOR inhibitors should be used to treat individuals with LAM if there is evidence of progressive LAM lesions or loss of lung function ie if lung function falls more quickly than expected for age.(99) Patients with progressive disease or specific complications for which no other therapy is available should be considered for treatment with an mTOR inhibitor. Those likely to benefit from an mTOR inhibitor according to current evidence are those with progressive deterioration in lung function and those with chylous complications.

Skin – recommendations and results of consensus

Delphi consensus

There was consensus to perform a detailed clinical dermatological inspection/exam using Wood's light in newly diagnosed or patients with suspected TSC. Then, an annual clinical dermatological inspection/examination should be performed. There was consensus that facial angiofibromas can be treated with topical mTOR inhibitors.

Expert opinion

Currently, funding is not available for topical mTOR inhibitors for all patients in the UK. Some patients are obtaining this treatment via Individual Funding Request (IFR) through TSC clinics.

Laser treatment can also be used for treating facial angiofibromas.(100, 101) Surgical excision can be considered for larger solitary lesions. Surgical excision can be considered for ungual fibromas which are causing problems.

Teeth

Teeth – recommendations and results of consensus

Expert opinion

Periodic oral evaluation should occur every 6-12 months, consistent with surveillance recommendations for all individuals in the general population. Periodic preventive measures as well as oral hygiene education, especially in patients with learning disabilities, is important.

Dental pits can be treated with restorative treatments if the patient is at high cavity risk, although they rarely cause symptoms or an increased incidence of dental decay.(102)

Oral fibromas can be excised surgically if symptomatic or if interfering with oral hygiene. Oral fibromas may recur once excised; therefore, periodic oral evaluation is encouraged.(103)

Heart

Heart – recommendations and consensus

Delphi consensus

There was consensus that a baseline electrocardiogram (ECG) should be performed in all patients to check for arrhythmia and conduction abnormalities such as Wolff–Parkinson–White syndrome (WPW).(104) There was consensus that all children and symptomatic adults at baseline should be offered an echocardiogram to evaluate for rhabdomyomas.

There was consensus to repeat echocardiogram in asymptomatic patients with rhabdomyomas. 53% of the responders suggested that an echocardiogram should be repeated every 1-3 years in asymptomatic patients, until either there is complete regression of cardiac rhabdomyomas or the first signs of regression.

In addition, there was consensus to perform a 12- lead ECG at a minimum of every 3-5 years.

Expert opinion

The natural history of these lesions is spontaneous regression. It may not be necessary to continue scanning these patients until complete regression. Continuous scanning is not beneficial in the absence of cardiac symptoms.

In patients with clinical symptoms, additional risk factors, or significant abnormalities on routine echocardiogram or ECG, more frequent interval assessment may be needed and may include ambulatory event monitoring. Patients with hemodynamic instability and or life threatening arrhythmia should be treated with antiarrhythmic medications, surgery or mTOR inhibitors depending on the situation.(105)

Eye

Eye – recommendations and results of consensus

Delphi consensus

There was consensus to perform a baseline ophthalmologic evaluation, including fundoscopic evaluation for all individuals diagnosed with TSC to evaluate for hamartomas and hypo-pigmented lesions of the retina.

There was no agreement on whether routine ophthalmic assessment should just be fundoscopy by direct ophthalmoscopy or a detailed ophthalmological review by an ophthalmologist.

Expert opinion

It may be unachievable to aim to offer a detailed ophthalmology review regularly. It is very rare for retinal hamartomas to cause problems. It is necessary that these patients receive a regular fundoscopy examination during their clinic visits to check for papilloedema. Symptomatic changes due to retinal hamartomas are very rare. Macular oedema and vitreous haemorrhage have been thought to associated with retinal hamartomas. More frequent assessment, including in those treated with vigabatrin, is of no proven benefit and not recommended unless new clinical concerns arise.(106)

Table 2.6.2 and 2.6.3 show surveillance and management recommendations for newly diagnosed or suspected TSC, and for patients already diagnosed with TSC.

Table 2.6.2: Surveillance and management recommendations for newly diagnosed or suspected tuberous sclerosis complex (TSC)

Genetic screening at baseline	
Genetic testing should be offered at baseline to all patients	consensus
If it is impossible to offer genetic testing to all patients, then genetic testing should be offered for reproductive counselling or when a TSC diagnosis is likely but cannot be clinically confirmed	expert opinion
Obtain three-generation family history	expert opinion
First-degree relatives of individuals with TSC should be offered clinical assessment and where possible genetic testing	expert opinion
Ensure that the availability of preimplantation, prenatal and non-invasive prenatal diagnosis options is discussed where appropriate	expert opinion

Central nervous system screening at baseline	
Perform MRI of the brain for all patients	consensus
Perform a baseline assessment for neuropsychiatric / neurodevelopmental disorders	consensus
Obtain a standard EEG in individuals with suspected epileptic seizure activity	consensus
Perform 24-hr video EEG, if changes in sleep, behaviour, or cognitive or neurological function are not explained by a standard EEG	expert opinion
Teach parents to recognise infantile spasms and focal seizures and repeat EEG urgently if there is a suspicion of seizures. Paediatric neurologists should be involved in their management	expert opinion

Kidney screening at baseline	
Perform MRI of the abdomen.	consensus
If MRI is contraindicated, perform CT or ultrasound scan	expert opinion
Check blood pressure and glomerular filtration rate (GFR)	consensus

Lung screening at baseline	
Perform baseline high-resolution chest computed tomography (HRCT) in females of child-bearing age	consensus
Adult males, if symptomatic, should also undergo HRCT	expert opinion
Provide counselling on oestrogen use in adolescent and adult females	expert opinion
Provide counselling on smoking risks in all patients	expert opinion

Skin screening at baseline	
Perform a detailed clinical dermatological inspection/exam using Wood's light	consensus

Dental screening at baseline	
Perform a detailed clinical dental inspection/exam looking for abnormal tooth eruption, dental pits and oral fibromas	expert opinion

Heart screening at baseline	
Perform a baseline ECG	consensus
Perform a baseline echocardiogram in all children and symptomatic adults.	consensus

Eye screening at baseline	
Perform a complete ophthalmologic evaluation, including dilated fundoscopy, to assess for retinal lesions and visual field deficits.	consensus

Table 2.6.3: Surveillance and management recommendations for patients already diagnosed with definite or possible tuberous sclerosis complex (TSC)

Genetic surveillance	
First-degree relatives of individuals with TSC should be offered clinical assessment and where possible genetic testing.	expert opinion
Ensure that the availability of preimplantation, prenatal and non-invasive prenatal diagnosis options is discussed where appropriate.	expert opinion

SEGA surveillance	
Perform MRI of the brain every 1-3 years in asymptomatic TSC patients, without SEGA, younger than age 25.	consensus
Perform MRI of the brain every 1-3 years in asymptomatic TSC patients, with SEGA	consensus
There was no consensus as to when to stop routine brain MRI scans in individuals with SEGA lesions. However, most responders suggested that this should be judged case by case, depending on their learning disabilities, communication abilities, and the SEGA interval growth.	consensus
Patients with large or growing SEGA, or with SEGA causing ventricular enlargement who remain asymptomatic, should undergo MRI scans more frequently, and the patients and their families should be educated regarding the potential of new symptoms	expert opinion
Growing SEGAs should be discussed at MDT with oncologists, neurologists, neuro-radiologists, neurosurgeons and TSC experts	consensus
The patient or family should be involved in this discussion.	expert opinion
The first line of treatment for growing SEGAs is surgery	consensus
Surgical resection should only be performed in a specialised centre with expertise in resecting intraventricular lesions if possible	expert opinion
Everolimus can be offered as per NHS England commissioning criteria	expert opinion

Surveillance for neurodevelopmental and Neuropsychiatric disorders	
At each annual clinical review, the TAND check list should be used	consensus
In-depth neuropsychology and neuropsychiatric assessments should be undertaken when indicated	consensus
Treatment should follow the NICE guidelines	expert opinion
Developmental status should be formally evaluated at key developmental time points and periods of transition, which are infancy (0-3 years), preschool (3-6 years), middle school (6-11 years), adolescence (12-18 years), and as clinically indicated	consensus
TSC clinics should have established links and care pathways with developmental paediatric, educational and CAMHS specialist services to help ensure a seamless, integrated, responsive and timely multidisciplinary approach, which includes consultation and liaison	expert opinion

Epilepsy surveillance	
Obtain a standard EEG in individuals with suspected epileptic seizure activity	consensus
Perform 24-hour video EEG, if changes in sleep, behaviour, or cognitive or neurological function are not explained by a standard EEG	expert opinion
Vigabatrin is the recommended first-line therapy for infantile spasms. Hormonal treatments should be used if treatment with vigabatrin is unsuccessful	expert opinion
Everolimus should be offered, if possible, to individuals with treatment resistant focal seizures	expert opinion
Epilepsy surgery should be considered for TSC patients with refractory epilepsy.	expert opinion
Families and patients should be counselled about the risk of SUDEP	expert opinion

Kidney surveillance	
Kidney imaging should be repeated regularly	consensus
If a GA is not required, patients with renal lesions, should have annual MRI of the abdomen to assess for the progression of AML, renal cystic disease and occurrence of the rare renal cancer throughout the lifetime of the patient. If MRI scan is impossible to perform annually and if on MRI the anatomy and pathology are judged to be easy to interpret by ultrasound scan, then the next surveillance scan or two could be an US, or fast low dose CT scan could be used	expert opinion
In the absence of renal lesions, the scans should be repeated every 1-3 years through childhood and early adult life	expert opinion
If GA is required and on MRI the anatomy and pathology are judged to be easy to interpret by US scan, then the next surveillance scan or two could be an US, or the new fast low dose CT scan could be used if this avoids an anaesthetic	expert opinion
Assess renal function (including determination of glomerular filtration rate [GFR]) and blood pressure annually in adults and children with renal lesions	consensus
Embolization covered by corticosteroids is first-line therapy for AMLs presenting with acute haemorrhage	expert opinion
Every attempt should be made to avoid nephrectomy	expert opinion
For asymptomatic, growing AML measuring larger than 3 cm in diameter, treatment with an mTOR inhibitor is the recommended first-line therapy	consensus
Selective embolization or kidney-sparing resections are possible second-line therapies for asymptomatic AMLs	expert opinion

Lung surveillance	
Provide counselling on oestrogen use in adolescent and adult females	expert opinion
Provide counselling on smoking risks in all patients	expert opinion
Obtain HRCT every 5-10 years in asymptomatic females of childbearing age, if there is no evidence of LAM on their baseline HRCT. This should be performed until menopause	consensus
Individuals with LAM detected on HRCT should have annual pulmonary function testing (pulmonary function testing and 6-minute walk test) and more often if they have progressive disease	consensus
mTOR inhibitors should be used to treat individuals with LAM if there is evidence of progressive loss of lung function	consensus

Skin surveillance	
Perform a detailed clinical dermatologic inspection/exam annually	consensus
Patients and families should be counselled to use sunblock (SPF 30+) routinely	expert opinion
Facial angiofibromas should be treated with topical mTOR inhibitors	consensus
Laser treatment can be used to treat these lesions	expert opinion
Surgical excision can be considered for larger solitary lesions	expert opinion
Surgical excision can be considered for ungual fibromas that are causing problems	expert opinion

Teeth surveillance	
Periodic oral evaluation should occur every 6-12 months, consistent with surveillance recommendations for all individuals in the general population. Periodic preventive measures as well as oral hygiene education especially in patients with learning disabilities are important	expert opinion
Symptomatic or deforming dental lesions, oral fibromas, and bony jaw lesions can be treated with surgical excision or curettage when present	expert opinion

Heart surveillance	
Echocardiogram should be repeated every 1-3 years in asymptomatic patients until either complete regression of cardiac rhabdomyomas or until first sign of cardiac rhabdomyomas regression	consensus
12- lead ECG is recommended at minimum every 3-5 years	consensus
In patients with clinical symptoms, additional risk factors, or significant abnormalities on routine echocardiogram or ECG, more frequent interval assessment may be needed and may include ambulatory event monitoring	expert opinion
Patients with hemodynamic instability and or life threatening arrhythmia should be treated	expert opinion

Eye surveillance	
Regular fundoscopy by direct ophthalmoscopy examination during each clinic visit should be performed	consensus
More frequent assessment, including those treated with vigabatrin, is of no proven benefit and not recommended unless new clinical concerns arise	expert opinion

Liver and pancreatic surveillance	
Liver and pancreas should be assessed for lesions during annual abdominal MRI scan	consensus

2.2.7 Limitations

There are limitations to both the methodology and applicability of the guidelines. They are based on consensus and expert opinion. We have also tried to refer to evidence, however, there is not enough robust evidence to rely on for all the aspects of TSC management. It is important that these guidelines are regularly updated based on new evidence. There is a danger that clinicians may follow existing guidelines without referencing to current evidence. The TSC field is rapidly progressing and new evidence is emerging regularly. We have also tried to be inclusive and make sure that all the relevant experts in this field were part of this process.

These guidelines can cause challenges to some clinicians as they may not be able to offer the monitoring and surveillance investigations and treatment to their patients due to lack of recourses. It is important that we do not shy away from good practice because there are not enough resources. We are hopeful that these guidelines will put pressure on funding authorities to allocate appropriate funding so that the patients receive appropriate care and treatment. These guidelines have the possibility to create some medicolegal issues. It would be expected of clinicians that they would provide the level of surveillance recommended. If a patient was to develop complications, clinicians and NHS trusts may face liability if they have not adhered to these guidelines. There are several medicolegal cases in relation to missed SEGA monitoring. Patients have developed life changing disabilities such as blindness due to inappropriate SEGA management. We are hopeful that these guidelines will change the natural history of the disease.

2.3 Published papers and Dissemination

- First part of this chapter “Causes of mortality in individuals with tuberous sclerosis complex”

    - Was published in a peer reviewed journal.
      Amin S, Lux A, Calder N, Laugharne M, Osborne J, O'callaghan F Causes of mortality in individuals with tuberous sclerosis complex. Dev Med Child Neurol. 2017 Jun;59(6):612-617.

    - I presented the paper at the annual meeting of Royal College of Paediatric and Child Health – oral presentation.
      S Amin, N Calder, M Laugharne, J Osborne, F O'Callaghan. Causes of mortality in individuals with tuberous sclerosis complex. British Association for Child and Adolescent Public Health and British Association of General Paediatrics. Arch Dis Child 2016;101:Suppl 1 A191

    - I also presented the paper at the European Paediatric Neurology Society – oral presentation.
      S. Amin, N. Calder, J. Merrifield, F. O'Callaghan. Causes of death in individuals with tuberous sclerosis complex. European Journal of Paediatric Neurology Volume 19, Supplement 1, May 2015, Pages S90

    - I also presented the paper at the British Paediatric Neurology Association annual meeting – oral presentation.
      Amin S, Lux A, Calder N, Laugharne M, Osborne J, O'callaghan F Causes of mortality in individuals with tuberous sclerosis complex. Dev Med Child Neurol 2015

    - I also presented the paper at the International TSC conference – poster presentation.
      Amin S, Lux A, Calder N, Laugharne M, Osborne J, O'callaghan F Causes of mortality in individuals with tuberous sclerosis complex. Lisbon 2016

- Second part of this chapter “UK guidelines for management and surveillance of Tuberous Sclerosis Complex”

    - Was published in a peer reviewed journal.
      Amin S, Kingswood JC, Bolton PF, Elmslie F, Gale DP, Harland C, Johnson SR, Parker A, Sampson JR, Smeaton M, Wright I, O'Callaghan FJK. The UK guidelines for management and surveillance of Tuberous Sclerosis Complex. QJM. 2018 Sep 21.

    - I presented the paper at the annual meeting of British Paediatric Neurology Association. The UK guidelines for management and surveillance of Tuberous Sclerosis Complex Dev Med Child Neurol. 2018

    - I also presented the paper at the Annual meeting of the Royal College of Paediatric and Child Health. Delphi consensus process for the UK guidelines for management and surveillance of tuberous sclerosis complex. Arch Dis Child 2018

Chapter 3

3.Quality of life in patients with Tuberous Sclerosis Complex

Purpose

The purpose of this study was to evaluate the impact of Tuberous Sclerosis complex on patients' quality of life. Results of such a study may help clinicians and policy makers decide where best to focus efforts and resources to improve the quality of life of patients with TSC. In addition, the results can be used to service plan, and to measure the impact (in terms of health gains) of clinical and social interventions.

Recent advances in medicine have changed the way that medicine is practiced. The emphasis is not only on diagnosis and treatment but also on controlling chronic conditions, and recognising that the efficacy of treatment is also reliant on the quality of life of the patient. Tuberous Sclerosis Complex (TSC) is a chronic condition which can cause epilepsy, learning, behavioural and psychosocial difficulties and other physical problems. Quality of life (QoL) in patients with TSC has not been studied before. I conducted this study to investigate the impact of the disease on the QoL of children and adults.

3.1 Assessment of quality of life in patients with Tuberous Sclerosis Complex

3.1.1 Background

PedsQL

There are several tools that have been used in assessing children's quality of life. A systematic review identified 43 quality of life assessment measures, which included disease specific and generic, and concluded that the PedsQL is the most promising tool. (110) PedsQL is a short, standardised, generic assessment tool which methodically evaluates patients' and parents' perceptions of health related quality of life in paediatric patients with chronic conditions. PedsQL is more acceptable than other assessment measures because of its brevity, reliability, validity, availability of age appropriate versions and equivalent forms for child and parent. In addition, it has core and modular designs which make it flexible to be used in a range of research and clinical settings for children with chronic conditions. (111)

The PedsQL was originally derived from data collected from a group of US children with cancer, and their parents, at different stages of treatment. The original version of PedsQL has gone through several improvements to achieve a more sensitive rating scale, a wider age range, and to match the core dimensions defined by WHO.

In this study, I used the UK version of PedsQL 4.0. This version has been assessed for its performance in a group of UK healthy children and children with chronic conditions and their parents. It has been shown that the UK-English version of PedsQL™ performance is as good as the original PedsQL™ and is recommended for assessment of paediatric health related quality of life in the UK. (108)

The questionnaire is designed for children to self report (age range 5– 18 years) and also for parent/carers to provide proxy-reports (age range 2–18 years). Each item on the form is similar between the self and proxy questionnaires. The language is developmentally suitable and first or third person tense is used. The US version was translated into UK English according to translation guidelines.(112)

This version is believed to be reliable and valid. The Child and Parent Reports of the PedsQL 4.0 Generic Core Scales are for 3 age groups including children aged 5-7, 8-12 and 13 to 18. It is composed of 23 items comprising 4 dimensions: Physical, emotional, social and school functioning. The physical domain has 8 items, emotional 5, social 5 and school 5. Scores are transformed on a scale from 0 to 100.

Each item asks the question of how much of a problem each item has been during the past four weeks and answers are made on a five point scale ranging from 0 to 4. 0 is being never a problem, 1 almost never a problem, 2 sometimes a problem, 3 often a problem and 4 almost always a problem.

Items are reverse scored and linearly transformed onto a 0-100 scale as follows: 0=100, 1=75, 2=50, 3=25, 4=0. If more than 50% of the items were missing the scale scores should not be computed. The physical health summary score is calculated as the sum of the items in the physical domain divided by the number of items. Psychosocial Health Summary Score is calculated as sum of the items divided by the number of items answered in the Emotional, Social, and School Functioning Scales. Higher scores indicate better health related quality of life.(108)

3.1.2 Participants

In this study, we investigated the QoL of 91 patients (35 children and 56 adults) with a definite diagnosis of TSC, as defined by the International Tuberous Sclerosis Complex Consensus Group(73) who attended the Bath TS clinic from February 2014 to August 2014. No patients were excluded from this study. Parents and the children were asked to complete a questionnaire during their clinic appointment at the TS clinic in the presence of TS specialists. Parents completed the assessments for those children who were unable to complete them themselves, due to LDs. The PedsQL form was used to assess the quality of life of children with TSC. The SF-36 form was used to assess the quality of life of adults with TSC. The form was completed by parents or carers on behalf of adults with learning disabilities.

This is a clinic cohort in which there was heterogeneity in terms of administered investigations. For example, not all patients were administered psychometric tests in a consistent fashion, but some tests were requested and administered when an initial clinical screen of abilities identified areas of concern. Patients were given the diagnosis

of learning disability based on their abilities to understand new or complex information or to learn new skills, or their ability to cope or live independently, and with such inabilities having started before adulthood. Although these categorisations were made on the basis of clinical assessment rather than according to any measurement tool, they broadly concur with the definitions of learning disability defined by the UK Department of Health(107). Ethical approval was not required as the assessment was being used as part of patient's routine care.

Children Healthy population

The normative data for PedsQL which is used in this study is derived from the Upton et al study in 2005. We compared the quality of life of our cohort of TSC patients with the healthy population. Upton et al recruited children from 23 schools in South Wales to obtain a baseline quality of life for a healthy population. 1034 children self-completed the QOL questionnaire and 665 parents completed proxy reports. The psychometric properties of this version were similar to those reported for the original US version of PedsQL. Internal reliability exceeded 0.70 for all proxy and self-report sub-scales. Discriminant validity was established for proxy and self-report with higher HRQL being reported for healthy children than those with health problems. Sex differences were noted on the emotional functioning subscale, with females reporting lower HRQL than males. Proxy and self-report correlation was higher for children with health problems than for healthy children(108).

Adult healthy population

The normative data for SF36 which is used in this study is derived from the Oxford Healthy Life Survey 1992. The Oxford Healthy Life Survey was conducted in Central England. The questionnaire was sent by post to 13800 randomly selected subjects between the ages of 18–64 inclusive. The individuals were identified through their General Practice. The survey achieved a response rate of 64.4%. Internal consistency of the different dimensions of the questionnaire were found to be high(109).

TSC with and without epilepsy population

These patients were categorized into this group during recruitment at their clinic appointments between February 2014 to August 2014. TSC patients without epilepsy were defined as patients who have never had epilepsy.

TSC with and without learning disabilities

We divided this cohort into two groups: those with and those without learning disabilities. Patients were given the diagnosis of learning disability based on their ability to understand new or complex information or to learn new skills, or their ability to cope or live independently, and with such inabilities having started before adulthood. Although these categorizations were made on the basis of clinical assessment rather than according to any measurement tool, they broadly concur with the definition of learning disability defined by the UK department of Health(107).

CHILD REPORT (ages **8-12**) **PedsQL questionnaire**

D# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

CHILD REPORT (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

CHILD REPORT (ages **8-12**) **PedsQL questionnaire**

*In the past **ONE month**, how much of a **problem** has this been for you …*

About My Health and Activities ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

About My Feelings ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

How I Get Along with Others ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I have trouble getting along with other kids	0	1	2	3	4
2. Other kids do not want to be my friend	0	1	2	3	4
3. Other kids tease me	0	1	2	3	4
4. I cannot do things that other kids my age can do	0	1	2	3	4
5. It is hard to keep up when I play with other kids	0	1	2	3	4

About School ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my schoolwork	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

PARENT REPORT for **CHILDREN** (ages **8-12**) **PedsQL questionnaire**

D# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **CHILDREN** (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month,** how much of a **problem** has your child had with …*

Physical Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

Emotional Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

Social Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Getting along with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

School Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

TEENAGER REPORT (ages **13-18**) **PedsQL questionnaire**

_____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

TEENAGER REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

TEENAGER REPORT (ages **13-18**) **PedsQL questionnaire**

*In the **PAST MONTH**, how much of a **problem** has this been for you …*

About My Health and Activities ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard for me to walk more than a couple of streets (about 100 metres)	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activities or exercise	0	1	2	3	4
4. It is hard for me to lift heavy things	0	1	2	3	4
5. It is hard for me to have a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I have aches and pains	0	1	2	3	4
8. I feel tired	0	1	2	3	4

About My Feelings ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

How I Get On with Others ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I have trouble getting on with other teenagers	0	1	2	3	4
2. Other teenagers do not want to be my friend	0	1	2	3	4
3. Other teenagers tease me	0	1	2	3	4
4. I cannot do things that other teenagers my age can do	0	1	2	3	4
5. It is hard to keep up with other teenagers my age	0	1	2	3	4

About School / College ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my school / college work	0	1	2	3	4
4. I miss school / college because of not feeling well	0	1	2	3	4
5. I miss school / college to go to the doctor or hospital	0	1	2	3	4

PARENT REPORT for TEENAGERS (ages **13-18**)
PedsQL questionnaire

_____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for TEENAGERS (ages 13-18)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

PARENT REPORT for TEENAGERS (ages 13-18)
PedsQL questionnaire

*In the past **ONE month**, how much of a **problem** has your teenager had with …*

Physical Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1.Walking 100 metres	0	1	2	3	4
2.Running	0	1	2	3	4
3.Participating in sports activities or exercise	0	1	2	3	4
4.Lifting something heavy	0	1	2	3	4
5.Taking a bath or shower by him or herself	0	1	2	3	4
6.Doing chores around the house	0	1	2	3	4
7.Having aches or pains	0	1	2	3	4
8.Feeling tired	0	1	2	3	4

Emotional Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1.Feeling afraid or scared	0	1	2	3	4
2.Feeling sad	0	1	2	3	4
3.Feeling angry	0	1	2	3	4
4.Trouble sleeping	0	1	2	3	4
5.Worrying about what will happen to him or her	0	1	2	3	4

Social Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1.Getting on with other teenagers	0	1	2	3	4
2.Other teenagers not wanting to be his or her friend	0	1	2	3	4
3.Getting teased by other teenagers	0	1	2	3	4
4.Not being able to do things that other teenagers his or her age can do	0	1	2	3	4
5.Keeping up with other teenagers	0	1	2	3	4

School Functioning ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1.Paying attention in class	0	1	2	3	4
2.Forgetting things	0	1	2	3	4
3.Keeping up with schoolwork	0	1	2	3	4
4.Missing school because of not feeling well	0	1	2	3	4
5.Missing school to go to the doctor or hospital	0	1	2	3	4

PedsQL PA - United Kingdom/English - Version of 14 Mar 11 - Mapi Research Institute.
ID6014 / PedsQL-4.0-Core-PA_AU4.0_eng-GB.doc

Scoring PedsQL

The **Child and Parent Reports** of the **PedsQL™** 4.0 Generic Core Scales for:

- Children (ages 8-12),
- And Teens (ages 13-18),

are composed of 23 items comprising 4 dimensions.

DESCRIPTION OF THE QUESTIONNAIRE:

Dimensions	Number of Items	Cluster of Items	Reversed scoring	Direction of Dimensions
Physical Functioning	8	1-8	1-8	Higher scores indicate better HRQOL.
Emotional Functioning	5	1-5	1-5	
Social Functioning	5	1-5	1-5	
School Functioning	5	1-5	1-5	

SCORING OF DIMENSIONS:

Item Scaling	5-point Likert scale from 0 (Never) to 4 (Almost always) 3-point scale: 0 (Not at all), 2 (Sometimes) and 4 (A lot) for the Young Child (ages 5-7) child report
Weighting of Items	No
Extension of the Scoring Scale	Scores are transformed on a scale from 0 to 100.
Scoring Procedure	**Step 1: Transform Score** Items are reversed scored and linearly transformed to a 0-100 scale as follows: 0=100, 1=75, 2=50, 3=25, 4=0. **Step 2: Calculate Scores** Score by Dimensions: • If more than 50% of the items in the scale are missing, the scale scores should not be computed, • Mean score = Sum of the items over the number of items answered. Psychosocial Health Summary Score = Sum of the items over the number of items answered in the Emotional, Social, and School Functioning Scales. Physical Health Summary Score = Physical Functioning Scale Score **Total Score**: Sum of all the items over the number of items answered on all the Scales.
Interpretation and Analysis of Missing Data	If more than 50% of the items in the scale are missing, the Scale Scores should not be computed. If 50% or more items are completed: Impute the mean of the completed items in a scale.

SF36

In this study, I used SF36v2 for adults with TSC with and without learning difficulties. This tool was initially used in the US in a health insurance experiment.(113) It was then translated into more than 100 languages and used in many countries. It is a generic, multipurpose, short survey, with 36 questions which assesses health status, and it has been proven to be useful in general and specific populations.

The SF-36 contains 36 item scales, which measure eight domains of health status including:

physical functioning (10 items); physical role limitations (four items); bodily pain (two items); general health (five items); energy/vitality (four items); social functioning (two items); emotional role limitations (three items) and mental health (five items).

Scoring SF36

Scoring 36-Item Health Survey is a two-step process. First, precoded numeric values are recoded per the scoring key shown in Table 3.3.1. All items are scored so that a high score reflects a more favourable quality of life. In addition, each item is scored on a 0 to 100 range so that the lowest and highest possible scores are 0 and 100, respectively. Scores represent the percentage of total possible score achieved. In step 2, items in the same scale are averaged together to create the 8 scale scores. Table 3.3.2 shows the items averaged together to form each scale. Items that are left unanswered (missing data) are taken out when calculating the scale scores. Hence, scale scores represent the average for all items in the scale that the respondent answered. (114)

For example, item 23 and 29 are used to score the measure of energy/fatigue which have 6 response choices each. A high score (response choice 6) on item 23 indicates the absence of energy, while a high score (response choice 6) on item 29 indicates the presence of energy. To score both items in the same direction, Table 3.3.1 shows that responses 1 through 6 for item 23 should be recoded to values of 100, 80, 60, 40, 20 and 0, respectively. Responses 1 through 6 for item 29 should be recoded to values of 0, 20, 40, 60, 80 and 100, respectively. Table 3.3.2 shows that these two recoded items should be averaged together to form the Energy. If the respondent is missing one of the two items, the person's score will be equal to that of the non-missing item.

Table 3.3.1: step 1 recording items

Item numbers	Change original response category *	To recoded value of:
1, 2, 20, 22, 34, 36	1 →	100
	2 →	75
	3 →	50
	4 →	25
	5 →	0
3, 4, 5, 6, 7, 8, 9, 10, 11, 12	1 →	0
	2 →	50
	3 →	100
13, 14, 15, 16, 17, 18, 19	1 →	0
	2 →	100
21, 23, 26, 27, 30	1 →	100
	2 →	80
	3 →	60
	4 →	40
	5 →	20
	6 →	0
24, 25, 28, 29, 31	1 →	0
	2 →	20
	3 →	40
	4 →	60
	5 →	80
	6 →	100
32, 33, 35	1 →	0
	2 →	25
	3 →	50
	4 →	75
	5 →	100

Table 3.3.2: step 1 averaging items to form scales

Scale	Number of items	After recoding per Table 1, average the following items
Physical functioning	10	3 4 5 6 7 8 9 10 11 12
Role limitations due to physical health	4	13 14 15 16
Role limitations due to emotional problems	3	17 18 19
Energy/fatigue	4	23 27 29 31
Emotional well-being	5	24 25 26 28 30
Social functioning	2	20 32
Pain	2	21 22
General health	5	1 33 34 35 36

SF36 questionnaire

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. ***Thank you for completing this survey!***

For each of the following questions, please tick the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very Good	Good	Fair	Poor
1	2	3	4	5

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same as one year ago	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a) Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐	☐	☐
b) Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐	☐	☐
c) Lifting or carrying groceries	☐	☐	☐
d) Climbing several flights of stairs	☐	☐	☐
e) Climbing one flight of stairs	☐	☐	☐
f) Bending, kneeling, or stooping	☐	☐	☐
g) Walking more than a mile	☐	☐	☐
h) Walking several hundred yards	☐	☐	☐
i) Walking one hundred yards	☐	☐	☐
j) Bathing or dressing yourself	☐	☐	☐

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a) Cut down on the amount of time you spent on work or other activities	☐	☐	☐	☐	☐
b) Accomplished less than you would like	☐	☐	☐	☐	☐
c) Were limited in the kind of work or other activities	☐	☐	☐	☐	☐
d) Had difficulty performing the work or other activities (for example, it took extra effort)	☐	☐	☐	☐	☐

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a) Cut down on the amount of time you spent on work or other activities	☐	☐	☐	☐	☐
b) Accomplished less than you would like	☐	☐	☐	☐	☐
c) Did work or other activities less carefully than usual	☐	☐	☐	☐	☐

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐	☐	☐	☐	☐

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe
☐	☐	☐	☐	☐	☐

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐	☐	☐	☐	☐

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a) Did you feel full of life?	☐	☐	☐	☐	☐
b) Have you been very nervous?	☐	☐	☐	☐	☐
c) Have you felt so down in the dumps that nothing could cheer you up?	☐	☐	☐	☐	☐
d) Have you felt calm and peaceful?	☐	☐	☐	☐	☐
e) Did you have a lot of energy?	☐	☐	☐	☐	☐
f) Have you felt downhearted and low?	☐	☐	☐	☐	☐
g) Did you feel worn out?	☐	☐	☐	☐	☐
h) Have you been happy?	☐	☐	☐	☐	☐
i) Did you feel tired?	☐	☐	☐	☐	☐

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐	☐	☐	☐	☐

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a) I seem to get ill more easily than other people	☐	☐	☐	☐	☐
b) I am as healthy as anybody I know	☐	☐	☐	☐	☐
c) I expect my health to get worse	☐	☐	☐	☐	☐
d) My health is excellent	☐	☐	☐	☐	☐

Thank you for completing these questions

Statistical analysis

The one sample t test was used to compare the means of different domains of PedQL and SF36 of patients with TSC compared with the means of the normal population and children with other conditions. This was also used to compare the means of children and adults with TSC with and without epilepsy and learning disabilities. Paired t test was used to compare the means of QoL of children's self reports and their parents' reports.

3.1.3 Results

The QoL of 91 patients was assessed. There were 35 children and 56 adults. The median age of the children was 12 and of the adults was 34. There was no gender difference amongst the adults and the male to female ratio in children was 19/16.

Forty-three out of 56 adults had epilepsy, of whom 35 had learning disabilities. All patients with LD had epilepsy. 29 out of 35 children had epilepsy, of whom 19 had learning disabilities. Again, all children with LD had epilepsy.

Twenty-one out of 56 adults had no learning disabilities and completed the self-reported questionnaire. The proxy reported questionnaire was completed by parents and carers for the 35 adults with learning disabilities. 16 out of 35 children did not have learning disabilities and completed the self-reported QoL questionnaire. The 19 children who had learning disabilities were unable to complete the self QoL questionnaire. Parents completed the proxy questionnaire for all the 35 children.

Comparison to healthy control children (see table 3.3.3)

In comparison with the sample of healthy children, there is a significant difference in the child self-report and proxy-report in the total scale scores. The total mean self-reported score for children with TSC was 71. It is 84 for healthy UK children p=0.020 (CI -23 to -23). The total mean proxy report for TSC children was 48. It is 85 for healthy children p<0.0005 (CI -45.24 to -28.76). The total mean for psychosocial domain of self-reported score for children with TSC was 67. It is 82 for healthy UK children p<0.0005 (CI -45 to -28). The mean proxy report for psychosocial domain for TSC children was 42. It is 82 for healthy children p<0.0005 (CI -45 to -28). In addition, the children and their parents reported a significant difference of QoL in the physical domain compared with the healthy population. The total mean for physical domain of self-reported scores for children with TSC was 78. It is 88 for healthy UK children p=0.076 (CI -21 to 1). The mean proxy report for TSC children was 56. It is 89 for healthy children p<0.0005 (CI -42 to -23). All the other self and proxy reported QoL domains were significantly lower than the healthy population, except the self-reported emotional domain. The mean for emotional domain of self-reported scores for children with TSC was 71. It is 78 for healthy UK children p=0.161 (CI -17 to 3). This remained insignificant after adjusting for epilepsy and learning disabilities. The self-reported total mean score for children with TSC and normal intellect was 71, and the score reported by their parents was 48, p<0.005 (CI 9.9 to 36).

	Healthy population N= 1698	TSC population N=35		TSC without epilepsy N=6		TSC with epilepsy N=29		TSC without learning disabilities N=16		TSC with learning disabilities N=19		TSC without epilepsy and learning disabilities N=6	
PedsQL scales	Mean (SD)	Mean (SD)	*P and CI value	Mean (SD)	*P value	Mean (SD)	*P value	Mean (SD)	*P value	Mean (SD)	*P value	Mean (SD)	*P value
Child self-report	*n=1033*	*n=16*		*n=6*		*n=10*		*n=16*		*n=0*		*n=6*	
Total score	84 (11)	71 (20)	0.020 -23 to -2.3	79 (17)	0.503 -22 to 12	67 (21)	0.030 -32 to -1	71 (21)	0.025 -24 to -1	NA	NA	79 (17)	0.503 -22 to 12
Psychosocial	82 (13)	67 (22)	0.010 -26 to -3	74 (17)	0.301 -25 to 9	62 (24)	0.027 -37 to -2	67 (23)	0.019 -27 to -2	NA	NA	74 (17)	0.301 -25 to 9
Physical	88 (11)	78 (21)	0.076 -21 to 1	88 (19)	0.296 -32 to 12	72 (20)	0.032 -30 to -1	77 (21)	0.053 -22 to 0	NA	NA	88 (19)	1 -19 to 19
School	79 (15)	60 (28)	0.016 -33 to -4	63 (36)	0.326 -53 to 21	58 (25)	0.026 -38 to -3	60 (29)	0.019 -34 to -3	NA	NA	63 (36)	0.326 -53 to 21
Social	88 (16)	72 (27)	0.031 -30 to -1	81 (22)	0.471 -30 to 16	67 (29)	0.047 -41 to -0	73 (27)	0.042 -29 to -0	NA	NA	81 (22)	0.471 -30 to 16
Emotional	78 (17)	71 (19)	0.161 -17 to 3	78 (13)	1 -13 to 13	68 (22)	0.184 -25 to 5	71 (20)	0.181 -17 to 3	NA	NA	78 (13)	1 -13 to 13
Parent proxy-report	*n=665*	*n=35*		*n=6*		*n=29*		*n=16*		*n=19*		*n=6*	
Total scores	85 (11)	48 (24)	<0.0005 -45 to -28	74 (18)	0.194 -29 to 7	43 (21)	<0.0005 -49 to -34	65 (22)	0.002 -31 to – 8	33 (14)	<0.0005 -58 to -45	74 (18)	0.194 -29 to 7
Psychosocial	82 (12)	42 (21)	<0.0005 -47 to -32	67 (20)	0.125 -35 to 5	36 (17)	<0.0005 -52 to -39	54 (22)	0<.0005 -39 to -16	31 (11)	<0.0005 -56 to -45	67 (20)	0.125 -35 to 5
Physical	89 (12)	56 (28)	<0.0005 -42 to -23	85 (19)	0.628 -23 to 15	50 (27)	<0.0005 -49 to -28	73 (26)	0.039 -25 to -0.7	39 (22)	<0.0005 -60 to -39	85 (19)	0.408 -26 to 12
School	82 (16)	41 (26)	<0.0005 -49 to -32	67 (25)	0.201 -41 to 11	36 (24)	<0.0005 -55 to -36	59 (25)	0.002 -36 to -9	27 (15)	<0.0005 -62 to -47	67 (25)	0.201 -41 to 11
Social	87 (15)	41(28)	<0.0005 -50 to -31	71 (23)	0.149 -40 to 8	35 (26)	<0.0005 -61 to -42	57 (29)	0.000 -45 to -14	29 (18)	<0.0005 -66 to -49	71 (23)	0.149 -40 to 8
Emotional	78 (15)	47 (24)	<0.0005 -39 to – 22	72 (16)	0.400 -22 to 10	42 (23)	<0.0005 -44 to -27	60 (22)	0.005 -29 to -6	35 (18)	<0.0005 -51 to -34	72 (16)	0.400 -22 to 10

Table 3.3.3: shows the total mean and all different domains of PedsQL self and proxy reported scores for children with TSC with and without epilepsy, with and without learning disabilities and healthy children. * compares the means with healthy population.

