THE STATUS EPILEPTICUS IN CHILDHOOD OUTCOMES STUDY (STEPSOUT): LONG-TERM MORTALITY, NEUROLOGICAL, COGNITIVE AND NEUROIMAGING OUTCOMES

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DECLARATION

I, Suresh Pujar, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed.....

ABSTRACT

Childhood convulsive status epilepticus (CSE) is associated with considerable short-term mortality and morbidity, but data on long-term outcomes are uncertain. In addition, while the current evidence suggests that prolonged febrile seizures (PFS) can result in acute hippocampal injury, whether this evolves into mesial temporal sclerosis (MTS) and cause temporal lobe epilepsy (TLE) in the long-term is uncertain.

In the status epilepticus in childhood outcomes study (STEPSOUT), prospective follow-up of a previously identified unique population-based cohort was performed to investigate mortality, morbidity, and structural consequences within 10 years after childhood CSE.

Data from STEPSOUT suggest that aetiology is the main determinant of long-term outcomes following CSE in childhood, and CSE characteristics including seizure duration did not affect outcomes. Mortality within 10 years after childhood CSE was 46 times higher than expected in the reference population, but was predominantly seen in children with pre-existing neurological impairments at CSE presentation and the cause of death was usually not seizure related. Similarly, the long-term outcome was generally less favourable for children who had epilepsy and/or neurological impairments at CSE presentation, but was surprisingly good for those who were neurologically normal prior to their initial CSE and survived beyond 30 days after CSE.

Children with PFS had a generally favourable outcome, and only a small minority developed TLE and/or MTS. Neuroimaging data demonstrated little evidence of evolving MTS, but suggested that children with PFS may have a higher prevalence of hippocampal developmental malformations. In addition, widespread diffusion tensor imaging changes reflecting disruption of white matter microstructure were apparent in children with PFS.

The novel findings in this work are in some instances, contrary to the long-standing perceptions/beliefs about childhood CSE. The implications of these findings for our understanding of the natural history and long-term prognosis after childhood CSE will be further explored in the body of this thesis.

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Papers in preparation

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Pujar S, Martinos M, Neville B, Scott R, Chin R. Long-term seizure and neurological prognosis of childhood status epilepticus: A population-based study.

Published abstracts

Platform presentations

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Pujar S, Martinos M, Neville B, Clark C, Chin R, Scott R. Are prolonged febrile seizures associated with long-term changes in hippocampus? A quantitative hippocampal

volumetric analysis. *Epilepsy Currents* 2013;13(S1):1-492. (American Epilepsy Society Meeting, San Diego)

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Poster presentations

Pujar S, Martinos M, Neville B, Clark C, Chin R, Scott R. Does childhood convulsive status epilepticus (cse) result in long-term hippocampal damage? A quantitative hippocampal volumetric analysis. *Epilepsy Currents* 2014;14(S1):378. (American Epilepsy Society Meeting, Washington- Abstract selected for pressroom briefing/release and presentation at the Investigator's Workshop poster session)

Chin R, Pujar S, Krishnan A, Chong W, Neville B, Scott R. Prevalence of hippocampal malrotation (HIMAL) is not higher in children with prolonged febrile seizures (PFS) compared to age-matched controls: A population-based study. *Epilepsy Currents* 2014;14(S1):373. (American Epilepsy Society Meeting, Washington)

Pujar S, Seunarine K, Martinos M, Neville B, Scott R, Chin R, Clark C. White matter microstructural abnormalities are present 8 years after prolonged febrile seizures: Results from a population-based study. *Epilepsia* 2014, 55 (S2):240. (European Congress On Epileptology, Stockholm)

CHAPTER 1 INTRODUCTION

1.1 Rationale for the study

Convulsive status epilepticus (CSE) is the most common medical neurological emergency in childhood with an incidence of approximately 20/100,000 children/year (Raspall-Chaure et al., 2007; Chin et al., 2006). It is widely accepted that childhood CSE is associated with increased short-term and long-term mortality and morbidity (Raspall-Chaure et al., 2006). However, there is wide variability in reported adverse outcome rates, primarily due to differences in factors such as study design and methodological quality (population-/hospital-based, prospective/retrospective), CSE definition, definition of adverse outcomes and the length of follow-up (Raspall-Chaure et al., 2006). The generalisability of much existing literature in understanding the natural history and prognosis following childhood CSE is therefore uncertain.

The reported thesis work will advance the understanding of long-term outcomes following childhood CSE by providing population-based estimates of the incidence and prevalence of adverse neurological, cognitive, behavioural, and educational outcomes within 10 years after the episode. In addition, this study investigates the long-term hippocampal and extra-hippocampal structural consequences following childhood CSE using magnetic resonance imaging (MRI). Population-based estimates of adverse outcomes and identification of risk factors for adverse outcomes could help development of guidelines for optimising acute seizure management, early diagnosis and intervention for adverse outcomes following childhood CSE. Clarification of the nature and prevalence of adverse outcomes, and development of strategies for early diagnosis and intervention could potentially reduce the long-term negative impact of CSE in children (Holt and Mikati, 2011; Robinson et al., 2014).

1.2 Definition of status epilepticus

Status epilepticus (SE) has been recognised as a fatal condition since antiquity, and the description of the condition was found on a Babylonian cuneiform tablet dated around 600 to 700 BC (Shorvon, 1994). The phrase *état de mal*, the predecessor of the current term "status epilepticus" is believed to have evolved from the slang used by patients with epilepsy in the Parisian asylums. The first appearance of the expression "status epilepticus" was in Bazire's English translation of Trousseau's lectures in clinical medicine in 1868 (Shorvon, 1994).

The initial definitions described SE as a state of prolonged seizure activity, but did not specify seizure duration. Clark and Prout in 1903/4 defined true SE as "the maximum development of epilepsy, in which one paroxysm follows another so closely that the coma and exhaustion are continuous between seizures" (Neligan and Shorvon, 2009). During the Xth Marseille Colloquium held in 1962, Henri Gastaut proposed a clinical definition of SE as, "a term used whenever a seizure persists for a sufficient length of time or is repeated frequently enough to produce affixed or enduring epileptic condition." This definition was later included in the International Classification of Seizure Type published in 1969/70 (Gastaut, 1970). Although he later suggested a duration of 60 minutes for the diagnosis of SE, this was not specified in the definition.

The International League Against Epilepsy (ILAE) has over the past five decades revised the definition of SE several times but not provided a precise definition of the duration of SE (Gastaut, 1970; Engel, 2001; Berg et al., 2010; 1981). It was only in 2015 the ILAE Task Force on Classification of Status Epilepticus included seizure duration in the definition and proposed new definition of SE as "*a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms, which lead to abnormally prolonged seizures (after time point t1). It is a condition, which can have long-term consequences (after time point t2), including neuronal death, neuronal injury, and alteration of neuronal networks, depending on the type and duration of seizures*" (Trinka et al., 2015). The Task Force defined operational time point t1 as 5 minutes and time point t2 as 30 minutes for convulsive SE. Time point t1 and t2 for focal SE with impaired consciousness were defined as 10 minutes and 60 minutes respectively, but this is based on limited evidence.

The ambiguity of definition of SE has resulted in investigators using various time definitions for SE, from 60 minutes in earlier studies to 30 minutes and 15-20 minutes (Raspall-Chaure et al., 2007; Aicardi and Chevrie, 1970). Epidemiological data suggest that the underlying aetiology is a major determinant of seizure duration (Berg et al., 1999b; Maytal et al., 1989; Stroink et al., 2007; Sillanpaa and Shinnar, 2002; Metsäranta et al., 2004). Also, evidence from animal studies suggest progressive neuronal damage with longer seizure duration, due to a combination of excitotoxic injury and physiologic factors such as hypoxia, ischaemia and acidosis (Meldrum and Brierley, 1973; Meldrum and Horton, 1973; Wasterlain et al., 1993; Chen et al., 2007). Thus, the outcomes could potentially vary depending on the seizure duration and therefore the definition of SE should be taken into account when comparing outcome studies.

The most widely used definition of SE is, "a seizure lasting more than 30 minutes, or intermittent seizures lasting more than 30 minutes without a return of normal function in between" (Lowenstein, 1999). The 30-minute duration is meant to demarcate the transition point when SE becomes established and unlikely to terminate without treatment and also, when seizure–induced neuronal injury takes place (Lowenstein, 1999; DeLorenzo et al., 1999; Chen et al., 2007; Meldrum and Brierley, 1973). However, epidemiological data suggest that, the longer a seizure lasts, the less likely it is to stop or even to respond to antiepileptic drug therapy (Lowenstein, 1999; Shinnar et al., 2001a; DeLorenzo et al., 1999). The likelihood of a seizure stopping spontaneously substantially decreases after 5 minutes, and therefore it would be reasonable to initiate treatment after 5 minutes.

There is clearly a need for different definitions of SE tailored for different purposes (Raspall-Chaure et al., 2007; Lowenstein, 1999; Trinka et al., 2015). An operational definition (5-minute-duration) would be useful as a guide for treatment of prolonged seizures, and the commonly used textbook definition (30-minute-duration) for evaluating the incidence and outcomes of CSE in epidemiological studies and is the definition that will be adopted for this thesis.

1.3 Classification of SE

The ILAE Task Force on Classification of Status Epilepticus recently proposed SE to be categorised according to the following four axes: (1) semiology, (2) aetiology, (3) EEG correlates, and (4) age (Trinka et al., 2015). However, as acknowledged by the Task Force, this is not always possible, especially in epidemiological studies. Thus, status epilepticus could be broadly classified as convulsive (CSE) and non-convulsive (NCSE), and may be further classified based on seizure semiology and electrophysiological features. As aetiology of SE is an important determinant of outcome, an aetiological classification of SE could potentially be useful for prognosis.

1.3.1 Classification according to semiology

1.3.1.1 Convulsive and non-convulsive SE

CSE refers to a SE with prominent motor manifestations, whereas NCSE refers to continuous or near continuous generalized electrical seizure activity lasting for at least 30 min, but without prominent motor symptoms (Walker et al., 2005).

As NCSE is infrequent in children and requires electroencephalogram (EEG) for reliable diagnosis, epidemiological studies in children are mostly restricted to patients with CSE (Bauer and Trinka, 2010; Raspall-Chaure et al., 2007; Raspall-Chaure et al., 2006; Chin et al., 2006). In keeping with this, this thesis is restricted to the outcomes of CSE.