Comparison to children with chronic conditions(108)

TSC and diabetes

Both children with TSC and their parents, reported a significantly worse quality of life compared with the quality of life reported by children with diabetes and their parents. The self-reported total mean score for children with diabetes was 82, compared to 71 for the TSC children $p<0.0005$ (CI -16.17 to -5.83). The proxy mean score for the diabetic children was 77, and 48 for the children with TSC $p<0.0005$ (CI -37.24 to -20.76). The self-reported psychosocial mean score for children with diabetes was 81, and 67 for the TSC children $p=0.0224$ (CI -25.72 to -2.28). The proxy report for diabetics was 75, and the score for TSC patients was 42, $p<0.0005$ (CI -40.21 to -25.79)

TSC and asthma

In comparison to children with asthma, the self-report for total scores and the psychological scores showed an insignificant difference. The self-reported total mean score for children with asthma was 75, whilst the total score for children with TSC was 71, $p=0.4362$ (CI -14.66 to 6.66). The proxy mean score for the asthmatic children was 72, and 48 for the children with TSC $p<0.0005$ (CI -32.24 to -15.76). The self-reported psychosocial mean score for children with asthma was 75, and 67 for TSC children $p=0.1664$ (CI -19.72 to 3.72). The proxy report for asthma was 71, and the score for TSC patients was 42 $p<0.0005$ (CI -36.21 to -21.79).

TSC and cancer

Similar to the above group, the parents of children with TSC reported a worse quality of life compared with reports by parents of children with cancer. The self-reported total mean score for children with cancer was 76, compared to 71 for the TSC children p=0.3332 (CI-15.66 to 5.66). The proxy mean score for the children with cancer was 71, and 48 for the children with TSC p<0.0005 (CI -31.24 to -14.76). The self-reported psychosocial mean score for the children with cancer was 74, and 67 for the TSC children p=0.222 (CI -18.72 to 4.72). The proxy report for those with cancer was 69, and the score for TSC patients was 42 p<0.0005 (CI -34.21 to -19.79).

TSC and IBD **(Inflammatory Bowel Disease)**

The self-reported total mean score for children with IBD was 74, compared to 71 for the TSC children p=0.557 (CI -13.66 to 7.66). The proxy mean score for the children with IBD was 73, and 48 for children with TSC p<0.0005 (CI -33.24 to -16.76). The self-reported psychosocial mean score for the children with IBD was 74, and 67 for the TSC children p=0.222 (CI -18.72 to 4.72). The proxy report for those with IBD was 73, and the score for TSC patients was 42, p<0.0005 (CI -38.21 to -23.79).

QoL in adults with TSC and healthy population(113) (see table 3.3.4)

Adult patients with TSC reported a poorer quality of life compared to the healthy population. Regardless of the presence of learning disabilities and epilepsy, there is a highly significant difference between all the domains of SF36 in TSC patients and the healthy population, apart from the mental health domain. The Mental Health score for adults with TSC was 71. It is 75 for the healthy UK population, p=0.050 (CI -8 to 0.02). The mental health scores remained insignificant compared to healthy adults regardless of whether the patients had epilepsy or learning disabilities.

The total mean score for body pain for patients with learning disabilities was 81 out of 100, whilst the mean score for adults without learning disabilities was 62, and is 87 for the general population. The higher the score, the less pain patients are believed to experience.

The higher the score, the less pain patients are believed to experience; the difference in body pain score for the adults with TS and LD was of borderline significance compared with the healthy population, whereas body pain score was significantly lower in adults with TS without LD, i.e. they experienced more pain. Table 3.3.4

	healthy population	TSC N=56		TSC patients without epilepsy N=13		TSC patients with epilepsy N=43		TSC patients without learning disabilities N=21		TSC patients with learning disabilities N=35		TSC patients without epilepsy and learning disabilities N=13	
SF36 scales	**Mean (SD)**	**Mean (SD)**	***P values**	**Mean (SD)**	***P values**	**Mean (SD)**	***P values**	**Mean (SD)**	***P values**	**Mean (SD)**	***P values**	**Mean (SD)**	***P values**
Physical Functioning	94 (12) n=4962	70 (33)	<0.0005 -32 to -15	78 (34)	0.115 -36 to 4	67 (33)	<0.0005 -37 to -16	81 (31)	0.069 -27 to 1	62 (33)	<0.0005 -43 to -20	78 (34)	0.115 -36 to 4
Role Physical	93 (13) n=5052	72 (36)	<0.0005 -30 to -11	83 (34)	0.309 -30 to 10	69 (36)	<0.0005 -35 to -12	79 (35)	0.081 -29 to 1	67 (36)	<0.0005 -38 to -13	83 (34)	0.309 -30 to 10
Body Pain	87 (16) n=5078	74 (29)	<0.0005 -20 to -5	66 (35)	0.051 -42 to 0.1	77 (27)	0.019 -18 to -1	62 (34)	0.003 -40 to -9	81 (24)	0.148 -14 to 2	66 (35)	0.051 -42 to 0.1
General Health	78 (15) n=4999	68 (21)	<0.0005 -15 to -4	67 (29)	0.028 -37 to -2	69 (19)	0.003 -14 to -3	68 (27)	0.105 -22 to 2	68 (18)	0.002 -16 to -3	67 (29)	0.196 -28 to 6
Vitality	62 (17) n=5076	57 (21)	0.080 -10.62 to 0.62	57 (28)	0.531 -21 to 11	58 (19)	0.174 -9 to 1	56 (25)	0.284 -17 to 5	58 (19)	0.221 -10 to 2	57 (28)	0.531 -21 to 11
Social Functioning	88 (18) n=5069	71 (27)	<0.0005 -24 to -9	80 (26)	0.289 -23 to 7	69 (28)	<0.0005 -27 to -10	75 (26)	0.032 -24 to -1	68 (28)	0.000 -29 to -10	80 (26)	0.289 -23 to 7
Role Emotion	89 (16) n=5058	74 (32)	<0.0005 -23 to -6	79 (28)	0.222 -26 to 6	73 (33)	0.002 -26 to -5	75 (30)	0.045 -27 to -0.3	73 (34)	0.008 -27 to -4	79 (28)	0.222 -26 to 6
Mental Health	75 (16) n=5073	71 (15)	0.050 -8 to 0.02	71 (18)	0.438 -14 to 6	71 (14)	0.068 -8 to 0.31	69 (19)	0.163 -14 to 2	72 (12)	0.148 -7 to 1.1	71 (18)	0.438 -14 to 6

Table 3.3.4: shows the quality of life of adult TSC patients with and without learning disabilities, and with and without epilepsy in this study compared with healthy adult population. * compares the means with healthy population.

3.1.4 Discussion

This is the first study investigating the quality of life of patients with TSC. We note that quality of life is significantly reduced in both adults and children with TSC, compared with the healthy population. The psychosocial domain showed the biggest reduction, compared to the other domains of quality of life. It also highlighted that children and adults with TSC without epilepsy and learning disabilities, report a poorer QoL. Pain was reported less in adults with LD than in adults with normal intellect. The quality of life of children with TSC was reported to be lower than children who suffer from asthma, diabetes, cancer and IBD.

There are limitations with this study. The study was based on a clinic population. Supra-regional specialist clinics tend to care for patients who are more severely affected by the disease, and we would therefore anticipate that this cohort may report a poorer quality of life, due to a greater severity of medical issues and comorbid factors than the overall population of patients with TSC. The gender balance and prevalence of learning disabilities in our clinic cohort, however, was similar to previously reported population based TSC cohorts, suggesting that this clinic population was not grossly dissimilar from the TSC population at large(39). Another limitation is that the quality of life questionnaires for patients with severe learning disabilities were completed by parents and carers. This method of quality of life assessment is less reliable than self-reported outcomes, but it is the only practicable method of assessment in people with learning disability.

The body pain scores were an interesting finding in this study. The higher the score, the less pain patients are believed to experience. The total mean score for body pain for patients with learning disabilities was 81 out of 100, whilst the mean score for adults

without learning disabilities was 62, and is 87 for the general population. This tells us that carers and parents of adults with TSC with learning disabilities report less pain compared to adults with TSC who have no learning disabilities. This makes the reliability of parents' and carers' reports on pain questionable. Clinicians tend to rely on parents and carers reports of pain. Some of the surveillance monitoring checks are based on the presence of pain in patients with TSC. For example, SEGA complications/growth such as hydrocephalus can present with headaches, and angiomyolipoma complications such as bleeding can manifest as loin pain. We may have to be cautious when relying on proxy reports for pain. One could argue that individuals with learning disabilities should be screened more carefully, as these complications cannot be easily detected based on history. Perhaps more frequent monitoring by imaging could be offered to this group. We know from experience that individuals with TSC and learning disabilities are more likely to develop fatal complications compared to those without learning disabilities(93).

In this study, 76% of adults had epilepsy, and 82% of the children had epilepsy. One could argue that the quality of life of these patients in this cohort is worse than the healthy population because of their epilepsy, and not because of TSC as a whole. Epilepsy is a significant morbidity and can have a significant impact on patient's quality of life(115). However, in our study, patients without epilepsy also showed poorer QoL scores compared with the general population. In this study, 62% of adults and 54% of children had learning disabilities. The presence of learning disabilities is another comorbidity which can inversely affect an individual's quality of life(116). However, children and adults without learning disabilities also reported a poorer quality of life compared to the healthy UK population.

Both the self and proxy reported scores for children with TSC were significantly lower than the general population. Proxy report by parents for the psychosocial domain was 25% worse than the self report. Despite the difference between self-reported and proxy scores, they still appear reliable, as they both have reported a significantly poorer QoL compared with the general population. This is similar to other studies that often report lower quality of life by proxy reports than by self report(117). Higher agreement between self and proxy reports is seen for observable physical aspects of QoL, compared to emotional or social aspects(110).

It is interesting to show that the point difference in quality of life domains, especially the social, in both children and adults, compares similarly with the general population. The self-reported total mean score for social functioning in adults with TSC was 80, and is 88 in the general population (point difference 8). The self-reported total mean score for social functioning in children with TSC was 73, and is 88 in the general population (point difference 15). One explanation for adults reporting better social scores than children, is that the adults have learned to cope with TSC and have learnt strategies to help minimise the burden of the disease on their psychology. We noted that the parents reported worse psychosocial domain scores than their children. Having said that, the quality of life assessment of children and adults were performed using two different assessment tools (PedsQL for children and SF36 for adults) and therefore it may not be reliable to compare the results of these two QoL assessment tools.

This study has also highlighted that the burden of TSC on patients is more than the burden of other medical conditions; asthma, diabetes, cancer and inflammatory bowel disease. This is an important finding, as a lot of patients with TSC do not have access

to appropriate treatment. For example, Neuropsychology assessments and interventions play a crucial part in the management of TSC patients, but many do not have access to these services. We have seen in this study that the psychosocial burden is significant, and therefore, it is important that all patients are offered this neuropsychology assessment as early as possible. It may be most efficient for the supra-regional TSC centres to have a psychologist as part of the clinical team, so the patients can access regular psychology review.

3.2 Published papers and Dissemination

- This chapter “Quality of life in patients with Tuberous Sclerosis Complex (TSC)” has been published in the EJPN.

Sam Amin, Andrew A Mallick, Andrew Lux, Finbar O'Callaghan. Quality of life in patients with Tuberous Sclerosis Complex (TSC). Eur J Paediatr Neurol. 2019 Nov;23(6):801-807.

- I presented this paper at the annual British Paediatric Neurology society. Oral presentation.

  S AMIN, A LUX, AA MALICK, F O'CALLAGHAN· Quality of life in patients with tuberous sclerosis complex (TSC). Dev Med Child Neurol Volume 59, Issue Supplement S1 Pages 1–113.

- I also presented this paper at the International TSC meeting, Lisbon, Nov 2016

- I have presented this paper at the European Paediatric Neurology Society Congress.

Chapter 4

4. Topical Sirolimus treatment for facial angiofibroma

4.1 Introduction

4.Topical Sirolimus treatment for facial angiofibroma

4.1 Introduction

4.1.1 Background – facial angiofibromas

Purpose

The purpose of this study was to evaluate the effect of sirolimus ointment on facial angiofibromas in children and adults with TSC. I was also keen to investigate the effect of this drug on patients' quality of life.

Angiofibromas start as small red nodules on the face, mainly on the cheeks and nose, and they usually become obvious at around 4-6 years of age. They can grow larger and more numerous over time and the surrounding skin becomes erythematous. They can spread over the whole face. The facial angiofibroma rash often gets worse and becomes disfiguring around puberty, which can cause severe distress to the affected person and their families. In addition, these tumours contain a higher than normal number of blood vessels and may bleed profusely if scratched, especially in children and adults with learning disabilities. Facial angiofibromas can aggravate anxiety, and can increase a sense of difference and isolation in people with TSC, especially in teenagers with normal intellect.

TOSCA (TuberOus SClerosis registry to increase disease Awareness) is an international, multicentre disease registry to record natural history, clinical manifestations, interventions, and outcomes in patients with TSC. The registry includes a 'core' section and subsections or 'petals'. The 'core' section is designed to record general information on patients' background, collected at baseline and updated annually. Subsections are to record additional data related to specific disease manifestations and are updated annually. TOSCA has recruited 2216 subjects with

TSC. Facial angiofibromas were reported in 1302 (58.8%); the median age of diagnosis was 6 years (Range from 0-67). In this cohort, 442 (33.9%) patients received treatment, some of which was laser therapy alone (152), and laser therapy in combination with other treatments was most common (220). Topical mTOR inhibitor treatment was also reported as a single agent in 76 or was combined with other treatment in 113. It was believed that facial angiofibromas are underreported, as most of these patients were from neurology and nephrology clinics. (118)

People with TSC commonly have severe epilepsy, variable degrees of intellectual impairment and autistic spectrum disorder, making it difficult to socialize and form relationships with non-family members. The disfiguring nature of the rash affects how other people interact with those with TSC and markedly reduces self-confidence in those affected, interfering with social relationships still further. This toxic mixture of physical disfigurement, psychological distress, reduced ability to cope and difficulty with social relationships, all cause marked impairment in the quality of life of people with TSC and their families.

Jozwiak et al investigated the prevalence and natural course of skin lesions in individuals with TSC. One hundred and six children with TSC (47 boys and 59 girls) aged 1 month-18 years from 1984 to 1995 were recruited to this study. The most frequently reported skin lesions were hypomelanotic macules, which were seen in 103 patients out of 106 (97.2%). The macules were present at birth in 66 children. In 20 patients their presentation was delayed until the first few months of life. They reported that facial angiofibromas were seen in 79 (74%) patients. Shagreen patch was seen in 51, café-au-lait macules in 30 patients. They also reported molluscum fibrosum

pendulum in 24 (22%), forehead fibrous plaques in 20 (18.9%), periungual fibromas in 16 (15.1%) and "confetti-like" macules in 3 (2.8%). (119)

Tuberous sclerosis complex is caused by a germline variant in one of two tumour suppressor genes, TSC1 and TSC2. Variant in these genes causes formation of benign tumours in various organs such as brain, lungs, kidneys, skin, etc. It is believed that these benign tumours are formed as a result of somatic second-hit in TSC tumours. However, the exact mechanism is unknown. Tyburczy et al grew fibroblast-like cells from 29 TSC skin tumours from 22 TSC subjects and identified germline and second-hit variants in TSC1/TSC2 using next-generation sequencing. They noted that 18 of 22 (82%) subjects had a variant, and 8 of the 18 (44%) subjects were mosaic with mutant allele frequencies of 0 to 19% in normal tissue DNA. Four patients had multiple tumours, and in each case, second-hit variants in TSC2 were distinct, indicating they developed independently. Most remarkably, 7 (50%) of the 14 somatic point variants were CC>TT ultraviolet 'signature' variants, never seen as a TSC germline variant. These occurred exclusively in facial angiofibroma tumours from sun-exposed sites. They suggested that UV-induced DNA damage is a cause of second-hit variants and development of TSC facial angiofibromas and they also suggested that measures should be put in place to limit UV exposure in TSC children and adults to reduce the frequency and severity of these lesions. (120)

The best treatment options previously have been laser therapy and surgery. The laser technique essentially burns individual lesions reducing the size of the red lumps. However, the larger the area treated, the more painful this is and the longer the recovery. Approximately 50% of patients with TSC have learning disabilities and therefore they require general anaesthetic for laser treatment. Most of these patients

require extensive or repeated laser sessions. In addition, laser therapy does not improve the background erythema and the rash gradually worsens again as new lesions form over the next 2-3 years. Surgery can also be used for single very large lesions but this leaves the rest of the rash unaffected. Some patients opt for laser therapy and ask for repeat treatments, but most feel it is too traumatic and ultimately futile.

4.1.2 Systemic Sirolimus (mTOR inhibitor)

Rapamycin and Everolimus

Rapamycin (also known as sirolimus) is a macrolide which was discovered in the soil of Easter Island (Rapa Nui) in 1965. It was a product of the bacterium Streptomyces hygroscopicus and appeared to have some antifungal activity. However, its use as an antifungal medication was soon abandoned when it was discovered to have potent immunosuppressive and antiproliferative properties due to its ability to inhibit the mTOR pathway. The name was a conjunction of Rapa (from Rapa Nui) and mycin (from streptomycin). (121)

Two decades later it was discovered that rapamycin was a potent mTOR complex 1 (mTORC1) inhibitor. Rapamycin was licensed by the FDA as an immunosuppressive agent for use following solid organ transplants in 1999. Its availability was crucial in working out the pathophysiology of TSC and in the rapid progress towards molecularly targeted treatments for TSC. After the fundamental discovery that TSC was an mTOR over-activation condition, preclinical and early-phase clinical research started to investigate the role of mTOR inhibitors in TSC. In recent years, systemic mTOR inhibitors such as Everolimus and rapamycin have been used to treat TSC related complications such as angiomyolipomas, sub ependymal giant cell astrocytoma,

lymphangioliomyomatosis and epilepsy, both in clinical trials and clinical settings. It has been shown that these systemic mTOR inhibitors improve facial angiofibromas in TSC individuals. However, their use is limited by concerns about systemic side effects. (52, 56)

4.1.3 Topical Sirolimus

Following the success of systemic mTOR inhibitors use in facial angiofibromas, some families, especially in the United States, obtained Sirolimus intravenous liquid formulation and applied it onto the faces of their children with facial angiofibromas. In addition, there have been several case reports of topical sirolimus for the treatment of facial angiofibromas, where different sirolimus ointment/cream strength, preparation and regimes have been used. None of these case reports/studies have used a validated tool to assess the severity of the rash and its response to treatment.

Koenig et al conducted a study investigating the safety of topically applied rapamycin in individuals with TSC, and also to determine its effectiveness in treating facial angiofibromas. All patients were over the age of 13 years and had a diagnosis of tuberous sclerosis complex. This was a double-blind, randomized study. The patients were divided into 3 groups, (i) no rapamycin; (ii) 1 mg of rapamycin per 30 cc (0.003%); or (iii) 5 mg of rapamycin per 30 cc (0.015%). They were given this treatment for 6 months. The patients had their bloods checked for sirolimus level. At the end of the study, the patients were asked if the formulation had improved the appearance of their facial angiofibroma. Twenty-three patients completed the study. There was no evidence of systemic absorption of rapamycin in their blood. Seventy-three percent of subjects in the treatment arms versus 38% of subjects in the placebo arm reported a

subjective improvement in the appearance of their facial angiofibromas. They concluded that the application of low-dose topical rapamycin (0.003-0.015%) to the face can safely decrease the appearance of facial angiofibromas in patients with tuberous sclerosis complex. The limitations with study were that the dose of topical sirolimus was very small, and that the assessment of change in rash was based on patient's report, which is a major limitation as this assessment was done subjectively. (41)

Tanaka et al investigated the efficacy of topical rapamycin 0.2% in individuals with TSC in a left-right comparative study between rapamycin 0·2% topical formulation and vehicle. They recruited 11 patients with TSC. Two formulations, an ointment and a gel, were prepared and in vitro percutaneous absorption of rapamycin was determined. They reported that in vitro percutaneous absorption of rapamycin was significantly greater with the gel compared with the ointment. In the clinical study, the rapamycin-treated cheek showed significant improvements relative to the vehicle-treated cheek in all outcome measures after 12 weeks of treatment. They reported no adverse events due to this treatment and there was no evidence of systemic absorption of rapamycin in the blood of the patients. They concluded that topical rapamycin was significantly effective against angiofibromas. Both formulations used were effective and safe. The 0·2% gel is especially useful because of its better skin penetration and low irritancy. The efficacy of this treatment was subjectively assessed which makes the reliability of the results questionable. In addition, the dose was probably suboptimal. (122)

There have been other small case series and reports on topical sirolimus formulations in TSC. None of these studies have used an objective assessment in determining the

efficacy of sirolimus. In addition, these studies have not assessed the impact of this treatment on patient's quality of life. (123-127)

Rauktys et al investigated topical rapamycin in a mouse model for TSC-related tumours. They applied 0.4% and 0.8% rapamycin ointments to nude mice bearing subcutaneous, TSC-related tumours. The topical treatments were compared with injected rapamycin and topical vehicle. They also measured the rapamycin levels in blood and tumours to assess systemic drug levels in all the animals. They reported that treatment with topical rapamycin improved survival and reduced tumour growth. In addition, topical rapamycin treatment resulted in systemic drug levels within the known therapeutic range and was not as effective as injected rapamycin. They concluded that topical rapamycin inhibits TSC-related tumour growth. They also suggested that these results could lead to a novel treatment approach for facial angiofibromas and other TSC skin lesions. (128)

4.2 Study "Sirolimus Ointment for Facial Angiofibromas in Individuals with Tuberous Sclerosis Complex"

4.2.1 Aims

We set up this pilot study to review the effectiveness and safety of topical sirolimus ointment 0.1% in our clinic. We also assessed the effect of treatment on quality of life.

4.2.2 Methods and Materials

Fourteen sequential patients with a definite diagnosis of TSC, as defined by the International Tuberous Sclerosis Complex Consensus Group, (73) from our TS clinic

at the Royal United Hospital in Bath started using sirolimus ointment from May 2014. Any patients who were suitable were offered the treatment. We had 14 patients suitable for this treatment at the time. Topical 0.1% sirolimus ointment was used. The patients were advised to apply a thin coating to the affected areas on the face, once a day in the evening. Each ointment pot contained 30 mg of sirolimus in 30 grams of ointment, and this consisted of 15 tablets of sirolimus (rapamycin® 2 mg tablets) crushed and mixed with white soft paraffin.

One pot was given for 6 weeks**.** Each pot costed approximately £180. The treatment was not funded by any pharmaceutical companies. The cost was covered by the NHS as this treatment was part of patient's standard care. An information leaflet about topical sirolimus therapy was given to our patients at the start of the therapy. Patients were advised to use hydrocortisone cream should they suffer with irritation or a burning sensation. Digital photography was undertaken at baseline, and then at six months. Six photographs were taken by a digital camera from each patient at each visit. Three photographs were taken with the camera flash on and the other three are taken with the flash in automatic mode. One photograph was taken in full face view directly facing the camera and the other two were side profiles. The preferred facial expression was natural with both eyes open. However, a facial smile was acceptable. Patients were advised not to wear makeup before having their photo taken. None of the patients in this series wore make up during the visits.

During each visit, patients, parents and carers were asked to report on the degree of improvement. The treating physicians were also reporting the effect of the ointment. In order to obtain a more objective assessment of the treatment a consultant dermatologist analysed the photographs and scored treatment response in a blinded

fashion. The dermatologist was given the pre and post treatment photos for each patient without knowing whether they were pre or post treatment photos. The Facial Angiofibroma Severity Index (FASI) was used by the dermatologist to assess the severity of the rash.

The FASI is an objective clinical tool that assesses the severity of facial angiofibromas and treatment response. The index has good inter-rater reliability (correlation coefficient s > 0.98; range 0.97-0.99). (129) The score is obtained by summing the partial scores assigned to each of three features: erythema, size, and extent of lesions. Erythema and lesion size are scored from 0 to 3, while the extent is scored 2 to 3. Mild, moderate and severe facial angiofibroma corresponds with activity score ranges on the FASI of ≤ 5, 6–7 and ≥ 8, respectively. (Table 1)

We use the Pediatric Quality Of Life Inventory (PedsQL™) in our clinic to monitor children's quality of life. PedsQL is a reliable and valid assessment tool for quality of life in children. It is also concise, has age appropriate versions and has parallel forms for children and parents. (123) For the adult patients, we use the Short Form 36 Health Survey Questionnaire (SF-36) to assess quality of life. (113)

Statistical analysis

FASI scores pre and post treatment were compared using Wilcoxon rank sum test. Mann-Whitney U Test was used to compare the FASI scores between children and adults. Quality of life scores were compared using student paired t test.

Ethical approval

Ethical approval was not required as this treatment was being used as part of patient's routine care. This is not a clinical trial. This treatment was available to all patients who were suitable. Consent was taken from each patient for taking facial photographsand for publication.

Patient Characteristics

Fourteen patients (9 children and 5 adults; 7 male) were in this cohort. The median age was 16, and range from 9 to 40 years. Five cases had learning difficulties. Six

cases had already received laser therapy or surgery for their facial rash in the past but significant angiofibromatosis remained. None received systemic mTOR inhibitors during the course of the study. Before the treatment, 4 patients had the maximum FASI score of 9, 7 patients scored 8 and 3 had a FASI score of 7 (Table1).

4.2.3 Results

Treatment Response

Based on the FASI photographic assessments by our dermatologist, FASI scores were improved in twelve out of fourteen patients (Figs 1, 4 and Table 1) (2 tailed-Wilcoxon Signed-Rank test p = 0.002). Of the remaining 2 patients, 1 had improvement in rash but no FASI score change. One had no detectable response. The treating physicians reported improvement in 13 out of 14 patients. Thirteen out of fourteen patients and their parents or carers reported facial angiofibroma improvement.

No one had worsening of facial angiofibromas after the therapy. Children (age 0-16 years) in this cohort responded better to the treatment than the adults. Median FASI score improvement after treatment were three points for children and one point for adults (Mann-Whitney U Test p=0.053). All the children had an improvement in their facial angiofibromas after 6 months compared with four out of five adults. The presence of learning disabilities had no apparent relationship with response to treatment. The median FASI score improvement after treatment were 2 points for both patients with and without learning disabilities. Both female and male patients were equally responsive to this treatment.

Quality Of Life

PedsQL was used in nine patients to assess their quality of life. Six children had no learning difficulties and completed the self-reported PedsQL. The self-reported scores for total psychosocial domain improved in five out of six patients (Fig 2A). The parents of all the nine paediatric patients completed the proxy PedsQL. The proxy reported scores for total psychosocial domain improved significantly after treatment (paired *t*-Test: t= -3.09, p= 0.014), with individual scores improving in five patients, staying the same in two and marginally declining in two. (Fig 2B).

Four adult patients had their quality of life assessed using SF36. The vitality/energy domain scores of SF36, improved markedly in two patients and stayed the same in two (Fig 3A). The social functioning domain scores improved in three patients and declined in one (Fig 3B).

The patient who had an unchanged FASI score but still reported rash improvement, also reported improvements in quality of life. His social function domain scores improved from 50 to 100 and vitality/energy from 56 to 69. The patient who had neither FASI nor facial angiofibroma improvement also had no improvement in quality of life.

Adverse Reactions

One patient developed facial redness two weeks after commencing the treatment. Hydrocortisone cream was used successfully to treat the reaction. He continued with sirolimus ointment and had no further reaction. No other patients reported any adverse events.

4.2.4 Discussion

This report shows that the facial angiofibromas, assessed by a dermatology assessment of photographs, using FASI, showed improvement in 12/14 TSC cases after 6 months of sirolimus ointment 0.1% treatment. The strength of this study is that it has attempted to objectively assess response of facial angiofibromatosis to topical mTOR inhibition using a validated instrument and an assessor blinded to treatment status. It has also attempted to describe the impact of treatment on the quality of life of patients. Sirolimus ointment and cream have been used in smaller case series. However, none of these reports have been from the UK and none have objectively assessed effectiveness and/or quality of life. (122, 125)

One of the patients who did not show FASI score improvement, had a very severe rash before treatment and it remained very severe after treatment. His rash had improved but not enough to change the FASI score. It may be that the FASI score is not sensitive enough to pick up improvement in patients with very severe rash. In particular, this patient's rash was less erythematous after treatment. Erythema is an important component of facial angiofibromatosis and it is the component that demonstrated the greatest improvement in the FASI scores. The degree of erythema noticed on visual inspection is reliably correlated with blood flow detected by Doppler scans. (130) It is probable that the topical mTOR inhibitor has been especially successful in reducing the vascularity of the angiofibromatosis. It is known that systemic mTOR inhibitors have a potent effect on the vascularity of other TSC lesions such as renal angiomyolipomas.

The better response amongst children compared to adults with this treatment is

possibly due to the fact that the children's rash was less severe before the treatment compared with the adults or because the rash is still developing and therefore is more susceptible to intervention. We know from clinical experience that facial angiofibromas tend to stabilise in adulthood. The median FASI scores for children improved from 8 to 5.5 whilst for adults the scores were from 9 to 8. These results suggest that early intervention may be more effective and therefore justifiable.

The treatment has improved the quality of life of the children and adults in this study. The treatment specifically showed a significant impact on the psychosocial domain component of the quality of life scores. The psychological impact of facial angiofibromas on TSC patients may be under-appreciated by healthcare professionals. In our experience, a lot of young children with TSC, who are mildly affected by the facial rash still get teased or picked on at school by their peers. This can have a significant bearing on their school progress, which can also be associated with numerous physical, mental, and social harms. (131) The physical domains of quality of life were also assessed as they are part of the PedsQL and SF36 questionnaires. However, there were no difference in the physical domains pre and post treatment in these patients. The parents and carers of one adult patient in this study have not completed an SF36 form on behalf of the patient as they found the questions difficult to answer. SF36 is a reliable tool and has content validity. (113) However, it may not be so reliable for adults with learning difficulties. There is no standardised and validated tool for quality of life assessment in adults with severe learning difficulties.

There are limitations with this case series report. Firstly, this is not a randomised placebo controlled trial and therefore the results may be subject to bias and there is certainly likely to be a lack of power in any statistical analyses. Secondly, we have not systematically measured compliance to treatment and therefore we cannot say that lack of response to treatment is not due to lack of compliance. However, it is reasonable to suggest that the improvement in facial angiofibromatosis demonstrated in this series is likely to be due to the topical treatment because we know from clinical experience that facial angiofibromas generally do not improve without intervention. Another limitation to the study is that the quality of life questionnaires for patients with severe learning difficulties were completed by parents and carers. This method of quality of life assessment is less reliable than self-reported outcome, but it is the only practicable method of assessment in people with learning disability, and it remains of interest. It is possible that the improvement we have seen in quality of life is due to a placebo effect or close follow up. However, there is good correlation between response of rash to treatment and improvement in quality of life. All the patients who had facial angiofibroma improvement also had improvement in quality of life. In addition, we have seen more improvement in psychosocial domains rather than physical domains after the treatment.

All of the patients treated with this ointment have requested to continue the treatment and this high retention rate further suggests that it is an effective intervention with limited inconvenience or adverse effects. It will be interesting to see whether long term treatment results in continued improvement. We do not know if stopping treatment results in recurrence. We will continue to review them regularly as this treatment constitutes part of their ongoing clinical care. Unfortunately, not many patients in the

UK have access to this ointment due to funding. We are hoping that reporting our experience of sirolimus ointment use will support family groups in streamlining funding for this treatment.

Conclusion

Sirolimus ointment 0.1% once a day was effective in treating facial angiofibromas in our clinical cohort. It also appeared to be safe, well tolerated, and had a positive significant impact on patients' quality of life. We saw greater effect in children than adults and therefore early treatment may be advisable. The positive outcomes from this case series suggest that a larger prospective placebo controlled randomised controlled trial is justified. The safety and efficacy of 0.1% sirolimus in this group sets a baseline platform for a larger national study to compare 0.1% strength with a higher strength and placebo. There is a need for a national study to determine optimal dosing regimen of topical sirolimus for facial angiofibromas in TSC, to facilitate EMA licence application for this indication and to facilitate NHS funding for this treatment. Currently, this treatment is not available through the NHS apart from through limited Tuberous Sclerosis clinics in the United Kingdom.