1.3.1.2 Focal or generalised seizure onset

- Focal- First clinical and electroencephalographic changes indicate origin of seizures from a discrete anatomic location in one hemisphere. Motor manifestations are unilateral.

- Primarily generalised- First clinical and electroencephalographic changes indicate initial involvement of both hemispheres. Motor manifestations are bilateral from the onset.

- Secondarily generalised- Seizures begin focally, but spread to other hemisphere. Motor manifestations begin unilaterally, and may later generalise.

1.3.1.3 Pattern of seizure activity

- Continuous- A single seizure lasting at least 30 minutes

- Intermittent- Two or more seizures without complete recovery of consciousness between them lasting at least 30 minutes

1.3.2 Aetiological classification

The aetiological classification used to classify CSE in our inception cohort is presented in Table 1.1 (Chin et al., 2006).

CSE category	Definition
Prolonged febrile seizure	CSE in a previously neurologically normal child aged between 6 months and 5 years during a febrile (temperature >38˚C) illness, and in the absence of defined central nervous system (CNS) infection.
Acute symptomatic	CSE in a previously neurologically normal child, within a week of an identified acute neurological insult including CNS infection, head trauma, metabolic derangements, drug-related effects, hypoxia or anoxia, cerebrovascular disease.
Remote symptomatic	CSE in the absence of an identified acute insult but with a history of a pre-existing CNS abnormality more than 1 week previously.
Acute on remote symptomatic	CSE that occurred within a week of an acute neurological insult or febrile illness and occurred in a child with a history of previous neurological abnormality, including epilepsy. This category included children with cerebral palsy with a febrile illness not of CNS origin, and children with obstructed ventriculoperitoneal shunts for hydrocephalus.
Idiopathic epilepsy related	CSE that is not symptomatic (see above) and occurred in children with a previous diagnosis of idiopathic epilepsy or when the episode of CSE is the second unprovoked seizure that has led to a diagnosis of idiopathic epilepsy.
Cryptogenic epilepsy related	CSE that is not symptomatic (see above) and occurred in children with a previous diagnosis of cryptogenic epilepsy or when the episode of CSE is the second unprovoked seizure that has led to a diagnosis of cryptogenic epilepsy.
Unclassified	CSE that cannot be classified into any other group.

Table 1.1 Aetiological classification of childhood CSE (Reproduced from Chin et al., 2004)

1.4 Epidemiology of CSE in children

1.4.1 Incidence

The incidence of CSE in children according to published population-based studies is presented in Table 1.2. The geographic differences in the reported incidence reflect differences in the ethnicity, socioeconomic status, prevalence of childhood infections and study methodology.

Region, Country	Number of cases	Incidence rate (per 100,000/year)
London, UK (Chin et al., 2006)	226	17-23
Kilifi, Kenya (Sadarangani et al., 2008)	388	35 (confirmed) 178 (confirmed plus probable)
Rochester, USA (Hesdorffer et al., 1998a)	69	24
Richmond, USA (DeLorenzo et al., 1996)	29	38
Okayama, Japan (Nishiyama et al., 2007)	106	37.1
French-speaking cantons, Switzerland (Coeytaux et al., 2000)	64	21
Ferrara, Italy (Govoni et al., 2008)	9	49.1

Table 1.2 Incidence of childhood SE from population-based studies

There is a bimodal distribution to the incidence of CSE with peaks in early childhood and in old age. In paediatric populations, the majority of episodes occur in children under the age of 4 with maximum incidence during infancy (Raspall-Chaure et al., 2007; Chin et al., 2006). In addition to age, ethnicity, genetic predisposition, and socioeconomic status play an important role in the incidence of SE in the population (Raspall-Chaure et al., 2007; Coeytaux et al., 2000; DeLorenzo et al., 1996; Chin et al., 2009).

About 10% of children with new-onset seizures/newly diagnosed epilepsy present with CSE (Berg et al., 1999b; Singh et al., 2010; Stroink et al., 2007; Sillanpaa and Shinnar, 2002). A further 10-20% will have at least one episode of CSE during the course of the disease, with most occurring in the first few years of epilepsy onset (Berg et al., 1999b; Berg et al., 2004a; Sillanpaa and Shinnar, 2002). The majority of children (62-88%) with first-ever episodes of CSE in population-based studies did not have prior epilepsy (Raspall-Chaure et al., 2007).

1.4.2 Aetiology

The aetiology of CSE varies across age groups and there is a strong correlation between age at the time of CSE and aetiology. Prolonged febrile seizures (PFS) and acute symptomatic CSE are the most common aetiologies in children younger than 2 years, whereas cryptogenic and remote symptomatic aetiologies are more common in older children (Raspall-Chaure et al., 2007; Chin et al., 2006; Shinnar et al., 1997).

PFS is the most common cause of CSE during childhood, accounting for approximately a third of all cases (Raspall-Chaure et al., 2007; Chin et al., 2006). It is however important to remember that about 20% of children presenting with first ever episode of CSE associated with fever may have acute CNS infection (bacterial meningitis or viral encephalitis) and the consequences of missing the diagnosis could be devastating (Chin et al., 2006; Chin et al., 2005). Each of the other aetiologies account for 15-20% of total, however, there are important geographic differences in the incidence (Table 1.2), and the aetiology of CSE (Figure 1.1) (Raspall-Chaure et al., 2007; Scott, 2010).

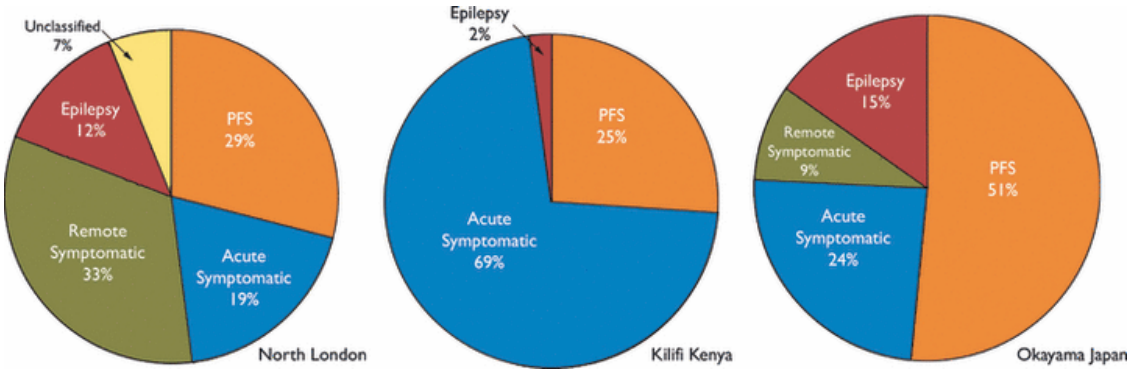


Figure 1.1 Distribution of aetiologies of childhood CSE in three geographically disparate population-based samples (Adapted from Scott, 2010)

That PFS is the most common cause of childhood CSE attracts special interest because of the long-standing hypothesis suggesting the causative role of PFS in hippocampal damage and later development of mesial temporal sclerosis (MTS), a common pathology in patients with temporal lobe epilepsy (TLE) (Patterson et al., 2014; Raspall-Chaure et al., 2006; Shukla and Prasad, 2012). Before discussing the potential mechanisms of seizure-induced hippocampal injury and epileptogenesis, an understanding of the

normal structure and function of the hippocampus, the pathology in MTS and the clinical features of TLE would be useful.

1.5 The hippocampus, MTS and TLE

1.5.1 Hippocampal anatomy

1.5.1.1 Shape and location

Hippocampus (from ancient Greek, *hippos* meaning "horse" and *kampos* meaning "sea monster"), so termed due to its appearance similar to "seahorse", is a component of the human limbic system (Figure 1.2). It is a complex anatomic structure located within the medial temporal lobe. The hippocampus is arc shaped with an enlarged anterior extremity and a narrow posterior extremity. It is 4 to 4.5 cm long, and divided into a transversely oriented anterior part (head), sagittally oriented middle part (body), and transversely oriented posterior part (tail) (Duvernoy, 2005).

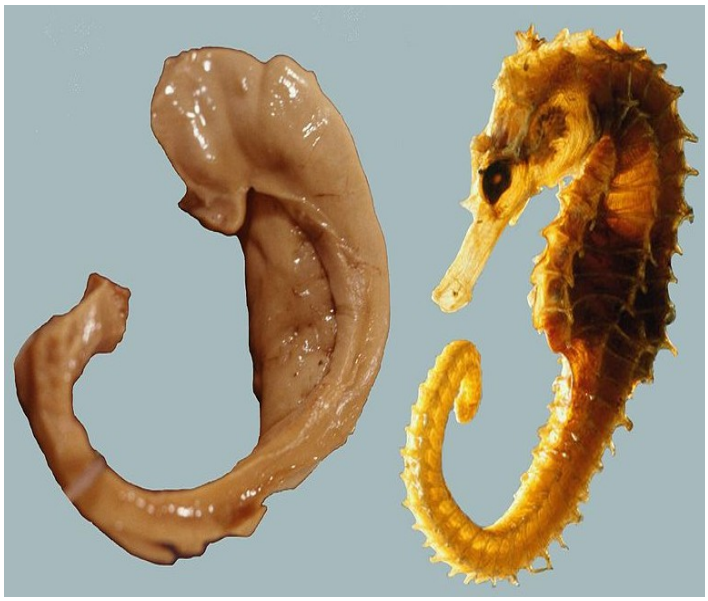


Figure 1.2 Gross appearance of hippocampus (left) resembling a seahorse (right)

1.5.1.2 Anatomical boundaries

The anterior end of the head of the hippocampus curves back medially to form the uncus, and the thickened anterior foot (pes hippocampi) is characterised by the internal and external digitations. The head of the hippocampus is separated from the amygdala

by the uncal recess of the temporal horn of the lateral ventricle anteriorly, and the alveus superiorly. The medial border of the head is formed by the ambient gyrus, parahippocampal gyrus, and cerebrospinal fluid in the transverse fissure. The white matter of the parahippocampal gyrus defines the inferior border, and the temporal horn of the lateral ventricle the lateral border of the head of hippocampus.

The body of hippocampus is bordered laterally and superiorly by the lateral ventricle, inferiorly by the parahippocampal gyrus and medially by the ambient cistern.