Figure legends:

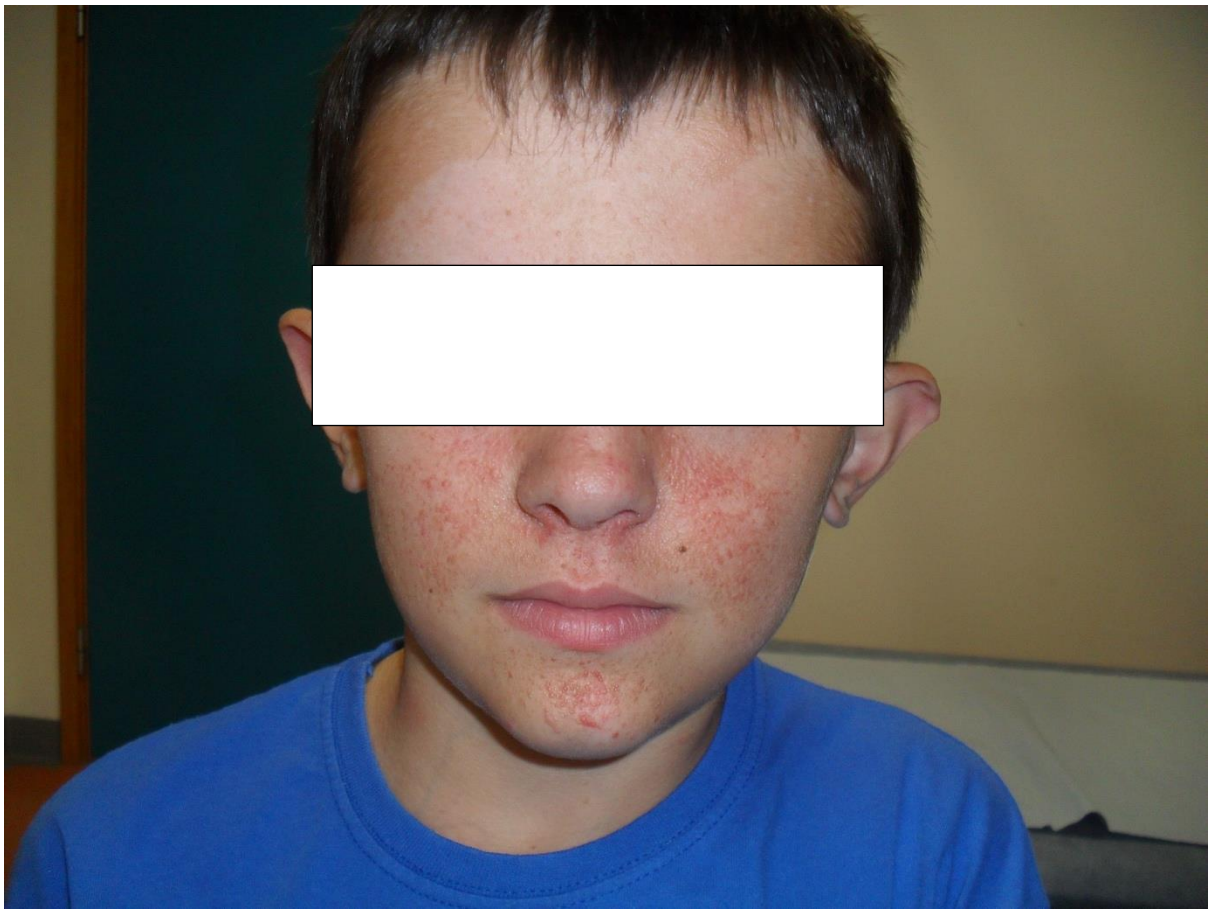


Figure 4.3.1: Before treatment

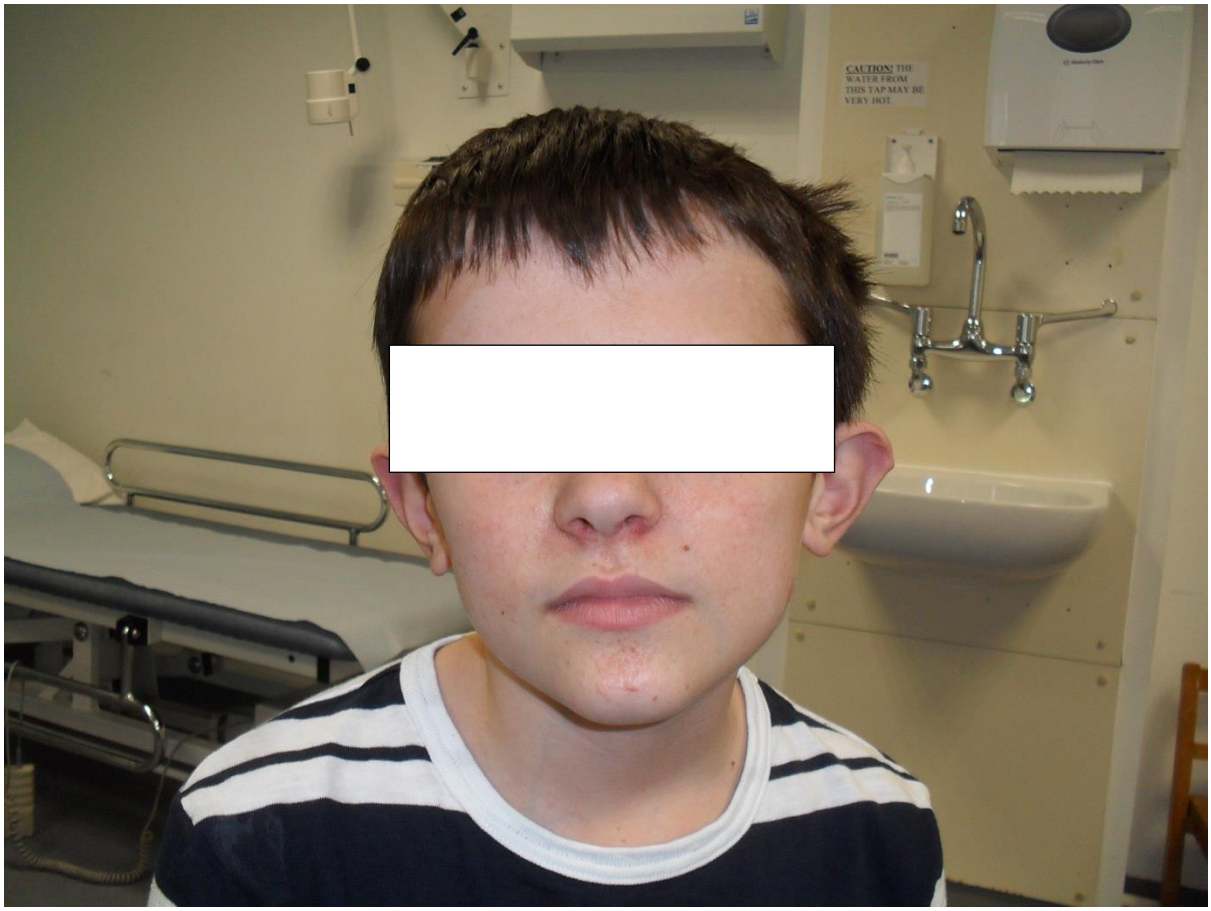


Figure 4.3.2: After treatement

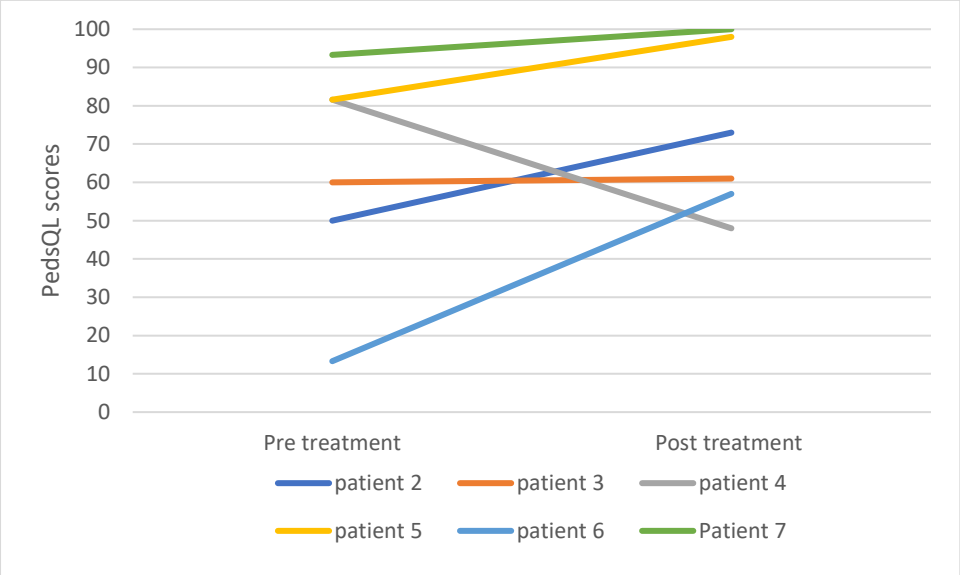


Figure 4.3.3: shows self-report responses by the children to the PedsQL psychosocial domain before and 6 months after the treatment.

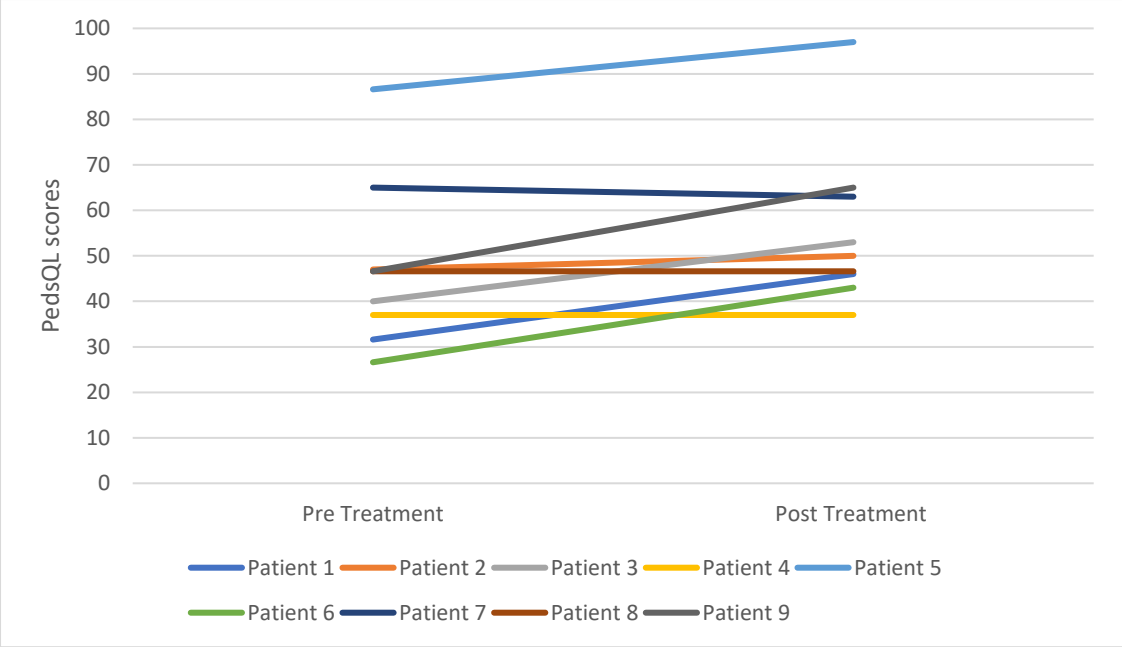


Figure 4.3.4: shows proxy-report responses by the parents of all the children to the PedsQL psychosocial domain before and 6 months after the treatment.

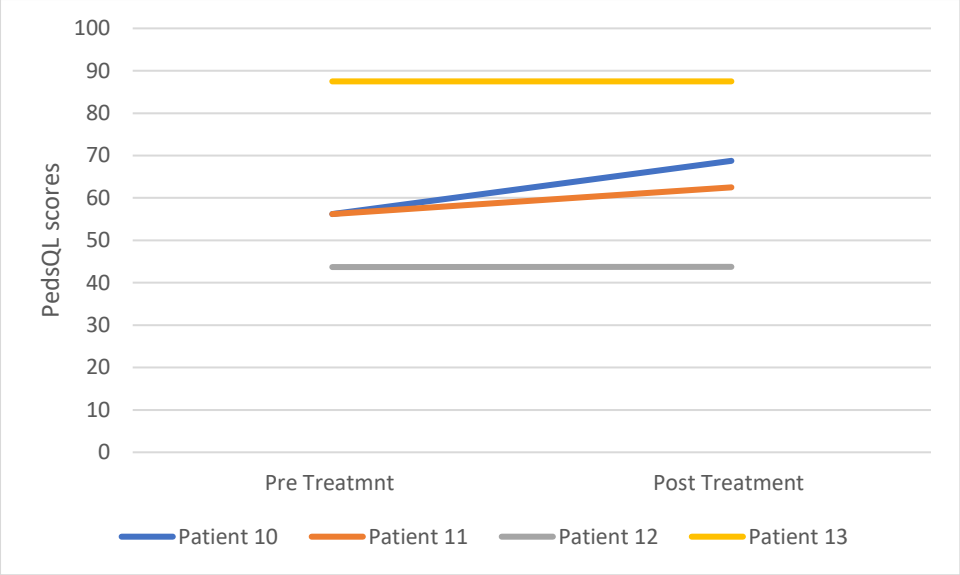


Figure 4.3.5: shows SF36 vitality/energy scores for adult patients before and after the treatment.

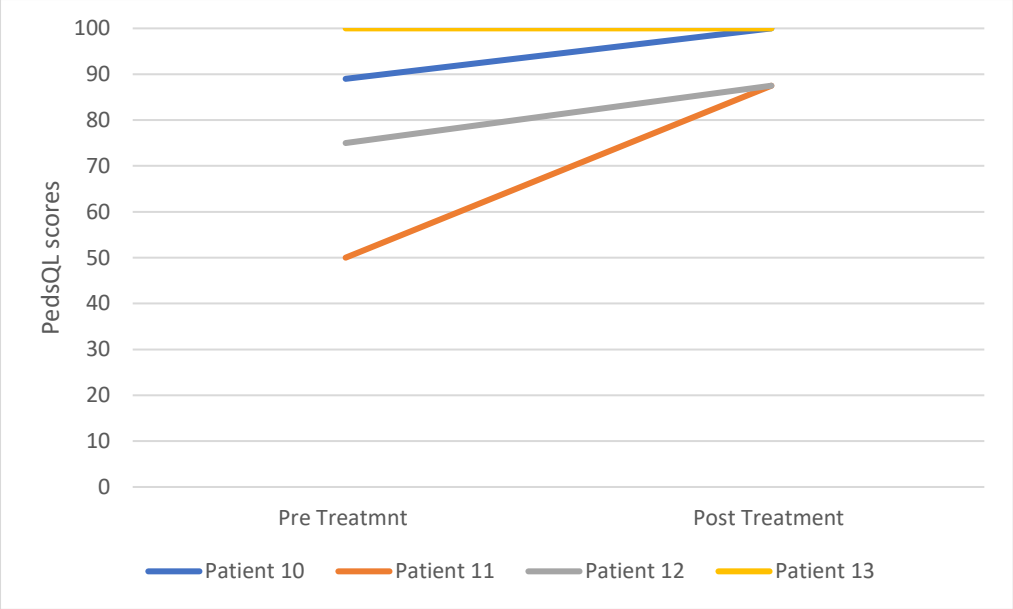


Figure 4.3.6: shows SF36 social functioning scores for the adult patients before and after the treatment.

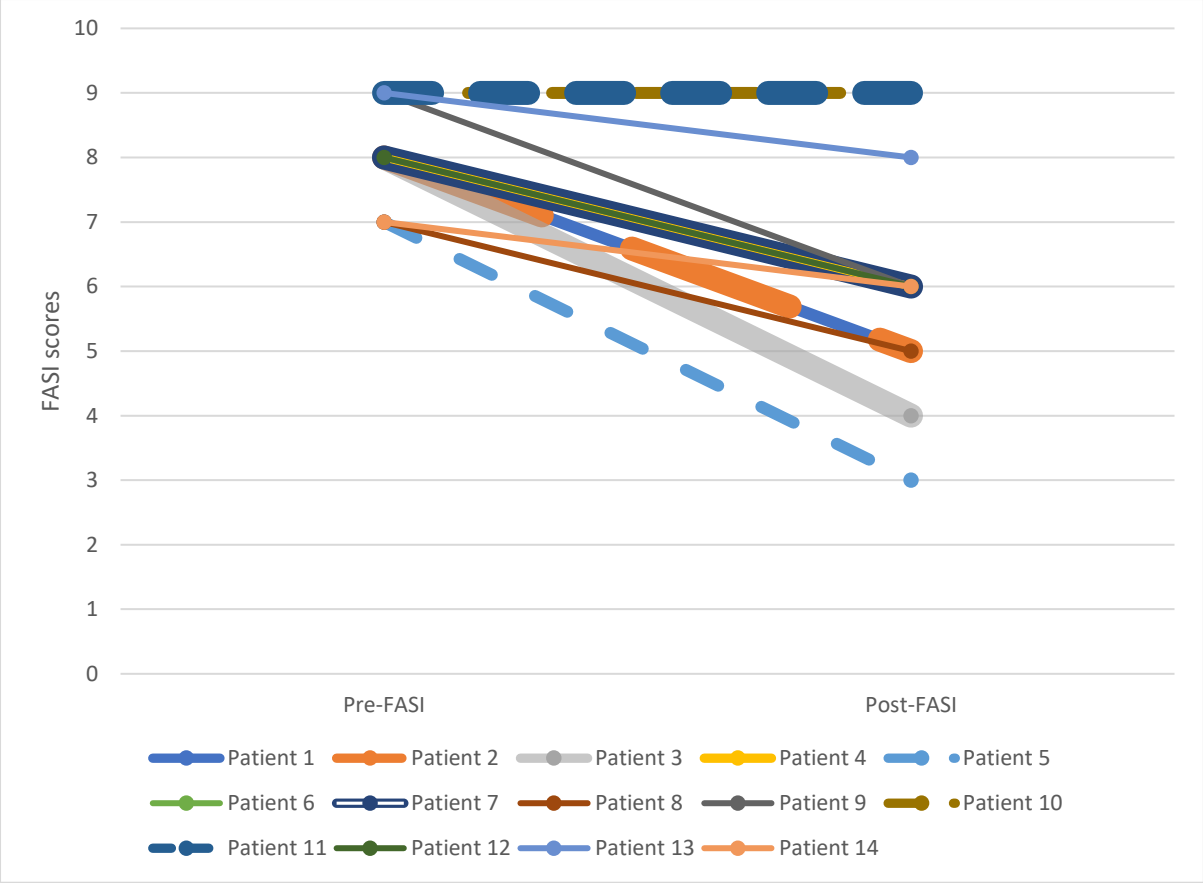


Figure 4.3.7: Shows FASI scores before and after treatment.

Patient No	Age	Sex	Learning disabilities-Present	Pre-Erythema (0/1/2/3)	Pre-Size (1/2/3)	Pre-Extension (2/3)	Pre-FASI	Post-Erythema (0/1/2/3)	Post-Size (1/2/3)	Post-Extension (2/3)	Post-FASI
1	12	♀	Yes	2	3	3	**8**	0	2	3	**5**
2	11	♂	No	2	3	3	**8**	1	2	2	**5**
3	13	♂	No	2	3	3	**8**	0	1	3	**4**
4	14	♀	No	2	3	3	**8**	1	2	3	**6**
5	16	♂	No	2	2	3	**7**	0	1	2	**3**
6	9	♀	No	2	3	3	**8**	1	2	3	**6**
7	17	♀	No	2	3	3	**8**	1	2	3	**6**
8	14	♀	Yes	2	2	3	**7**	1	2	2	**5**
9	9	♂	Yes	3	3	3	**9**	2	2	2	**6**
10	23	♂	No	3	3	3	**9**	3	3	3	**9**
11	27	♂	No	3	3	3	**9**	3	3	3	**9**
12	24	♀	No	2	3	3	**8**	1	2	3	**6**
13	22	♂	Yes	3	3	3	**9**	2	3	3	**8**
14	40	♀	Yes	1	3	3	**7**	0	3	3	**6**

Erythema	Size	Extension	FASI Score
Skin color-0 Light Red-1 Red-2 Dark Red/Purple-3	Small (<5mm)=1 Large(>5mm)=2 Confluent=3	<50% Cheek area=2 >50% Cheek area=3	< or = 5 – Mild 6-7 = Moderate = or > 8 – Severe

Table 4.4.1: Shows patients characteristics and Facial Angiofibroma Severity Index (FASI) score before and after treatment

4.5 Published papers and Dissemination

I published “Sirolimus Ointment for Facial Angiofibromas in Individuals with Tuberous Sclerosis Complex” in a peer reviewed journal.
S. Amin, A. Lux, A. Khan, F. O’Callaghan. Sirolimus Ointment for Facial Angiofibromas in Individuals with Tuberous Sclerosis Complex. Int Sch Res Notices. 2017 Nov 15;2017: 8404378.

I presented “Sirolimus ointment for facial angiofibromas in individuals with tuberous sclerosis complex (TSC)” at the annual BPNA meeting. The abstract was published.
S AMIN, A LUX, A KHAN, F O’CALLAGHAN. Sirolimus ointment for facial angiofibromas in individuals with tuberous sclerosis complex (TSC). Dev Med Child Neurol Volume 59, Issue Supplement S1 Pages 1–113.

I presented “Novel treatment option of Sirolimus ointment for facial angiofibromas in individuals with Tuberous Sclerosis Complex (TSC)” at the annual RCPCH conference. The abstract was published.
S Amin, A Lux, A Khan, F O’Callaghan. Novel treatment option of Sirolimus ointment for facial angiofibromas in individuals with Tuberous Sclerosis Complex (TSC). British Society for Paediatric and Adolescent Rheumatology and British Society of Paediatric Dermatology. Arch Dis Child 2016;101:Suppl 1 A143

Chapter 5

5. Metformin treatment for Tuberous Sclerosis Complex

5.1 Abstract

5 Metformin treatment for Tuberous Sclerosis Complex

5.1 Abstract

Metformin was used in patients with influenza and malaria. In some developed countries, sanction on its use was only removed two decades ago. Currently, metformin is the most widely prescribed anti-diabetic drug in the world, and it is classed as an essential drug on the World Health Organization (WHO) list.

Our knowledge of the effect of metformin on human health is increasing. In addition to its ability to improve the control of hyperglycaemia, metformin has been shown to reduce the burden of ageing via effects on damaged DNA and the process of apoptosis. Studies have shown that metformin may reduce the risk of cardiovascular disease through influences on body weight, blood pressure, cholesterol levels and the progression of atherosclerosis. Studies also suggest that metformin may be beneficial for neuro-psychiatric disorders, erectile dysfunction, cognitive impairment and in reducing the risk of dementia. *In vivo* and *in vitro* studies have shown that metformin has anti-cancer properties, and population studies have suggested that metformin may reduce the risk of cancer or improve cancer prognosis. It is thought that it exerts its anti-neoplastic effect through the inhibition of the mammalian target of rapamycin (mTOR) signaling pathway, by activating the AMPK (adenosine monophosphate-activated protein kinase) regulator and the tumour protein p53 (also known as TP53). Metformin has also been shown to reduce the disease progression in Duchenne Muscular Dystrophy which is a progressive neuromuscular disorder.

We investigated metformin in a randomised double blind placebo controlled trial. We demonstrated that metformin is safe and well tolerated in children and adults with TSC. Patients on metformin had a significant reduction in SEGA volume compared with placebo. Metformin did not reduce AML size but growth appeared slower than in the placebo group, although this difference was not statistically significant. There may be a role for metformin in slowing or reversing growth of life-threatening hamartomas in TSC. Further study is justified.

5.1.1 Discovery and early use of metformin

Metformin was originally discovered from the plant *Galega officinalis,* part of the Faboideae subfamily. The Latin term *Gala* derives from ‘milk’ and *ago* meant ‘to bring on’, and it was believed that ingesting the plant improved lactation in some animals. (132, 133) It is also known as ‘goat’s rue’, ‘French lilac’, ‘Italian fitch’ and ‘professor weed’. The Galega plant originated in the Middle East and is also found in Europe and Asia.

This plant was used in conventional medicine. It was used to treat influenza and malaria. It was believed to have effects that today we would describe as bacteriostatic, antiviral, antimalarial, antipyretic and analgesic. (134)

It was found that patients with diabetes who were taking goat’s rue had lower blood glucose. In addition, this plant relieved urinary frequency in some individuals. This led scientists to investigate the plant and find the glucose lowering agent. In 1922, Emil Werner and James Bell, chemists in Dublin, Ireland, found that the active ingredient in this plant was guanidine (CH_6ClN_3).

The biguanides are derivatives of guanidine. (135). (136) Biguanide is an organic compound with the formula $HN(C(NH)NH_2)_2$. Biguanides are metformin, phenformin and buformin.

Emil Werner and James Bell found that dicyanodiamide is precursor to guanidine derivatives. They noted that dicyanodiamide converts to dimethyldiguanide, which is metformin, in the presence of dimethylammonium chloride in acidic conditions for 3-4 hours.

In 1929, Slotta and Tschesche found that metformin has sugar-lowering action in rabbits. They noted that this was the most potent amongst other compounds they studied. (137) The discovery of insulin has overshadowed these results.

Frederick Banting and Charles Best started working on insulin in 1921. They showed that there is a substance produced by pancreas reduces the blood sugar. The substance removes sugar from a dog's urine whose pancreas had been removed. However, this substance was not pure enough as it was causing fever in diabetic patients. (138) Soon after this, James Collip joined the team and he produced insulin from beef pancreas and this was pure enough to treat diabetic patients. (139)

In the 1950s, metformin attracted attention again, as it was found to have no harmful effect on blood pressure and heart rate in animals compared with other anti-hypoglycaemic agents.

During the same decade, scientists in Paris reinvestigated the blood sugar reducing activity of metformin in patients with diabetes. Metformin was called a 'glucose eater' (Glucophage). The group published first trial of metformin in humans with diabetes.

Phenformin was the first biguanide available for clinical use. However, it was soon withdrawn from use due to toxic effects. There were numerous case reports of lactic acidosis in 1970 due to phenformin, hence it was removed from the market. Buformin was also found to have toxic effects and it was removed from the clinical use. Metformin was the only biguanide to remain safe to use. However, some countries such as Australia put

severe restrictions on metformin and phenformin, not taking into account the different pharmacokinetics of the two drugs.

Metformin became more popular when it was trialed in the UK and was shown to be effective in type 2 diabetes, causing no significant hypoglycemia or weight loss. It took a long time for the rest of the world to realize this. (140, 141)

The Canadians then approved metformin in 1972 after the withdrawal of the other biguanides. The Americans did not rediscover metformin until 1990 and then the Australians removed sanction on its use. The Food and Drug Administration (FDA) approved metformin for type 2 diabetes in 1994. (142)

Large studies in the UK showed that metformin use is associated with reduced risks of myocardial infarction, stroke and mortality irrespective of glucose control. Hence it became the first drug of choice for type 2 diabetes. Consequently, in 2012, it was declared in The United States of America and Europe that metformin should be the first drug of choice for all individuals with type 2 diabetes. (143, 144)

Currently, metformin is the most widely prescribed anti-diabetic drug in the world, and it is classed as an essential drug on the World Health Organization (WHO) list. In addition, metformin has been used in patients with polycystic ovarian disease and gestational diabetes. Because of its protective effect on lipids, glucose levels, and weight, it has been used in patients who receive anti-psychiatric drugs.

5.1.2 Chemical structure

The chemical names of metformin are metformin; 1,1-Dimethylbiguanide; Glucophage; 657-24-9; Glumetza; and Dimethylbiguanide. The chemical structure of metformin is C4H11N5. Figure 1. Two guanide molecules joined together are known as biguanides. Metformin is a bigunaide. It is a white crystalline compound which has a molecular weight of 129 gm /mol. It is water soluble, and insoluble in chemical agents such as ether, chloroform or acetone.

Figure 5.4.1: shows metformin chemical structure

5.1.3 Absorption and Bioavailability

In humans, metformin is estimated to be absorbed over approximately 6 hours, and its bioavailability is approximately 50-60% under fasting conditions. (145) Absorption may occur through a saturable process, and therefore the level of absorption is not dose related. The saturable process begins when a drug is given orally, the drug then enters the stomach. It begins to dissolve in the gastrointestinal fluid. Most of the absorbed drug enters the liver via the hepatic portal vein to reach the systemic circulation. If the drug becomes saturated in any of these systems, increase in dispensed dose will not match the increase in amount of drug absorbed into the body. Taking metformin with food, delays its absorption. (146, 147) If one tablet of metformin is taken with food the mean peak plasma concentration (C_{max}) is lowered by a mean of 40%, and the time to peak plasma concentration (T_{max}) is prolonged by approximately 35 minutes. The amount of fat in food does not affect the drug pharmacokinetics.

The intestinal absorption of metformin may be facilitated by plasma membrane monoamine transporters (PMATs). These transporters are encoded by the *SLC29A4* gene and they are expressed on the luminal side of enterocytes. (147) Other genes, known as organic cation transporters, are thought to facilitate the transfer of metformin into the interstitial fluid (145, 148), for example, the *OCT1* (*SLC22A1*) and *OCT3* (*SLC22A3*) genes, which are expressed on the brush border and cytoplasm of the enterocytes. The *OCT2* gene plays a role in facilitating metformin uptake from the systemic circulation into renal epithelial cells. (147) *SLC29A4* and *OCT1* gene expression also occurs in kidney cells. (148, 149)

Genetic polymorphisms in these transporter genes are likely to have an impact on metformin pharmacokinetics and variability in drug responses between individuals. Trough steady-state metformin plasma concentrations have been shown to range from 54 to 4133 ng/ml and demonstrate significant inter-individual variability in metformin pharmacokinetics. (150)

Inhibitors of the OCT1, 2 and 3 transporters, such as proton pump inhibitors, can inhibit metformin uptake. Oral antidiabetic drugs such as repaglinide and rosiglitazone can also inhibit OCT1 and therefore inhibit metformin uptake. (151, 152)

5.1.4 Distribution

The apparent volume of distribution is the theoretical volume that the body would require if it were to contain the total amount of an administered drug at the same concentration as that found in the blood plasma.

The volume distribution of metformin is 654 L for metformin 850 mg administered as a single dose. The volume of distribution following intravenous administration is 63-276 L. This is probably due to less binding in the gastrointestinal tract. It may also be due to the way different methods are used to determine volume of distribution.

Some anti-hypoglycemic agents, such as sulfonylureas, are more than 90% protein bound, whereas metformin is negligibly bound to plasma proteins. The steady state plasma concentration of metformin is reached within 24 to 48 hours, and is <1 µg/mL. The steady state is reached at a time of 4 to 5 times the half-life, after regular administration is commenced. In other words, the rate of elimination is equal to the rate of input.(146)

5.1.5 Metabolism and Elimination

Following oral metformin administration, metformin is excreted, without chemical modification, in the urine. The major route for metformin excretion is via the renal tubules. Its renal clearance is 3.5 times greater than that of creatinine. In addition, more than 90% of the absorbed drug is excreted through the kidneys within the first 24 hours following oral administration. The mean plasma half-life is 6.2 hours (range 8-12 hours). Half-life is the time that a drug takes to lose its pharmacologic and physiologic effect. (153)

5.1.6 Pharmacogenomics

Studies have shown that genetic polymorphisms can have an effect on the pharmacokinetics of metformin. A study of a group of volunteers showed that individuals who carry the reduced function *SLC22A1 (OCT1)* alleles have a higher area under the concentration– time curve (AUC), higher maximal plasma concentration C_{max}, and a lower oral volume of distribution compared with volunteers who were carrying wild-type alleles (154). Genetic variants in *SLC22A2 (OCT2)* have also been shown to have an effect on metformin pharmacokinetics. Studies have shown that the variants were associated with an increase in AUC and C_{max}. (155) These genetic variants can also affect the pharmacodynamics of metformin. In healthy volunteers, reduced function of *SLC22A1 (OCT1)* variants has a significant clinical effect, causing impaired response to a glucose tolerance test. (154) Variations in other genes, such as *MATE2-K (SLC47A2)*, another organic cation transporter, have also been shown to have an effect on metformin response. Diabetic patients who were homozygous for the *MATE2-K* genetic variant g.-130G>A (rs12943590) showed a significantly poorer response to metformin treatment assessed as relative change in glycated hemoglobin (HbA1c). (156)

Studies have shown that the *ATM* (ataxia telangiectasia mutated) gene which is involved in DNA repair and cell cycle control, has a role in the effect of metformin upstream of AMPK. It has been shown that variation in this gene alters glycaemic response to metformin. Two large cohorts of patients with diabetes have shown that some variants in the *ATM* gene locus were associated with a better glycemic response to metformin. The minor allele (C) of the most strongly associated Single Nucleotide Polymorphism (SNP), *rs11212617*, had a population frequency of 44% and was associated with treatment success. The treatment success was classed as achievement of HbA1c < 7%.(157)

Studies have shown that variations in other genes such as *LKB/ STK11 (rs8111699)* are associated with ovulatory response to treatment with metformin, with the C allele associated with a significantly decreased chance of ovulation in patients with polycystic ovary disease who are treated with metformin(158).

5.1.7 Blood Brain Barrier

Orally administered metformin has been shown to cross the blood-brain barrier. The distribution is not normally distributed in the central nervous system. Metformin has been shown to have an anti-inflammatory effect on the central nervous system. (159-161) It has also been demonstrated that metformin prolongs survival time in a Huntington mouse model. (162) It has been demonstrated in animal studies that after a single dose of 50mg/kg of metformin, the drug crosses the blood brain barrier and is seen in the brain. (163, 164)

In addition, in other conditions, such as multiple sclerosis and Alzheimer's disease, where AMPK activity is disturbed, metformin has been shown to restore the activity of AMPK in the central nervous system. (165)

Labuzek et al used the Wistar rats inflammatory model to study the level of metformin in different parts of the brain, cerebrospinal fluid and plasma. Inflammation was induced by injecting lipopolysaccharide intraperitoneally. There were two experiments involved in this study, acute and chronic. A single dose of metformin of 150 mg/kg was given orally for the acute experiment group and 300 mg/kg daily was given to the chronic group.

The maximum level of metformin in plasma (mean 27.8 SD 3.3 μmol/l) was 1 hour after administration and maximum level in the brain (mean 13.5 SD 2.3 nmol/g) was 6 hours after administration. The authors also reported that the concentration of metformin in the brain tissue was similar to the plasma concentration. This was achieved by looking at the brain-to-plasma ratio. It was noted that metformin was not equally distributed in the brain.

The cerebellum was found to have the highest level of metformin, and the striatum had the least. (166)

5.1.8 Side effects of metformin

Lactic acidosis

Lactic acidosis is a rare, but sometimes lethal side effect of metformin. It is characterized by accumulation of lactic acid and decreased pH levels. Lactic acidosis occurs when the blood lactate level reaches >5 mmol/L and the pH goes below the normal range. The incidence of MALA (Metformin Associated Lactic Acidosis) is around 0.03 cases/1000 patient-years with 50% mortality.(142)

Lactic acidosis is primarily seen in patients with cardiovascular, renal and liver disease, due to tissue hypo-perfusion and hypoxemia. MALA is seen in patients who are taking metformin, especially in the presence of hypoxia and poor tissue perfusion. MALA has not been reported in any clinical trials. This is probably because most patients who take part in clinical trials are likely to be stable and have regular blood test monitoring. (167) However, MALA has been reported in patients with normal kidney function. It is believed to be reversible in patients with no comorbidities. (168)

Patients who are aged 80 and older are also at risk of developing lactic acidosis due to metformin. This drug should only be given to this group of patients if their kidney function is within the normal range. In order to avoid this side effect, metformin should be stopped immediately in the presence of sepsis, dehydration and hypoxia. (169)

Lactic acidosis due to metformin may occur due to anaerobic stimulation of lactate production by intestinal cells, with reduced elimination of lactate from the liver especially in patients with liver failure. Accumulation of metformin in renal failure, overdose and liver failure contributes to this.(170)

The main stay of treatment in MALA is supportive. Metformin should immediately be stopped if MALA is suspected. Hemodialysis has been recommended as a protective measure by many intensive care doctors. (171) Continuous renal replacement therapy (CRRT) has also been recommended and it has been shown to be safe and effective in this situation. (172)

Other side effects

Chronic administration of metformin has been associated with a decrease of vitamin B12 absorption with decrease of serum levels. This should be considered if a patient presents with megaloblastic anaemia. (173)

Metformin can cause taste disturbance and is thought to be secreted into saliva via the OCT3 solute carrier. OCT3 (organic cation transporter-3) is a polyspecific drug transporter in the solute carrier 22 family which is highly expressed in the salivary glands. It is localized at both basolateral (blood-facing) and apical (saliva-facing) membranes of salivary gland acinar cells. Studies have shown that in wild-type mice, metformin is transported with a high level of accumulation in the salivary glands, whilst *Oct* (-/-) mice, have been shown to lack uptake and accumulation of metformin in the salivary glands. (174)

Metformin causes gastrointestinal side effects such as abdominal pain, diarrhoea, vomiting, nausea and loss of appetite. These side effects occur most frequently during introduction of the drug and tend to improve in most cases. The gastrointestinal side effects of metformin have been associated with the reduced-function alleles of *SLC22A1* and serotonin reuptake transporter (*SERT*). (175) *SLC22A1* is the approved symbol for solute carrier family 22 member 3 by the HUGO Gene Nomenclature Committee.

5.1.9 Pharmacodynamics of metformin

5.1.10 Metformin and diabetes

Metformin reduces blood sugar levels by reducing hepatic glucose synthesis, reducing absorption of glucose in the gastrointestinal tract and increasing the insulin sensitivity.

Hepatic glucose synthesis

The hepatic suppression of gluconeogenesis is meditated by insulin. Metformin increases the activity of the insulin receptors and stimulates glucose uptake by cells through the translocation of glucose transporters such as GLUT-1 (*SLC2A1* gene). (176) Metformin can also block the effect of glucagon hormone which leads to reduction in glucose production.(177) As a result, metformin reduces the blood sugar levels by inhibiting the hepatic glucose production.

Glucose uptake in peripheral tissue

Metformin can also increase the activity of the insulin receptors and stimulates glucose uptake in skeletal muscles through the translocation of glucose transporters such as GLUT-4 (*SLC2A4* gene). As a result, metformin leads to an increase in the uptake of glucose in skeletal muscles.

Metformin and the pancreas

Metformin is not known to cause significant hypoglycaemia as it does not increase the level of insulin in the circulation. In fact, it reduces the level of insulin.(178) in non-diabetic patients, metformin causes a reduction in blood glucose level which leads to a reduction in insulin secretion. Some patients with diabetes may develop hypoglycemia secondary to metformin as this mechanism may be less effective in diabetic patients.(179)

5.1.11 Metformin beyond control of blood sugar

5.1.11.1 Metformin and ageing

It has been postulated that metformin reduces the burden of ageing. Two large clinical trials, the Investigation of Metformin in Pre-Diabetes on Atherosclerotic Cardiovascular OuTcomes (VA-IMPACT), and the Targeting Aging with Metformin (TAME), are aiming to assess the effects of metformin in non-diabetic patients, and particularly its effects on the ageing process.

The process of ageing is complex but a crucial element is DNA damage and cell death, processes which are initiated via mechanisms that include Inflammatory markers including interleukins.