The body of hippocampus continues posteriorly as the tail and ends with the subsplenial gyrus. The medial, lateral, and the inferior borders are the same as for the body. The superior border of the tail is formed by the white matter of the fornix anteriorly, and the temporal horn of the lateral ventricle posteriorly.

1.5.1.3 Internal structure

The hippocampus is a bilaminar structure consisting of the cornu Ammonis (or hippocampus proper), rolled into the dentate gyrus (or fascia dentata) (Figure 1.3 & Figure 1.4).

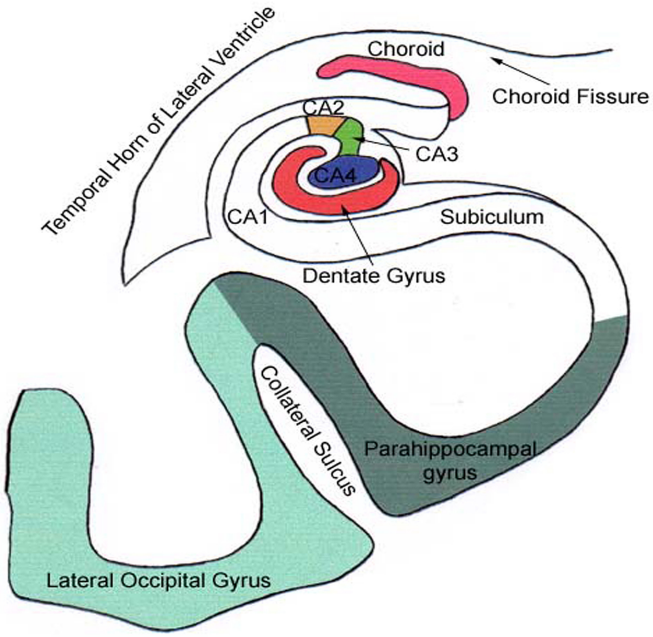


Figure 1.3 Anatomy of hippocampus and adjacent structures in mesial temporal lobe

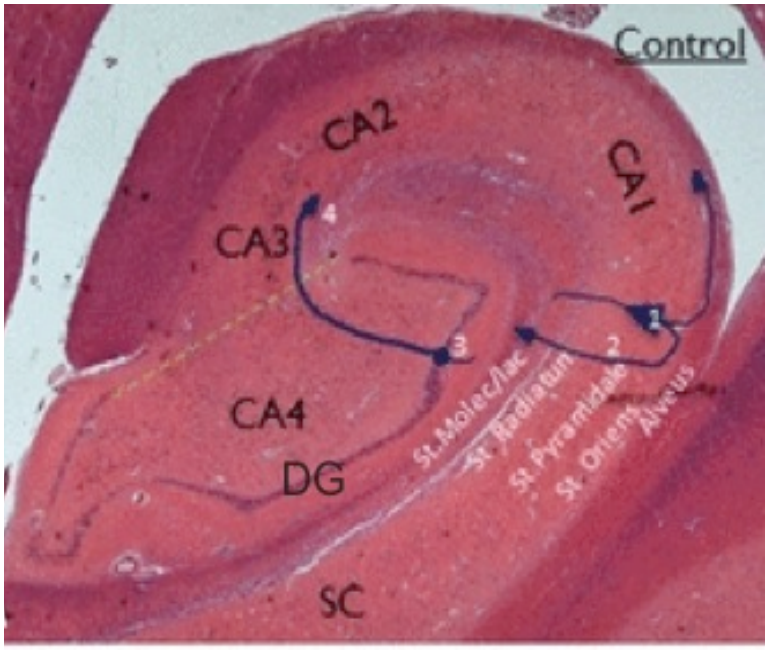


Figure 1.4 Haematoxylin & eosin section showing internal structure of a normal hippocampus. Hippocampal subfields (CA1 to CA4), subiculum (SC), dentate gyrus (DG) and the laminar layers of hippocampus are labelled. (1) indicates the position and orientation of a normal CA1 pyramidal cell with its apical dendrite extending through the deeper layers (2) and its outgoing axon (arrow) in the alveus, and (3) marks the position of a normal granule cell and its trajectory to CA3 in the mossy fibre pathway (4). (Adapted from Malmgren and Thom, 2012)

The cornu Ammonis (named after the curved ram's horns of Amun in Egyptian mythology) is a heterogenous structure consisting of six layers (Figure 1.4) (Duvernoy, 2005). The *alveus* contains the axons of the hippocampal and subicular neurons, the main efferent pathway, and then enters the fimbria. The *stratum oriens* is composed of scattered nerve cells (basket cells) and is crossed by the axons of the pyramidal neurons. The *stratum pyramidale* is the main layer of the cornu Ammonis and contains the typically triangular pyramidal neurons. The *stratum radiatum* consists mainly of apical dendrites from the pyramidal neurons that connect with Schaffer collaterals, fibres from septal nuclei, and commissural fibres. The *stratum lacunosum* is formed mainly of perforant fibres and Schaffer collaterals. The *stratum moleculare* contains few interneurons and arborisations of apical dendrites of pyramidal neurons, and blends with that of the dentate gyrus.

The cornu Ammonis is divided into four subfields (CA1 to CA4) based on the structure of the pyramidal cell layer (Figure 1.3 & Figure 1.4). CA1 is the largest subfield in humans, and is in continuity with the subiculum. It consists of typically triangular, small, and scattered pyramidal neurons. CA2 is narrower with densely packed large ovoid neurons. CA3 corresponds to the curve (or genu) of cornu Ammonis, with neurons like those in CA2, but less densely packed. CA3 is characterised by the presence of fine, non-myelinated fibres (the mossy fibres), which arise from the dentate gyrus. CA4 is situated within the concavity of the dentate gyrus, and comprises of scattered ovoid neurons. CA4 is characterised by the presence of intertwined large mossy myelinated fibres.

The dentate gyrus derives its name from the characteristic toothed appearance of *margo denticularis*, a narrow segment of dentate gyrus, on the temporal lobe surface. The 3-layer molecular structure of dentate gyrus is simpler than that of cornu Ammonis. The *stratum granulosum* is the main layer and contains the bodies of granule cells. The mossy axons of the granule cells traverse the polymorphic layer to CA4 and CA3. The thick *stratum moleculare* receives fibres from the perforant pathway in the external two thirds, and commissural and septal fibres occupy its inner third. The *polymorphic layer* connects the granular layer to CA4 and is crossed by the axons of granule cell neurons.

1.5.2 Hippocampal connections and functions

There are two main neuronal circuits within the hippocampus: the primitive *polysynaptic or the perforant pathway*, mainly involved in episodic and spatial memory, and the *direct pathway,* the most important in humans, mainly involved in semantic memory (Duvernoy, 2005).

The *polysynaptic pathway* is composed of a long neuronal chain involving the entorhinal cortex, the dentate gyrus, the cornu Ammonis, and the subiculum. As these structures form a functional unit, they are sometimes grouped together as the "hippocampal formation". The *polysynaptic pathway* originates in layer 2 of the entorhinal cortex, perforates the subiculum (hence the name *perforant pathway*) to reach the molecular layer of the dentate gyrus. The axons of the granule cells, the mossy fibres, synapse with the neurons in CA3 and CA4 (Figure 1.4). The axons of CA3 and CA4 emit the Schaffer collaterals before entering the alveus. The Schaffer collaterals synapse with the apical dendrites of CA1, the axons of which then enter the alveus and produce collaterals to the subiculum. The subiculum sends the response through fibres in the alveus and then the

fimbria. The outputs from the polysynaptic pathway follow the fimbria, fornix, the anterior thalamic nucleus (directly or through the mamillothalamic tract) and reach the cortex. The input from the cortex is received through fibres from the posterior parietal association cortex to the entorhinal area.

In the *direct pathway*, the fibres originate in layer 3 of the entorhinal cortex and directly reach neurons in CA1. The CA1 neurons then project onto the subiculum, the axons of which return to entorhinal cortex. The output from the direct pathway is believed to reach areas of the temporal cortex and prefrontal cortex, and the main input comes from the inferior temporal association cortex, through the perirhinal cortex.

Although the complete function of the hippocampus is yet to be understood, current evidence from human and animal studies suggests that hippocampus plays a key role in memory and learning, and in regulation of emotional behaviour (Eichenbaum, 2004; Squire, 2004; Squire et al., 2004; Fanselow and Dong, 2010). It is critical in formation and consolidation of *semantic memory* (memory of facts and concepts), *episodic memory* (permits conscious recollection of events and the relations between them), and *spatial memory* (recognition of spatial location). Structural or functional disruption of hippocampal network will result in impairment of memory dependent tasks. Hippocampus also plays a key role in regulation of emotional behaviour, and hippocampal abnormalities have been reported in psychiatric disorders such as post-traumatic stress disorder, anxiety disorder, and major depression (Pitman et al., 2012; Femenía et al., 2012).

1.5.3 The effect of CSE on hippocampal formation

Some hippocampal neurons are highly vulnerable to damage from CSE (e.g. CA1 and CA3 pyramidal cells and dentate hilar neurons), whereas others are relatively resistant (e.g. CA2 neurons and dentate gyrus granule cells) (Wasterlain et al., 1993; Scantlebury et al., 2007; Rajasekaran et al., 2010). The precise reason for the selective vulnerability is yet to be determined. It is hypothesized that CSE-induced neuronal injury activates a cascade of processes such as mossy fibre sprouting, neurogenesis, and gliosis resulting in aberrant network organisation, which is the basis for epileptogenesis and hippocampal network dysfunction (Rakhade and Jensen, 2009; Scantlebury et al., 2007; McClelland et al., 2011; Rajasekaran et al., 2010; Jansen et al., 2008).

1.5.4 Mesial temporal sclerosis (MTS) or hippocampal sclerosis (HS)

In his landmark paper published in 1880, Sommer for the first time described Ammon's horn (hippocampal) sclerosis in patients with epilepsy (Sommer, 1880). The association between HS and TLE was first described by Stauder in 1935, and Sano and Malamud in 1953 confirmed the relationship between HS and EEG evidence of seizures of TLE semiology (Sano and Malamud, 1953; Stauder, 1935). Surgical series in 1950s and 60s described the hippocampal pathology in patients with TLE and established HS as the most frequent pathology in TLE patients undergoing lobectomy (Margerison and Corsellis, 1966; Meyer et al., 1954; Cavanagh and Meyer, 1956). Several decades later, HS still represents the most common pathologic finding in adults with pharmaco-resistant TLE undergoing epilepsy surgery (Blümcke, 2009).

1.5.4.1 Pathology of MTS/HS

Although the terms MTS and HS are often used interchangeably in clinical literature, HS denotes pathology limited to the hippocampus, where as in MTS (or "HS plus") there is involvement of extrahippocampal mesial temporal lobe structures such as entorhinal cortex and amygdala in addition to HS (Blümcke et al., 2013).

The histopathological diagnosis of HS is based on the identification of pyramidal neuron loss and chronic fibrillary gliosis involving mainly CA1, CA4 and CA3 subfields as well as other hilar neurons (Figure 1.5) (Malmgren and Thom, 2012; Blümcke et al., 2013). Granule cell dispersion, mossy fiber sprouting, and alterations to interneurons may also be seen in some cases. In addition, extrahippocampal temporal lobe changes, subtle focal cortical dysplasias (FCDs) or microdysgenesis and a second lesion in the temporal lobe, such as a low grade tumor (so called "dual pathology") have been reported in association with HS (Blümcke et al., 2012; Malmgren and Thom, 2012).

HS can be classified into subtypes based on the histopathology findings. The most common type is *classical HS*, with severe neuronal loss primarily in CA1 subfield, accompanying neuronal loss in CA4, and relative preservation of CA2 subfield (Figure 1.5). In *total or severe HS,* the neuronal loss also involves CA2 and the granule cell layer of the dentate gyrus. The atypical or non-classical HS patterns, *end-folium HS* (neuronal loss restricted to CA4) and *CA1 HS* (neuronal loss restricted to CA1) are seen in about a fifth of cases (Malmgren and Thom, 2012). In a recently proposed consensus classification of HS in TLE, the International League Against Epilepsy (ILAE)

Commission on Diagnostic Methods classifies classical and total or severe HS as ILAE type 1, CA1 HS as ILAE type 2, end-folium (CA4 predominant) HS as ILAE type 3, and "no hippocampal sclerosis with gliosis only" as no-HS (Figure 1.5) (Blümcke et al., 2013).

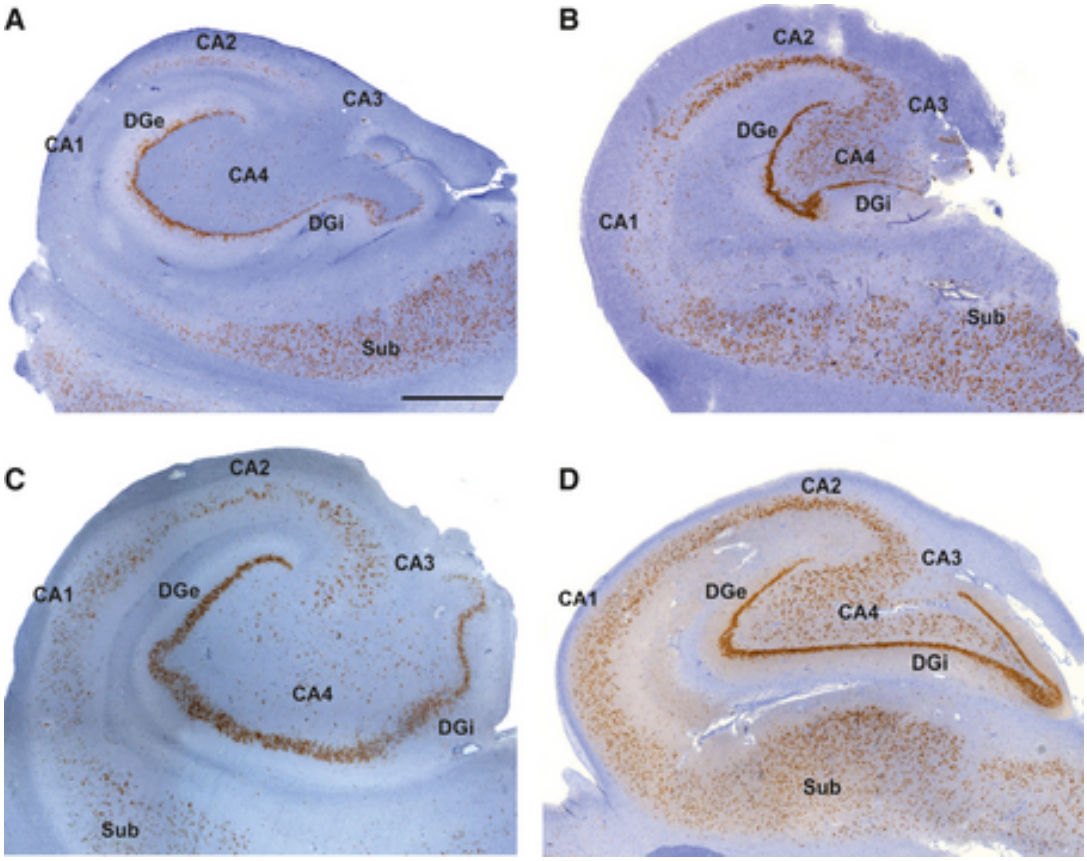


Figure 1.5 Histopathologic subtypes of HS (NeuN immunohistochemistry with haematoxylin counterstaining). (A) ILAE HS type 1 shows pronounced neuronal loss in CA1 and CA4 subfields, variable cell loss in dentate gyrus, and relative sparing of CA2. (B) ILAE HS type 2 shows predominant neuronal loss in CA1. (C) ILAE HS type 3 shows predominant neuronal loss in CA4. (D) No-HS shows no significant neuronal loss in any subfield. Sub, subiculum: DGe/DGi, external/internal limbs of dentate gyrus. Adapted from Blümcke et.al. 2013.

Data on clinicopathological and prognostic correlation for HS types is confounded by differences in study designs, surgical procedures and the duration of follow-up (Blümcke et al., 2013; Blümcke et al., 2012). There is some suggestion from retrospective studies that early febrile seizures are more often associated with ILAE HS type 1 than with type 2, 3, or no-HS in patients with TLE. TLE patients with ILAE HS type

1 have been reported to have the best surgical outcome (60-80% seizure freedom 1-2 years after surgery), whereas the outcome is considered to be poor for those with type 2, 3, and no-HS (Blümcke et al., 2013).

1.5.4.2 Radiological features of MTS/HS

The hallmark of MTS on MR Imaging are hippocampal atrophy and increased signal intensity on FLAIR and T2-weighted sequences (Figure 1.6) (Bronen, 1998; Woermann and Vollmar, 2009). The other MRI findings in MTS may include loss of the normal internal architecture of the hippocampus, temporal lobe volume loss, dilatation of the temporal horn of the lateral ventricle, smaller fornix, and an atrophic mamillary body (all ipsilateral to the side of MTS). Some cases of MTS may show temporal or extratemporal abnormalities such as focal cortical dysplasia or tumor (dual pathology). Experienced neuroradiologists can visually diagnose MTS in 80 to 90% of cases, and the sensitivity can be further enhanced with quantitative methods such as hippocampal volumetry and T2 relaxometry (Woermann and Vollmar, 2009).

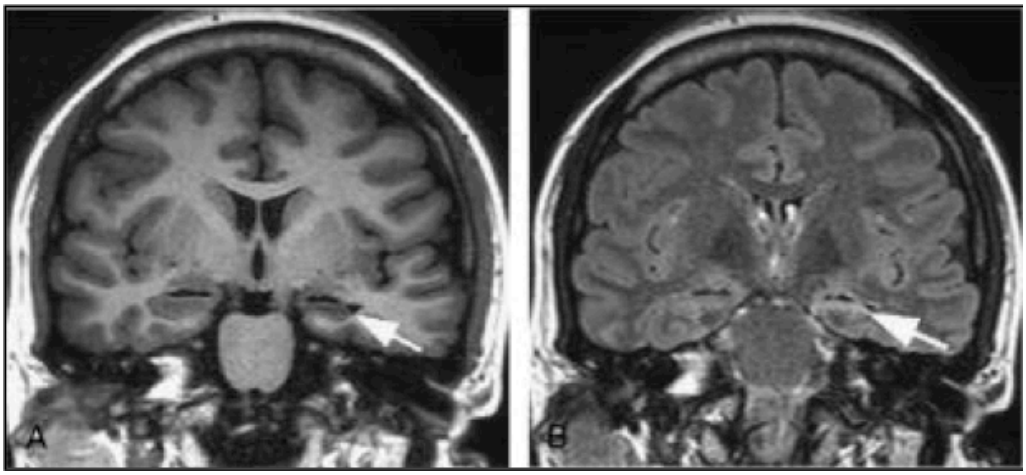


Figure 1.6 Coronal MR images showing (A) atrophy of left hippocampus on T1-W (arrow), and (B) atrophy and increased signal in left hippocampus (arrow) on FLAIR sequence.

Although MTS has rarely been reported as an incidental finding in some individuals without clinical seizures, it is most frequently found in patients with temporal lobe epilepsy (TLE) (Kobayashi et al., 2002; Benbadis et al., 2002; Moore et al., 1999; Morris et al., 2009; Labate et al., 2010). It is therefore believed that MTS is epileptogenic and most people who have MTS will develop seizures.

1.5.5 Temporal lobe epilepsy (TLE)

TLE is a form of epilepsy characterised by recurrent unprovoked seizures originating from either the medial or the lateral temporal lobe (Wallace et al., 1989). The ILAE classification offers description of seizures originating in the amygdalo-hippocampal area (mesial TLE, mTLE) and those coming from the lateral temporal area (neocortical TLE, nTLE). The seizures can be focal or secondarily generalised. The seizures in mTLE are characterised by rising epigastric discomfort, nausea, marked autonomic signs and symptoms, including borborygmi, belching, pallor, fullness/ flushing of the face, tachycardia, respiratory arrest, pupillary dilatation, fear, panic, and olfactory-gustatory hallucinations. Lateral temporal seizures are characterised by auditory hallucinations or illusions or dreamy states, visual misperceptions, or language disorders in case of language dominant hemisphere focus. The ictal manifestations vary according to the location of seizure onset and also the age and brain maturity. One of the hallmarks of TLE in adults and older children is the occurrence of auras and complex automatisms. The automatisms observed in younger children are typically simpler than those seen in older children and adults, and a history of aura is often difficult to obtain due to limited verbal communication skills (Nickels et al., 2012). The seizures in TLE are generally brief, but tend to be quite frequent.