Metformin inhibits the translocation of the transcription factor NF-kB to the nucleus and prevents the phosphorylation of IκB and IKKα/β, which are required for activation of NF-kB pathway.(180) Activation of NF-kB pathway leads to production of inflammatory cytokines. It has been suggested that chronic inflammation leads to aging and this phenomenon is known as inflammaging. Metformin exhibits its antiaging effect via the inhibition of the NF-kB pathway. (180)

Metformin can also minimise the production of reactive oxygen species (ROS) in respiratory chain complex 1, which prevents oxidative stress related cell death. ROS are an endogenous source for DNA damage. (181)

Ceramides are found within cell membranes and can play a role in cellular signaling, including cell differentiation, proliferation, and programmed cell death. Ceramides are

believed to have a role in the ageing process through the cell death programme. They inhibit myoblast proliferation and cell cycle regulation in skeletal muscles. Metformin has been shown to minimise the harmful effect of ceramides. (182)

Metformin may also have a role in cardio-protection via its effect on the endothelial nitric oxide synthase dependent pathway, which can stimulate ischemia induced revascularization. It can also be neuroprotective, reducing neuronal damage by preventing etoposide-induced apoptosis in neurons (183, 184)

5.1.11.2 Metformin and cardiovascular disease

Human studies have shown that metformin reduces the risk of cardiovascular disease in diabetic patients. The cardiometabolic effect has also been evaluated in diabetic patients. Studies have shown that those individuals who were taking metformin had a reduction in body weight, LDL cholesterol, and atherosclerosis, based on carotid artery intima-media thickness analysis.(185, 186)

5.1.11.3 Metformin and weight loss

Studies have shown that individuals with diabetes, without diabetes, and those at risk of diabetes, are likely to lose a small but beneficial amount of weight when taking metformin. Metformin has not been approved for weight loss but some clinicians use it in over-weight patients who are at risk of diabetes.(187-189)

5.1.11.4 Metformin and neuropsychiatric disorders

Depression

A positive effect of metformin in depression has been reported. Guo et al reported that patients with type 2 diabetes who were on metformin showed improvement in depression. This is may be because these patients achieved better glycemic control.(190)

Cognitive abilities

Studies have shown that individuals who take metformin are likely to show a reduction in mild cognitive impairment compared with those patients who are not taking metformin, or those taking other hypoglycemic agents. The risk of dementia has also been reported to be lower in those who take metformin. (191, 192)

One could argue that there are a lot of cofounding factors with these patients such as cardiovascular disease, age, diabetes, and other comorbidities. However, this study by Luchsinger et al has suggested that metformin also improves cognition in individuals without diabetes.(193)

Metformin and erectile dysfunction

There are three possible mechanisms involved in the pathogenesis of erectile dysfunction, such as endothelium-dependent vasodilatory impairment, sympathetic nerve activity elevation and atherosclerotic luminal narrowing. Metformin exerts its effect via these pathways. Animal studies have shown that treatment with metformin in rats and rabbits

restores the transcription of endothelial nitric oxide production in the penile tissue, thus improving the endothelium dependent vasodilatory impairment. (194)

Metformin has also been shown to improve the endothelial dependant vasodilatation in diabetic and non-diabetic patients. (195) Sympathetic over-activity is another possible mechanism for erectile dysfunction. Metformin has been shown to reduce the level of norepinephrine which is a marker of sympathetic activity. (196)

Atherosclerotic luminal narrowing is another possible mechanism for erectile dysfunction which is linked to high blood pressure. Metformin has been shown to attenuate hypertension, thus improving or reducing the occurrence of atherosclerotic luminal narrowing. (197)

5.1.11.5 Metformin and Duchenne Muscular Dystrophy (DMD)

DMD is an X-linked recessive neuromuscular disorder that affects 1 in 3,500–6,000 male births. Generally, children with DMD present in early childhood with proximal muscle weakness and become wheelchair-dependent by adolescence. This is a progressive muscle degeneration disorder which is caused by a variant in the *DMD* gene. Variant in this gene leads to an absence of the protein dystrophin. (198, 199) Dystrophin is located primarily in skeletal and heart muscle, where it helps stabilize and protect muscle fibres. Loss of dystrophin leads to loss of cytoskeletal integrity.(200) This in turn leads to dysregulation of calcium homeostasis and increased production of reactive oxygen species (ROS), which results in protein and membrane damage. Mitochondria are the main source for cellular ROS. High production of cellular ROS implies altered mitochondrial function in DMD. (201) Biopsy samples of DMD patients have shown reduced rates of cellular respiration and lower activities of enzymes of the mitochondrial respiratory chain. (202) These findings have also been seen in DMD mouse models. (203)

Loss of dystrophin in DMD is associated with a significant reduction in neuronal nitric oxide (NO) activity. (204) NO activation is essential for mitochondrial function in order to minimise oxidative stress and to improve fat usage for energy production. (205) NO plays a role in regulating muscular energy. Activation of AMP-activated protein kinase (AMPK) stimulates NO synthesis. (206) Animal studies have shown that in DMD animal models, activation of AMPK reduces muscle fatigability and improves muscle functions. (207)

Hafner et al used metformin 250mg twice a day with L-arginine for 16 weeks in five ambulatory children with DMD. The authors reported no serious side effects and none of the patients dropped out of the study. It was noted in the muscle biopsy samples before

treatment that there was a significant reduction in the mitochondrial protein expression and an increase in oxidative stress. They reported that there was a significant increase in the mitochondrial electron transport chain and a reduction in oxidative stress after treatment. They also reported a reduction in resting energy expenditure rates, and energy substrate use shifted from carbohydrates to fatty acids. It was concluded that pharmacological stimulation of the nitric oxide pathway with metformin leads to an improvement in mitochondrial function and a slowing of disease progression. (208)

5.1.11.6 Metformin and Cancer

Population studies have suggested that metformin may reduce the risk of cancer or improve cancer prognosis. This led scientists to further investigate metformin. (209, 210)

Vivo and in vitro studies have shown that metformin has anti-cancer properties. The mechanism by which metformin exhibits its anti-neoplastic effect is through the inhibition of the mammalian target of rapamycin (mTOR) signaling pathway, by activating the AMPK regulator (adenosine monophosphate-activated protein kinase) and p53.

In vitro experiments have been carried out to investigate the effect of metformin on epithelial cells in breast cancer cells. The aim was to ensure that this effect is a direct effect of metformin rather than through insulin levels. It has been noted that metformin acts as a growth inhibitor for MCF-7 human breast cancer cells. MCF-7 is the acronym of Michigan Cancer Foundation-7. These cell lines were discovered in 1970s. These cells are known to be responsive to insulin. The effect of metformin has been through the suppression of phosphorylation of p70S6K at Thr389 rather than through insulin or insulin growth factors (IGF). Activation of the AMPK pathway by metformin is observed in epithelial breast cancer cells. Activation of AMPK leads to inhibition of the mTOR pathway, reduction in proliferation, S6 kinase inactivation, reduction in mRNA translation and protein synthesis in these cancer cells. (211)

Other studies have investigated the effect of metformin on other cancer cells**,** such as those from colon cancers. A group studied the effect of metformin on human colonic carcinoma cell lines. They used paired isogenic human colonic carcinoma cell lines, HCT116 p53+/+ and p53−/− in nude mice. The right flank of the mice was injected with

p53−/− cells and the left flank with HCT116 p53+/+. The animals were given intraperitoneal metformin or saline solution, four days post cell injection. It is known that loss of wild-type p53−/− accelerates tumour formation in untreated animals. The group reported a significant tumour volume reduction in the metformin group in the HCT116 p53−/− cells compared with the growth of the tumours from p53+/+ cells in the opposite flank. The tumour volume after one month for the p53−/− xenografts was >50% smaller than p53−/− xenografts from the animals treated with saline solution. (212)

Another group evaluated the effects of metformin on renal cell carcinoma and its main mechanisms. They reported that metformin was able to cycle arrest and inhibit renal cell carcinoma growth in vitro and vivo via the activation of AMPK, and inhibition of mTOR signalling in renal carcinoma cells. They investigated the effect of metformin on cancer cell proliferation by treating 786-O and OS-RC-2 renal carcinoma cells with different metformin concentrations. It was noted that metformin was able to significantly inhibit the proliferation of renal cell carcinoma. They also observed that metformin is able to prevent cell colony formation of the renal cell carcinoma cells. (213)

Metformin has also been shown to inhibit cell proliferation and migration in the oral squamous cell carcinoma cell line model. It has been reported that metformin reduces HIF-1a mRNA and protein levels. It has also been observed that metformin increases the level of PDH (Pyruvate Dehydrogenase) in hypoxic conditions. It is believed that metformin has an anti-proliferative effect, and can inhibit migration in squamous cell carcinoma. In addition, it increases the number of apoptotic cells and the transcription of caspase 3. (214)

Scientists have attempted to use nano particles to deliver metformin to cancer cells. It is an attractive idea as these particles can potentially deliver drugs directly to the affected tissue, in high doses, without systemic adverse effects. (215) Snima et al developed nano particles which contained metformin, and assessed the effect of metformin on both a pancreatic cancer cell line and a normal cell line. The nano particles were measuring 240 SD 50 nm and were made through the ionic-gelation method. This consisted of a coat of O-carboxymethyl chitosan which easily incorporated metformin molecules because of the electrostatic attraction between the carboxymethyl negative charges of the chitosan derivative and the NH4 positive charges of metformin molecules. The degree of release of metformin from the nano particles was pH dependant. An acidic environment caused faster release of the drug than an alkaline environment. As a tumour environment is more acidic, it may retain and attract more metformin.(216) The long term safety of these particles is unknown. The particles may change the permeability of red cell membranes, by forming conduction pores or by modifying the activity of sodium/potassium or calcium/magnesium pumps, and therefore their safety may be questionable. (217) Metformin containing nano particles were shown to be haemocompatible in Wistar rats and the particles had a haemolytic ratio of less than 5 %, thus confirming their safety in the case of oral administration (217). In addition, the kidney and liver function of the rats remained unchanged in spite of the presence of the particles in the kidney and liver tissue.(218)

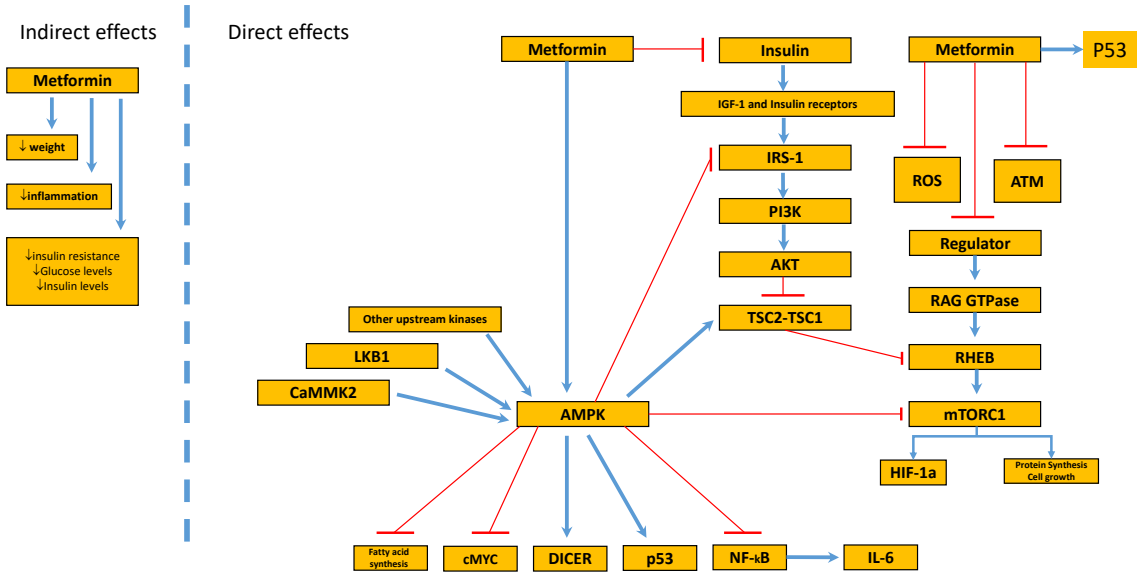
5.1.11.7 Metformin and cancer in human studies

Human studies have shown that metformin may aid cancer prevention and possibly avoid tumour recurrence. A large study tested the hypothesis that metformin reduces the risk of cancer in people with type 2 diabetes. This was an observation cohort study in Scotland. Patients with type 2 diabetes who were taking metformin from 1994-2000 were identified. These patients were compared with another group of diabetic patients who were not taking metformin. The groups were matched by year of diabetes diagnosis. They investigated the ratio of cancer diagnosis in the two groups. It was noted that cancer was seen in 7.3% of 4,085 metformin users compared with 11.6% of 4,085 diabetic patients who were not receiving metformin. They reported an unadjusted hazard ratio (95% CI) for cancer as 0.46 (0.40-0.53). The ratio was adjusted for several cofounding factors such as sex, age, BMI, Hb A1C, deprivation, smoking, and other drug use, and reported it to be 0.63 (0.53-0.75). The authors concluded that metformin may have a role in reducing the risk of cancer. (219)

Another study investigated the link between tumour complete response rate and metformin in diabetic patients with breast cancer who were receiving neoadjuvant chemotherapy. The Authors identified 2,529 patients who were given neoadjuvant chemotherapy for breast cancer from 1990 to 2007. The group consisted of 68 diabetic patients who were receiving metformin, 87 diabetic patients were not receiving metformin and 2,374 nondiabetic patients. The complete response rate was noted to be 24% in the metformin group, 8.0% in the group who were not receiving metformin, and 16% in the nondiabetic group ($p= .02$). This study concluded that diabetic patients who had breast cancer and were receiving metformin had a higher complete tumour response rate than the other group. (220)

Another study investigated the effect of metformin in diabetic patients who had colorectal cancer. In this study, 86 patients with colorectal cancer had diabetes. This group was divided into two groups, metformin and non-metformin. It was noted that metformin enhanced the anti-proliferative effects of 5-fluorouracil on CD133+ in cancer stem cells. It was reported that the distant metastasis rate in the patients receiving metformin was significantly lower than in the non-metformin group (5.60% vs 21.6%, p=0.035). In addition, less patients in the metformin group had differentiated adenocarcinoma than in the other group (2.78% vs 16.0%, p=0.048). These results supported the results of the previous studies. Better outcomes of patients with colorectal cancer on metformin was contributed to the inhibitory effect of metformin on CD133+ colonic cancer stem cells.(221)

Figure 5.4.2: Metformin and the mTOR pathway–This is a reproduced diagram. The original diagram was created by Pernicova et al. Written permission has been obtained from the senior author, Dr Korbonits. (222)



5.1.12 Metformin and mTOR pathway

The exact mechanism of action of metformin is not well understood. As illustrated in figure 1, metformin can exert its inhibitory effect on the mTOR pathway. (223)

Both complexes mTORC1 and mTORC2 contain a catalytic subunit mTOR. These two complexes are crucial for cellular growth and receive stimuli from various energy and hormonal signaling.

mTORC1 receives signals from different signalling pathways such as insulin, IGF1 (Insulin like Growth Factor 1), IGF2 and via AMPK (AMP-activated protein kinase) as shown in figure 1. (13)

Inhibition of mTORC1 by metformin via the AMPK pathway is through the activation of tumour suppressor genes *TSC1* and *TSC2* genes which code for tuberin and hamartin protein respectively. In addition, metformin can inhibit mTORC1 directly via AMPK and this is achieved by AMPK inhibiting RAPTOR (regulatory-associated protein of mTOR). AMPK phosphorylates RAPTOR and this phosphorylation is required for the inhibition of mTORC1 complex. RAPTOR is an adaptor protein and positive regulator within the mTORC1 complex. (224)

Metformin can also inhibit mTORC1 via IGF-1 and the insulin signalling pathway. In order to promote cellular growth, both insulin and IGF 1 block the TSC1 and TSC2 which lead to activation of mTORC1. Blocking IGF and insulin by metformin means that TSC1 and TSC2 can exert their inhibitory effect on mTORC1. (225)

Metformin can also induce p53, a tumour suppressor protein that can inhibit mTORC1. p53 can sense genotoxic stresses such as DNA damage, which could change the genetic material of cells, in turn stimulating p53 in order to stop cellular growth and proliferation. p53 activates AMPK, and subsequently TSC1 and TSC2, which then inhibit mTORC1. The activation of AMPK by p53 is by formation of a complex, LKB-1-p53. This complex has been shown to regulate and activate AMPK. (226)

Metformin increases the expression of the *DICER1* gene, which codes for the *DICER1* enzyme of the RNase III family. It cleaves double-stranded RNA and pre-microRNA into short double stranded RNA fragments which are microRNA and small interfering RNA. Variant in *DICER1* gene leads to a complex tumour syndrome. Hence metformin has an antineoplastic effect via induction of DICER expression. (227)

Metformin has also been shown to inhibit the proto-oncogene *c-MYC*. *c-MYC* is overexpressed in many cancers. It plays a crucial role in growth control, differentiation and apoptosis. (228)

Metformin is also an inhibitor of HIF-1α (hypoxia inducible factor) via AMPK and mTORC1. HIF-1α is an oxygen sensitive transcriptional activator which mediates the tissue response to low oxygen. It facilitates adaptation and survival of cells during changes in oxygen levels. It also has a key role in metabolic transformation in cancer. (212, 214)

The drug metformin has also been shown to inhibit the expression of fatty acid synthase, which is a multi-enzyme protein that catalyzes fatty acid synthesis. Fatty acid synthase

has been shown to be an oncogene and is upregulated in cancer cells. (229, 230) Growth factors and amino acids activate mTORC1 through the RAG GTPases, independently of AMPK. Activation of the RAG GTPases by the Ragulator complex leads to the mobilization of mTORC1 to the lysosomal surface. The complex is then activated by RHEB. Metformin can also inhibit mTOR complex 1 through direct inactivation of the Ragulator complex, which will inhibit RAG GTPases, leading to dissociation of mTORC1 from its activator RHEB.(157, 228)

Metformin reduces production of ROS (reactive oxygen species), oxidative stress and DNA damage through the inhibition of mitochondrial complex I. (181, 214, 231)

It has been postulated that metformin inhibits ATM, serine-protein kinase. This is because it has been noted that variation in glycaemic control in patients with type 2 diabetes has been linked to the presence of common genetic variants adjacent to the ataxia telangiectasia mutated *(ATM)* gene. Ataxia telangiectasia is a neurological condition caused by variant in the *ATM* gene. Patients with this condition are susceptible to cancer and type 2 diabetes. The *ATM* gene encodes for a tumour suppressor protein which is important for DNA repair and cell cycle control. (232)

5.1.13 Metformin in animal TSC models

The efficacy of metformin has been trialed in TSC animal models. $Tsc2^{+/-}$ mice models can develop renal cystadenomas which increase in size as the animal gets older. The lesions can progress to renal cell carcinoma. These murine models of *Tsc2* were developed by using a gene targeting approach. It has been shown that *Tsc2–/–* is embryonic lethal at days 9.5–12.5 from hepatic hypoplasia. *Tsc2+/–* mice develop a wide variety of neoplastic growths.

These lesions grow slowly and are less likely to become malignant neoplasms. The group showed that a 15 month old *Tsc2+/–* has approximately 100 cystadenomas lesions in each kidney. Renal cell carcinoma was seen in 3 animals out of 150 at the age of 12 months. Only one had lung metastasis. All the other growths in the lung, liver and limbs show a slow growth rate.(231)

The authors claimed that this model may be better than the previously described TSC animal models as the gene disruption is better characterized in these models. The range of growths that have been seen in the $Tsc2^{+/-}$ mice differs from that in patients with TSC. However, the slow growth rate and the limited change to malignant neoplasm make these models suitable as TSC related lesions in patients with TSC behave similar to these.

The frequency of kidney cystadenomas in these models is not dissimilar to the Eker rat *Tsc2+/-*. However, Eker rats develop haemangiomas in their spleen and uterus, rather than liver. (232) In addition, pituitary tumours and cerebral hamartomas are seen in Eker rats, whilst $Tsc2^{+/-}$ mice don't tend to develop these lesions. The rats can also have lung

lesions. The scientists suggest that the differences in phenotype between the mice and rats is probably due to the difference in their genetic make up rather than due to differences in the way the genes are expressed or the function of tuberin. (233)

A group in Boston investigated the effect of metformin in *Tsc2*$^{+/-}$ A/J mice on renal cystadenomas. A/J mice are commonly used to model cancer as they have high susceptibility to carcinogen induced tumours. These animals were given one of five treatment regimens. The first group of 9 mice were given rapamycin intraperitoneally at 6mg/kg for 3 days per week. The second group of five mice were given vehicle on the same schedule. In the third group, Bortezomib was given to 8 mice at 0.8 mg/kg subcutaneously for two days per week. Bortezomib is a proteasome inhibitor which aggravates ER (endoplasmic reticulum) stress in cancer cells lacking *TSC* genes. It has been approved for multiple myeloma. It is believed that this drug kills the myeloma cells via the induction of apoptosis.(234, 235)

In the fourth group, metformin was given in 5% sucrose drinking water at 300 mg/kg per day to 10 mice. The fifth group of 8 mice were given 5% sucrose drinking water as a control group.

The first three groups were treated simultaneously, as were the last two groups. Rapamycin was only given for one month as it has been shown in a previous study to be effective in reducing tumour volume in Tsc2$^{+/2}$. (236)

Bortezomib was also given for month because of its potential significant side effects and toxicity. Metformin was given for 4 months. The dose of metformin was obtained from a

study which investigated the effect of metformin on prevention of tumours in a combined LKB1-PTEN mouse model. (237)

The animals were killed at the age of 5 months. Rapamycin and Bortezomib were given to mice aged 4 months, whilst metformin was given to mice aged 1 month.

The rapamycin group showed a significant difference in the tumour extent before and after treatment compared with the vehicle and other treatment group. This was assessed by gross observation. Based on the microscopic assessment, the rapamycin group showed a significant difference in tumour extent when compared with the vehicle group and the other treatment groups.

The tumour volume per kidney was not significantly reduced for the other two treatment groups, bortezomib or the metformin group. Both bortezomib and metformin showed pharmacodynamic effects as the authors expected. The authors concluded that neither bortezomib nor metformin have a significant benefit in the native $Tsc2^{+/-}$ mouse model. This suggested that these treatment options may have limited benefits in treating TSC related hamartomas. (238)

Shen et al investigated the effect of metformin on kidney lesions in a $Tsc1^{+/-}$ mouse model of tuberous sclerosis complex. In this study, the mice were randomly allocated to either metformin or drinking water. There were 10 mice in each group. The treatment baseline was commenced at 6 months and stopped at 15 months of age. Metformin at a dose of 150 mg/kg was given in the first 7 months and was then increased to 600mg/kg for 2 months. The mice had MRI scans at the end of the trial. Interestingly the weight change

was not significant between the groups. The mean weight gain for the treatment group and placebo were +2.26g and +2.30g respectively. MRI scans were used to assess the renal lesions. There was no significant difference between the total number of kidney lesions for each mouse, or the number of type specific renal lesions, such as cysts, papillary or solid lesions. In addition, the mean and individual volume of renal lesions were not significantly different between the groups. They also examined the activity of the mTOR pathway in the tissue samples. The level of pS6 (Ser235/236) was noted to be marginally lower in kidney tissues of those animals who received metformin. However, there was no consistent difference in pS6 (Ser235/236) level in tumour cells between the control and treatment groups. They also investigated the phosphorylation status of other metformin targets such as pAMPK (Thr172) , pACC (Ser79), pAkt (Ser473) and pRaptor(Ser792). There was no noticeable effect of metformin on the phosphorylation level of pAkt(Ser473) or pRaptor(Ser792) in Tsc1+/-mouse kidney tissues.

The authors have also studied the essential transporter for metformin uptake. SLC22A2 is a crucial organic cation transporter in the kidneys. It was noted that this transporter was significantly reduced in renal tumour cells compared with normal renal cells in both Tsc1+/ and Tsc2 +/-mice. It was concluded that metformin reduces mTORC1 signaling in Tsc1+/- normal kidney tissue but not in the tumour cells. The lack of efficacy of metformin may be due to the suppression of the expression of organic cation transporters such as SLC22A1, SLC22A2 and SLC22A3.(239)

5.1.14 Metformin in humans with TSC

TSC is a relatively common genetic disorder with a prevalence of 4–9 per 100,000. (82) (240) It is characterized by the development of tumours (hamartomas) throughout the body. Hamartomas can occur in almost any tissue but particularly in the kidneys, brain, skin and heart. TSC is associated with difficult epilepsy (approximately 75% of patients); learning difficulties (approximately 50%, 30% with profound learning disability IQ<21); (82) and a range of psychological and behavioural problems including autism. (241, 242)

Tumours on the skin and nails can be significantly disfiguring. Many adults with TSC are unable to live independently and require state or family care. (243) Tumours affecting the heart (244) kidneys (39) and brain can cause life-threatening complications. (33)

Kidney tumours tend to increase in number and size with increasing age, and can be associated with symptomatic bleeding, sometimes life-threatening, in 10%. Patients are likely to require lifelong health-care follow-up.

The two genes responsible for TSC (called *TSC1* and *TSC2*) were identified in the 1990s. Since then there has been considerable progress in elucidating the molecular mechanisms by which they exert their influence. [103] They play a central role in regulating an insulin driven cell signalling pathway (IRS-PI3Kinase-mTOR-S6 Kinase) that promotes cell growth. (245) Variants in *TSC1/2* mean that their gene products do not inhibit mTOR effectively. This allows the promotion of unregulated cell growth that leads to the development of tumours. It is postulated that drugs that inhibit mTOR will inhibit or reverse tumour development in TSC. (246)

Rapamycin and everolimus are drugs that are known to inhibit mTOR and recent studies have shown that they can reduce TSC-related lesions such as kidney AMLs, brain SEGAs and facial angiofibromas (52, 54, 59, 247, 248). Spontaneous regression of these hamartomas is not reported. (248-250)

A recently published Phase III trial has shown that everolimus, when used as an adjunctive therapy, significantly reduced treatment-resistant focal seizures in individuals with TSC compared with placebo. 64% of patients who were on everolimus had stomatitis compared with 9% on placebo. Diarrhoea was reported in 22% vs 5% and hypercholesterolaemia in 7% vs 1%. (59)

In a phase III (EXIST 1) international, multicentre, double-blind, randomized, placebo-controlled trial, Franz et al evaluated the efficacy and safety of everolimus in patients with SEGA. Everolimus was associated with a significantly greater overall SEGA response rate (>/= 50% shrinkage), compared with placebo (35% vs. 0%). The most common adverse events were mouth ulceration (25 [32%] in the everolimus group *vs* two [5%] in the placebo group), stomatitis (24 [31%] *vs* eight [21%]), convulsion (18 [23%] *vs* ten [26%]), and pyrexia (17 [22%] *vs* six [15%]).(251)

Exist-2 was a placebo controlled phase 3 randomized controlled trial investigating the efficacy and safety of everolimus in treating renal AMLs > 3cm in adults. (252) The angiomyolipoma response rate (Defined as >/= 50% shrinkage) was 42% (33 of 79 [95% CI 31–53%]) for everolimus and 0% (0 of 39 [0–9%]) for placebo. The most common adverse events in the everolimus and placebo groups were stomatitis (48% [38 of 79], 8%

[3 of 39], respectively), nasopharyngitis (24% [19 of 79] and 31% [12 of 39]), and acne-like skin lesions (22% [17 of 79] and 5% [2 of 39]). (253).

Metformin is a drug that potentially offers the benefit of mTOR inhibition without the side effect and cost profile of other mTOR inhibitors. It is used by millions of people with type 2 diabetes and has a benign side effect profile. (254)

We treated 51 TSC patients with either placebo or metformin in a multi-centre randomized, double-blind, parallel group, placebo-controlled trial of metformin. The patients were treated for 12 months. The mean kidney (angiomyolipoma) AML volume increase from baseline was 25.5% for the placebo group and 9.6% for the metformin group. Difference in response, 15.9% (95% CI -9% to 41%) p=0.221. The mean SEGA volume increased from baseline by 37% in the placebo group but reduced from baseline by 23.3% in the metformin group. Difference in response, 60.3% (95% CI -0.4% to 111, p=0.048). We reported three serious adverse events that reflected the underlying disease. We concluded that metformin is safe and well tolerated in children and adults with TSC. Patients on metformin had a significant reduction in SEGA volume compared with placebo. We also saw seizure reduction in the metformin group. The growth of AML in the treatment group was slower than in the placebo group. (84) This trial is discussed in greater depth in section 5.2.

Table 5.5.1: shows a summary of the current and potential uses of metformin in clinical practice.

Metformin
Currently used • Type 2 Diabetes mellitus • Polycystic ovary disease Potential use • Cardiovascular disease • Weight loss • Depression • Cognitive abilities • Erectile dysfunction • Anticancer • Anti-ageing • Tuberous Sclerosis Complex • Duchenne Muscular Dystrophy

5.2 A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex

5.2.1 Aims

We set up A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex to investigate the efficacy and safety of metformin in Tuberous Sclerosis Complex.

This was a multicentre randomized, double-blind, parallel group, placebo-controlled trial of metformin in children and adults with TSC.

The study hypothesis was that treatment with metformin will reduce the size of kidney tumours in people with TSC. Secondary aims were to investigate the side-effect profile of metformin in people with TSC; the effect of metformin on SEGA, facial and nail tumours, severity of epilepsy, behaviour, and quality of life.

5.2.2 Methods and Materials

The eligibility and exclusion criteria for this study were as follows:

Eligibility criteria

- Male or female
- Aged 10–65 years.
- All the patients had to have a definite diagnosis of TSC, as defined by the International Tuberous Sclerosis Complex Consensus Group. (73)
- Patients had a minimum of one renal AML ≥ one centimeter in diameter
- Signed informed consent.

Exclusion criteria

- A serious inter-current illness or an uncontrolled disease that could compromise participation in the study.
- Impairment of renal function*
- The use of x-ray contrast medium containing iodine within the last 30 days
- Multiple AMLs that cannot be distinguished separately on magnetic resonance imaging (MRI)
- Renal haemorrhage within the preceding year as patients with high risk of renal haemorrhage may require frequent assessment and intervention.
- Patients with known conservatively managed renal aneurysm(s) >10mm.
- Liver insufficiency which is a contraindication for metformin.
- Acute or chronic disease which may cause tissue hypoxia and increase the risk of lactic acidosis e.g. cardiac/respiratory failure, recent myocardial infarction.
- Patients with diabetes.
- Any patients on treatment with injected or oral hypoglycaemic drugs.
- The use of any investigational drug within the last 30 days
- Pregnancy, planning to become pregnant, or breastfeeding.

* Renal impairment is here defined as being creatinine levels that exceed the level that are currently viewed by NICE guidelines as needing caution before administration of Metformin i.e.
Children 8-11 yrs: 40-80 mmol/L
Children >12yrs: 75-120 mmol/L
Adults: >130 mmol/l or an estimated glomerular filtration rate (eGFR)
<45 ml/minute/1.73 m2

5.2.3 Ethics and consent

The ethical application was reviewed by NHS/HSC Research and Development offices, Research Ethics Committee and Medicines and Healthcare products Regulatory Agency (MHRA) – Medicines. Ethical approval was obtained from research ethics committee Yorkshire and the Humber – Leeds West – reference number 11/YH/0295.

EudraCT code: 2011-001319-30 and ISRCTN code: ISRCTN92545532.

Consent was obtained from the parents or legal guardians of children. Assent from the children was also sought were possible. Consent was obtained from adults with normal intellect. Parents cannot give consent on behalf of their children once they are over 18, even if they have learning disabilities. For this group of patients, consent was obtained from all the parties who were involved in the patients' care such as parents, guardians, carers, legal representatives, general practitioners, case workers, support workers and hospital specialists, via best interest decision. The investigators communicated with these parties by email, phone, or face to face meetings, to explain the study and obtain written consent from them. This was in line with ethical approval.

Recruitment

Participants were recruited from three specialist TSC clinics in Bath, Bristol and London in the United Kingdom. Prior to their standard clinic appointment, all clinic patients (or their parents/carers) were sent a letter introducing the study (including participant information sheets and study team contact details). They were offered the opportunity to discuss the study at an appointment directly after their next clinic visit.

We have enclosed the information sheets and consent forms for the following:

Adults with normal intellect,

Children,

Parents,

General practioners

MiTS: Metformin in Tuberous Sclerosis Complex

A randomized, double-blind, parallel group, placebo-controlled trial.

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Information for Adults

You are being invited to take part in a research study. Before you decide, it is important to understand why the research is being done, and what it will involve for you. Please take some time to read this information carefully, and discuss it with your family, friends, and GP if you wish. Ask us if anything isn't clear, or if you would like more information.

What is the research about?

This research is about Tuberous Sclerosis Complex (TSC). This is the genetic condition that you have. Our bodies are made up of millions of cells. Normally our body controls and organises how the cells grow. In people with TSC, the body has less control of how cells grow. Too many cells grow in places they don't need to. This is what makes the tumours that grow on the skin, kidneys, brain, and throughout the body. In this research, we are finding out if the drug metformin will help the body control cell growth and shrink the size of the tumours.

What happens? What would I have to do?

Half of the people in this study will be taking metformin, and half will be taking a placebo (a pill that looks the same, but has no medicine in it). Everyone takes their treatment for one year, and has six appointments (over 18 months) for assessments of their TSC. You would have scans of your kidneys and brain, photographs of any tumours on your face and fingernails, assessments of your epilepsy, of your learning, and how well you feel in yourself. You would also have three small blood tests. (On page 3 there is a schedule showing exactly what happens and when.)

Why do some people just get the placebo? And why is it randomly decided?

We don't know yet if metformin makes a difference in TSC. We need to see if people who take metformin do better than people who take just a placebo. So we put people into these two groups, and then compare the results. To make sure the groups are similar to start with, each person's group is randomly decided (as if by flipping a coin). You have a 50% chance (1 in 2) of getting metformin. Neither you nor the investigator can control the decision – it is done independently by a computer programme.

Both you and the investigator are kept 'blind' to the group you are in, until the end of the study. (They can find out which group you are in if there is any medical reason to know.)

What are the advantages of being in the study?

If you are taking metformin, it is possible that the tumours on your skin, nails, kidneys and brain will shrink. It is also possible that your learning could improve, you could have less seizures, and generally feel better in yourself.

At the moment we don't know how much the metformin might reduce your tumours, and we don't know how much it might improve other aspects of your TSC. By being in the study, you would be helping us find out. We hope you would also benefit from the extra care you receive by being in the study.

What are the risks and disadvantages of being in the study?

The medicine:

- Metformin is a very safe medicine. It is used by millions of people worldwide for type 2 diabetes, to help control blood sugar. It does **not** change your blood sugar if you are not diabetic. It is common for people starting treatment to find that it upsets their stomach at first. Taking metformin with a meal should help, if you find it upsets your stomach.
- A very rare, but serious side effect of metformin can be lactic acidosis (a build up of lactic acid in the bloodstream). This usually only affects people with serious heart or kidney disorders. (We make sure no one with these risks takes part in the study.) Signs of lactic acidosis are abnormal breathing, dizziness, drowsiness and confusion. It is important to stop taking the medicine and get urgent medical advice if you have these signs.

If you are worried about the treatment at any time please call us at one of the numbers at the bottom of the leaflet, or your GP / emergency services if you need urgent care.

The assessments:

- MRI scans (magnetic resonance imaging) are quite noisy, and make some people feel claustrophobic.
- Blood tests can sometimes cause soreness and swelling around the puncture site, but this should just be temporary.
- The questionnaires to assess your learning and how you feel can be time-consuming, but people often find them fun too.

What if something goes wrong?

If you have a concern about any part of this study, please contact us and we will do our best to answer your concerns. Our contact details are at the front of this booklet. If you are still unhappy and wish to complain formally, you can contact the Patient Advice and Liaison Service (PALS) on [0117 342 3604]. This is the NHS organisation to support patients with any concerns.

If something goes wrong and you are harmed during the research, and this is due to someone's negligence, then you may have grounds for a legal action for compensation

against [University Hospitals Bristol NHS Foundation Trust] but you may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

Do I have to be in the study?

No – it is your choice to be in the study or not. Your doctor will continue to look after you as normal whether you are in the study or not. You would be free to stop being in the study at any time, without having to explain why.

Confidentiality – who would know about me being in the study?

The researcher team will have access to your information. Regulatory Authorities and Research Monitors may see your information when they check on the study. All the information we collect would be kept safely and confidentially, and you would not be named in any report published from the study. With your permission, we would let your GP know that you are taking part in the study.

Who is funding this study? Who has reviewed it?

This study is funded by the UK's National Institute for Health Research.
This study has been reviewed and approved by the National Research Ethics Service in the UK.

What do I do now?

Please take some time to think about whether you would like to be in the study.
Feel free to ask us any questions at any time.

Your schedule: what happens and when

1	**Starting assessments**: ▪ MRI scan of your kidneys and brain, to measure the size of your tumours ▪ Photographs of any tumours on your face and fingernails. ▪ Assessment of your cognitive abilities, behaviour, and quality of life (questionnaires). ▪ Assessment of your epilepsy (you would also have a diary to record your seizures). ▪ A blood test to check that your liver and kidneys are working fine, and that your blood sugar is fine. **Starting treatment**: ▪ Half of the people in the study will be taking metformin, and half will be taking a placebo (a dummy pill). This is randomly decided, and you and the researchers both find out at the end of the study which group you were in. Your doctor will always be able to find out if you are taking metformin if they need to know.
2	**At 6 weeks:** ▪ A second blood test to check that your liver and kidneys are working fine, and that your blood sugar is fine.
3	**At 6 months:** ▪ An ultrasound scan of your kidneys. ▪ Photographs of any tumours on your face and fingernails. ▪ Assessment of your epilepsy. ▪ A raise in your dose of treatment (from two pills per day to three) as long as you are well.
4	**6 weeks later:** ▪ The last blood test to check that your liver and kidneys are working fine, and that your blood sugar is fine.
5	**At 12 months:** ▪ You stop taking the study medicine. ▪ MRI scan of your kidneys and brain, to measure the size of your tumours ▪ Photographs of any tumours on your face and fingernails. ▪ Assessment of your cognitive abilities, behaviour, and quality of life (questionnaires). ▪ Assessment of your epilepsy
6	**At 18 months:** ▪ An ultrasound scan of your kidneys. ▪ Photographs of any tumours on your face and fingernails. ▪ Assessment of your epilepsy. *END OF THE STUDY* When the results have been analysed, we will write to you to let you know what we have found.

Research team contact details

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Researcher: Dr Sam Amin, 0117 342 0187

Study Coordinator: Hannah Edwards, 0117 342 0160

University Hospitals Bristol NHS Foundation Trust 24-hr line: 0117 923 0000

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Consent form for Adults

Name of Participant:…….

Study ID:……..

		Please write your initials in each box
1	I have read and understood the information sheet dated 2nd September 2011 for this study. I have had a chance to think about the study, ask questions, and I have had my questions answered satisfactorily.	
2	I understand that taking part is voluntary, and that I am free to stop at any time without needing to explain why, and without my medical care and legal rights being affected in any way.	
3	I understand that the data collected will be seen by researchers on this study, and may be seen by regulatory authorities and research monitors. I give my permission for these people to have access to my information.	
4	I agree for my GP to be informed about my participation in the study.	
5	I agree to take part in this study.	
	Optional extra consent:	
6	I am happy for the research team to contact me in the future, to invite me to take part in other research about TSC.	