Although TLE may have several causes, MTS is the most common pathologic finding in pharmacoresistant TLE (Blümcke et al., 2013). TLE associated with MTS is often refractory to medical treatment, but has good seizure outcome following surgical resection, with 60-80% achieving seizure freedom (Spencer and Huh, 2008; Semah et al., 1998; Blümcke et al., 2013). In addition to pharmacoresistant epilepsy, children with TLE may have increased co-morbidity such as cognitive difficulties, memory impairment, and psychological/psychiatric problems, which can have adverse impact on quality of life (MacAllister and Schaffer, 2007; Nickels et al., 2012).

As MTS is the most common cause of TLE, and there is a long-standing hypothesis proposing a causal relationship between PFS and later development of MTS and TLE, it is important to investigate this further and review the evidence to confirm or refute the hypothesis (Patterson et al., 2014; Raspall-Chaure et al., 2006; Shukla and Prasad, 2012).

1.6 CSE-induced neuronal injury and PFS-MTS-TLE hypothesis

1.6.1 The PFS-MTS-TLE hypothesis

It was Meyer, who first proposed that an initial event of a prolonged complex febrile seizure, occurring between 6 months and 5 years could result in HS and eventually lead to habitual TLE (Cavanagh and Meyer, 1956). The "Meyer's hypothesis" was widely debated at the Marseille Colloquium of 1955, and is still debated today. This hypothesis was based on the observation in retrospective studies from tertiary epilepsy centres of higher incidence of complex febrile seizures in patients who had pharmacoresistant TLE secondary to MTS (Cavanagh and Meyer, 1956; Taylor and Ounsted, 1971; Falconer, 1974; Sagar and Oxbury, 1987; Kuks et al., 1993; Cendes et al., 1993; Miura et al., 1992). Febrile seizures occur in 2-5% of children, of which about 5% are prolonged (Nelson and Ellenberg, 1976; Verity and Golding, 1991; Berg and Shinnar, 1996). Although the exact incidence of TLE is not known, it is significantly less frequent than PFS (Téllez-Zenteno and Hernández-Ronquillo, 2012; Nickels et al., 2012). If PFS directly led to development of MTS/TLE, then why does this process take place only in some individuals and not in others? Could there be other underlying factors (such as genetic or structural) that predispose to the development of MTS/TLE after PFS? Alternatively, whether both MTS/TLE and PFS have a common predisposing factor? Or indeed, could PFS be the first manifestation of MTS/TLE in those predisposed? In the following sections the candidate will review published data on CSE-induced neuronal injury and evidence for Meyer's hypothesis.

1.6.2 CSE-induced neuronal injury & epileptogenesis

While vast amount of research on animal models has enhanced our understanding of the pathophysiology of CSE, it is important to acknowledge the inherent limitations of animal studies while interpreting the data and extrapolating the finding to humans. In animal models, CSE is often induced by the administration of noxious agents which themselves might result in neuronal injury and subsequent adverse outcomes. Also, the nature of injury and the outcomes may be species specific.

The term "epileptogenesis" is often used to describe the process by which the previously normal brain is functionally and/or structurally altered and biased towards the generation of the abnormal electrical activity that subserves chronic seizures (Goldberg and Coulter, 2013). In the context of CSE, epileptogenesis occurs during the latency

period between the episode of CSE and the development of spontaneous seizures. This implies that effective intervention during this period could potentially halt this process and prevent the adverse consequences of CSE.

1.6.2.1 Mechanism and nature of CSE-induced neuronal injury

The hippocampus appears to be particularly vulnerable to CSE-induced injury. Some hippocampal neurons are highly vulnerable to damage from CSE (e.g. CA1 and CA3 pyramidal cells and dentate hilar neurons), whereas others are relatively resistant (e.g. CA2 neurons and dentate gyrus granule cells) (Wasterlain et al., 1993). Experimental evidence suggests that glutamate plays a major role in seizure-induced neuronal injury and this injury is calcium mediated (Wasterlain et al., 1993; Scantlebury et al., 2007; Naylor, 2010). In addition, the systemic and metabolic complications of CSE, such as hypotension, reduced cerebral perfusion, lactic acidosis, hypoxia, and hypoglycaemia may also contribute to the neuronal injury (Wasterlain et al., 1993; Meldrum and Horton, 1973; Meldrum and Brierley, 1973). Recent data suggest that alterations in gene expression of ion channels (e.g. hyperpolarization-activated cation channels), molecular signalling pathways, and inflammation involving interleukin-1β (IL-1β) pathway may play a major role in epileptogenesis by driving the formation of aberrant neuronal circuitry (Vezzani et al., 2009; Auvin et al., 2010; Dube et al., 2010; Goldberg and Coulter, 2013; McClelland et al., 2011; Pitkänen and Lukasiuk, 2011).

1.6.2.2 Data from animal models

The classical studies by Meldrum and co-workers in primates showed that experimentally induced CSE results in extensive neuronal injury predominantly in the neocortex and the hippocampal formation, even in paralyzed and ventilated animals (though less severe) (Meldrum and Horton, 1973; Meldrum and Brierley, 1973). Subsequent animal experiments (mostly in rodents) have provided insights into the diversity of behavioural, structural, and functional changes that occur as a result of CSE. Results from most of these studies have revealed that the consequences of CSE are largely dependent on the developmental stage of the animal when CSE occurs (Scantlebury et al., 2007; Rajasekaran et al., 2010). Evidence of neuronal injury, epileptogenesis and impairment of hippocampal dependent learning and memory following CSE are predominantly seen in prepubescent and adult rats, and rarely in the young.

Status epilepticus induced by electrical or chemo-convulsant stimulation in adult rodent models results in neuronal death, gliosis and volume loss, changes resembling those observed in human MTS (Engelhorn et al., 2007b; Covolan and Mello, 2000; Rakhade and Jensen, 2009). However, early-life seizure models have consistently failed to demonstrate significant neuronal death in the hippocampus (Rakhade and Jensen, 2009; Rajasekaran et al., 2010; Scantlebury et al., 2007). Similarly, experiments on febrile seizure models demonstrated transient early neuronal injury in the hippocampus after prolonged seizures, but no significant cell loss was observed even in rats that became epileptic (McClelland et al., 2011; Dube et al., 2010; Jansen et al., 2008). Whereas subtle loss of some neurons (e.g. hilar interneurons) cannot be excluded, the results suggest that a significant neuronal loss is probably not a prerequisite for epileptogenesis following PFS. Mossy fibre sprouting appears to be an important process in epileptogenesis and has consistently been demonstrated in experimental models (Scantlebury et al., 2005; Rakhade and Jensen, 2009; Laitinen et al., 2010; Jansen et al., 2008; Kuo et al., 2008).

Dube et al. reported that the probability of developing TLE following experimental FS in rats increased by 30% after a 64-min-long FS compared with a 24-min-long FS, and also spontaneous seizures in rats with prolonged FS lasted longer (median 7s v 91s, $p<0.05$), and had more motor manifestations (Dubé et al., 2006; Dube et al., 2010). Although prolonged FS were associated with longer duration of interictal activity, interictal activity itself did not predict subsequent development of spontaneous seizures.

1.6.2.3 Data from human studies

Reports of histologic analyses of post mortem tissue from individuals with CSE demonstrate significant neuronal loss in the hippocampus, particularly in CA1 and CA3 regions (DeGiorgio et al., 1992; Corsellis and Bruton, 1983). While there are several isolated case reports suggesting development of MTS/TLE following PFS and CSE, they are subject to potential bias and cannot provide information necessary for epidemiological analyses (Sokol et al., 2003; Tanabe et al., 2011; Merkenschlager et al., 2009; Gong et al., 2008; VanLandingham et al., 1998).

The advent of magnetic resonance Imaging (MRI) has enabled researchers to study the consequences of CSE non-invasively. Prospective MRI studies in children have shown imaging changes suggestive of acute hippocampal oedema 2 to 5 days following PFS (Scott et al., 2002; Natsume et al., 2007; Provenzale et al., 2008; VanLandingham et al.,

1998; Shinnar et al., 2012). However, these changes seem to be transient and soon disappear. A small proportion of children may show a reduction in hippocampal volume, but without other radiological features of MTS, within 12 months after PFS (Scott et al., 2003; Yoong et al., 2013a). Others have reported a high incidence of MTS 9 to 12 months after PFS, but only in those with hippocampal T2 signal abnormalities on initial MRI (Provenzale et al., 2008; Lewis et al., 2014). In the only reported prospective, long-term follow-up study Tarkka et al. found no radiological evidence of MTS in 24 children with PFS after a mean follow-up of 12.5 years (Tarkka et al., 2003). However, children in the PFS group had significantly lower right-left hippocampal volume difference compared to controls and in 3 (12.5%) children the right-left volume difference was more than 2 standard deviations below that of the controls.

Collectively, current data suggest that children may acutely develop hippocampal oedema following PFS, which resolves after a few days and a small proportion may show hippocampal volume reduction. The majority of children do not develop MTS after PFS, but a subgroup with abnormal hippocampal T2 signal on acute imaging appear to be at a higher risk of developing later MTS. It is uncertain whether the small hippocampi and the T2 signal abnormalities predate the seizures or develop as a consequence. Also, whether or not the small hippocampi will progress to MTS (with or without TLE) over a longer-term period is yet to be investigated. It is therefore possible that PFS is either a marker for pre-existing abnormality (structural or genetic) and/or a cause of additional damage to an already predisposed brain.

1.6.3 The two-hit hypothesis

The available data suggest that only a minority of children are susceptible to developing MTS following PFS. The two-hit hypothesis could explain why some children are susceptible and others are not. It proposes that there are two factors involved in the aetiology of MTS, an initial precipitating injury (IPI) such as PFS, plus some other factor ("dual pathology") that increases the child's vulnerability to hippocampal injury (Lewis, 2005; McClelland et al., 2011; Sanon et al., 2012; Hofman et al., 2011). The second factor could be a structural abnormality such as a microdysgenesis in the hippocampus/ temporal lobe or a genetic predisposition that enhances susceptibility to develop MTS.