Date:……..Signature:……...

Name of person taking consent
……

Date:……..Signature:……

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Information for Adults without capacity

You are being invited to take part in a research study.

Before you decide, it is important to understand why we are doing the study.

Someone will explain the study to you. Please ask us if you have any questions.

TSC

What is the study about?

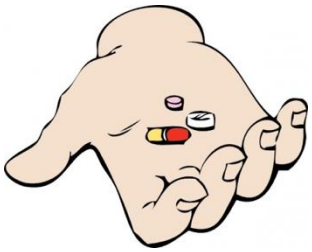
The study is about Tuberous Sclerosis Complex (TSC).

This is the condition that you have.

In TSC, there are some bits in your body that grow too much (tumours). These can sometimes be a bad thing for you.

There is a medicine that might help stop your tumours growing.

This study is to find out if taking the medicine stops your tumours growing.

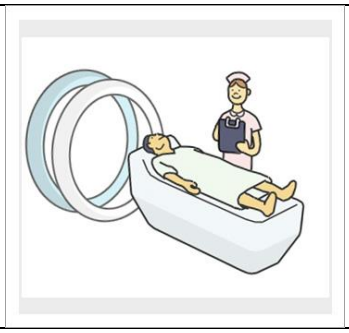
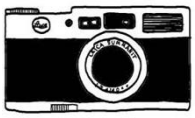
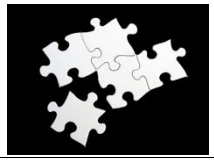


What would I have to do?

In the study you take a medicine for 1 year.

You might be taking the medicine (called metformin) OR you might be taking just a pill with no medicine in it.

You would have some checks and tests at the hospital.

		Scans to measure the size of your tumours.
		Photos of any tumours on your fingernails and marks on your face.
		Questions about your fits (epilepsy). Questions about how you feel.
		Games to check your thinking.
		Some blood tests.
	**What is good about the study?** The medicine might make your tumours smaller. This might help keep you well. Finding out would help other people with TSC too.	
	What are the bad bits? Is it dangerous? The medicine is safe. Lots of people take it to help with <u>diabetes.</u> Sometimes it can make people feel sick, or make your tummy hurt. This might not happen. If it does, eat some food when you take the medicine.	

If you feel dizzy or your breathing is strange, it is important to talk to a doctor right away.
The blood tests might make your arm hurt a bit. But it will go away quickly. We can use a special cream that stops it hurting if you like.

?

Do I have to do it?

No. You choose if you want to be in the study or not.
Your doctor will keep on looking after you,
whether you say yes or no.
If you don't like it, you can stop at any time.

?

Who would know I'm in the study?

We only tell people if they need to know.

When we write about the study, we don't say your name.

?

How do I know it's safe?

A group of people (the 'National Research Ethics Service') check all studies to make sure they are safe.
They have looked at this study and say it is safe for people to be in it.

What do I do now?

Think about what it would be like to be in the study.
Would you be happy to do it?
Or would you not like it?
Ask us questions if you don't feel sure.

Who do I talk to?

You can call any of these people:

The doctor in charge of the study:	Dr Finbar O'Callaghan 0117 342 0167 Or 0117 342 0160
The hospital in Bristol	0117 923 0000
Emergency help	999

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Upper Maudlin Street, Bristol BS2 8AE
Tel: +44 (0)117 342 0167
Fax: +44 (0)117 342 0186
www.bristolcns.org

Assent form for Adults without capacity

Name of Participant: ……

Study ID: ……

		Tick the box if you agree
1	Someone has explained the study to me. I have thought about it and asked questions.	☐
2	I would like to be in the study.	☐
3	I know I can stop any time if I don't like it.	☐
	(Optional)	
4	The researchers might call me in the future to tell me about other studies. I don't mind if they do this.	☐

Please write your name here if you would like to be in the study:
…….....

Name of carer / legal guardian: ……...…

Date: ……..Signature :…….

Name of person taking consent ……...

Date: ……..Signature: ……

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Information for Carers / Legal Guardians of Adults without capacity

The person in your care is being invited to take part in a research study.
You are being asked to give an opinion on whether you think they would be happy to take part, or whether doing so could upset them. Before you decide, it is important to understand why the research is being done, and what it will involve for them. Please take some time to read this information carefully, and discuss it with the family, friends, and GP of the person in your care if you wish.
Ask us if anything isn't clear, or if you would like more information.

What is the role of an individual consenting on behalf of someone else?

The person in your care lacks the full capacity to give their consent to participate in research. As their representative, you are being approached to potentially provide consent on their behalf. You need to consider their interests, wishes and feelings, and judge whether the person who lacks full capacity would be happy to take part, or whether doing so could upset them.

What is the research about?

This research is about Tuberous Sclerosis Complex (TSC). This is the genetic condition that the person in your care has. Our bodies are made up of millions of cells. Normally our body controls and organises how the cells grow. In people with TSC, the body has less control of how cells grow. Too many cells grow in places they don't need to. This is what makes the tumours that grow on the skin, kidneys, brain, and throughout the body. In this research, we are finding out if the drug metformin will help the body control cell growth and shrink the size of the tumours.

What happens?

Half of the people in this study will be taking metformin, and half will be taking a placebo (a pill that looks the same, but has no medicine in it). Everyone takes their treatment for one year, and has six appointments (over 18 months) for assessments of their TSC. The person in your care would have scans of their kidneys and brain, photographs of any tumours on their face and fingernails, assessments of their epilepsy, of their learning, and how well they feel in themselves. They would also have three

small blood tests. (On page 3 there is a schedule showing exactly what happens and when.)

What are the advantages of being in the study?

If the person in your care is taking metformin, it is possible that the tumours on their skin, nails, kidneys and brain will shrink. It is also possible that their learning could improve, they could have less seizures and they could generally feel better in themselves.

At the moment we don't know how much the metformin might reduce their tumours, and we don't know how much it might improve the other aspects of their TSC. By being in the study, they would be helping us find out. We hope they would also benefit from the extra care they receive by being in the study.

What are the risks and disadvantages of being in the study?

The medicine:

- Metformin is a very safe medicine. It is used by millions of people worldwide for type 2 diabetes, to help control blood sugar. It does **not** change your blood sugar if you are not diabetic.
- It is common for people starting treatment to find that it upsets their stomach at first. Taking metformin with a meal should help.
- A very rare, but serious side effect of metformin can be lactic acidosis (a build up of lactic acid in the bloodstream). This usually only affects people with serious heart or kidney disorders. We make sure no one with these risks takes part in the study.) Signs of lactic acidosis are abnormal breathing, dizziness, drowsiness and confusion. It is important for the person in your care to stop taking the medicine and get urgent medical advice if they have these symptoms.

  If you are concerned about the treatment at any time please call us at one of the numbers at the bottom of the leaflet, or a GP / emergency services if the person in your care needs urgent care.

The assessments:

- MRI scans (magnetic resonance imaging) can be noisy, and make some people feel claustrophobic. Some people need general anaesthetics for these scans, and any general anaesthetic comes with some risks. These are scans that the person in your care would normally have every year to monitor their TSC.
- The blood tests can sometimes cause soreness and swelling. We can use a cream to numb the arm if the person in your care would prefer this.
- The questionnaires to assess learning and how the person feels can be time-consuming, but people often find them fun too.

Do they have to be in the study?

No – it is your choice for the person in your care to be in the study or not. Their doctor will continue to look after them as normal whether they are in the study or not. They would be free to stop being in the study at any time, without either of you having to explain why.

Confidentiality – who would know about this person being in the study?

The researchers will look at their medical records, and Regulatory Authorities and Research Monitors may see their information when they check on the study. All the information we collect would be kept safely and confidentially, and they would not be named in any report published from the study. With your permission, we would let the GP of this person know that they are taking part in the study.

Who is funding this study? Who has reviewed it?

This study is funded by the UK's National Institute for Health Research.
This study has been reviewed and approved by the National Research Ethics Service in the UK.

What do I do now?

Please take some time to think about whether you think the person in your care would be happy to be in the study, or if doing so could upset then. Please feel free to ask us any questions at any time.

Research team contact details

Chief Investigator: Dr Finbar O'Callaghan
0117 342 0167

Researcher: Dr Sam Amin
0117 342 0187

Study Coordinator: Hannah Edwards
0117 342 0160

University Hospitals Bristol NHS Foundation Trust 24-hr line:
0117 923 0000

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Consent form for Carers / Legal Guardians Of Adults without capacity

Name of participant: ……

Study ID: ……

Please write your initials in each box

1 I have read and understood the information sheet dated [20th June 2011] for this study. I have had a chance to think about the study, ask questions, and I have had my questions answered satisfactorily. ☐

2 I understand that taking part is voluntary, and that we are free to stop at any time without needing to explain why, and without the medical care and legal rights of the person in my care being affected in any way. ☐

3 I understand that the data collected will be seen by researchers on this study, and may be seen by regulatory authorities and research monitors. I give my permission for these people to have access to information about the person in my care. ☐

4 I agree for the GP of the person in my care to be informed of their participation in the study. ☐

5 In my opinion, the person in my care would have no objection to taking part in this study. I agree for the person in my care to take part in this study. ☐

Optional extra consent:

6 I am happy for the research team to contact me in the future, to invite the person in my care to take part in other research. ☐

Name of carer / legal guardian: ……...……
Date: ……..Signature: ……
Name of person taking consent ……...
Date: ……..Signature: …….

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Information for General Practitioners

One of your patients has decided to take part in our research study. They or their parent/legal guardian have given consent to participate, and they are free to withdraw at any time.

Patient name: ……

Date of birth: ……
Address: ……

This is a randomised, double-blind, parallel-group, placebo-controlled trial investigating whether treatment with metformin reduces the size of renal angiomyolipomata in people with Tuberous Sclerosis Complex (TSC).

Rationale for the trial:
In people unaffected by TSC, the products of the TSC genes (hamartin and tuberin) help to regulate cell growth. They do this by inhibiting an intracellular molecule called mTOR. In people with TSC, these genes are damaged, and so some of this natural inhibition of cell growth is lost. This is what leads to the development of tumours throughout the body. As metformin *also* inhibits the intracellular molecule mTOR, it may provide an alternative way to control the growth of tumours in people with TSC.

Treatment
Participants in this trial will be treated for 12 months with either metformin or placebo. Starting dose for adults will be 500mg twice a day, raised to 500mg three times a day at 6 months (if treatment is well tolerated). Starting dose for children (10-16 years) will be 500mg once a day for the first two weeks of treatment, and will follow the adult schedule thereafter. All participants will undergo brain and kidney scans (MRI and ultrasound), photographs of facial and nail tumours, and assessments of cognition, behaviour, quality of life and epilepsy.

Adverse Events:
We are required to report and document all adverse events, whether they are related to the study or not. With the patient's permission, we may need to contact you for information regarding their medical history. If you have any questions please feel free to contact us at any time.

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Information for Parents

You and your child are being invited to take part in a research study. Before you decide, it is important to understand why the research is being done, and what it will involve for you. Please take some time to read this information carefully, and discuss it with your family, friends, and GP if you wish. Ask us if anything isn't clear, or if you would like more information.

What is the research about?

This research is about Tuberous Sclerosis Complex (TSC). This is the genetic condition that your child has. Our bodies are made up of millions of cells. Normally our body controls and organises how the cells grow. In people with TSC, the body has less control of how cells grow. Too many cells grow in places they don't need to. This is what makes the tumours that grow on the skin, kidneys, brain, and throughout the body. In this research, we are finding out if the drug metformin will help the body control cell growth and shrink the size of the tumours.

What would it involve for us?

Half of the people in this study will be taking metformin, and half will be taking a placebo (a pill that looks the same, but has no medicine in it). Everyone takes their treatment for one year, and has six appointments (over 18 months) for assessments of their TSC. You child would have scans of their kidneys and brain, photographs of any tumours on their face and fingernails, assessments of their epilepsy, their learning, and how well they feel in themselves. They would also have three small blood tests. (On page 3 there is a schedule showing exactly what happens and when.)

What are the advantages of being in the study?

If your child is taking metformin, it is possible that the tumours on their skin, nails, kidneys and brain will shrink. It is also possible that their learning could improve, they could have less seizures, and generally feel better in themselves.

At the moment we don't know how much the metformin might reduce their tumours or help other parts of their TSC. By being in the study, they would be helping us find out. We hope they would also benefit from the extra care they receive by being in the study

What are the risks and disadvantages of being in the study?

<u>The medicine:</u>

- Metformin is a very safe medicine. It is used by millions of people worldwide for type 2 diabetes, to help control blood sugar. It does **not** change your blood sugar if you are not diabetic. It is common for people starting treatment to find that it upsets their stomach at first. Taking metformin with a meal should help, if you find it upsets your stomach.
- A very rare, but serious side effect of metformin can be lactic acidosis (a build up of lactic acid in the bloodstream). This usually only affects people with serious heart or kidney disorders. (We make sure no one with these risks takes part in the study.) Signs of lactic acidosis are abnormal breathing, dizziness, drowsiness and confusion. It is important to stop taking the medicine and get urgent medical advice if your child has these signs.

If you are worried about the treatment at any time please call us at one of the numbers at the bottom of the leaflet, or your GP / emergency services if you need urgent care.

The assessments:

- MRI scans (magnetic resonance imaging) are quite noisy, and make some people feel claustrophobic.
- Blood tests can sometimes cause soreness and swelling around the puncture site, but this should just be temporary.
- The questionnaires to assess your child's learning and behaviour can be time-consuming, but people often find them fun too.

Do we have to be in the study?

No – it is your choice for your child to be in the study or not. Your child's doctor will continue to look after them as normal whether they are in the study or not. You would be free to stop being in the study at any time, without having to explain why.

Confidentiality – who would know about my child being in the study?

The researchers will look at your child's medical records, and Regulatory Authorities and Research Monitors may see your child's information when they check on the study. All the information we collect on your child would be kept safely and confidentially, and they would not be named in any report published from the study. With your permission, we would let your child's GP and hospital doctor know that they are taking part in the study.

Who is funding this study? Who has reviewed it?

This study is funded by the UK's National Institute for Health Research.

This study has been reviewed and approved by the National Research Ethics Service in the UK.

What do I do now?

Please take some time to think about whether you would like your child to be in the study. Please feel free to ask us any questions at any time.

Research team contact details

Chief Investigator: Dr Finbar O'Callaghan
0117 342 0187

Researcher: Dr Sam Amin
0117 342 0176

Study Coordinator: Hannah Edwards

Your schedule: what happens and when

1	**Starting assessments**: ▪ MRI scan of kidneys and brain, to measure the size of tumours ▪ Photographs of any tumours on the face and fingernails. ▪ Assessment of learning, behaviour, and quality of life (questionnaires). ▪ Assessment of epilepsy (you would also have a diary to record their seizures). ▪ A blood test to check that their liver and kidneys are working fine, and that their blood sugar is fine. **Starting treatment**: ▪ Half of the people in the study will be taking metformin, and half will be taking a placebo (a dummy pill). This is randomly decided, and you and the researchers both find out at the end of the study which group your child was in. Your doctor will always be able to find out if they are taking metformin if they need to know.
2	**At 6 weeks:** ▪ A second blood test to check that their liver and kidneys are working fine, and that their blood sugar is fine.
3	**At 6 months:** ▪ An ultrasound scan of the kidneys. ▪ Photographs of any tumours on the face and fingernails. ▪ Assessment of epilepsy. ▪ A raise in the dose of treatment (from two pills per day to three) if they are tolerating the treatment well.
4	**6 weeks later:** ▪ The last blood test to check that their liver and kidneys are working fine, and that their blood sugar is fine.
5	**At 12 months:** ▪ They <u>stop</u> taking the study medicine. ▪ MRI scan of kidneys and brain, to measure the size of the tumours ▪ Photographs of any tumours on the face and fingernails. ▪ Assessment of cognitive abilities, behaviour, and quality of life (questionnaires). ▪ Assessment of epilepsy.
6	**At 18 months:** ▪ An ultrasound scan of the kidneys. ▪ Photographs of any tumours on the face and fingernails. ▪ Assessment of epilepsy. *END OF STUDY* When the results have been analysed, we will write to you and your child to let you know what we have found.

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Consent form for Parents

Name of Child: ……

Study ID: …….

		Please write your initials in each box
1	I have read and understood the information sheet dated 20th June 2011 for this study. I have had a chance to think about the study, ask questions, and I have had my questions answered satisfactorily.	
2	I understand that taking part is voluntary, and that we are free to stop at any time without needing to explain why, and without my or my child's medical care and legal rights being affected in any way.	
3	I understand that the data collected will be seen by researchers on this study, and may be seen by regulatory authorities and research monitors. I give my permission for these people to have access to my child's information.	
4	I agree for my child to take part in this study.	
5	I agree for my child's GP to be informed about our participation in the study.	
	Optional extra consent:	
6	I am happy for the research team to contact me in the future, to invite me and my child to take part in other research.	

Name of parent: ……...

Date: ……..Signature: …….
Name of person taking consent ……...
Date: ……..Signature: ……

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Information for Young People (10-16)

You are being invited to take part in a research study.
Before you decide, it is important to understand why the research is being done, and what it means for you. Please read this information carefully, and discuss it with your family and friends. Just ask us if anything isn't clear, or if you would like more information.

What is the study about?

This study is about Tuberous Sclerosis Complex (TSC). This is the genetic condition that you have.

Your body is made up of millions of cells. Normally your body controls how much your cells grow. In TSC, you don't have as much control as normal over how much your cells grow. This is why tumours (which are extra cells) grow on your kidneys, brain, skin, and other parts of your body too.

In this study we are finding out if the drug metformin will help to **stop** the tumours growing.

We are also finding out if metformin improves other parts of your TSC, like your epilepsy, and how you feel generally, at home and at school.

What would I have to do?

Being in the study would mean you take a medicine for one year.

- This might be the medicine metformin, or it might be a 'placebo' (a dummy pill). We won't be able to tell you which one you were taking until the end of the study.
- Why doesn't everyone get the 'real' medicine? If everyone in the study had metformin, it would be hard to tell if it was really the medicine that helped your TSC, or if just being in the study helped. By having some people taking just the dummy pill, we will be able to see if metformin has extra positive effects.

You would also have some appointments at the hospital for tests and check-ups. Here are the checks you would have:

1. A scan of your brain and kidneys, to measure the size of your tumours. This happens once when you start taking the medicine, and once when you stop. You would also have 2 ultrasound scans of just your kidneys.

2. Photos of any tumours on your skin and fingernails. This happens 4 times – once every 6 months.

3. Tests of your thinking and understanding, your behaviour, and how you feel generally. This happens once when you start taking the medicine, and once when you stop.

4. An assessment of your epilepsy. This happens 4 times, once every 6 months. You and your parents also keep a diary of any seizures you have while you are in the study.

5. A blood test to check that your kidney and liver are working normally. This happens once at the start, once after you have been taking the medicine for 6 weeks, and once more about 6 months later.

What are the good things about it?

If you are taking metformin, it is possible that some of your tumours will shrink.

Also, it is possible that your learning could improve, you might have less seizures, and you might generally feel better in yourself.

At the moment we don't know how much the metformin might help your TSC – but by being in the study, you would be helping us find out. What we learn should help other people with TSC in the future.

What are the risks?

The medicine:

Metformin is a very safe medicine. Millions of people all over the world take this every day to help control diabetes. People sometimes find that it makes them feel sick or upsets their stomach at first, but this usually clears up quickly. Taking metformin with a meal should help.

Very rarely, taking metformin can mean that something called 'lactic acid' builds up in your blood. This is serious, but it usually only happens if someone has serious problems with their heart or kidneys. We make sure that no-one with these problems takes part in the study.
Signs of lactic acidosis are: starting to breathe differently, feeling dizzy and being very tired. If you think you have these, it's important to stop taking the medicine and ask for urgent medical advice.

If you are worried about the treatment at any time please call us at one of the numbers at the bottom of the leaflet, or your GP / emergency services if you need urgent care.

The tests:

The scans you would have of your brain and kidneys can be noisy, and you need to lie still for these. You might need a general anaesthetic for this (to make you fall asleep for a short time) and these can make you feel a bit sick sometimes.

The blood tests can sometimes feel sore. We can use a cream to make your arm numb for this if you want.

The questionnaires to test your learning, and how you feel in general, can take a long time (about an hour) but most people find them fun.

Do I have to be in the study?

No – you can choose. Take some time to think about it, and talk with your friends and family. It is your choice to be in the study or not. Your doctor will continue to look after you as normal whether you are in the study or not. It's fine to stop at any time. If you want to stop, you don't need to explain why.

Who would know that I'm in the study?

We keep all information about you safely and confidentially. We only share your information with people who need or have a right to know. We don't give your name when we write reports about the study.

Who pays for the study? How do I know it's safe?

This study is funded by the UK's National Institute for Health Research.
It has been reviewed and approved by the National Research Ethics Service in the UK.

What do I do now?

Please take some time to think about whether you would like to be in the study.
Feel free to ask us any questions at any time.

Research team contact details

Chief Investigator: Dr Finbar O'Callaghan
0117 342 0187

Researcher: Dr Sam Amin
0117 342 0176

Study Coordinator: Hannah Edwards
0117 342 0210

University Hospitals Bristol NHS Foundation Trust
24-hr line: 0117 923 0000

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Assent form for Young People

Name of Participant: ……

Study ID: …….

		Please tick the box if you agree
1	Someone has explained this study to me, and I have had a chance to think about the study. I have had a chance to ask questions, and I have had my questions answered satisfactorily.	
2	I know that I can stop being in the study at any time, and no-one will mind.	
3	I understand the researchers will collect information about me, and that I will be having some tests and scans.	
4	I would like to be in this study. .	
	Optional extra consent:	
5	I am happy for the research team to contact me in the future, to invite me to take part in other research.	

Please write your name here if you would like to be in the study:

……..

Name of parent: ……...

Date: ……..Signature: …….

Name of person taking consent ……...

Date: ……..Signature: ……..…

Assessments

Vineland Adaptive Behaviour Scales – interview form

Communication Domain - Receptive

Response Options: 2 = Usually, 1 = Sometimes or Partially, 0 = Never, DK = Don't Know

1.	Turns eyes and head toward sound.	2 1 0 DK
2.	Looks toward parent or carer when hearing parent's or carer's voice.	2 1 0 DK
3.	Responds to his or her name spoken (for example, turns toward speaker, smiles, etc.).	2 1 0 DK
4.	Demonstrates understanding of the meaning of no, or word or gesture with the same meaning (for example, stops current activity briefly).	2 1 0 DK
5.	Demonstrates understanding of the meaning of yes, or word or gesture with the same meaning (for example, continues activity, smiles, etc.).	2 1 0 DK
6.	Listens to story for at least 5 minutes (that is, remains relatively still and directs attention to the storyteller or reader).	2 1 0 DK
7.	Points to at least three major body parts when asked (for example, nose, mouth, hands, feet, etc.).	2 1 0 DK
8.	Points to common objects in a book or magazine as they are named (for example, dog, car, cup, key, etc.).	2 1 0 DK
9.	Listens to instructions.	2 1 0 DK
10.	Follows instructions with one action and one object (for example, “Bring me the book”; “Close the door”; etc.).	2 1 0 DK
11.	Points to at least five minor body parts when asked (for example, fingers, elbows, teeth, toes, etc.).	2 1 0 DK
12.	Follows instructions with two actions or an action and two objects (for example, “Bring me the crayons and the paper”; “Sit down and eat your lunch”; etc.).	2 1 0 DK
13.	Follows instructions in “if-then” form (for example, “If you want to play outside, then put your things away”; etc.).	2 1 0 DK
14.	Listens to a story for at least 15 minutes.	2 1 0 DK
15.	Listens to a story for at least 30 minutes.	2 1 0 DK
16.	Follows three-part instructions (for example, “Brush your teeth, get dressed, make your bed”; etc.).	2 1 0 DK
17.	Follows instructions or directions heard 5 minutes before.	2 1 0 DK
18.	Understands sayings that are not meant to be taken word for word (for example, “Button your lip”; “Hit the road”; etc.).	2 1 0 DK

19. Listens to an informational talk for at least 15 minutes. 2 1 0 DK

20. Listens to an informational talk for at least 30 minutes. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Receptive Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Communication Domain – Expressive

1.	Cries or fusses when hungry or wet.	2 1 0 DK
2.	Smiles when you smile at him or her.	2 1 0 DK
3.	Makes sounds of pleasure (for example, coos, laughs, etc.).	2 1 0 DK
4.	Makes nonword baby sounds (that is, babbles).	2 1 0 DK
5.	Makes sounds or gestures (for example, waves arms) to get parent's or carer's attention.	2 1 0 DK
6.	Makes sounds or gestures (for example, shakes head) if he or she wants an activity to stop or keep going.	2 1 0 DK
7.	Waves good-bye when another person waves or parent or carer tells him or her to wave.	2 1 0 DK
8.	Says “Da-da”, “Ma-ma” or another name for parent or carer (including parent's or carer's first name or nickname).	2 1 0 DK
9.	Points to object he or she wants that is out of reach.	2 1 0 DK
10.	Points or gestures to indicate preference when offered a choice (for example, “Do you want this one or that one?”; etc.).	2 1 0 DK
11.	Repeats or tries to repeat common words immediately upon hearing them (for example, ball, car, go, etc.).	2 1 0 DK
12.	Names at least three objects (for example, bottle, dog, favourite toy, etc.).	2 1 0 DK
13.	Says one-word requests (for example, up, more, out, etc.).	2 1 0 DK
14.	Uses first names or nicknames of brothers, sisters or friends, or says their names when asked.	2 1 0 DK
15.	Answers or tries to answer with words when asked a question.	2 1 0 DK
16.	Names at least 10 objects.	2 1 0 DK
17.	States own first name or nickname (for example, Alison, Little Sister, etc.) when asked.	2 1 0 DK
18.	Uses phrases with a noun and a verb (for example,“Katie stay”; “Go home”; etc.).	2 1 0 DK
19.	Asks questions by changing inflection of words or simple phrases (for example, “Mine?”; “Me go?”; etc.); grammar is not important.	2 1 0 DK
20.	Says at least 50 recognisable words.	2 1 0 DK
21.	Uses simple words to describe things (for example, dirty, pretty, big, loud, etc.).	2 1 0 DK
22.	Asks questions beginning with what or where (for example, “What's that?”; “Where doggie go?”; etc.).	2 1 0 DK

23. Uses negatives in sentences (for example, “Me no go”; “I won't drink it”; etc.); grammar is not important. 2 1 0 DK

24. Tells about experiences in simple sentences (for example, “Ginger and I play”; “Dan read me a book”; etc.). 2 1 0 DK

25. Says correct age when asked. 2 1 0 DK

26. Says at least 100 recognisable words. 2 1 0 DK

27. Uses in, on or under in phrases or sentences (for example, “Ball go under chair”; “Put it on the table”; etc.). 2 1 0 DK

28. Uses and in phrases or sentences (for example, “Mum and Dad”; “I want ice cream and cake”; etc.). 2 1 0 DK

29. Says first and last name when asked. 2 1 0 DK

30. Identifies and names most common colours (that is, red, blue, green, yellow, orange, purple, brown and black). 2 1 0 DK
    Scoring Tip:
    Mark a “2” if the individual names 6 to 8 colours; mark a “1” if the individual names 2 to 5 colours; mark a “0” if the individual names 0 or 1 colour.

31. Asks questions beginning with who or why (for example, “Who's that?”; “Why do I have to go?”; etc.). 2 1 0 DK

32. Uses present tense verbs ending in ing (for example, “Is singing”; “Is playing”; etc.). 2 1 0 DK

33. Uses possessives in phrases or sentences (for example, “That's her book”; “This is Charlie's ball”; etc.). 2 1 0 DK

34. Uses pronouns in phrases or sentences; must use correct gender and form of the pronoun, but sentences need not be grammatically correct (for example,“He done it”; “They went”; etc.). 2 1 0 DK

35. Asks questions beginning with when (for example, “When is dinner?”; “When can we go home?”; etc.). 2 1 0 DK

36. Uses regular past tense verbs (for example, walked, baked, etc.); may use irregular past tense verbs ungrammatically (for example, “I runned away”; etc.). 2 1 0 DK

37. Uses behind or in front of in phrases or sentences (for example, “I walked in front of her”; “Terry is behind you”; etc.). 2 1 0 DK

38. Pronounces words clearly without sound substitutions (for example, does not say “wabbit” for “rabbit”, “Thally” for “Sally”, etc.). 2 1 0 DK

39. Tells basic parts of a story, fairy tale or television show plot; does not need to include great detail or recount in perfect order. 2 1 0 DK

40. Says month and day of birthday when asked. 2 1 0 DK

41. Modulates tone of voice, volume and rhythm appropriately (for example, does not consistently speak too loudly, too softly or in a monotone, etc.). 2 1 0 DK

42. Tells about experiences in detail (for example, tells who was involved, where activity took place, etc.). 2 1 0 DK

43. Gives simple directions (for example, on how to play a game or how to make something). 2 1 0 DK
    Scoring Tip:
    Mark a “2” if the directions are clear enough to follow; mark a “1” if the individual articulates directions but they are not clear enough to follow; mark a “0” if the individual never attempts to articulate directions.

44. Uses between in phrases or sentences (for example, “The ball went between the cars”; etc.). 2 1 0 DK

45. Says own telephone number when asked. 2 1 0 DK

46. Easily moves from one topic to another in conversation. 2 1 0 DK

47. Stays on topic in conversations; does not go off on tangents. 2 1 0 DK

48. Explains ideas in more than one way (for example, “This was a good book. It was exciting and fun to read”; etc.). 2 1 0 DK

49. Has conversations that last 10 minutes (for example, relates experiences, contributes ideas, shares feelings, etc.). 2 1 0 DK

50. Uses irregular plurals correctly (for example, children, geese, mice, women, etc.). 2 1 0 DK

51. Says complete home address (that is, street or road, flat number, city and county), with or without postcode, when asked. 2 1 0 DK

52. Describes a short-term goal and what he or she needs to do to reach it (for example, says, “I want to get an A on my test, so I'm going to study hard”; etc.) 2 1 0 DK

53. Gives complex directions to others (for example, to a distant location, for recipe with many ingredients or steps, etc.). 2 1 0 DK
    Scoring Tip:
    Mark a “2” if the directions are clear enough to follow; mark a “1” if the individual articulates directions but they are not clear enough to follow; mark a “0” if the individual never attempts to articulate directions.

54. Describes a realistic long-range goal that can be done in 6 months or more (for example, says, “I want to buy a bike, so I'll babysit and run errands to earn enough money to buy it”; etc.). 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Expressive Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Communication Domain – Written

1.	Identifies one or more alphabet letters as letters and distinguishes them from numbers.	2 1 0 DK
2.	Recognises own name in printed form.	2 1 0 DK
3.	Identifies at least 10 printed letters of the alphabet.	2 1 0 DK
4.	Prints or writes using correct orientation (for example, in English from left to right; in some languages from right to left or top to bottom).	2 1 0 DK
5.	Copies own first name.	2 1 0 DK
6.	Identifies all printed letters of the alphabet, upper- and lowercase.	2 1 0 DK
7.	Prints at least three simple words from example (for example, cat, see, bee, etc.).	2 1 0 DK
8.	Prints or writes own first and last name from memory.	2 1 0 DK
9.	Reads at least 10 words aloud.	2 1 0 DK
10.	Prints at least 10 simple words from memory (for example, hat, ball, the, etc.).	2 1 0 DK
11.	Reads simple stories aloud (that is, stories with sentences of three to five words).	2 1 0 DK
12.	Prints simple sentences of three or four words; may make small errors in spelling or sentence structure.	2 1 0 DK
13.	Prints more than 20 words from memory; may make small spelling errors.	2 1 0 DK
14.	Reads and understands material of at least Year 3/P3 level.	2 1 0 DK
15.	Puts lists of words in alphabetical order.	2 1 0 DK
16.	Writes simple correspondence at least three sentences long (for example, postcards, thank-you notes, e-mail, etc.).	2 1 0 DK
17.	Reads and understands material of at least Year 5/P5 level.	2 1 0 DK
18.	Writes reports, papers or essays at least one page long; may use computer.	2 1 0 DK
19.	Writes complete postal and return addresses on letters or packages.	2 1 0 DK
20.	Reads and understands material of at least Year 7/P7 level.	2 1 0 DK
21.	Edits or corrects own written work before handing it in (for example, checks punctuation, spelling, grammar, etc.).	2 1 0 DK
22.	Writes advanced correspondence at least 10 sentences long; may use computer.	2 1 0 DK
23.	Reads and understands material of at least Year 10/S3 level.	2 1 0 DK
24.	Reads at least two newspaper articles weekly (print or electronic version).	2 1 0 DK
25.	Writes business letters (for example, requests information, makes complaint,	

places order, etc.); may use computer. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Written Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Daily living skills – personal

1.	Opens mouth when food is offered.	2 1 0 DK
2.	Eats solid foods (for example, cooked vegetables, chopped meats, etc.).	2 1 0 DK
3.	Sucks or chews on finger foods (for example, crackers, biscuits, toast, etc.).	2 1 0 DK
4.	Drinks from a cup or glass; may spill.	2 1 0 DK
5.	Lets someone know when he or she has wet or soiled nappy or trousers (for example, points, vocalises, pulls at nappy, etc.).	2 1 0 DK
6.	Feeds self with spoon; may spill.	2 1 0 DK
7.	Sucks from straw.	2 1 0 DK
8.	Takes off clothing that opens in the front (for example, a coat or cardigan); does not have to unbutton or unfasten the clothing.	2 1 0 DK
9.	Pulls up clothing with elastic waistbands (for example, underwear or tracksuit bottoms).	2 1 0 DK
10.	Feeds self with fork; may spill.	2 1 0 DK
11.	Drinks from a cup or glass without spilling.	2 1 0 DK
12.	Feeds self with spoon without spilling.	2 1 0 DK
13.	Urinates in toilet or potty chair.	2 1 0 DK
14.	Puts on clothing that opens in the front (for example, a coat or cardigan); does not have to fasten or button the clothing.	2 1 0 DK
15.	Asks to use toilet.	2 1 0 DK
16.	Defecates in toilet or potty chair.	2 1 0 DK
17.	Is toilet-trained during the day.	2 1 0 DK
18.	Fastens zips that are fastened at the bottom (for example, in trousers, on backpacks, etc.).	2 1 0 DK
19.	Wipes or blows nose using tissue or handkerchief.	2 1 0 DK
20.	Is toilet-trained during the night.	2 1 0 DK
21.	Puts shoes on correct feet; does not need to tie laces.	2 1 0 DK
22.	Fastens poppers.	2 1 0 DK
23.	Holds spoon, fork and knife correctly.	2 1 0 DK
24.	Washes and dries face using soap and water.	2 1 0 DK
25.	Brushes teeth.	2 1 0 DK

26. Buttons large buttons in front, in correct buttonholes. 2 1 0 DK

27. Covers mouth and nose when coughing and sneezing. 2 1 0 DK

28. Buttons small buttons in front, in correct buttonholes. 2 1 0 DK

29. Connects and fastens zips that are not fastened at the bottom (for example, in jackets, sweatshirts, etc.). 2 1 0 DK

30. Turns taps on and adjusts temperature by adding hot or cold water. 2 1 0 DK

31. Wears appropriate clothing during wet or cold weather (for example, raincoat, boots, jumper, etc.). 2 1 0 DK

32. Bathes or showers and dries self. 2 1 0 DK

33. Finds and uses appropriate public toilet for his or her gender. 2 1 0 DK

34. Washes and dries hair (with towel or hair dryer). 2 1 0 DK

35. Cares for minor cuts (for example, cleans wound, puts on a bandage, etc.). 2 1 0 DK

36. Takes medicine as directed (that is, follows directions on label). 2 1 0 DK

37. Uses thermometer to take own or another's temperature. 2 1 0 DK

38. Seeks medical help in an emergency (for example, recognises symptoms of serious illness or injury, such as shortness of breath, chest pain, uncontrolled bleeding, etc.). 2 1 0 DK

39. Follows directions for health care procedures, special diet, or medical treatments. 2 1 0 DK

40. Keeps track of medication (nonprescription and prescription) and refills them as needed. 2 1 0 DK

41. Makes appointments for regular medical and dental check-ups. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Personal Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Daily living skills – domestic

1. Is careful around hot objects (for example, the stove or oven, an open fire, etc.). 2 1 0 DK
2. Helps with simple household chores (for example, dusts, picks up clothes or toys, feeds pet, etc.). 2 1 0 DK
3. Clears unbreakable items from own place at table. 2 1 0 DK
4. Cleans up play or work area at end of an activity (for example, finger painting, model building, etc.). 2 1 0 DK
5. Puts away personal possessions (for example, toys, books, magazines, etc.). 2 1 0 DK
6. Is careful when using sharp objects (for example, scissors, knives, etc.). 2 1 0 DK
7. Clears breakable items from own place at table. 2 1 0 DK
8. Helps prepare foods that require mixing and cooking (for example, baking a cake or macaroni cheese, etc.). 2 1 0 DK
9. Uses simple appliances (for example, a toaster, tin opener, bottle opener, etc.). 2 1 0 DK
10. Uses microwave oven for heating, baking or cooking (that is, sets time and power setting, etc.). 2 1 0 DK
11. Puts clean clothes away in proper place (for example, in drawers or wardrobe, on hooks, etc.). 2 1 0 DK
12. Uses tools (for example, a hammer to drive nails, a screwdriver to screw and unscrew screws, etc.). 2 1 0 DK
13. Washes dishes by hand, or loads and uses dishwasher. 2 1 0 DK
14. Sweeps, mops or vacuums floors thoroughly. 2 1 0 DK
15. Clears table completely (for example, scrapes and stacks dishes, throws away disposable items, etc.). 2 1 0 DK
16. Uses household products correctly (for example, laundry detergent, furniture polish, glass cleaner, etc.) 2 1 0 DK
17. Prepares basic foods that do not need mixing but require cooking (for example, rice, soup, vegetables, etc.). 2 1 0 DK
18. Cleans one or more rooms other than own bedroom. 2 1 0 DK
19. Uses sharp knife to prepare food. 2 1 0 DK
20. Uses stove or oven for heating, baking or cooking (that is, turns rings on and off, sets oven temperature, etc.). 2 1 0 DK
21. Prepares food from ingredients that require measuring, mixing and cooking. 2 1 0 DK
22. Washes clothing as needed. 2 1 0 DK