Data from animal studies and some human studies support the role of cortical dysgenesis in increasing the susceptibility to febrile seizures and development of MTS (Lewis, 2005; Hofman et al., 2011; McClelland et al., 2011; Sanon et al., 2012; Park et al.,

2010). A second pathology, commonly cortical dysplasia, has often been observed in association with MTS in surgical specimens of TLE patients undergoing temporal lobectomy (Park et al., 2010; Bocti et al., 2003; Blümcke et al., 2013). Dual pathology has been shown to predispose the brain to PFS and also to increased severity of seizure-induced hippocampal neuronal damage (Park et al., 2010; Sanon et al., 2012; Scantlebury et al., 2005; Gibbs et al., 2011). A recent study reported significantly higher prevalence of developmental malformations, hippocampal malrotation (HIMAL) in particular, in children with PFS compared to simple febrile seizures (Shinnar et al., 2012). However, due to conflicting data on the prevalence of HIMAL in the general population its clinical significance is still debated (Gamss et al., 2009; Connolly, 2013; Bajic et al., 2009).

Both febrile seizures and epilepsy are known to have a strong genetic predisposition (Abou-Khalil et al., 2007; Heuser et al., 2011; Depondt et al., 2002). Some genetic conditions such as Dravet syndrome and generalised epilepsy with febrile seizures plus (GEFS+) predispose to both febrile seizures and epilepsy. Although born with a sodium channel mutation (SCN1A), patients do not exhibit spontaneous seizures in the first few months of life, but often experience PFS. It is hypothesised that PFS can trigger a secondary cascade of events leading to neuronal plasticity and epileptogenesis (Goldberg and Coulter, 2013). Studies on familial mesial TLE have demonstrated that MRI features of MTS and hippocampal malformations may be seen in unaffected family members who never had febrile seizures or TLE (Kobayashi et al., 2003; Kobayashi et al., 2002; Kobayashi et al., 2001; Depondt et al., 2002). There are also studies showing an association between the IL-1β511T polymorphism and TLE with MTS (Kauffman et al., 2008). Therefore, it is plausible that genetic predisposition could potentially influence the development of MTS after PFS.

In summary, data from the animal experiments suggest that prolonged seizures may cause hippocampal neuronal injury and the injured neurons/neuronal-networks may subsequently become epileptogenic with impairment of hippocampal-dependent functions. It is uncertain whether a similar process follows CSE in children. Results from case series in humans indicate that children may sustain transient hippocampal injury immediately after CSE and a minority will show hippocampal volume reduction few months later, but the longer-term evolution of these changes have not been investigated.

In chapter 2 the candidate will review current data on the outcomes following childhood CSE and discuss the rationale for the current study.

CHAPTER 2 A REVIEW OF OUTCOMES FOLLOWING CHILDHOOD CSE

2.1 Introduction

In a landmark study published in 1970, Aicardi and Chevrie reported the outcome of CSE in children as "grave, mental or neurological residua or both being present in at least 57% of patients" (Aicardi and Chevrie, 1970). Subsequent prospective studies however have reported better outcomes. While it is widely accepted that childhood CSE has associated morbidity and mortality, the role of CSE itself on subsequent adverse outcomes is still debated. Furthermore, there is considerable variability in the reported frequencies of adverse outcomes following childhood CSE (Raspall-Chaure et al., 2006). A systematic review of all published studies to rigorously assess for methodological quality and systematically analyse the results would be valuable in establishing the generalisability of the findings in the context of considerable heterogeneity and uncertainty of reported outcomes. However, two high quality systematic reviews were conducted recently, one addressing outcomes of all forms of childhood CSE, and the other addressing mortality and morbidity of febrile seizures, including febrile status epilepticus (Chungath and Shorvon, 2008; Raspall-Chaure et al., 2006). In addition, a further systematic review addressing the morbidity and mortality in CSE, including CSE in children, was conducted more recently (Neligan and Shorvon, 2011; Neligan and Shorvon, 2010). With all three recent systematic reviews reaching similar conclusions for outcomes of childhood CSE, conducting another systematic review on the same topic is unnecessary. Thus, the review presented in this chapter entails a comprehensive literature search to identify subsequently reported studies, assess their methodology, and synthesize the new data into what is already known.

The purpose of the review in this chapter is to identify and synthesize reported data on outcomes of childhood CSE toward establishing current knowledge, highlight methodological limitations, and to identify deficiencies in knowledge that need addressing in future research. The discussion of the review is presented in 3 sections: (1) consideration of methodological differences and definitions, (2) clinical outcomes after childhood CSE; namely, mortality, subsequent epilepsy, CSE recurrence, neurological and cognitive sequelae, behavioural problems, and educational difficulties, and (3) neuroimaging findings that include structural and quantitative MRI data investigating the consequences of childhood CSE.

2.2 Consideration of methodological differences and definitions

The analysis of published studies reveals considerable heterogeneity in study design (prospective vs. retrospective), study type (population-based vs. hospital-based), CSE definition, definition of outcome variables, and the length of follow-up, resulting in varied estimates of adverse outcomes. In the systematic review of outcomes of childhood CSE, Raspall-Chaure and colleagues assessed the methodological quality of each eligible study using a scoring system and found that adverse outcomes were significantly affected by the methodological differences (Raspall-Chaure et al., 2006). While prospective population-based studies are less likely to provide biased information compared to retrospective and hospital-based studies, conducting such studies is often logistically more complex and typically cost more (Szklo, 1998). Therefore, published literature on outcomes of childhood CSE is predominated by data from hospital-based studies. Both prospective population-based cohort studies and well-conducted prospective hospital-based studies with clear comparison groups and using clear definition for outcome variables have the potential to provide reliable, prognostically useful information (Raspall-Chaure et al., 2006; Szklo, 1998).

By definition, this review is restricted to studies reporting childhood-onset (between age 1 month and 18 years) CSE and using 30-minute or longer duration criteria for CSE definition. Data on outcomes for seizures lasting <30 minutes are discussed where relevant, but not included in data synthesis. Most studies use Maytal and colleagues' modification of Hauser and colleagues' classification to classify CSE aetiology, but the criteria used for assigning subjects into aetiological groups differ between some studies (Hauser et al., 1982; Maytal et al., 1989).

Consideration of methodological differences in CSE classification is particularly relevant for status epilepticus associated with a febrile illness, and not due to a CNS infection. Some authors (usually from North America) include children with prior neurological abnormalities and/or developmental delay in this group, whereas authors from the UK do not (Chin et al., 2006; MacDonald et al., 1999; Maytal and Shinnar, 1990; Maytal et al., 1989; Nelson and Ellenberg, 1976; Scott et al., 2002; Shinnar et al., 2008; Verity and Golding, 1991; Verity et al., 1993; Yoong et al., 2013a). Also, various terminology such as "*febrile status epilepticus*", "*prolonged febrile seizures*", "*prolonged febrile convulsions*", and "*lengthy febrile convulsions*" have been used to describe febrile seizures lasting longer than 30 minutes (Chin et al., 2006; Maytal and Shinnar, 1990; Shinnar et al., 2008; Verity and Golding, 1991; Verity et al., 1993; Yoong et al., 2013a). Furthermore, most

studies report outcomes for simple and complex febrile seizures, but not separately for febrile seizures >30 minutes (Chungath and Shorvon, 2008). A *complex febrile seizure* is defined as a febrile seizure with one or more of the following characteristics: more than 15 minutes duration, more than one seizure in 24 hours, or presence focal features (Nelson and Ellenberg, 1978). Therefore, it is often difficult to decipher the effect of seizure duration on outcomes from those studies. The term "febrile status epilepticus (FSE)" is subject to variable interpretation as CSE in a febrile child could be manifestation of a febrile seizure, or CNS infection (acute symptomatic), or fever-induced seizure in a previously abnormal brain (acute on remote symptomatic). Although "prolonged febrile seizures" and "lengthy febrile convulsions" are sometimes used interchangeably, more authors prefer the term "prolonged febrile seizures (PFS)" and thus is the terminology used in this and the following chapters. Further methodological differences that need to be taken into consideration while interpreting the results will be discussed in relevant sections.

2.3 Clinical outcomes

In addition to increased mortality, the reported adverse outcomes following childhood CSE include subsequent epilepsy (with/without MTS), CSE recurrence, motor deficits, cognitive impairment, behavioural problems, and educational difficulties.

In their systematic review of outcomes of childhood CSE, Raspall-Chaure et al. found that the reported adverse outcomes were significantly affected by the study design (prospective v retrospective), type of study (population-based v hospital-based), CSE definition, and the length of follow-up (Raspall-Chaure et al., 2006). The authors also assessed the methodological quality of each eligible study using a scoring system and observed that the reported morbidity and mortality were considerably lower in high quality studies.

2.3.1 CSE and mortality

2.3.1.1 Short-term mortality

Childhood CSE is associated with significant short-term mortality (death during hospital admission or within the first 30 to 60 days after an episode of CSE).

In the review by Raspall-Chaure et al., the short-term mortality after CSE was 2.7-5.2% in high quality studies (Raspall-Chaure et al., 2006). Mortality was 5-8% if only children

admitted to paediatric intensive care units (PICU) were included. Most deaths during hospitalization were seen in children with acute or remote symptomatic causes and mortality in PFS/FSE was negligible. In their systematic review of outcomes after febrile seizures, Chungath and Shorvon calculated a summated mortality rate of 1.6% for FSE (14 deaths in 876 patients from 18 studies) (Chungath and Shorvon, 2008). The authors however point out the possibility that some of the children who died were misclassified as having FSE, when in fact might have had an underlying symptomatic cause for CSE.

More recent studies from resource-rich countries report short-term mortality after CSE of 0-1.2%, compared with 7.1-23.2% reported in studies from resource-poor countries (Siddiqui et al., 2008; Molinero et al., 2009; Sadarangani et al., 2008; Hussain et al., 2007; Kravljanac et al., 2011; Lin et al., 2009; Loddenkemper et al., 2012; Maegaki et al., 2015; Nishiyama et al., 2015; Yoong, 2012). Higher prevalence of acute CNS infections, delay in accessing emergency medical care and lack of intensive care facilities (reflected as longer seizure duration in these studies) could be the reasons for increased mortality following CSE in resource-poor countries. Conversely, lower prevalence of CNS infections, use of standardized protocols for pre-hospital and in-hospital management of seizures, and better intensive care facilities are the probable reasons for lower mortality reported in studies from resource-rich countries. Similar to the observation in previous reviews, short-term mortality was mostly seen in children with acute or remote symptomatic causes (Kravljanac et al., 2011; Lin et al., 2009; Loddenkemper et al., 2012; Maegaki et al., 2015; Molinero et al., 2009; Sadarangani et al., 2008; Siddiqui et al., 2008; Komur et al., 2012). No deaths were reported during hospitalization in children with PFS/FSE in recent studies (Kravljanac et al., 2011; Lin et al., 2009; Maegaki et al., 2015; Molinero et al., 2009; Hussain et al., 2007; Nishiyama et al., 2015; Komur et al., 2012).