23. Performs maintenance tasks as needed (for example, replaces light bulbs, changes vacuum cleaner bag, etc.). 2 1 0 DK

24. Plans and prepares main meal of the day. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Domestic Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Daily living skills – community

1.	Demonstrates understanding of function of telephone (for example, pretends to talk on phone, etc.).	2 1 0 DK
2.	Talks to familiar person on telephone.	2 1 0 DK
3.	Uses TV or radio without help (for example, turns equipment on, accesses channel or station, selects programme, etc.).	2 1 0 DK
4.	Counts at least 10 objects, one by one.	2 1 0 DK
5.	Is aware of and demonstrates appropriate behaviour while travelling in a car (for example, keeps seat belt on, refrains from distracting driver, etc.).	2 1 0 DK
6.	Demonstrates understanding of the function of money (for example, says, "Money is what you need to buy things at the shop"; etc.).	2 1 0 DK
7.	Uses pavement (where available) or shoulder of road when walking or using wheeled equipment (for example, skates, scooter, tricycle, etc.).	2 1 0 DK
8.	Demonstrates understanding of function of clock (for example, says, "Clocks tell time"; "What time can we go?"; etc.).	2 1 0 DK
9.	Follows household rules (for example, no running in the house, no jumping on the furniture, etc.).	2 1 0 DK
10.	Demonstrates computer skills necessary to play games or start programs with computer turned on; does not need to turn computer on by self.	2 1 0 DK
11.	Summons to the telephone the person receiving a call or indicates that the person is not available.	2 1 0 DK
12.	Identifies one, two, five, ten, twenty, etc. pence coins by name when asked; does not need to know the value of coins.	2 1 0 DK
13.	Looks both ways when crossing streets or roads.	2 1 0 DK
14.	Says current day of the week when asked.	2 1 0 DK
15.	Demonstrates understanding of right to personal privacy for self and others (for example, while using toilet or changing clothes, etc.).	2 1 0 DK
16.	Demonstrates knowledge of what phone number to call in an emergency when asked.	2 1 0 DK
17.	Tells time using a digital clock or watch.	2 1 0 DK
18.	States value of one, two, five, ten, twenty, etc. pence coins.	2 1 0 DK
19.	Discriminates between notes of different denominations (for example, refers to £5 note, £10 note, etc., in conversation; etc.).	2 1 0 DK

20. Obeys traffic lights and green man and red man signals. 2 1 0 DK

21. Points to current or other date on calendar when asked. 2 1 0 DK

22. Demonstrates understanding that some items cost more than others (for example, says, “I have enough money to buy chewing gum but not a chocolate bar”; “Which pencil costs less?”; etc.). 2 1 0 DK

23. Tells time by the half hour on analogue clock (for example, 1:30, 2:00, etc.). 2 1 0 DK

24. Makes telephone calls to others, using standard or mobile phone. 2 1 0 DK

25. Orders a complete meal in a fast-food restaurant. 2 1 0 DK

26. Carries or stores money safely (for example, in wallet, purse, money belt, etc.). 2 1 0 DK

27. Tells time by 5-minute segments on analogue clock (for example, 1:05, 1:10, etc.). 2 1 0 DK

28. Obeys curfew parent or carer sets. 2 1 0 DK

29. Watches or listens to programmes for information (for example, weather report, news, educational programme, etc.). 2 1 0 DK

30. Counts change from a purchase. 2 1 0 DK

31. Demonstrates computer skills necessary to carry out complex tasks (for example, word processing, accessing the Internet, installing software, etc.). 2 1 0 DK

32. Evaluates quality and price when selecting items to purchase. 2 1 0 DK

33. Obeys time limits for breaks (for example, lunch or coffee breaks, etc.). 2 1 0 DK

34. Travels at least 5 to 10 miles to familiar destination (that is, cycles, uses public transport or drives self). 2 1 0 DK

35. Demonstrates understanding of right to complain or report legitimate problems when dissatisfied with services or situations. 2 1 0 DK

36. Notifies school or supervisor when he or she will be late or absent. 2 1 0 DK

37. Uses savings or current account responsibly (for example, keeps some money in account, tracks balance carefully, etc.). 2 1 0 DK

38. Travels at least 5 to 10 miles to unfamiliar destination (that is, bikes, uses public transport or drives self). 2 1 0 DK

39. Earns money at part-time job (that is, at least 10 hours a week) for 1 year. 2 1 0 DK

40. Attempts to improve job performance after receiving constructive criticism from supervisor. 2 1 0 DK

41. Manages own money (for example, pays most or all own expenses, uses cheques or money orders for purchases as needed, etc.). 2 1 0 DK

42. Has held full-time job for 1 year. 2 1 0 DK

43. Budgets for monthly expenses (for example, utilities, rent, etc.). 2 1 0 DK

44. Applies for and uses personal credit card responsibly (for example,

does not exceed credit limit, pays on time, etc.). 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Community Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Socialization – interpersonal relationships

1. Looks at face of parent or carer. 2 1 0 DK
2. Watches (that is, follows with eyes) someone moving by cot or bed for 5 seconds or more. 2 1 0 DK
3. Shows two or more emotions (for example, laughs, cries, screams, etc.). 2 1 0 DK
4. Smiles or makes sounds when approached by a familiar person. 2 1 0 DK
5. Makes or tries to make social contact (for example, smiles, makes noises, etc.). 2 1 0 DK
6. Reaches for familiar person when person holds out arms to him or her. 2 1 0 DK
7. Shows preference for certain people and objects (for example, smiles, reaches for or moves toward person or object, etc.). 2 1 0 DK
8. Shows affection to familiar persons (for example, touches, hugs, kisses, cuddles, etc.). 2 1 0 DK
9. Imitates or tries to imitate parent's or carer's facial expressions (for example, smiles, frowns, etc.). 2 1 0 DK
10. Moves about looking for parent or carer or other familiar person nearby. 2 1 0 DK
11. Shows interest in children the same age, other than brothers or sisters (for example, watches them, smiles at them, etc.). 2 1 0 DK
12. Imitates simple movements (for example, claps hands, waves good-bye, etc.). 2 1 0 DK
13. Uses actions to show happiness or concern for others (for example, hugs, pats arm, holds hands, etc.). 2 1 0 DK
14. Shows desire to please others (for example, shares a snack or toy, tries to help even if not capable, etc.). 2 1 0 DK
15. Demonstrates friendship-seeking behaviour with others the same age (for example, says, “Do you want to play?” or takes another child by the hand, etc.). 2 1 0 DK
16. Imitates relatively complex actions as they are being performed by another person (for example, shaving, putting on makeup, hammering nails, etc.). 2 1 0 DK
17. Answers when familiar adults make small talk (for example, if asked, “How are you?” says, “I'm fine”; if told, “You look nice”, says, “Thank you”; etc.). 2 1 0 DK
18. Repeats phrases heard spoken before by an adult (for example, “Honey, I'm home”; “No dessert until you clean your plate”; etc.). 2 1 0 DK
19. Uses words to express own emotions (for example, “I'm happy”; “I'm scared”; etc.). 2 1 0 DK
20. Has best friend or shows preference for certain friends (of either sex) over others. 2 1 0 DK

21. Imitates relatively complex actions several hours after watching someone else perform them (for example, shaving, putting on makeup, hammering nails, etc.). 2 1 0 DK

22. Uses words to express happiness or concern for others (for example, says, “Yeah! You won”; “Are you all right?”; etc.). 2 1 0 DK

23. Acts when another person needs a helping hand (for example, holds door open, picks up dropped items, etc.). 2 1 0 DK

24. Recognises the likes and dislikes of others (for example, says, “Chow likes soccer”; “Susie doesn't eat pizza”; etc.). 2 1 0 DK

25. Shows same level of emotion as others around him or her (for example, does not downplay or overdramatise a situation, etc.). 2 1 0 DK

26. Keeps comfortable distance between self and others in social situations (for example, does not get too close to another person when talking, etc.). 2 1 0 DK

27. Talks with others about shared interests (for example, sports, TV shows, summer plans, etc.). 2 1 0 DK

28. Starts small talk when meets people he or she knows (for example, says, “How are you?”; “What's up?”; etc.). 2 1 0 DK

29. Meets with friends regularly. 2 1 0 DK

30. Chooses not to say embarrassing or mean things or ask rude questions in public. 2 1 0 DK

31. Places reasonable demands on friendship (for example, does not expect to be a person's only friend or to have the friend always available, etc.). 2 1 0 DK

32. Understands that others do not know his or her thoughts unless he or she says them. 2 1 0 DK

33. Is careful when talking about personal things. 2 1 0 DK

34. Cooperates with others to plan or be part of an activity (for example, a birthday party, sports event, etc.). 2 1 0 DK

35. Demonstrates understanding of hints or indirect cues in conversation (for example, knows that yawns may mean, “I'm bored”, or a quick change of subject may mean, “I don't want to talk about that”; etc.). 2 1 0 DK

36. Starts conversations by talking about things that interest others (for example, says, “Peter tells me you like computers”; etc.). 2 1 0 DK

37. Goes on group dates. 2 1 0 DK

38. Goes on single dates. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

interpersonal relationships Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Socialization – Play and leisure time

1. Responds when parent or carer is playful (for example, smiles, laughs, claps hands, etc.). 2 1 0 DK
2. Shows interest in where he or she is (for example, looks or moves around, touches objects or people, etc.). 2 1 0 DK
3. Plays simple interaction games with others (for example, peekaboo, pat-a-cake, etc.). 2 1 0 DK
4. Plays near another child, each doing different things. 2 1 0 DK
5. Chooses to play with other children (for example, does not stay on the edge of a group or avoid others). 2 1 0 DK
6. Plays cooperatively with one or more children for up to 5 minutes. 2 1 0 DK
7. Plays cooperatively with more than one child for more than 5 minutes. 2 1 0 DK
8. Continues playing with another child with little fuss when parent or carer leaves. 2 1 0 DK
9. Shares toys or possessions when asked. 2 1 0 DK
10. Plays with others with minimal supervision. 2 1 0 DK
11. Uses common household objects or other objects for make-believe activities (for example, pretends a block is a car, a box is a house, etc.). 2 1 0 DK
12. Protects self by moving away from those who destroy things or cause injury (for example, those who bite, hit, throw things, pull hair, etc.). 2 1 0 DK
13. Plays simple make-believe activities with others (for example, plays dress-up, pretends to be superheroes, etc.). 2 1 0 DK
14. Seeks out others for play or companionship (for example, invites others home, goes to another's home, plays with others in the playground, etc.). 2 1 0 DK
15. Takes turns when asked while playing games or sports. 2 1 0 DK
16. Plays informal, outdoor group games (for example, tag, skipping rope, catch, etc.). 2 1 0 DK
17. Shares toys or possessions without being asked. 2 1 0 DK
18. Follows rules in simple games (relay races, spelling competitions, electronic games, etc.). 2 1 0 DK
19. Takes turns without being asked. 2 1 0 DK
20. Plays simple card or board games based only on chance (for example, Happy Families, Snap, Ludo™, etc.). 2 1 0 DK
21. Goes places with friends during the day with adult supervision (for example, to a shopping centre, park, community centre, etc.). 2 1 0 DK

22. Asks permission before using objects belonging to or being used by another. 2 1 0 DK

23. Refrains from entering group when nonverbal cues indicate that he or she is not welcome. 2 1 0 DK

24. 24 Plays simple games that require keeping score (for example, rounders, netball, etc.). 2 1 0 DK

25. Shows good sportsmanship (that is, follows rules, is not overly aggressive, congratulates other team on winning and does not get angry when losing). 2 1 0 DK

26. Plays more than one board, card or electronic game requiring skill and decision making (for example, Monopoly™, Cribbage, etc.). 2 1 0 DK

27. Goes places with friends in evening with adult supervision (for example, to a concert, lecture, sporting event, the cinema, etc.). 2 1 0 DK

28. Follows rules in complex games or sports (for example, football, rugby, volleyball, etc.). 2 1 0 DK

29. Goes places with friends during the day without adult supervision (for example, to a shopping centre, park, community centre, etc.). 2 1 0 DK

30. Plans fun activities with more than two things to be arranged (for example, a trip to a beach or park that requires planning transportation, food, recreational items, etc.). 2 1 0 DK

31. Goes places with friends in evening without adult supervision (for example, to a concert, lecture, sporting event, the cinema, etc.). 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Play and leisure time Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Socialization – coping skills

1. Changes easily from one at-home activity to another. 2 1 0 DK
2. Says “thank you” when given something. 2 1 0 DK
3. Changes behaviour depending on how well he or she knows another person (for example, acts differently with family member than with stranger, etc.). 2 1 0 DK
4. Chews with mouth closed. 2 1 0 DK
5. Says “please” when asking for something. 2 1 0 DK
6. Ends conversations appropriately (for example, says, “Good-bye”; “See you later”; etc.). 2 1 0 DK
7. Cleans or wipes face and hands during and/or after meals. 2 1 0 DK
8. Responds appropriately to reasonable changes in routine (for example, refrains from complaining, etc.). 2 1 0 DK
9. Says that he or she is sorry for unintended mistakes (for example, bumping into someone, etc.). 2 1 0 DK
10. Chooses not to taunt, tease or bully. 2 1 0 DK
11. Acts appropriately when introduced to strangers (for example, nods, smiles, shakes hands, greets them, etc.). 2 1 0 DK
12. Changes voice level depending on location or situation (for example, in a library, during a film or play, etc.). 2 1 0 DK
13. Says he or she is sorry after hurting another's feelings. 2 1 0 DK
14. Refrains from talking with food in mouth. 2 1 0 DK
15. Talks with others without interrupting or being rude. 2 1 0 DK
16. Accepts helpful suggestions or solutions from others. 2 1 0 DK
17. Controls anger or hurt feelings when plans change for reason(s) that cannot be helped (for example, bad weather, car trouble, etc.). 2 1 0 DK
18. Keeps secrets or confidences for longer than one day. 2 1 0 DK
19. Says he or she is sorry after making unintentional mistakes or errors in judgement (for example, when unintentionally leaving someone out of a game, etc.). 2 1 0 DK
20. Shows understanding that gentle teasing with family and friends can be a form of humour or affection. 2 1 0 DK
21. Tells parent or carer about his or her plans (for example, what time he or she is leaving and returning, where he or she is going, etc.). 2 1 0 DK
22. Chooses to avoid dangerous or risky activities (for example, jumping off high places, picking up a hitchhiker, driving recklessly, etc.). 2 1 0 DK

23. Controls anger or hurt feelings when he or she does not get his or her way (for example, when not allowed to watch television or attend a party; when suggestion is rejected by friend or supervisor; etc.). 2 1 0 DK

24. Follows through with arrangements (for example, if promises to meet someone, meets that person; etc.). 2 1 0 DK

25. Stops or stays away from relationships or situations that are hurtful or dangerous (for example, being bullied or made fun of, being taken advantage of sexually or financially, etc.). 2 1 0 DK

26. Controls anger or hurt feelings due to constructive criticism (for example, correction of misbehaviour, discussion of test score or grade, performance review, etc.). 2 1 0 DK

27. Keeps secrets or confidences for as long as needed. 2 1 0 DK

28. Thinks about what could happen before making decisions (for example, refrains from acting impulsively, thinks about important information, etc.). 2 1 0 DK

29. Is aware of potential danger and uses caution when encountering risky social situations (for example, binge drinking parties, Internet chat rooms, personal ads, etc.). 2 1 0 DK

30. Shows respect for co-workers (for example, does not distract or interrupt others who are working, is on time for meetings, etc.). 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Coping skills Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Motor skills – Gross motor

1.	Holds head erect for at least 15 seconds when held upright in parent's or carer's arms.	2 1 0 DK
2.	Sits supported (for example, in a chair, with pillows, etc.) for at least 1 minute.	2 1 0 DK
3.	Sits without support for at least 1 minute.	2 1 0 DK
4.	Creeps or moves on stomach across floor.	2 1 0 DK
5.	Sits without support for at least 10 minutes.	2 1 0 DK
6.	Raises self to sitting position and sits without support for at least 1 minute.	2 1 0 DK
7.	Crawls at least 1.5 metres on hands and knees, without stomach touching floor.	2 1 0 DK
8.	Pulls self to standing position.	2 1 0 DK
9.	Crawls up stairs.	2 1 0 DK
10.	Takes at least two steps.	2 1 0 DK
11.	Stands alone for 1 to 3 minutes.	2 1 0 DK
12.	Rolls ball while sitting.	2 1 0 DK
13.	Climbs on and off low objects (for example, chair, step stool, slide, etc.).	2 1 0 DK
14.	Crawls down stairs.	2 1 0 DK
15.	Stands for at least 5 minutes.	2 1 0 DK
16.	Walks across room; may be unsteady and fall occasionally.	2 1 0 DK
17.	Throws ball.	2 1 0 DK
18.	Walks to get around; does not need to hold on to anything.	2 1 0 DK
19.	Climbs on and off adult-sized chair.	2 1 0 DK
20.	Runs without falling; may be awkward and uncoordinated.	2 1 0 DK
21.	Walks up stairs, putting both feet on each step; may use bannister.	2 1 0 DK
22.	Kicks ball.	2 1 0 DK
23.	Runs smoothly without falling.	2 1 0 DK
24.	Walks down stairs, facing forward, putting both feet on each step; may use bannister.	2 1 0 DK
25.	Jumps with both feet off floor.	2 1 0 DK
26.	Throws ball of any size in specific direction.	2 1 0 DK

27. Catches beach ball-sized ball with both hands from a distance of 0.5 to 1 metre. 2 1 0 DK

28. Walks up stairs, alternating feet; may use bannister. 2 1 0 DK

29. Pedals tricycle or other three-wheeled toy for at least 1.8 metres. 2 1 0 DK

30. Jumps or hops forward at least three times. 2 1 0 DK

31. Hops on one foot at least once without falling; may hold on to something for balance. 2 1 0 DK

32. Climbs on and off high objects (for example, climbing frame, 1.2-metre slide ladder, etc.). 2 1 0 DK

33. Walks down stairs, alternating feet; may use bannister. 2 1 0 DK

34. Runs smoothly, with changes in speed and direction. 2 1 0 DK

35. Rides bicycle with stabilisers for at least 3 metres. 2 1 0 DK

36. Catches beach ball-sized ball (from at least 1.8 metres away) with both hands. 2 1 0 DK

37. Hops forward on one foot with ease. 2 1 0 DK

38. Skips at least 1.5 metres. 2 1 0 DK

39. Catches tennis or cricket-sized ball (from at least 3 metres away), moving to catch it if necessary. 2 1 0 DK

40. Rides bicycle with no stabilisers without falling. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Gross Motor Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Motor skills – Fine motor

1.	Reaches for toy or object.	2 1 0 DK
2.	Picks up small objects (no larger than 5 cm on any side); may use both hands.	2 1 0 DK
3.	Moves object from one hand to the other.	2 1 0 DK
4.	Squeezes squeaky toy or object.	2 1 0 DK
5.	Picks up small object with thumb and fingers.	2 1 0 DK
6.	Removes object (for example, a block or clothes peg) from a container.	2 1 0 DK
7.	Puts object (for example, a block or clothes peg) into container.	2 1 0 DK
8.	Turns pages of board, cloth or paper book, one at a time.	2 1 0 DK
9.	Stacks at least four small blocks or other small objects; stack must not fall.	2 1 0 DK
10.	Opens doors by turning doorknobs.	2 1 0 DK
11.	Unwraps small objects (for example, chewing gum or sweets).	2 1 0 DK
12.	Completes simple puzzle of at least two pieces or shapes.	2 1 0 DK
13.	Turns book or magazine pages one by one.	2 1 0 DK
14.	Uses twisting hand-wrist motion (for example, winds up toy, screws/ unscrews lid of jar, etc.).	2 1 0 DK
15.	Holds pencil in proper position (not with fist) for writing or drawing.	2 1 0 DK
16.	Colours simple shapes; may colour outside lines.	2 1 0 DK
17.	Builds three-dimensional structures (for example, a house, bridge, vehicle, etc.) with at least five small blocks.	2 1 0 DK
18.	Opens and closes scissors with one hand.	2 1 0 DK
19.	Glues or pastes two or more pieces together (for example, for art or science projects, etc.).	2 1 0 DK
20.	Uses tape to hold things together (for example, torn page, art project, etc.).	2 1 0 DK
21.	Draws more than one recognisable form (for example, person, house, tree, etc.).	2 1 0 DK
22.	Makes recognisable letters or numbers.	2 1 0 DK
23.	Draws circle freehand while looking at example.	2 1 0 DK
24.	Uses scissors to cut across paper along a straight line.	2 1 0 DK
25.	Colours simple shapes; colours inside the lines.	2 1 0 DK

26. Cuts out simple shapes (for example, circles, squares, rectangles, etc.). 2 1 0 DK

27. Uses eraser without tearing paper. 2 1 0 DK

28. Draws square freehand while looking at example. 2 1 0 DK

29. Draws triangle freehand while looking at example. 2 1 0 DK

30. Ties knot. 2 1 0 DK

31. Draws straight line using a ruler or straight edge. 2 1 0 DK

32. Unlocks dead-bolt, key, or combination locks that require twisting. 2 1 0 DK

33. Cuts out complex shapes (for example, stars, animals, alphabet letters, etc.). 2 1 0 DK

34. Uses keyboard, typewriter or touch screen to type name or short words; may look at keys. 2 1 0 DK

35. Ties secure bow. 2 1 0 DK

36. Uses a keyboard to type up to 10 lines; may look at the keys. 2 1 0 DK

Item Before Basal ____ X 2 =……

Basal Item Through Ceiling Item:

DK and/or Missing Total +……..

N/O Total +……

Sum of 2s and 1s +……..

Fine Motor Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Maladaptive behaviour – Internalising

1.	Is overly dependent (that is, clings to carer, teacher, brother or sister).	0 1 2
2.	Avoids others and prefers to be alone.	0 1 2
3.	Has eating difficulties (for example, eats too fast or too slowly, hoards food, overeats, refuses to eat, etc.).	0 1 2
4.	Has sleep difficulties (for example, sleepwalks, has frequent nightmares, sleeps significantly more or less than typical for his or her age).	0 1 2
5.	Refuses to go to school or work because of fear, feelings of rejection or isolation, etc.	0 1 2
6.	Is overly anxious or nervous.	0 1 2
7.	Cries or laughs too easily.	0 1 2
8.	Has poor eye contact (that is, does not look at or face others when speaking or spoken to).	0 1 2
9.	Is sad for no clear reason.	0 1 2
10.	Avoids social interaction.	0 1 2
11.	Lacks energy or interest in life.	0 1 2

Sum of 2s and 1s =

Internalising Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Maladaptive behaviour – Externalising

1.	Is impulsive (that is, acts without thinking).	0 1 2
2.	Has temper tantrums.	0 1 2
3.	Intentionally disobeys and defies those in authority.	0 1 2
4.	Taunts, teases or bullies.	0 1 2
5.	Is inconsiderate or insensitive to others.	0 1 2
6.	Lies, cheats or steals.	0 1 2
7.	Is physically aggressive (for example, hits, kicks, bites, etc.).	0 1 2
8.	Is stubborn or sullen.	0 1 2
9.	Says embarrassing things or asks embarrassing questions in public (for example, “You're fat”, or “What's that big red thing on your nose?”).	0 1 2
10.	Behaves inappropriately at the urging of others.	0 1 2

Sum of 2s and 1s =

Externalising Raw Score =

Vineland Adaptive Behaviour Scales – interview form

Maladaptive behaviour – Other

1.	Sucks thumb or fingers.	0 1 2
2.	Wets bed or must wear nappies at night.	0 1 2
3.	Acts overly familiar with strangers (for example, holds hands, hugs, sits on lap, etc.).	0 1 2
4.	Bites fingernails.	0 1 2
5.	Has tics (that is, involuntary blinking, twitching, head shaking, etc.).	0 1 2
6.	Grinds teeth during the day or night.	0 1 2
7.	Has a hard time paying attention.	0 1 2
8.	Is more active or restless than others of same age.	0 1 2
9.	Uses school or work property (for example, telephone, Internet access, office supplies, etc.) for unapproved personal purposes.	0 1 2
10.	Swears.	0 1 2
11.	Runs away (that is, is missing for 24 hours or longer).	0 1 2
12.	Is truant from school or work.	0 1 2
13.	Ignores or doesn't pay attention to others around him or her.	0 1 2
14.	Uses money or gifts to “buy” affection.	0 1 2
15.	Uses alcohol or illegal drugs during the school or work day.	0 1 2

Sum of 2s and 1s =

Other Raw Score =

Internalising Raw Score + =

Externalising Raw Score + =

Other Raw Score + =

Maladaptive Behaviour Index Raw Score =

Vineland Adaptive Behaviour Scales – Scoring

Basal and Ceiling Rules: For each subdomain, a basal of four consecutive items scored “2” and a ceiling of four consecutive items scored “0” should be established.

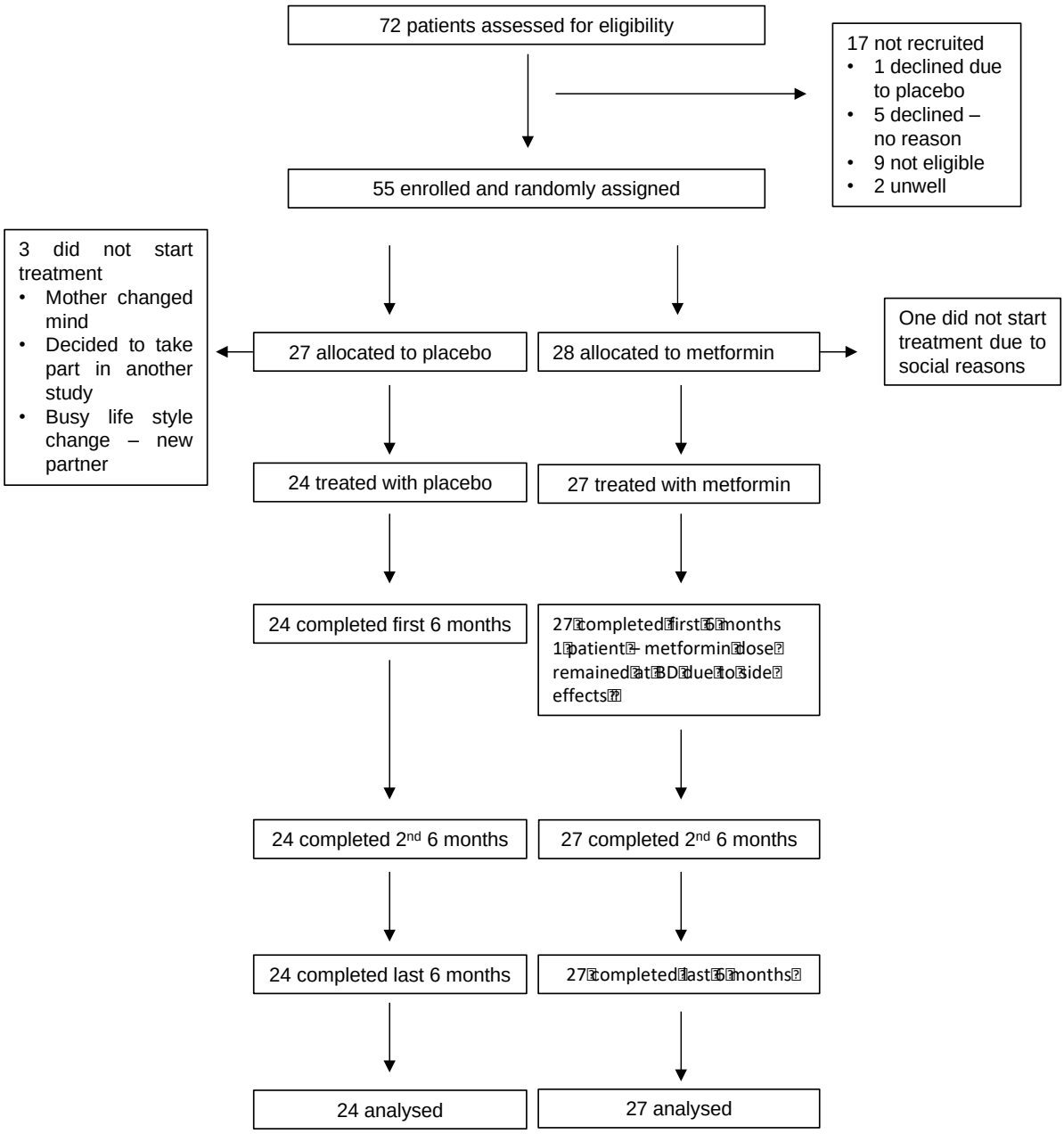
The basal item is defined as the highest item in the highest set of four consecutive items scored “2”.

The ceiling item is defined as the lowest item in the lowest set of four consecutive items scored “0”.

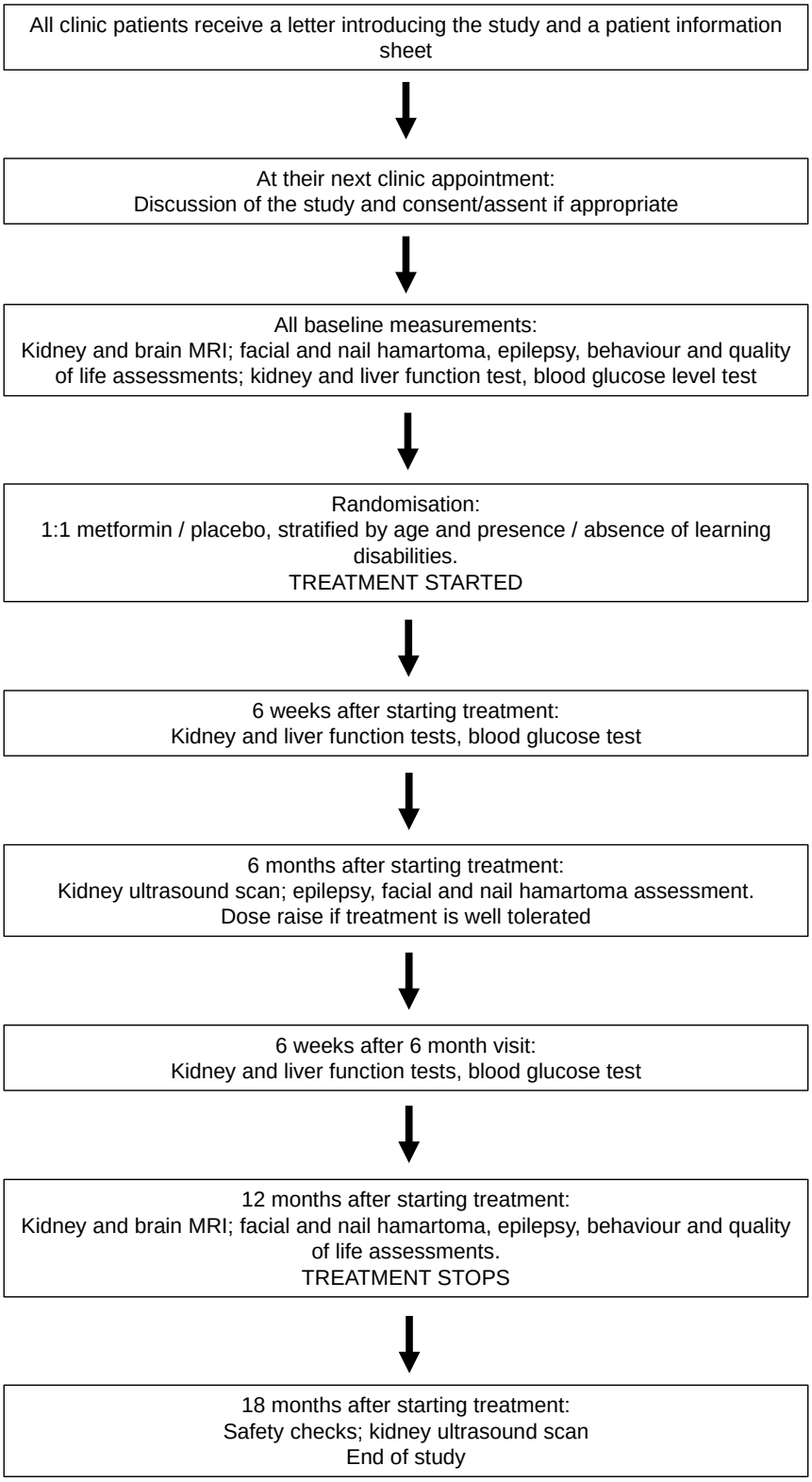
If no basal is established, treat Item 1 as the basal item. If no ceiling is established, treat the last item in the subdomain as the ceiling item.

We used VABS software to calculate the v-scale scores, % confidence interval, % rank, adaptive level and age equivalent for each subdomains and standard scores for each domain.

Table 5.5.2: shows trial profile.



Involvement for participants: Schedule of Assessments. Table 5.5.3



Study settings

This was a multicentre trial. Patients were recruited from Bristol, Bath and London TSC clinics for this study. The assessments were carried out in Bristol, Bath and London. They were carried out either as an in-patient when patients were having MRI scans, sometimes as outpatients, and in some circumstances at patient's family home or residential home to minimise family and patient stress.

Randomisation

Patients were randomly allocated (1:1) to placebo or metformin for 12 months. The randomisation was stratified by centre and minimised by age-group (10 to <20; 20 to <30; 30 to <40; and 40 to <65) and by the presence or absence of learning disabilities. The randomisation was concealed.

The investigators randomised patients online, and wrote randomisation number and treatment pack number on the study prescription form. The prescriptions were sent to the main trial pharmacy at the sponsor's site. The trial pharmacists receive the prescription forms, look up the treatment pack number on their (provided) spreadsheet. This lists the drug to dispense for each treatment pack number. The online randomisation system will also send an automatic email to all pharmacists confirming the recruited patient's ID, centre and drug allocation. The pharmacists make up the treatment pack and dispense. The online randomisation was performed by the (BRTC) Bristol Randomised Trials Collaboration Unit, School of Social and Community Medicine, University of Bristol.

Trial medication and intervention

Placebo and Metformin

We used standard licensed 500mg metformin tablets manufactured by Relonchem. The placebo tablets were manufactured by Essential Nutrition Ltd. They were matched for shape, size and colour. Both were labelled, and final Quantitative Pharmacology release was certified by the University Hospitals Bristol Pharmaceuticals.

For adult patients, the starting dose was 500mg twice a day orally. At 6 months, the dose was escalated to 500mg three times a day as long as the patient was tolerating the treatment. For children aged 10-16 years, the drug dosing started at 500mg once a day. After two weeks at this dose, it was escalated to 500mg twice a day. At 6 months the dose was escalated to 500mg three times a day as long as the patient was tolerating treatment well.

prescription chart

University Hospitals Bristol NHS
NHS Foundation Trust

Needed by __ : __ on __ /__ /__
- Researcher to collect [

MiTS study - 2011-001319-30

CLINICAL TRIAL PRESCRIPTION CHART
Chief Investigator: Dr Finbar O'Callaghan

N.B. ALWAYS REFER TO THE ORIGINAL PROTOCOL WHEN PRESCRIBING

VISIT: (please circle) 0 or 2

PATIENT NAME HOSPITAL NUMBER DATE OF BIRTH CONSULTANT	PATIENT TRIAL NO.	RANDOMISATION NO.	TREATMENT PACK NO.
	WEIGHT (Kg)	HEIGHT (cm)	
	ALLERGIES		
	BLOOD RESULTS Date:		

Hb	WBC	Plts.	Neuts	Cr	eGFR

PRESCRIBING INFORMATION:

Adults:
- Drug dosing will start at 500mg twice a day
- After 6 months there will be a dose escalation to 500mg three times a day, if the patient is tolerating treatment well.

Children 10-16 years:
- Drug dosing will start at 500mg once a day. After two weeks at this dose, it will be escalated to 500mg twice a day.
- After 6 months there will be a dose escalation to 500mg three times a day, if the patient is tolerating treatment well.

PLEASE DISPENSE STUDY MEDICATION AS FOLLOWS:

Study Drug: **METFORMIN 500mg TABLETS or PLACEBO**

To be taken ORALLY as indicated below

For 12 months total

Visit	Dosing Regimen	Tick	Dose Prescribed	Quantity to supply
Visit 0 (Baseline)	**Adults**		**ONE tablet TWICE DAILY**	1 month=60 tablets 7 months= **420 tablets**
	Children Aged 10 – 16 years		**ONE tablet ONCE DAILY for 2 weeks, Then ONE tablet TWICE DAILY thereafter.**	2 weeks supply=14 tablets 6 ½ months=390 tablets Total=390+14=**404 tablets**
Visit 2 (Month 6 +/- 4 weeks)	**Dose Escalation**		**ONE tablet THREE TIMES A DAY**	1 months=90 tablets 7 months=**630 tablets**
	Standard Dose		**ONE tablet TWICE DAILY**	1 month=60 tablets 7 months=**420 tablets**

PRESCRIBER:

Sign: _____ Name: _____ Date: _____ Bleep: _____

PHARMACY USE:

Clinical check by / date	Quantity dispensed	Dispensed by / date	Checked by / date
	Metformin 500mg/Placebo tablets x _____		

COLLECTED BY:
SIGNATURE:_____**NAME:**_____**DATE:**_____**TIME:**_____

Blinding

This was a double blind study. The participants, their carers and parents, healthcare providers, data collectors, outcome adjudicators, and data analysts were blinded.