Apart from acute and remote symptomatic aetiology, younger age at CSE, longer seizure duration, focal CSE and intermittent CSE have been reported as determinants of short-term mortality after childhood CSE. Most in-hospital deaths occur in children with acute or progressive symptomatic CSE (Raspall-Chaure et al., 2006; Sadarangani et al., 2008; Molinero et al., 2009; Kwong et al., 1995; Maytal et al., 1989; Callenbach et al., 2001; Chin et al., 2006; Kravljanac et al., 2011; Maegaki et al., 2005; Tabarki et al., 2001). The mortality in children with unprovoked CSE and PFS is less than 2%, compared with 12-27% in those with acute symptomatic CSE (Raspall-Chaure et al., 2006; Logroscino et al., 1997; Maegaki et al., 2005; Maytal and Shinnar, 1990; Maytal et al., 1989; Tabarki et al., 2001; Kravljanac et al., 2011; Lin et al., 2009; Molinero et al., 2009). Of acute

symptomatic causes, CNS infections and anoxic insult are particularly associated with high mortality (Kravljanac et al., 2011; Lacroix et al., 1994; Scholtes et al., 1996; Tabarki et al., 2001; Sadarangani et al., 2008).

Younger age at CSE onset (particularly under 2 years) has been associated with higher mortality in some studies (Raspall-Chaure et al., 2006; Aicardi and Chevrie, 1970; Cavazzuti and Lalla, 1984; Kwong et al., 1995; Logroscino et al., 1997; Tabarki et al., 2001; Dunn, 1988; Maegaki et al., 2015; Scholtes et al., 1996; Gulati et al., 2005). However, this association is probably due to higher incidence of acute symptomatic CSE in this age group. While an association between longer seizure duration (typically >60 minutes) and higher mortality has been reported in a few studies, data from other studies do not support a direct association (Dunn, 1988; Kravljanac et al., 2011; Logroscino et al., 1997; Maegaki et al., 2005; Maytal et al., 1989; Scholtes et al., 1996; Siddiqui et al., 2008; Towne et al., 1994; Gulati et al., 2005; Hussain et al., 2007). Heterogeneity of available data due to differences in study methodology, patient population, severity of underlying condition, and quality of treatment make it difficult to determine the direct impact of CSE on mortality. Two studies report higher mortality in children with continuous CSE and one study in those with intermittent CSE, but the difference was not statistically significant (Lin et al., 2009; Waterhouse et al., 1999; Chin et al., 2006). Similarly, data on seizure type and mortality are inconsistent as both focal and generalized CSE have been associated with higher mortality (Kravljanac et al., 2011; Sadarangani et al., 2008). Finally, children who develop systemic complications such as shock, hypoxia, and hypoglycaemia following CSE have higher mortality (Sadarangani et al., 2008; Barnard and Wirrell, 1999; Gulati et al., 2005; Loddenkemper et al., 2012; Maegaki et al., 2005).

Thus, current data suggest that short-term mortality is primarily determined by the severity of underlying aetiology, and the role of other potentially modifiable factors including duration and early treatment remain unclear.

2.3.1.2 Mortality within 5 to 10 years after CSE

As with short-term mortality, data on long-term mortality after childhood CSE is also variable depending on the study design and the duration of follow-up. Raspall-Chaure et al. reported estimates of long-term mortality after childhood CSE ranging from 5.4% to 17% (Raspall-Chaure et al., 2006). Cumulative mortality rates of 5.4% and 13% within

10 years after CSE were reported in two population-based studies (Logroscino et al., 1997; Logroscino et al., 2002; Verity et al., 1993). While the study from the UK was prospective and the aetiology of CSE was PFS in more than 50% of the cohort, the study from the USA was retrospective and the aetiology was PFS in 22%, which potentially explain the observed difference in mortality between the two studies. No deaths were observed in children with PFS in both these studies and also in another prospective population-based study which followed-up 74 children with PFS up to the age of 7 years (Nelson and Ellenberg, 1978).

In a recently reported prospective population-based study from rural Kenya, 23% of the children died within 4 years after CSE (Prins et al., 2014). Nearly all children who died during follow-up had generalized seizures and fever during their fatal illness. The causes of death were not reported in the paper and therefore it is difficult to determine whether or not the deaths were seizure related or were due to underlying illness. In another retrospective, hospital-based study from the USA, 6% of 149 children with CSE died at median 3.6 years follow-up (Fernández et al., 2014). The mortality in children with seizure duration ≥30 minutes was not significantly higher compared to that in children with seizure duration of 5-29 minutes (6% vs. 4.1%, p = 0.25). Similarly, in another large, population-based study with up to 28 years follow-up, the mortality in children with PFS was not significantly higher than that in children with seizure duration of 15 to 29 minutes or the control population, when children with pre-existing neurological disorders and epilepsy were excluded (Vestergaard et al., 2008).

As with short-term mortality, a higher mortality during follow-up is observed in those with symptomatic CSE compared with other causes (Logroscino et al., 2002; Barnard and Wirrell, 1999; Lacroix et al., 1994; Verity et al., 1993; Cavazzuti and Lalla, 1984). Whether CSE is just a marker of severity of underlying disease, or whether it has independent effect on long-term mortality above that related to underlying cause is unclear. Data from long-term follow-up epilepsy cohort studies suggest that CSE is not associated with increased risk of death after adjusting for underlying diagnosis (Berg et al., 2004a; Berg et al., 2004b; Callenbach et al., 2001; Camfield et al., 2002; Sillanpaa and Shinnar, 2002; Sillanpaa and Shinnar, 2010). CSE characteristics such as long seizure duration (>24 hours), acute symptomatic aetiology, and myoclonic seizure type have been associated with increased long-term mortality in adults, but this observation was not valid for the paediatric population (Logroscino et al., 2002). Thus, the association

between CSE characteristics and long-term mortality has not been investigated in paediatric population and needs to be addressed in future studies.

2.3.2 CSE and subsequent epilepsy

2.3.2.1 Epilepsy following childhood CSE due to all causes

Currently available estimates of epilepsy incidence following an episode of childhood CSE vary between 8% and 74% depending on study methodology, inclusion criteria and follow-up duration (Table 2.1). While hospital-based studies report an incidence of 23-36% by 3-4 years after CSE due to all causes, a lower incidence (15% in 110 children) was reported in a recent prospective population-based study from Kenya after similar length of follow-up (Prins et al., 2014; Aicardi and Chevrie, 1970; Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Kwong et al., 1995; Maytal et al., 1989; Tabarki et al., 2001). In another prospective population-based study from the UK, 82% of 17 children developed subsequent unprovoked seizures 5-10 years after afebrile CSE but the incidence of epilepsy was not reported (Verity et al., 1993). In addition, the authors do not report whether any of these children had prior unprovoked seizures/epilepsy or the unprovoked seizures developed after CSE in all.

The reported incidence of epilepsy after CSE of unknown cause (idiopathic) is 40% and 57% at 3.6 and 13 years follow-up (Awaya et al., 1992; Eriksson and Koivikko, 1997). A higher incidence of epilepsy is reported following acute and remote symptomatic CSE than unprovoked CSE (Raspall-Chaure et al., 2006; Awaya et al., 1992; Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Kwong et al., 1995; Maytal et al., 1989; Verity et al., 1993). Although focal epilepsy appears to be more common in those who develop epilepsy after CSE, other types have also been reported (Aicardi and Chevrie, 1970; Awaya et al., 1992; Cavazzuti and Lalla, 1984; Verity et al., 1993; Viani et al., 1987; Eriksson and Koivikko, 1997).

Reference	Study type/ design	CSE type	Follow-up	Incidence of epilepsy
Prins et al., 2014	Population/ Pros	All CSE	3-4y	16/110 (15%)
Barnard and Wirrell, 1999	Population/ Retro	All CSE	5.2y	5/14 (36%)
Verity et al., 1993	Population/ Pros	Non-PFS	5-10y	14/17 (82%)[$]
Hussain et al., 2007	Hospital/ Retro	All CSE	14.4m	7/88 (8%)
Maytal et al., 1989	Hospital/ Retro & Pros	All CSE	27.5m 8.1m	Overall 36/125 (29%)[$] Prospective 11/69 (16%)[$]
Kwong et al., 1995	Hospital/ Retro	All CSE	22.7m	4/25 (16%)
Eriksson and Koivikko, 1997	Hospital/ Retro	All CSE	3.6y	Overall 15/65 (23%) Idiopathic 6/15 (40%)
Aicardi and Chevrie, 1970	Hospital/ Retro	All CSE[£]	Unclear	66/184 (36%)
Tabarki et al., 2001	Hospital/ Retro	All CSE <2y	4y	34/117 (29%)
Cavazzuti and Lalla, 1984	Hospital/ Pros	All CSE <1y	5-10y	49/66 (74%)
Awaya et al., 1992	Hospital/ Pros	Idiopathic[£]	13y	8/14 (57%)

Pros = Prospective, Retro = Retrospective, [$]Reported as subsequent unprovoked seizures, [£]CSE defined as seizure duration of >60 minutes

Table 2.1 Incidence of epilepsy after childhood CSE, arranged according to study type/ design

2.3.2.2 Epilepsy following PFS/FSE

Most studies on prognosis after febrile seizures report significantly higher risk of subsequent epilepsy in children with complex febrile seizures (CFS) (duration >15 minutes, focal, or multiple episodes within the same febrile illness) compared with simple febrile seizures, but do not separately report risk of epilepsy for PFS/FSE (Annegers et al., 1979; MacDonald et al., 1999; Chungath and Shorvon, 2008; Pavlidou and Panteliadis, 2013; Sapir et al., 2000). In their systematic review of studies reporting the outcomes of febrile seizures, Chungath and Shorvon estimated a summated risk of subsequent epilepsy to be 2.5% for simple febrile seizures and 17.1% for CFS or PFS/FSE (Chungath and Shorvon, 2008).