The neuro-radiologists were blinded to the scans. The dermatologist was also blinded to the groups. The dermatologists did not know if the photographs were before or after treatment. The research team including doctors and nurses were also blinded. They did not know if the patients were on placebo or metformin. The trial pharmacists were the only people who were aware of the groups. The rest of the teams were blinded.

Assessments

Primary outcome – AML

The assessments were performed at baseline and 12 months after the initiation of treatment. The primary outcome was percentage change in renal AML volume measured via renal MRI. All patients had a renal MRI at baseline prior to initiation of treatment and 12 months after starting the treatment. Patients with learning disabilities, and some of the children, required general anaesthetic. The same MRI protocol was used in the three centres.

MRI 1.5 tesla was used with pre- and post-gadolinium intravenous agent. Fat saturated spoiled gradient echo sequences were performed in axial and coronal planes. Contrast bolus was adjusted by body weight according to manufacturer dose specifications.

The scans were analyzed by a radiologist who was blind to treatment allocation. Images were reviewed on a work-station (Fuji Synapse PACS, Fujifilm, Japan). Lesions were measured in three dimensions with baseline AMLs required to measure ≥1cm. Interval assessment of the index lesions was performed in the same axes. The volume measurements were performed as an approximated ellipsoid using the formula, width x depth x length x 0.523. The volume of the five largest AMLs per patient were identified from the baseline and 12 month scans. The mean volume was calculated for each patient at baseline and 12 months. The percentage change in AML volume was calculated for each patient.

MRI scan specifications

Abdomen + pelvis -

A) T1 VIBE FAT SAT PRE GAD AXIAL AND CORONAL SEQUENCES

B) T1 VIBE FAT SAT POST GAD AXIAL AND CORONAL SEQUENCES

HEAD – START WITH PREGAD SEQUENCES AS PER RPOTOCOL AND THEN PROCEED TO POST GAD ABDOMEN + PELVIS SEQUENCES FOLLOWED BY POST GAD HEAD SEQUENCES

SEQUENCE OF SCANNING

PRE GAD HEAD SEQUENCES

↓

ABDOMEN +PELVIS - T1 VIBE FAT SAT PRE GAD AXIAL AND CORONAL SEQUENCES

↓

ABDOMEN +PELVIS - T1 VIBE FAT SAT POST GAD AXIAL AND CORONAL SEQUENCES

↓

POST GAD HEAD SEQUENCES

Secondary outcome – SEGA

Each patient in the trial had a baseline cranial MRI and a follow-up cranial MRI 12 months after the initiation of treatment. Up to 2 SEGAs were identified per patient. The volume of SEGAs were calculated at baseline and 12 month scan. The scans were analyzed by a neuroradiologist who was blind to treatment allocation.

The two largest SEGA lesions were chosen in patients who had more than two SEGA lesions. SEGA was defined as a lesion at the Foramen of Monro which enhanced with contrast, and measured ≥ 0.5 cm in diameter.

Identical volumetric measurement methodology was used to the AML lesions.

Secondary outcome – Safety

Toxicity was graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events. Participants recorded a daily diary for the first 14 days, then a monthly diary thereafter. Following any dose escalation there was a further 14 days of daily record keeping. Clinical assessment including measurement of renal and liver function and blood glucose levels occurred at baseline, 6 weeks after starting treatment, and then 6 weeks after any dose change at 6 months.

Site code:	Participant ID:			VISIT no.	Date:	**Adverse Events Diary**
BRIS/BATH/CAMB				0	DD/MMM/YYYY	

	Adverse Events Diary (*first 14 days)*
	Day 1…….. …… …… Day 2…….. …… …… Day 3…….. …… …… Day4……... …… …… Day 5…….. …… …… Day 6…….. …… …… Day 7…….. …… …… Day 8…….. …… …… Day 9…….. …… …… Day 10…… …… …… Day 11…… …… …… Day 12…… ……... …… Day 13…… …… …… Day 14……

	Comment

Site code:	Participant ID:			VISIT no.	Date:	**Adverse Events Diary**
BRIS/BATH/CAMB					DD/MMM/YYYY	

	Adverse Events Diary *(----------month)*

	Comment

Secondary outcome – Facial and nail tumours

The facial angiofibromas and ungual fibromas were assessed at baseline and at 12 months after initiation of treatment using digital photography. These lesions were assessed both by patient/parent/carer report and by a dermatologist blinded to treatment allocation using physician's global assessment (PGA) of digital photographs.

During the baseline and 12 month visits, patients, parents and carers were asked to report on the degree of improvement. They were asked if the rash was "improved, the same or worse." For children and individuals with learning disabilities, parents and carers' reports were analyzed.

The same 10.2 mega pixels digital camera was used for all the patients, in all the three centres, by the same researcher. Six photographs were taken from each patient at baseline and at their 12 month visit. Three photographs were taken with the camera flash on and the other three are taken with the flash in automatic mode. One photograph was taken in full face view directly facing the camera and the other two were side profiles. Patients were advised not to wear makeup before having their photo taken. None of the patients in this series wore make up during the visits.

Assessments

Secondary outcome – Epilepsy

Patient epilepsy diaries were kept throughout the study. The total number of seizures was assessed.

Secondary outcome – Adaptive Behaviour

This was assessed at baseline, and 12 months after starting treatment. It was assessed using the Vineland Adaptive Behaviour Scale (VABS). The questionnaires were completed by the study researcher who was trained to conduct VABS interview. This was a face to face interview.

Secondary outcome – Quality of life (QOL)

Health related QOL was assessed at baseline and at 12 months using the Pediatric Quality of Life Inventory (PedsQL) for children (ages 10 to18 years), and the Short Form 36 Health Survey (SF-36) for adults (i.e. > 18 years).

Data collection forms

During each visit the data collection forms were completed and signed then the data was transferred into an electronic database which matched the paper copy data entry forms.

The following are the data collection forms for each visit throughout the study including the seizure and safety diary.

Site code:	Participant ID:	Visit no.	Date of visit:	**A: PARTICIPANT START-UP**
BRIS/BATH/CAMB		0 (Baseline)	DD/MMM/YYYY	

1	**INCLUSION CRITERIA**	*(please circle the answer)*	
	Age 10 to 65	YES	NO
	Clinically definite diagnosis of TSC	YES	NO
	One or more renal AML (>1cm diameter)	YES	NO
	Signed informed consent	YES	NO

2	**EXCLUSION CRITERIA**	*(please circle the answer)*	
	Serious inter-current illness that could compromise participation?	YES	NO
	Impaired renal function?	YES	NO
	Use of x-ray contrast medium containing iodine in last 30 days?	YES	NO
	Multiple AMLs where individual lesions cannot be distinguished?	YES	NO
	Renal haemorrhage within last year?	YES	NO
	Known renal aneurysms >10mm?	YES	NO
	Liver insufficiency?	YES	NO
	Acute or chronic disease that could cause tissue hypoxia?	YES	NO
	Diabetes?	YES	NO
	On treatment with any hypoglycaemic drug?	YES	NO
	Use of any investigational drug within last 30 days?	YES	NO
	Pregnant / breastfeeding / planning to become pregnant?	YES	NO

3	**INVESTIGATOR SIGN-OFF FOR PARTICIPANT TO ENTER TRIAL**
	Name.....…… Signature…… Date: DD/MMM/YYYY

4	**RANDOMISATION**	*(please circle the answer)*	
	Age-group:	10 to <20 20 to <30 30 to <40 40 to <65	
	Learning difficulties:	Present	Absent

5	**TREATMENT STARTED**	DD/MM/YYYY

Site code:	Participant ID:	Visit no.	Date of visit:	**B: MRI BRAIN & KIDNEYS**
BRIS/BATH/CAMB		0 / 4	DD/MMM/YYYY	

1	**MRI BRAIN**	DD/MMM/YYYY	LOCATION	✓	DIAMETER (mm)	VOLUME (mm^3)
		NONE				
	Subependymal Giant Cell Astrocytoma at the Foramen of Monro ≥ 0.5cm in diameter (SEGA)	SEGA 1	Left Lateral Ventricle			
			Right Lateral Ventricle			
			Third Ventricle			
		SEGA 2	Left Lateral Ventricle			
			Right Lateral Ventricle			
			Third Ventricle			

2	**MRI KIDNEYS**	DD/MMM/YYYY	LOCATION	✓	DIAMETER (mm)	VOLUME (mm^3)
		Lesion 1	Left Kidney			
			Right Kidney			
		Lesion 2	Left Kidney			
			Right Kidney			
		Lesion 3	Left Kidney			
			Right Kidney			
		Lesion 4	Left Kidney			
			Right Kidney			
		Lesion 5	Left Kidney			
			Right Kidney			

3	**COMMENTS**

4	**FORM COMPLETED BY**

	Name..... …… Date: DD/MMM/YYYY

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Site code:	Participant ID:	Visit no.	Date of visit:	**C1: ULTRASOUND KIDNEY**
BRIS/BATH/CAMB		2 (6m+/- 4wks)	DD/MMM/YYYY	

1	**ULTRASOUND KIDNEYS** DD/MMM/YYYY	**LOCATION** *(please circle)*		**DIAMETER (mm)**
	Lesion 1	L	R	
	Lesion 2	L	R	
	Lesion 3	L	R	
	Lesion 4	L	R	
	Lesion 5	L	R	

	Safe to continue in trial? YES / NO *(please circle)* Investigator / researcher initials:..... Signature:..... Date:.....

2	**COMMENTS**

3	**FORM COMPLETED BY**
	Name..... …… Date: DD/MMM/YYYY

Site code:	Participant ID:	Visit no.	Date of visit:	**C1: ULTRASOUND KIDNEY**
BRIS/BATH/CAMB		5 (18m+/-4wks)	DD/MMM/YYYY	

1	**ULTRASOUND KIDNEYS** DD/MMM/YYYY	**LOCATION** *(please circle)*		**DIAMETER (mm)**
	Lesion 1	L	R	
	Lesion 2	L	R	
	Lesion 3	L	R	
	Lesion 4	L	R	
	Lesion 5	L	R	

2	**COMMENTS**

3	**FORM COMPLETED BY**
	Name..... …… Date: DD/MMM/YYYY

Site code:	Participant ID:	Visit no.	Date of visit:	**D1: Facial, nail tumour & epilepsy assessment**
BRIS/BATH/CAMB		0 (Baseline)	DD/MMM/YYYY	

1	**FACIAL TUMOURS**	Photo taken
	Present: YES / NO *(please circle)*	YES / NO *(please circle)*

2	**NAIL TUMOURS**			**No. tumours**	Photo taken
	Present on Left Hand YES / NO *(please circle)*	***If yes,** record number of tumours on each digit.*	Digit 1		YES / NO *(please circle)*
			Digit 2		
			Digit 3		
			Digit 4		
			Digit 5		
	Present on Right Hand YES / NO *(please circle)*	***If yes,** record number of tumours on each digit.*	Digit 1		YES / NO *(please circle)*
			Digit 2		
			Digit 3		
			Digit 4		
			Digit 5		
	Present on Left Foot YES / NO *(please circle)*	***If yes,** record number of tumours on each digit.*	Digit 1		YES / NO *(please circle)*
			Digit 2		
			Digit 3		
			Digit 4		
			Digit 5		
	Present on Right Foot YES / NO *(please circle)*	***If yes,** record number of tumours on each digit.*	Digit 1		YES / NO *(please circle)*
			Digit 2		
			Digit 3		
			Digit 4		
			Digit 5		

3	**EPILEPSY ASSESSMENT** *(from patient seizure diary)*
	Patient has seizures: YES / NO *(please circle)* Total number of seizures in previous 4 weeks: Total number of seizure free days in last 4 weeks:

4	**COMMENTS**

5	**FORM COMPLETED BY**

	Name..... …… Date: DD/MMM/YYYY

Metformin in Tuberous Sclerosis Complex, Protocol no. CH/2011/3670 V3.0 EudraCT no. 2011-001319-30

Site code:	Participant ID:	Visit no.	Date of visit:	**D2: Facial, nail tumour & epilepsy assessment**
BRIS/BATH/CAMB		2 / 4 / 5	DD/MMM/YYYY	

1	**FACIAL TUMOURS**	Photo taken
	Present: YES / NO *(please circle)*	YES / NO *(please circle)*
	Study investigator's report: *(please circle - see key below to grade 0-6)*	0 1 2 3 4 5 6
	Patient report :	Improved / Same / Worse
	Parent / carer report:	Improved / Same / Worse

2	**NAIL TUMOURS**		**No. tumours**	Photo taken
	Present on Left Hand YES / NO *(please circle)*	***If yes,*** *record number of tumours on each digit.*	Digit 1	YES / NO *(please circle)*
			Digit 2	
			Digit 3	
			Digit 4	
			Digit 5	
	Present on Right Hand YES / NO *(please circle)*	***If yes,*** *record number of tumours on each digit.*	Digit 1	YES / NO *(please circle)*
			Digit 2	
			Digit 3	
			Digit 4	
			Digit 5	
	Present on Left Foot YES / NO *(please circle)*	***If yes,*** *record number of tumours on each digit.*	Digit 1	YES / NO *(please circle)*
			Digit 2	
			Digit 3	
			Digit 4	
			Digit 5	
	Present on Right Foot YES / NO *(please circle)*	***If yes,*** *record number of tumours on each digit.*	Digit 1	YES / NO *(please circle)*
			Digit 2	
			Digit 3	
			Digit 4	
			Digit 5	
	Study investigator's report: *(please circle - see key below to grade 0-6)*			0 1 2 3 4 5 6
	Patient report :			Improved / Same / Worse
	Parent / carer report:			Improved / Same / Worse

3	**EPILEPSY ASSESSMENT** *(from patient seizure diary)*
	Patient has seizures: YES / NO *(please circle)* Total number of seizures in previous 4 weeks:..... Total number of seizure free days in last 6 months:.....

4	**FORM COMPLETED BY**
	Name..... …… Date: DD/MMM/YYYY

KEY FOR INVESTIGATOR'S RESPONSE (PHYSICIAN'S GLOBAL ASSESSMENT)	
GRADE	**DESCRIPTION**
0: Completely clear	No evidence of disease / 100% improvement
1: Almost clear	Very significant clearance (>90 to <100%); only traces of disease remain
2: Marked improvement	Significant improvement (>75 to <90%); some evidence of disease remains
3: Moderate improvement	Intermediate between slight and marked improvement; (>50 to < 75%)
4: Slight improvement	Some improvement (>25% to <50%); but still significant evidence of disease
5: No change	Disease has not changed from baseline condition (+<25%)
6. Worse	Disease is worse than at baseline evaluation (>25% or more).

Site code:	Participant ID:	Visit no.	Date of visit:	**E: COGNITION**
BRIS/BATH/CAMB		0 / 4	DD/MMM/YYYY	

1	**COGNITIVE ASSESSMENT**				
	Assessment performed: VABS / WASI / not done *(please circle)* If not done, please state reason:				
	Either	**Vineland Adaptive Behaviour Scale (VABS)**			
		DD/MMM/YYYY	**Raw Score**	**v-Scale Score**	**Standard Score**
		Receptive			
		Expressive			
		Written			
		Communication standard score			
		Personal			
		Domestic			
		Community			
		Daily living skills standard score			
		Interpersonal relationships			
		Play and leisure time			
		Coping skills			
		Socialisation standard score			
		Gross motor			
		Fine motor			
		Motor skills standard score			
		Adaptive Behaviour Comp.			
		Internalizing			
		Externalizing			
		Other			
		Maladaptive behaviour index			
	Or	**Wechsler Abbreviated Scale of Intelligence (WASI)**			
		DD/MMM/YYYY	**Raw Score**	**T Score**	
		Vocabulary			
		Block design			
		Similarities			
		Matrix reasoning			
			Sum of T scores	**IQ**	**Percentile**
		Verbal			
		Performance			
		Full-4			
		Full-2			

2	**COMMENTS**
3	**FORM COMPLETED BY**

	Name.....…..…… …… **Date: DD/MMM/YYYY**

Site code:	Participant ID:	Visit no.	Date of visit:	**F: QUALITY OF LIFE**
BRIS/BATH/CAMB		0 / 4	DD/MMM/YYYY	

1	**ASSESSMENT PERFORMED**
	Assessment performed: PEDS-QL (for ages 10 to 18 years) *(please circle)* SF-36 (for ages ≥ 18 years) Not done If not done, please state reason:

2	**PEDIATRIC QUALITY OF LIFE INVENTORY (PEDS-QL) FOR AGES 10 TO 18**			
	Child report	DD/MMM/YYYY	**Scaled Score**	**Summary Score**
		Physical Functioning		
		Emotional Functioning		
		Social Functioning		
		School Functioning		
		Physical Health summary		
		Psychosocial Health summary		
		Total scale score		
	Parent report	DD/MMM/YYYY	**Scaled Score**	**Summary Score**
		Physical Functioning		
		Emotional Functioning		
		Social Functioning		
		School Functioning		
		Physical Health summary		
		Psychosocial Health summary		
		Total scale score		

3	**FORM COMPLETED BY**
	Name.....…..…… …… Date: DD/MMM/YYYY

G: Adverse Events (UH Bristol Investigator's Template for recording Adverse Events)

Full title of Study: A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex.			
Ethics No:	11/YH/0295	**UHB R&D no:**	CH/2011/3670

Sheet number : _____of_____

AE No:	Patient ID	Description of Event		Start date	Duration/End date	Outcome	**Sequelae
						☐ Resolved ☐ Ongoing ☐ Ongoing with sequelae**	
Assessment							
Intensity:		☐ mild ☐ moderate ☐ severe	Expectedness	☐ expected ☐ unexpected i.e. not described in protocol, product information or investigator brochure.			
Causality: Relationship to study drug/device/intervention		☐ not related ☐ unlikely to be related ☐ possibly related ☐ probably related ☐ definitely related	Seriousness	☐ Not serious ☐ Results in death* ☐ Life threatening* ☐ Results in hospitalisation or prolongation of existing hospitalisation* ☐ Results in disability or incapacity* ☐ Congenital anomaly or birth defect* ☐ Other (please specify)*			

AE No:	Patient ID	Description of Event		Start date	Duration/End date	Outcome	**Sequelae
						☐ Resolved ☐ Ongoing ☐ Ongoing with sequelae**	
Assessment							
Intensity:		☐ mild ☐ moderate ☐ severe	Expectedness	☐ expected ☐ not expected i.e. not described in protocol, product information or investigator brochure.			
Causality: Relationship to study drug/device/intervention		☐ not related ☐ unlikely to be related ☐ possibly related ☐ probably related ☐ definitely related	Seriousness	☐ Not serious ☐ Results in death* ☐ Life threatening* ☐ Results in hospitalisation or prolongation of existing hospitalisation* ☐ Results in disability or incapacity* ☐ Congenital anomaly or birth defect * ☐ Other (please specify)*			

UH Bristol Research Related Adverse Event Reporting Policy. Version 3.4
* Event is considered serious – report to the sponsor and UH Bristol R&I Department within 24 hours using the form provided.
Where none is provided use the UH Bristol Research Related SAE/SUSAR Initial Report Form

Site code:	Participant ID:	Visit no.	Date of visit:	**H: Blood tests**
BRIS/BATH/CAMB		0 / 1 / 3	DD/MMM/YYYY	

1	KIDNEY AND LIVER FUNCTION TEST	VALUE	REFERENCE RANGE
	Creatinine		8-11 yrs: 40-80 µmol/L ≥12 yrs (Male): 75-120 µmol/L ≥12 yrs (Female): 60-100 µmol/L
	Urea		≥ 6 weeks: 3.0-7.0 mmol/L
	Sodium		All ages: 133-143 mmol/L
	Potassium		All ages: 3.7-5.2 mmol/L
	Glomerular filtration rate (GFR)		eGFR >90ml/min/1.73m^2 = normal eGFR <60ml/min/1.73m^2 = chronic kidney disease *NB calculation not valid in children <16 years*
	Lactate dehydrogenase		**Male** **Female** 10-12 yrs: 120-325 iu/L 120-260 iu/L 13-15 yrs: 120-290 iu/L 100-275 iu/L 16-18 yrs: 105-235 iu/L 105-230 iu/L ≥18 yrs: 240-480 iu/L 240-480 iu/L
	Bicarbonate		All ages 21-34 mmol/L
	Alanine Aminotransferase (ALT)		**Male** **Female** 10-18 yrs: 5-30 U/L 5 -20 U/L ≥18 yrs: 10-50 U/L 10-35 U/L
	Aspartate Aminotransferase (AST)		5 years-adult: <50 iu/L
	Bilirubin		4 weeks-adult: < 21 µmol/L
	Gamma-glutamyltransferase (GGT)		**Male** **Female** All ages: 10-71 U/L 6-42 U/L

2	BLOOD GLUCOSE		
	Tested: YES / NO (please circle)		All ages: <6.1 mmol/L

3	COMMENTS

4	FORM COMPLETED BY

	Name..... …… Date: DD/MMM/YYYY

Metformin in Tuberous Sclerosis Complex, Protocol no. CH/2011/3670 V3.0 EudraCT no. 2011-001319-30

Site code:	Participant ID:	Visit no.	Date of visit:	**I1: Treatment compliance**
BRIS/BATH/CAMB		2 (6m+/-4wks)	DD/MMM/YYYY	

1	**TREATMENT COMPLIANCE**	
	Participant report:	
	Parent/carer report:	
	Number of tablets required since baseline (Visit 0):	
	No. tablets supplied at baseline:	
	Expected surplus tablets remaining by Visit 2:	
	Actual number of tablets remaining:	
	Discrepancy? **YES / NO** *(please circle)* Reason if Yes	

2	**DOSE INCREASE TO 500MG THREE TIMES A DAY**
	YES / NO *(please circle)* Reason if No: Investigator / researcher initials: ..... Signature: ..... Date: .....

3	**FORM COMPLETED BY**
	Name..... …… Date: DD/MMM/YYYY

Site code:	Participant ID:	Visit no.	Date of visit:	**I2: Treatment compliance**
BRIS/BATH/CAMB		4 (12m+/-4wks)	DD/MMM/YYYY	

1	**TREATMENT COMPLIANCE**	
	Participant report:	
	Parent/carer report:	
	Number of tablets required for treatment since Visit 2:	
	No. tablets supplied at Visit 2:	
	Expected surplus tablets remaining:	
	Actual number of tablets remaining:	
	Discrepancy? **YES / NO** *(please circle)* Reason if Yes:	

2	**TREATMENT STOPPED**
	Confirmed with the patient: **YES / NO** *(please circle)* Date stopped: DD/MMM/YYYY Investigator / researcher initials: ..... signature.....

3	**FORM COMPLETED BY**
	Name..... …… Date: DD/MMM/YYYY

Site code:	Participant ID:	Visit no.	Date of visit:	**J: Vital signs**
BRIS/BATH/CAMB		0 / 1 / 2 / 3 / 4 / 5	DD/MMM/YYYY	

1	VITAL SIGNS	VALUE		
	Blood pressure	Systolic :	Diastolic:	Mean:
	Pulse rate	bpm		
	Respiratory rate	breaths per minute		
	Weight	kg		
	Height	cm		

2	COMMENTS

3	FORM COMPLETED BY
	Name..... …… Date: DD/MMM/YYYY

Site code:	Participant ID:	Visit no.	Date of visit:	**K: Seizure Diary**
BRIS/BATH/CAMB		4 weeks before 0, then monthly	DD/MMM/YYYY	

Use your seizure diary to make a note of any seizure episodes. Circle Yes or No. If yes, state how many seizures happened on that day.

Day 1	Seizure Yes / No	If Yes, number of seizures:	
Day 2	Seizure Yes / No	If Yes, number of seizures:	
Day 3	Seizure Yes / No	If Yes, number of seizures:	
Day 4	Seizure Yes / No	If Yes, number of seizures:	
Day 5	Seizure Yes / No	If Yes, number of seizures:	
Day 6	Seizure Yes / No	If Yes, number of seizures:	
Day 7	Seizure Yes / No	If Yes, number of seizures:	
Day 8	Seizure Yes / No	If Yes, number of seizures:	
Day 9	Seizure Yes / No	If Yes, number of seizures:	
Day 10	Seizure Yes / No	If Yes, number of seizures:	
Day 11	Seizure Yes / No	If Yes, number of seizures:	
Day 12	Seizure Yes / No	If Yes, number of seizures:	
Day 13	Seizure Yes / No	If Yes, number of seizures:	
Day 14	Seizure Yes / No	If Yes, number of seizures:	
Day 15	Seizure Yes / No	If Yes, number of seizures:	
Day 16	Seizure Yes / No	If Yes, number of seizures:	
Day 17	Seizure Yes / No	If Yes, number of seizures:	
Day 18	Seizure Yes / No	If Yes, number of seizures:	
Day 19	Seizure Yes / No	If Yes, number of seizures:	
Day 20	Seizure Yes / No	If Yes, number of seizures:	
Day 21	Seizure Yes / No	If Yes, number of seizures:	
Day 22	Seizure Yes / No	If Yes, number of seizures:	
Day 23	Seizure Yes / No	If Yes, number of seizures:	
Day 24	Seizure Yes / No	If Yes, number of seizures:	
Day 25	Seizure Yes / No	If Yes, number of seizures:	
Day 26	Seizure Yes / No	If Yes, number of seizures:	
Day 27	Seizure Yes / No	If Yes, number of seizures:	
Day 28	Seizure Yes / No	If Yes, number of seizures:	
Day 29	Seizure Yes / No	If Yes, number of seizures:	
Day 30	Seizure Yes / No	If Yes, number of seizures:	

5.2.4 Statistical analysis

Sample size calculations for this study were difficult as there is no previous evidence, either in the experimental or clinical sphere, of metformin use in tuberous sclerosis that could inform the investigators of likely effect size. However, at the beginning of the study it was calculated that 36 patients would be needed in each arm to achieve a statistical power of 90% to detect a mean reduction in renal tumour volume of 20% at a significance level of 5%.

All the outcome variables were continuous. Initial comparisons between the active treatment group and placebo for primary and secondary outcomes were made with using either t-tests or Wilcoxon-Mann-Whitney (WMW) tests depending upon whether the data was normally or non-normally distributed. One-sided alternativess were used as the study hypothesis was that metformin based upon its documented action on the mTOR pathway would result in reduction of hamartoma volume compared to placebo. We tested assumptions of normality using the Shapiro-Wilk's test and homogeneity of variances with Bartlett's test. Statistical analyses were done with Stata IC, version 11.2, and R, version 3.4.2.

The trial is registered with The International Standard Randomised Controlled Trial Number (ISRCTN), number 92545532, and the European Union Drug Regulating Authorities Clinical Trials (EUDRACT) number 2011-001319-30

5.2.5 Funding

This study was funded by the National Institute for Health and Research (NIHR) through the the Research for Patient Benefit Programme (RfPB). The funding application was peer reviewed by the NIHR.

5.2.6 Results

Between 1st November 2012 and 30th September 2015 72 patients were assessed for eligibility, of whom 55 met the inclusion criteria and were randomly assigned (see Figure 1). 28 were allocated to metformin therapy and 27 to placebo. Three patients allocated to placebo and one allocated to metformin did not start therapy for social reasons, leaving 27 patients who started metformin therapy and 24 who started on placebo. All the patients who began treatment completed the 12-month treatment period and therefore 51 patients were analysed for primary and secondary outcomes.

The demographic details of the participating cohort are shown in Table 1. There were no clinically important differences observed between treatment groups with regard to baseline characteristics.

Table 5.5.4: Baseline patient demographic and disease characteristics

	Metformin (n=27)	Placebo (n=24)
Median age	30 year	26 year
Age	Number of patients	
10-20	9 (33%)	8 (33%)
>20-30	6 (22%)	6 (25%)
>30-40	7 (25.9%)	7 (29%)
>40-65	5 (18.5)	3 (12.5%)
Gender		
Men	16 (59%)	9 (37.5%)
Women	11 (40.7%)	15 (62.5%)
Presence of learning disabilities	18 (66%)	16 (66%)
Presence of SEGA	14 (51%)	13 (54%)
Mean diameter angiomyolipoma lesion		
≥8 cm	4 (14.8%)	4 (16.6%)
≥4 cm and <8 cm	12 (44.4%)	7 (29%)
≥3 cm and <4 cm	4 (14.8%)	1 (4%)
<3 cm	7 (25.9%)	12 (50%)
Unilateral angiomyolipoma	5 (18.5%)	5 (20.8%)
Number of angiomyolipoma lesions	89	78

Safety and tolerability

Two patients withdrew from the study before starting the trial drug. Three serious adverse events occurred that reflected the underlying disease. Two were AML haemorrhages. They were both in the metformin group. One of whom bled when he was off metformin, during the last 6 months of the trial. The other patient bled whilst on metformin treatment. Both patients continued the trial as it was believed that this complication was not due to the trial drug. One patient had worsening seizures requiring hospitalization. This patient continued the trial as worsening seizures is expected in this condition. This patient was on metformin. These three events were classed as serious adverse events because the patients required hospital admission. Table 2 shows adverse events in both groups.

In all the patients, liver function, and kidney function and blood glucose remained stable during the trial. One patient's creatinine level, which was 104 mmol/L at baseline, rose to 114 mmol/L after starting metformin and then came down to 106 mmol/L whilst on metformin. This patient completed the trial uneventfully. Table 6 shows adverse events in both groups.

The mean weight difference from baseline to 12 months was 2.5 kilogram for the placebo group, whereas the mean weight difference for the metformin group was -0.7 kilograms.

Table 5.5.5: shows adverse events in both groups

Placebo	**Metformin**
Backache (n=1)	Diarrhea (n=2)
Dental infection (n=1)	Food poisoning (n=1)
Headache (n=1)	Fall (n=1)
	Worsening Seizures (n=1)
	Gastric upset (n=1)
	Depression (n=1)
	UTI (n=1)
	AML bleeding (n=2)
	Hospital admission due to seizures (n=1)

Renal Angiomyolipomas:

All the patients in the trial had at least one angiomyolipoma ≥ one centimetre in diameter. The distribution of the percentage volume changes in participants of the trial on both metformin and placebo is shown in Figure 2 and the individual responses to treatment are depicted in the waterfall plots in Figure 3. The data were not normally distributed and therefore the difference in treatment effect was analysed using non-parametric statistics. The median percentage change in AML volume was +7.6% (IQR -1.8% to +42.6%) for the placebo group and +8.9% (IQR 1.3% to 19.5%) for the metformin group (WMW test, U=355, p = 0.28).

Figure 5.4.3: Distribution of percentage volume changes in AMLs on placebo and metformin

0 = Placebo 1 = Metformin

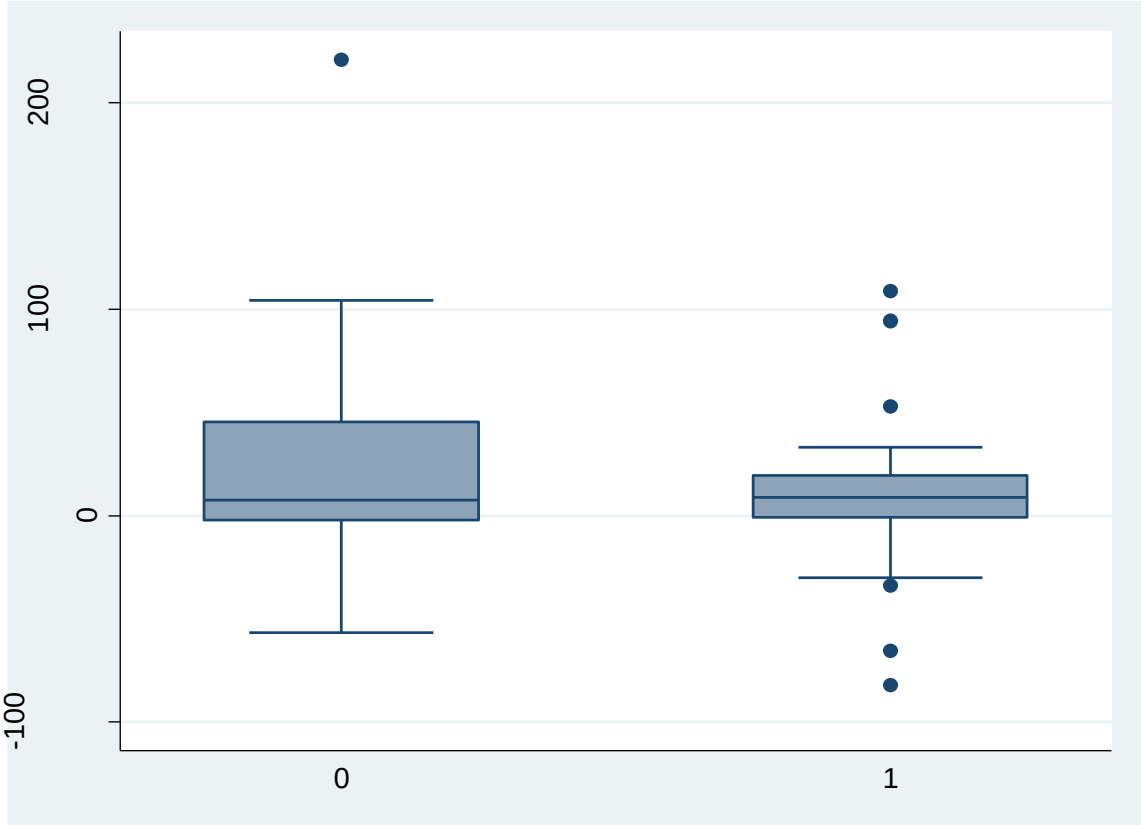


Figure 5.4.4: Waterfall Plot: AML Volume change on Metformin

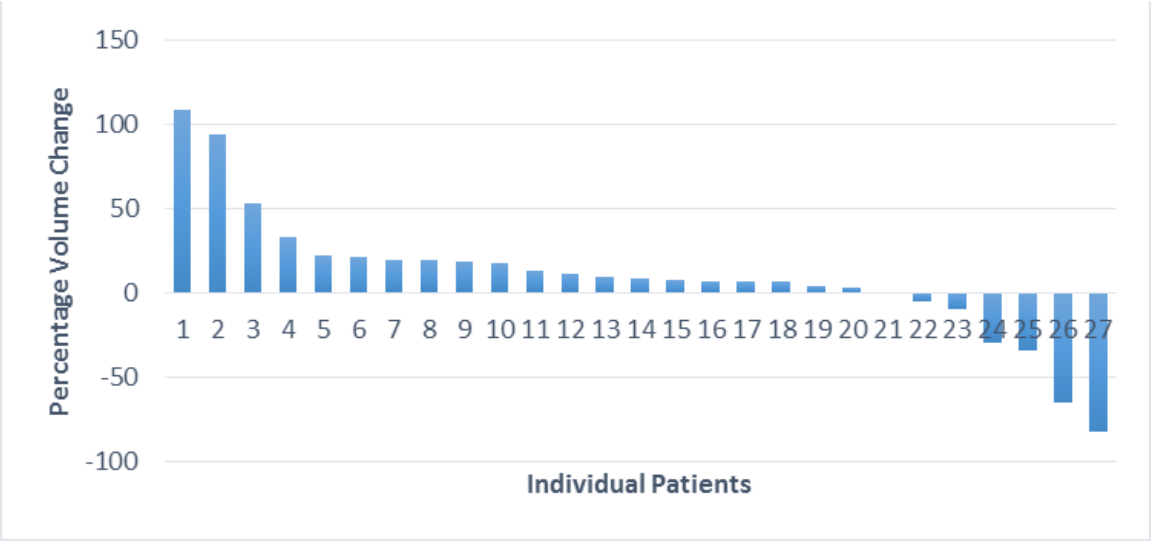
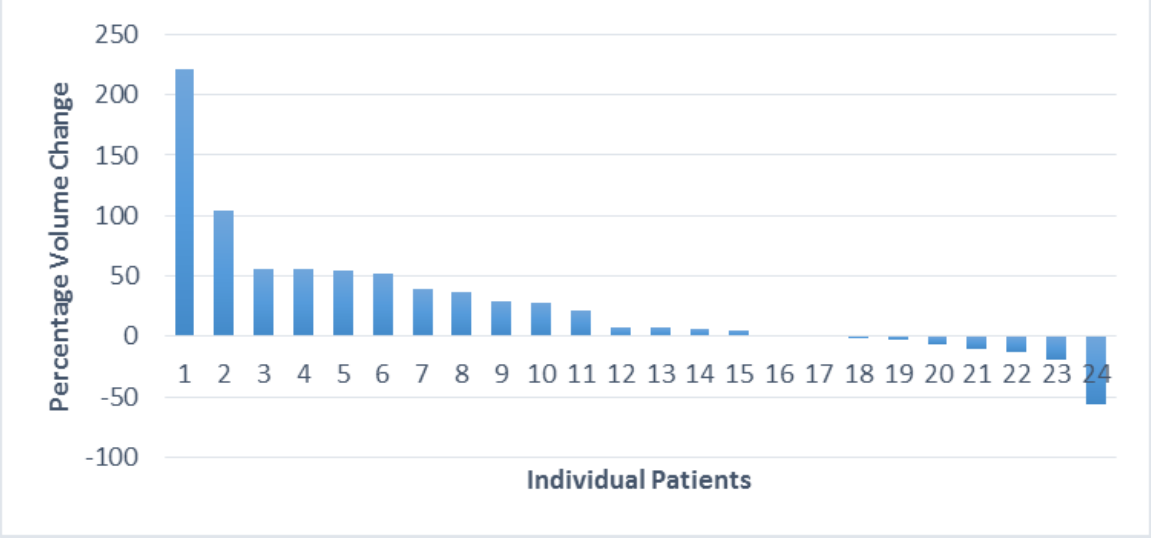


Figure 5.4.5: Waterfall Plot: AML Volume change on Placebo



Sub-ependymal Giant Cell Astrocytomas:

Twenty-seven patients in the trial had at least one subependymal giant cell astrocytoma. 13 were randomised to placebo and 14 were randomised to metformin. The distribution of the percentage volume changes in SEGAs in participants of the trial on both metformin and placebo is shown in Figure 4 and the individual responses to treatment are depicted in the waterfall plots in Figure 5. The data were not normally distributed and therefore the difference in treatment effect was analysed using non-parametric statistics. The median percentage change in SEGA volume was +3.0% (IQR -22.8% to +27.7%) for the placebo group and -20.8% (IQR -47.1% to -5.0%) for the metformin group (WMW test, U=130, p = 0.03). On a priori grounds we know that age is associated with risk of growth of SEGAs. SEGAs are known to grow in the first three decades of life and then become more quiescent such that current screening recommendations suggest screening only in the first three decades of life. In a retrospective analysis we sub-divided patients with SEGA into those who were either greater (n = 14) or less than (n = 13) 30 years of age in order to see whether metformin was more effective on SEGAs in younger patients because these were the lesions that are more biologically and clinically active. In those patients less than thirty years, the median percentage change in SEGA volume was + 11.4 % (IQR -22.8% to +162.4%) for the placebo group and – 28.0% (IQR -61.1% to -11.4%) for the metformin group (WMW test, p = 0.03) In those patients greater than thirty years, the median percentage change in SEGA volume was + 3.0% (IQR – 39.7% to + 14.4%) for the placebo group and – 10.3% (IQR – 49.9% to + 19.8%) for the metformin group (WMW test, p = 0.24)

Figure 5.4.6: Distribution of percentage volume changes in SEGAs on placebo and metformin

0 = Placebo 1 = Metformin

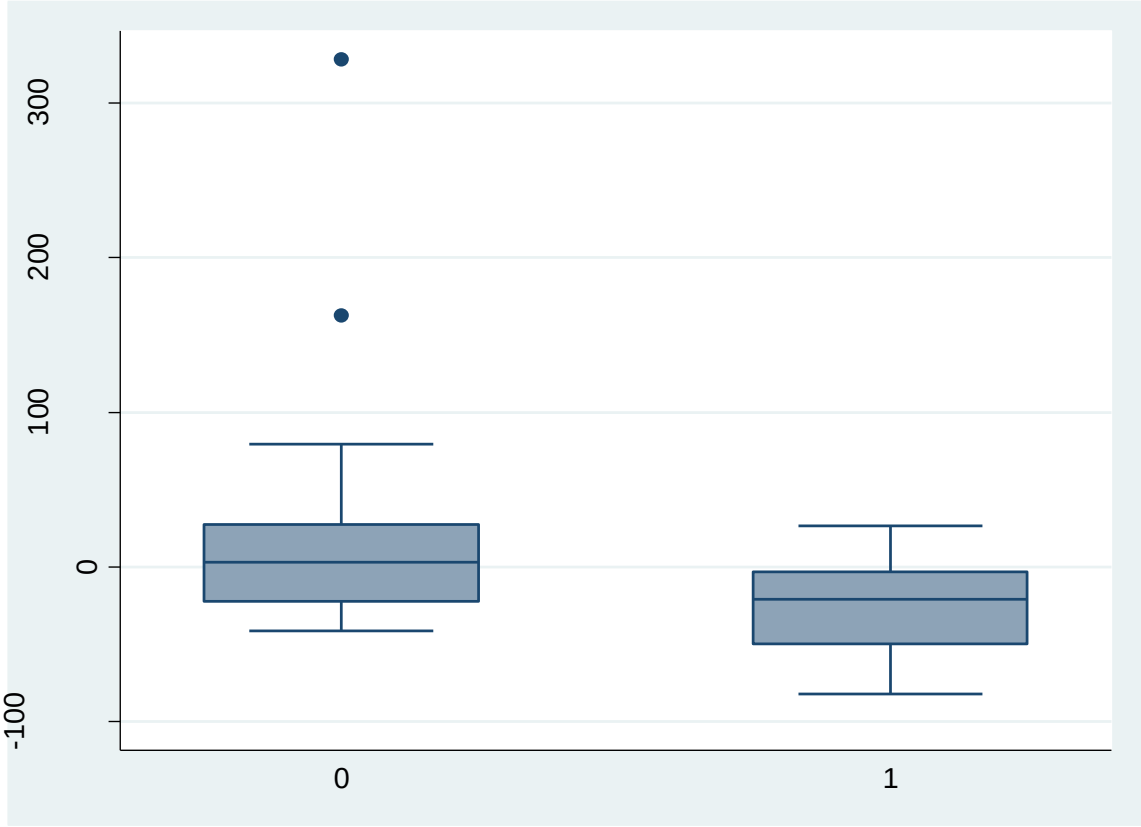


Figure 5.4.7: Waterfall Plot: SEGA Volume Change on Metformin

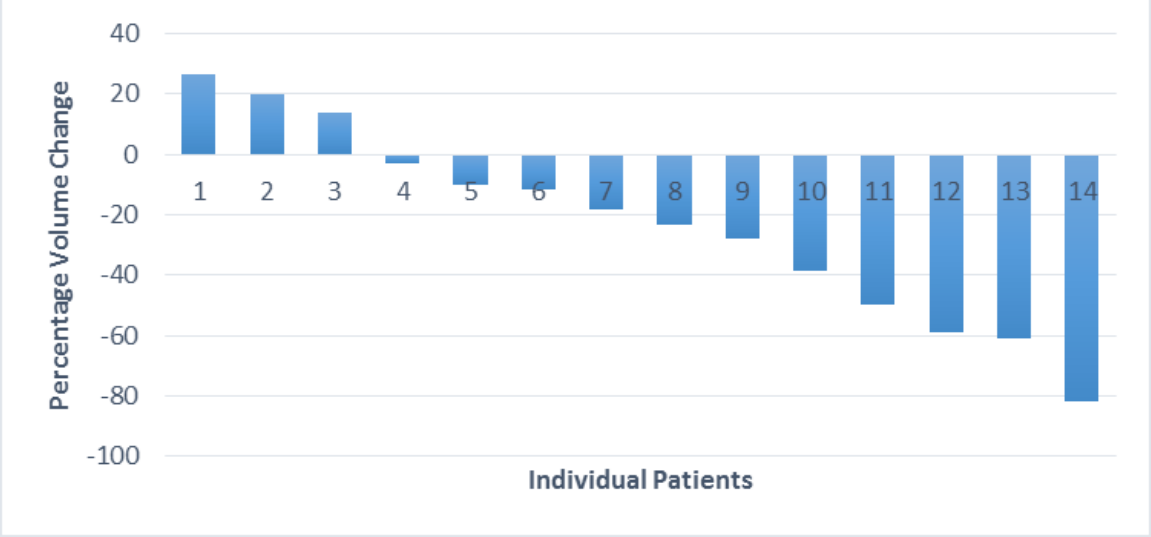
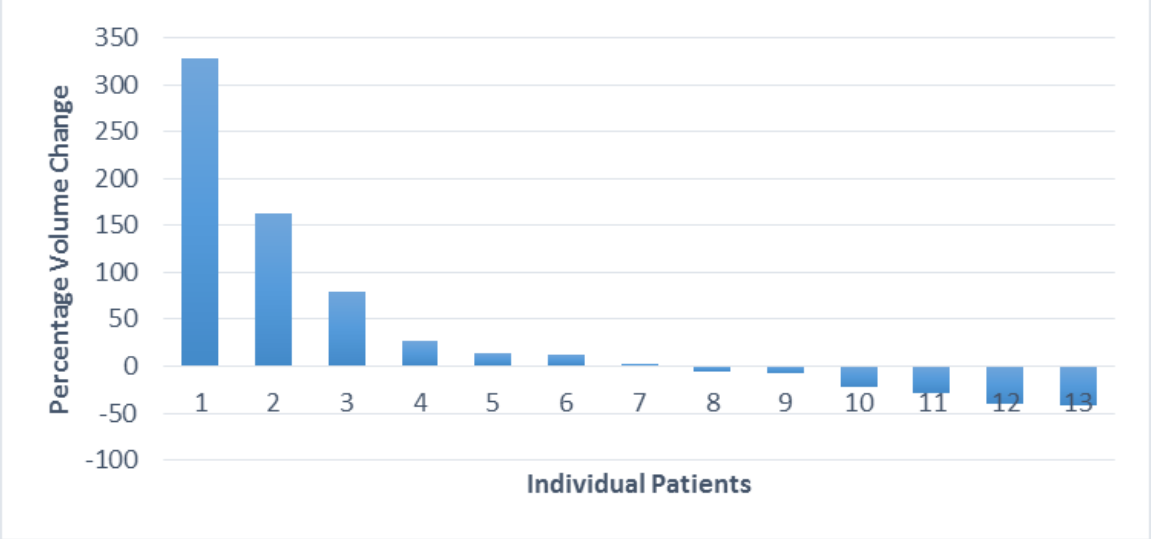


Figure 5.4.8 Waterfall Plot: SEGA Volume Change on Placebo



Epilepsy:

Twenty-nine patients had active epilepsy, of whom 14 were on placebo and 15 on metformin. Eight patients (3 on metformin and 5 on placebo) failed to complete the seizure diary and therefore data on seizure frequency was available in 21 patients. A mean reduction of 43.7% from baseline in seizures was observed in the metformin group and 3.1% in the placebo group, with a difference in response of 40.6% (95% CI -3.1% to +84.2%, t =1.95, p = 0.03). Nine out of twelve patients on metformin had a reduction in seizure frequency versus three out of nine patients reporting a reduction in seizure frequency on placebo. Three patients in the metformin group became seizure free at the 12-month assessments versus zero patients in the placebo group becoming seizure-free.

Figure 5.4.9: shows number of seizures at baseline and 12 months for those patients on placebo

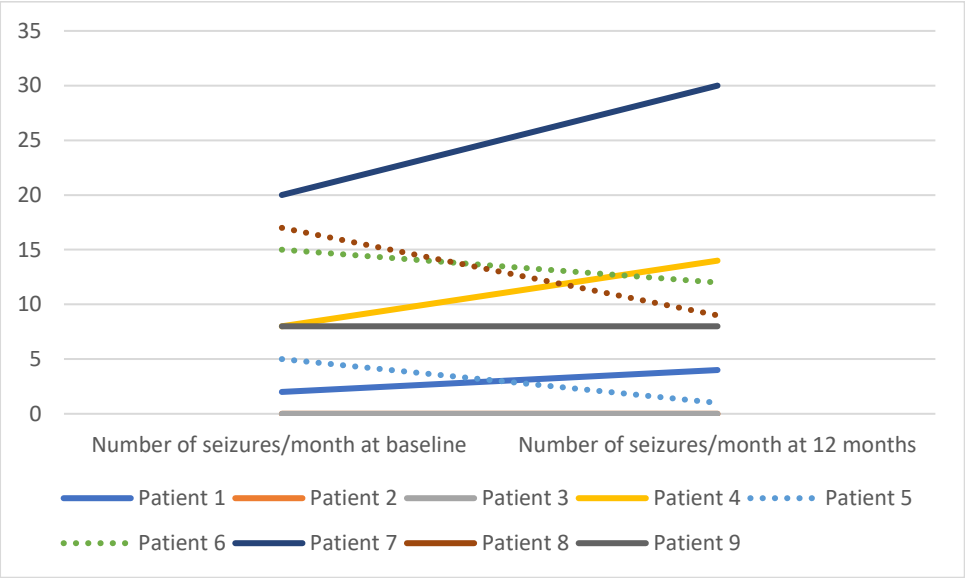
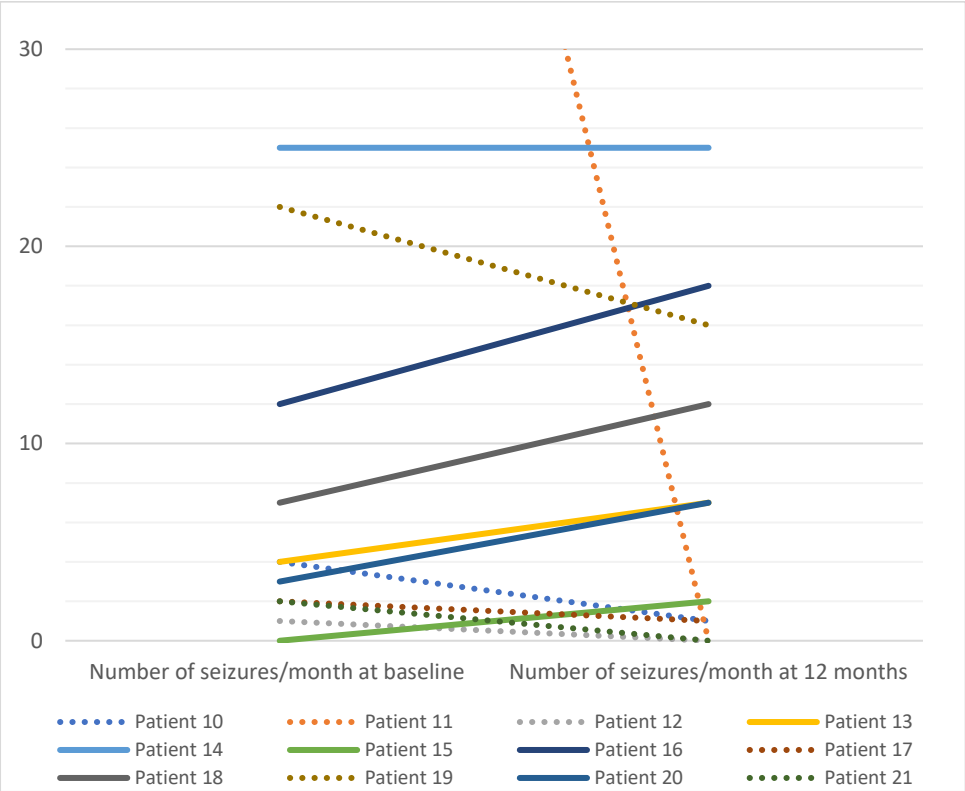


Figure 5.4.10: shows number of seizures at baseline and 12 months for those patients on metformin



Quality of life (QOL)

The mean SF36 mental health summary scores for the placebo group deteriorated from 49 at baseline to 46 at 12 months. Whilst for the metformin group, the scores were 47 at baseline and 51 at 12 months.

The mean SF36 physical health summary scores for the placebo group was 44 at baseline and at 46 12 months. Whilst for the metformin group, the scores were 55 at baseline and 55 at 12 months. The higher the score, the better reported quality of life.

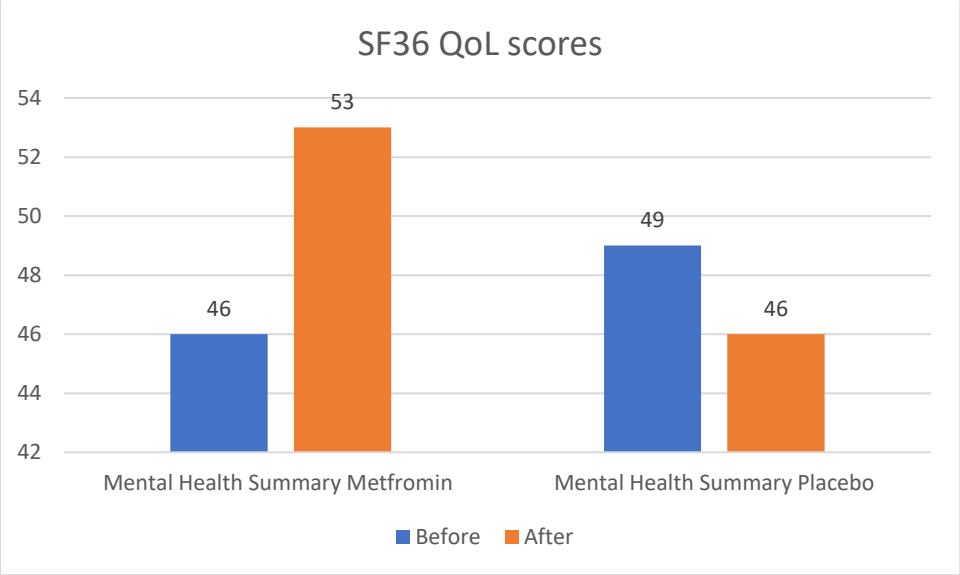


Figure 5.4.11: shows the median SF36 scores for adults on placebo and metformin from baseline to 12 months.

The total self-mean Peds QL scores for the placebo group reduced from 79 to 71, whilst for the metformin group it rose from baseline to 12 months from 76 to 79. The mean score for psychosocial domain for the placebo group reduced from 74 to 67 and increased from 74 to 75 for the metformin group. The physical domain for the placebo group reduced from 90 to 79 whilst the physical domain in the metformin group increased from 78 to 89.

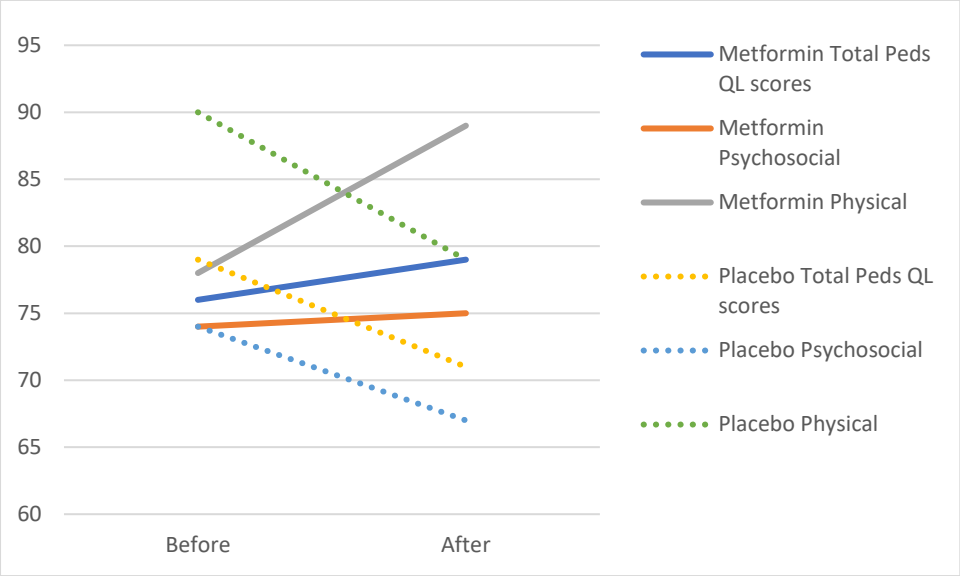


Figure 5.4.12: shows the mean PedsQL scores for children on placebo and metformin from baseline to 12 months.

Facial Angiofibromas:

Nineteen patients had facial angiofibromas in the placebo group and 23 in the metformin group. The mean PGA (Physician Global Assessment) score for facial angiofibroma at 12 months for the placebo group was 3.4 (95% CI 3.0 to 3.9) and 3.1 (95% CI 2.5 to 3.6) for the metformin group. A lower score indicates greater improvement but there was no meaningful difference between the two treatment groups. Two out of 23 patients/carers/parents reported improvement on metformin. One out of 19 reported improvement on placebo. There were no reports of worsening rash in either group.

Table 5.5.6: shows Physician Global Assessment (PGA) scores

PGA scores (255, 256)	
Completely clear	0
Almost clear	1
Marked improvement	2
Moderate improvement	3
Slight improvement	4
No change	5
Worse	6

Ungual Fibromas:

Thirty-eight patients had ungual fibromas, 18 were on placebo and 20 on metformin. The mean PGA scores at 12 months were 4.4 (95% CI 4.1 to 4.8) for the placebo group and 4.2 (95% CI 3.7 to 4.6) for the metformin group, indicating that there was no clinically meaningful difference between the two groups. None of the patients, carers or parents reported worsening or improving ungual fibromas in either group.

Vineland:

There was no appreciable change in Vineland scores over the course of the trial in either treatment group. The total mean adaptive behaviour scores for the placebo group at baseline was 53.5 (95% CI 38.4 to 68.6), and 54.2 (95% CI 37.4 to 71.0) at 12 months. The total mean adaptive behaviour scores for the metformin group was 52.7 (95% CI 36.4 to 69.0) at baseline, and 49.5 (95% CI 34.5 to 64.4) at 12 months.

5.2.7 Discussion

This randomised, double-blind, parallel group, placebo-controlled trial of metformin is the first to investigate the safety and efficacy of metformin in children and adults with TSC. This study showed that metformin was safe and well tolerated in this trial. Metformin did not reduce AML volume over the course of the trial. However, patients on metformin had a reduction in SEGA volume compared with placebo and also had a reduction in epileptic seizure frequency. There was no significant difference between the treatments with respect to the other secondary outcomes. The serious adverse events that occurred during the trial were apparently unrelated to the trial medication.

There is now an extensive literature describing the beneficial effects of metformin on a variety of different cancers.(209, 213) The beneficial effect is thought to be mainly due to metformin's inhibitory effect on the mechanistic target of rapamycin (mTOR) signalling pathway via activation of adenosine monophosphate-activated protein kinase (AMPK). (211) We formulated the hypothesis that treatment with metformin would result in reduction in size of renal angiomyolipomas in tuberous sclerosis patients. We chose to look at reduction in volume of angiomyolipomas as our primary outcome because they are one of the most prevalent hamartomas in TSC, they are associated with significant morbidity and mortality,(93) and previous trials of mTOR inhibitors such as rapamycin and everolimus have shown an effect on these lesions.(52) This trial did not show metformin to have a significant effect on AMLs in this patient population. One possible explanation for this negative result is that metformin simply does not have a clinically significant inhibitory effect on the mTOR pathway in TSC patients. Previous studies in a mouse model of TSC did not demonstrate any therapeutic effect of metformin in reducing the size of renal

cystadenomas that form in this model after loss of expression of the TSC2 gene. The authors speculate that the complete absence of TSC2 in the renal cystadenoma lesions in this model leads to strong activation of mTORC1 due to high levels of RHEB-GTP, which makes the lesions resistant to the lesser inhibitor effects of metformin.(238) However, the effect of metformin on SEGAs seen in this study argues that metformin may indeed have a clinically significant effect on some of the hamartomas in TSC. It may be that the dose of metformin used in this study is not adequate to produce a significant inhibitory effect in the renal lesions. The dose chosen for this study had no other rationale other than it was the dose used and tolerated in type 2 diabetes patients. Further studies using different dosing regimes may well be justified.

The effect of metformin in reducing the volume of SEGAs in TSC patients in this study is striking. SEGAs are another important cause of morbidity, and occasional mortality, in TSC patients. They are hamartomas that grow at the foramen of Monro and can cause obstruction to flow of cerebrospinal fluid and consequent hydrocephalus. They occur in approximately 10-20% of TSC patients. Metformin has been shown to have a beneficial effect in more aggressive brain tumours such as high grade gliomas. (257) Metformin effectively crosses the blood-brain barrier and is distributed in multiple brain regions after oral dosing. (166) The apparent effect of metformin in reducing SEGA volume in the patients in this study is clinically meaningful and is potentially important for the future treatment of TSC patients. It is certainly a finding that needs replication and further research. SEGAs are known to grow in the first three decades of life and then become more quiescent such that current screening recommendations suggest screening only up to the age of 25. (258) In a post-hoc analysis we subdivided the

patients in this study into those less than 30 years and therefore at an age when SEGA growth would be most likely and those greater than 30 years who might be less likely to have active lesions. The effect of metformin on the SEGAs of those patients less than 30 years was more marked than in the older patients.

There is emerging evidence from animal work that metformin may have an anti-epileptic and anti-epileptogenic effect. Metformin may have an anti-epileptic effect via a number of different pathways: inhibition of MTOR, activation of AMPK and prevention of oxidative damage induced by seizure activity. Metformin has been shown to suppress seizures in some rodent models and Bruegeman et al have recently demonstrated that metformin significantly suppressed seizure behaviour in a zebrafish PTZ-induced seizure model. (259-261) Metformin has not previously been studied in any clinical trial for human seizures or epilepsy. Consequently, the reduction of seizure activity seen in patients treated with metformin in this study is interesting. However, our study data were undermined by the failure to obtain seizure data in eight patients and we did not control for alterations in other anti-epileptic drugs. Nevertheless, the findings are sufficiently intriguing to justify further study of the possible anti-epileptic effects of metformin in tuberous sclerosis complex in an adequately powered clinical trial.

Everolimus and rapamycin are two mTOR inhibitors that have been shown to be effective at both reducing hamartoma size and improving refractory epilepsy in TSC. (55, 59, 97) However, these agents have significant side-effects. The most common side-effects of these agents are mouth ulceration and stomatitis but there is a risk of immunosuppression and severe infection. Two deaths in the recently reported EXIST-

3 study were attributed to treatment. (262) Metformin inhibits mTOR via a different mechanism than everolimus and rapamycin and it has a significantly more benign side-effect profile. At the dose used in this study, it does not appear to be as potent an mTOR inhibitor as rapamycin or everolimus and does not have such a dramatic effect on the hamartomas associated with TSC. However, given its better side-effect profile it may prove to be a more attractive option for TSC patients who may benefit from long-term mTOR inhibition to prevent the development of symptomatic SEGAs and to improve their long-term epilepsy control. It is also possible that it could be given in conjunction with other mTOR inhibitors possibly having a synergistic effect and possibly allowing use of lower doses of more toxic mTOR inhibitors and thus reducing the incidence of severe side-effects. These questions need to be explored in future research.

The obvious strengths of this trial are that treatment was randomised and that participants, families and carers, and investigators were blind to treatment allocation and therefore outcomes were assessed objectively and without bias. All the patients who started treatment in the trial were assessed for the primary outcome and all those who had SEGAs were assessed for SEGA growth at the end of the trial. There are, however, limitations with this study. The trial was small with just fifty-one participants. Although it was not possible to do a meaningful power calculation in this study because of a complete lack of data to support any assumptions re effect size, we had initially aimed to recruit 72 patients. Recruitment was difficult for several reasons. Firstly, the trial took place at the same time as heavily financed industry-sponsored trials of the mTOR inhibitor everolimus that were looking at the same population of patients. Secondly, the trial required annual MRI scans of brain and kidney and these required

general anaesthesia in learning disabled patients and sometimes carers were reluctant to submit individuals for general anaesthesia for a research study. Thirdly, the limited finance for the study prevented extension of the study to multiple sites beyond where the investigators worked. It is possible that the small numbers in the study has precluded seeing a significant difference between metformin and placebo with respect to angiomyolipoma growth, but there was certainly no evidence from this data to suggest metformin was causing angiomyolipomas to shrink. It is more likely that greater numbers in the study may have enabled us to see even more convincing effects of metformin on SEGA growth and on epilepsy control. Consequently we think there is a strong case for doing a larger study looking at these two outcomes in particular.

As with all clinical trials there is a possible issue with respect to external validity with this data. Sixty-eight per cent of the participants in this trial had learning disability compared with a rate of approximately 50% seen in epidemiological studies of tuberous sclerosis patients. However, the increased representation of learning disability patients in this trial may reflect the fact that more severe renal disease is more common in learning disabled TSC patients. (93) We should be aware, however, that the results from this trial may not map directly onto a general population of TSC patients.

One definite issue for the study is that seizure diary data was available for just 73% of the patients with epilepsy. The major reason for this was that some carers of individuals with learning disabilities in residential care homes were unable to complete the daily seizure diary due to short staffing issues. The results from this study suggest that metformin may have had a beneficial effect on seizure control but the

incompleteness of the dataset means that this result should be treated with caution. However, it strengthens the need to look at the effect of metformin on epilepsy in TSC in a larger trial in which epilepsy control is a primary outcome.

Metformin was well tolerated by children and adults in this study. Gastric upset is not uncommonly reported in diabetic patients. We encountered only one patient who complained of gastric upset and this settled quickly. This side effect was not commonly reported in our study probably because we did not use high doses and we gradually built the dose up over 6 months. The possible side effect of lactic acidosis with metformin use is still controversial. None of our patients developed lactic acidosis. In addition, none of the patients who were taking metformin developed hypoglycaemia. Metformin increases the sensitivity to insulin rather than insulin level, thus hypoglycaemia is not expected to occur due to metformin administration. [254]

Conclusions

Metformin is safe and well tolerated in children and adults with TSC. Metformin did not reduce AML size. Patients on metformin had a reduction in SEGA volume compared with placebo, which was more marked in younger (< 30 years of age) patients. Patients on metformin also appeared to have fewer epileptic seizures, although seizure diary data was complete for just 73% of patients. There may be a role for metformin in slowing or reversing growth of life-threatening hamartomas in TSC and for helping to control the frequency of epileptic seizures. Further study is justified.

5.3 Published papers and Dissemination

1) I presented “A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex” at the annual BPNA meeting. The abstract was published.

   S Amin, AA Mallick, H Edwards, A Lux, Marcus Likeman, M Laugharne, F O’Callaghan. A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex. Dev Med Child Neurol 2018.

2) I presented “A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex” at the Hospital Grand round at University Hospital Bristol.

3) I presented “A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex” at the EPNS (European Paediatric Neurology Society) research meeting.

4) I presented “A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex” at the regional neuroscience meeting, Southmead Hospital.

5) Accepted for oral presentation at the International child Neurology Conference. “A randomized, double-blind, parallel group, placebo-controlled trial of metformin in tuberous sclerosis complex”.

6) This paper “The Metformin in Tuberous Sclerosis (MiTS) Study: a randomised double-blind placebo controlled trial” has been accepted for publication by the eClinical Medicine published by The Lancet.

7) I published “The journey of metformin from glycaemic control to mTOR inhibition and the suppression of tumour growth” in a peer reviewed journal. British Journal of Clinical pharmacology

   Amin S, Lux A, O'Callaghan F. The journey of metformin from glycaemic control to mTOR inhibition and the suppression of tumour growth. Br J Clin Pharmacol. 2018 Oct 5.

6. Conclusion

For many patients TSC is a complex and severe condition which can have poor outcomes, especially for those individuals who have multiorgan involvement or have little or no access to high cost drugs and interventions. I am hopeful that the results of my project will have a significant impact on the natural history of this condition. I am hopeful that the results and subsequent new treatment option will modify the severity of this condition for many individuals in the world, reducing morbidity. Hopefully less people will lose their lives as a result of life-threatening complications of TSC.

The morbidity and morbidity study of chapter two has highlighted important points in relation to TSC care and has already made an impact on the way this condition is being managed. Policy makers and funding authorities are already citing this paper when investments are made, to reduce mortality in this cohort. NHS England referenced this paper when the decision was made to licence and fund everolimus in TSC to reduce mortality in TSC. This paper was rated amongst the top 10 of most frequently cited papers in Developmental Medicine and Child Neurology.

Because of its complexity, multiorgan involvement, lack of strong evidence for management and lack of resources, there was a need to unify the management of TSC in the UK. We are hopeful that the guidelines produced from this project will change the way TSC patients are manged. There will be challenges for some clinicians in implementing all aspects of these guidelines. We know that some people may not be able to offer regular MRI scans to their patients as per these guidelines, but this does not mean we should shy away from best practice. This is a pragmatic and safe guideline. It may lead to some medicolegal implications. I know that there are some

past missed SEGA cases which have resulted in life-long permanent disabilities for some individuals. We are hopeful that these guidelines will prevent these catastrophic complications in the future. TSA has created a patient friendly version of the guideline to be circulated amongst the TSC community. They have also funded open access to the journal, so that clinicians will be able to access the guideline easily. I am in the process of conducting a national audit to review whether or not this guideline has been implemented around the UK.

The quality of life study has highlighted the burden of TSC on patients. The study highlighted the impact of TSC on psychosocial domains. It clear that a lot of patients in the UK do not have access to appropriate services to alleviate the impact of mental health and psychological issues. We showed that the psychosocial burden is significant, and therefore, it is important that all patients are offered a neuropsychology assessment as early as possible. Following this study, I secured funding of approximately £100000 to conduct a study to find the most appropriate therapy for TSC related mental health issues. I will be conducting a randomised controlled trial of Acceptance and Commitment therapy in TSC. Acceptance and commitment therapy (ACT) is an evidence-based psychological therapy that has been successfully used to improve physical and mental health among children and adults with chronic conditions. It is a “third wave” cognitive behavioural therapy that encourages openness to and awareness of the present moment in order to help participants maintain behaviours consistent with their life goals. ACT fosters engagement with, rather than avoidance of, painful experiences to move towards acceptance of unchangeable difficulties alongside building a rich and meaningful life despite the presence of ongoing difficulties. This gives ACT strong face validity for application to TSC patients where

there can be permanent cognitive impairment and unavoidable ongoing physical symptoms and functional limitations.

The discovery of the mTOR pathway in the last couple of decades has opened up a stream of new opportunities for targeted therapies. I investigated the effect of mTOR inhibition, delivered in novel ways, on TSC related complications. Firstly, I studied the safety and effectiveness of using topical rapamycin in patients with facial angiofibromas. The publication of the results of this study has made this new therapy available to many children and adults with TSC. The study showed that this treatment can have a significant impact on patient's quality of life. Facial angiofibromas can have an immense psychological effect, especially in individuals with normal intellect. The study further highlighted the need for a larger study to determine optimal dosing regimen and strength of topical sirolimus for facial angiofibromas in TSC. I am in the process of setting up a study "SOFA – Sirolimus Ointment for Facial Angiofibroma". We plan to recruit 100 participants, fifty adults (18 years and over) and fifty children (< 18 years). We will be using 3 different sirolimus strengths 0.1%, 0.5% and 1%. This is a randomised placebo controlled and follow up (Phase 3) study of topical sirolimus treatment for facial angiofibromas in patients with TSC; to investigate efficacy improving the rash, quality of life, and safety.

Finally, I conducted a multi-centre double blind randomised placebo controlled trial of metformin in patients with TSC. Specifically, I investigated the safety and effectiveness of metformin on TSC-related lesions (renal angiomyolipomas, and cerebral subependymal giant cell astrocytomas), epilepsy, quality of life, and cognition. Metformin is a drug that potentially offers the benefit of mTOR inhibition without the

side effect and cost profile of other mTOR inhibitors. Both everolimus and rapamycin are immunosuppressant drugs and can cause significant side effects, however metformin has a very benign side effect profile. Treatment with everolimus or rapamycin is life-long, and costs thousands of pounds per year per patient. However metformin costs very little. Our study is the only treatment trial of metformin in TSC. It is the first trial to assess the safety and efficacy of metformin in individuals with TSC. This study suggests that treatment with metformin may be effective in reducing SEGA volume and frequency of epileptic seizures in children and adults with TSC. There may also be a role for metformin in slowing or reversing the growth of some life-threatening hamartomas in TSC and for reducing seizure frequency. Metformin appears to be a less potent inhibitor of mTOR compared to everolimus and rapamycin, but it has a more benign side-effect profile and is less expensive.

A larger study is required to assess the effect of metformin on kidney angiomyolipomas. Maybe a larger dose of metformin for a longer period is required to show an effect on kidney angiomyolipomas. There is no published information to suggest an optimal dose of metformin when the objective is to reduce tumour or hamartoma size. The dose used in this study was chosen because it was the dose that is used, shown to be effective, and tolerated when treating type 2 diabetes patients which is the current main indication for metformin use. We now know it is safe to use metformin in children and adults with TSC and therefore we should be able to trial a higher dose in this population. A recent animal study by Fang et al reported that metformin treatment effectively prevented aberrant kidney enlargement and cyst growth, inhibited inflammatory response, attenuated interstitial fibrosis, and protected

renal function in a mouse model of renal proximal tubule-specific TSC1 gene-knockout.

Another added advantage of Metformin is that it does not interact with the cytochrome P450 oxidase system. The potential safety issue with different drugs e.g. mTOR inhibitors and cannabidiol has recently been demonstrated with an inhibitory effect of cannabidiol on the metabolism of mTOR inhibitors. Franz et al reported that Cannabidiol resulted in increased serum levels of everolimus and/or sirolimus in patients with TSC. Obviously this is likely to cause significant side effects due to increasing the level of mTOR inhibitors. Metformin level is unlikely to be altered by Cannabidiol.

It would also be interesting to assess the effect of Metformin on TAND (TSC associated Neuropsychiatric Disorders). In this study we did look at adaptive behaviour using the Vineland Adaptive Behaviour Scale (VABS). This is a relatively crude instrument for looking at some aspects of TAND. For example, the socialisation domain and the maladaptive behaviour domain could theoretically give some insight into TAND. Interestingly there was no difference between metformin and placebo in overall Vineland scores or in scores in the socialisation and maladaptive domains. We accept that any future study should look at TAND in more detail and with more appropriate instruments.

We have not been able to establish if Metformin has a role in prevention of SEGA development. It may be possible to use Metformin as a prophylaxis in a long term study to investigate its effect on the development of infantile spasms, SEGA and other

TSC related lesions. We know that some patients with TSC may not develop these complications. However, as metformin is safe and cheap it is justifiable to trial it as prophylaxis. We also know that there is some evidence to suggest that Metformin may have a secondary benefit in cardiovascular disease, weight loss, mental health, cognitive abilities, anticancer and anti-ageing.

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