The reported risk of epilepsy within 5 years after PFS/FSE ranges from 0% to 11%, with the lowest (0%) at 1-year follow-up in a population-based study of 34 children from north London, and 11% at 4-year follow-up in a retrospective hospital-based study from Tunisia (Table 2.2) (Eriksson and Koivikko, 1997; Esch et al., 1996; Lewis et al., 2014; Maytal and Shinnar, 1990; Maytal et al., 1989; Scott et al., 2003; Tabarki et al., 2001; Hussain et al., 2007; Yoong, 2012). Few studies report epilepsy risk after 5 or more years of follow-up. In a prospective population-based study from the USA, 3/74 (4.1%) children with FSE developed epilepsy by the age of 7 years (Nelson and Ellenberg, 1978). While the epilepsy risk in the PFS group was substantially higher than in the general population, this was not significantly different when compared with brief febrile seizures (4.1% vs. 1.5%). In a similar prospective population-based study from the UK, although 4/19 children with PFS developed subsequent afebrile seizures, only 2 had recurrent afebrile seizures and thus 2/19 (10.5%) developed epilepsy after 5-10 years follow-up (Verity et al., 1993). Of the two studies with follow-up duration of over 10 years, the reported risk of epilepsy was 15% at 13 years in a prospective hospital-based study from Japan and 8% at 18 years in a retrospective population-based study from Rochester, USA (Annegers et al., 1979; Awaya et al., 1992). The Japanese study used 60-minute duration criteria for PFS definition. Thus, inclusion of more severe cases of PFS together with hospital-based study design could potentially be the reason for the reported high incidence of epilepsy in this study.

Reference	Study type/design	CSE type	Follow-up	Incidence of epilepsy
Yoong, 2012	Population/ Pros	Febrile	12m	0/34 (0%)
Nelson and Ellenberg, 1978	Population/ Pros	Febrile	Age 7y	3/74 (4%)
Verity et al., 1993	Population/ Pros	Febrile	5-10y	2/19 (10.5%)
Annegers et al., 1979	Population/ Retro	Febrile	18y	8%
Scott et al., 2003	Hospital/ Pros	Febrile	5.5m	1/14 (7%)
Lewis et al., 2014	Hospital/ Pros	Febrile	12m	16/226 (7%)
Hussain et al., 2007	Hospital/ Retro	Febrile	15.6m	1/47 (2%)
Maytal et al., 1989	Hospital/ Retro & Pros	Febrile	27.5m 8.6m	Overall 2/46 (4%)[$] Prospective 1/28 (4%)[$]
Esch et al., 1996	Hospital/ Retro	Febrile	21.8m	3/57 (5%)[§]
Maytal and Shinnar, 1990	Hospital/ Retro & Pros	Febrile	3.4y 2.7y	Overall 3/44 (7%)* Prospective 2/30 (7%)*
Eriksson and Koivikko, 1997	Hospital/ Retro	Febrile	3.6y	1/24 (4%)
Tabarki et al., 2001	Hospital/ Retro	Febrile <2y	4y	6/56 (11%)
Awaya et al., 1992	Hospital/ Pros	Febrile[£]	13y	6/40 (15%)

[$]Reported as subsequent unprovoked seizures, *Afebrile seizures seen only in children with neurological impairment prior to CSE, [§]53% received prophylactic phenobarbital therapy for a mean of 18 months after CSE, [£]CSE defined as seizure duration of >60 minutes

Table 2.2 Incidence of epilepsy after PFS/FSE arranged according to study type/design

2.3.2.3 The PFS – MTS – TLE hypothesis

As discussed in chapter 1, there is a long-standing hypothesis that PFS has a causal role in the development of MTS and associated TLE. Retrospective studies from tertiary epilepsy centres highlighted that as many as 40% of patients with refractory TLE secondary to MTS had a history of prolonged or complex febrile seizures (Cavanagh and Meyer, 1956; Taylor and Ounsted, 1971; Falconer, 1974; Sagar and Oxbury, 1987; Kuks

et al., 1993; Cendes et al., 1993; Miura et al., 1992; French et al., 1993; Abou-Khalil, 2010). However, prospective and population-based studies have failed to find a causal association between PFS/FSE and TLE (Verity et al., 1993; Annegers et al., 1987; Nelson and Ellenberg, 1978; Nelson and Ellenberg, 1976; Camfield et al., 1994; Berg and Shinnar, 1996). Epidemiological data also suggest that TLE is uncommon in children who develop epilepsy after febrile seizures (Berg et al., 1999a; Camfield et al., 1994; Lewis et al., 2014; MacDonald et al., 1999; Nelson and Ellenberg, 1978; Verity and Golding, 1991; Verity et al., 1993). Possible explanations for the reported discrepancy between retrospective/hospital-based studies and prospective/population-based studies include: (1) potential recall and selection bias in retrospective/hospital-based studies resulting in overestimation of risk, (2) long latency (up to 8-11 years) between PFS/FSE and the development of TLE, which is longer than the follow-up period in most prospective studies and therefore the risk may have been underestimated, (3) lack of neuroimaging in population-based studies and therefore hippocampal changes/MTS may have been missed, especially in those without epilepsy, and (4) insufficient sample size- the relative infrequency of PFS/FSE and MTS in humans will require a large sample size to demonstrate a modest effect (French et al., 1993; Mathern et al., 2002; Shukla and Prasad, 2012; Patterson et al., 2014; Maher and McLachlan, 1995; Shinnar, 2003). Prospective cohort studies with a longer-term follow-up and inclusion of neuroimaging in addition to longitudinal clinical assessments, as is the subject matter of this thesis, could enhance our understanding of the natural history following childhood CSE.

Neuroimaging data on short- and long-term structural consequences after childhood CSE, and PFS/FSE in particular, will be discussed later in this chapter.

2.3.2.4 Risk factors for development of epilepsy after CSE

The risk of subsequent epilepsy is lower in children with unprovoked CSE than in those with acute and remote symptomatic CSE (Raspall-Chaure et al., 2006; Awaya et al., 1992; Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Kwong et al., 1995; Maytal et al., 1989; Verity et al., 1993). Presence of neurological abnormalities prior to the episode of CSE is a known risk factor for developing epilepsy, and epilepsy after FSE was observed only in those with prior neurological abnormalities in one study (Cavazzuti and Lalla, 1984; Maytal and Shinnar, 1990; Maytal et al., 1989; Nelson and Ellenberg, 1976; Nelson and Ellenberg, 1978; Verity et al., 1993). Young age at CSE (particularly under 1) was associated with increased risk of epilepsy in some studies, but this could be due to higher incidence of acute symptomatic CSE in this age group (Aicardi and

Chevrie, 1970; Awaya et al., 1992; Eriksson and Koivikko, 1997; Hussain et al., 2007; Kwong et al., 1995; Lacroix et al., 1994; Maytal et al., 1989; Tabarki et al., 2001; Viani et al., 1987). An increased risk of epilepsy in children with focal CSE, and a family history of afebrile seizures/epilepsy is also reported, particularly with febrile seizures (Annegers et al., 1987; Awaya et al., 1992; Nelson and Ellenberg, 1978; Pavlidou and Panteliadis, 2013; Verity and Golding, 1991; Verity et al., 1993).

A direct association between long seizure duration and risk of subsequent epilepsy has long been debated. While a few studies report higher risk of epilepsy with longer seizure duration (usually >120 minutes), evidence from other studies do not support this association (Aicardi and Chevrie, 1970; Awaya et al., 1992; Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Hussain et al., 2007; MacDonald et al., 1999; Maytal et al., 1989; Nelson and Ellenberg, 1978; Pavlidou and Panteliadis, 2013; Sahin et al., 2001; Scholtes et al., 1996; Verity and Golding, 1991; Viani et al., 1987; Hayakawa et al., 1979). Furthermore, children with symptomatic CSE tend to have longer seizures compared to those with other causes, and therefore it is difficult to separate the effect of seizure duration from that of underlying cause (Eriksson and Koivikko, 1997; Kwong et al., 1995; Tabarki et al., 2001). To determine whether longer seizure duration increased the risk of subsequent afebrile seizures, Hesdorffer and colleagues investigated a population-based cohort 10 years after an episode of acute symptomatic seizure and found the risk with prolonged seizures (>30 minutes) to be 3.3-fold (95% CI 1.8-6.1) higher than with brief seizures (Hesdorffer et al., 1998b). However, the authors did not report separate results for the paediatric population. Similarly, two prospective population-based studies compared the risk of subsequent unprovoked seizures 5 to 10 years after prolonged febrile seizures (>30 minutes) with that after brief febrile seizures and found differing results. Although the risk of developing subsequent afebrile seizures was greater in children with prolonged seizures than in those with brief seizures in both studies, the difference was statistically significant (21% vs 3·4%, p<0·005) in the UK study but not (4.1% vs 1.5%, p value not reported) in the USA study (Nelson and Ellenberg, 1978; Verity et al., 1993). Thus, current data on the association between seizure duration and the risk of developing epilepsy after CSE is inconsistent and needs to be explored further in future studies.

2.3.3 CSE recurrence

Data from the highest-scored quality studies suggest the overall recurrence of CSE due to all causes to be 10-15% by 2 years and about 25% by 5 to 10 years (Cavazzuti and

Lalla, 1984; Chin et al., 2006; Dunn, 1988; Kwong et al., 1995; Maegaki et al., 2005; Mah and Mah, 1999; Maytal and Shinnar, 1990; Shinnar et al., 1992; Verity et al., 1993; Raspall-Chaure et al., 2006). A higher recurrence rate is reported in epilepsy cohort studies with longer follow-up. Stroink and colleagues reported CSE recurrence in 32% of children with epilepsy by 5 years, Berg and colleagues 32% by 8 years, and Sillanpaa and Shinnar 56% by 23 years of follow-up (Berg et al., 2004a; Sillanpaa and Shinnar, 2002; Stroink et al., 2007). The highest recurrence is reported in those with remote symptomatic (44%) and progressive symptomatic (67%) aetiology (Raspall-Chaure et al., 2006; Shinnar et al., 1992; Berg et al., 1999b; Berg et al., 2004a; Chin et al., 2006; Maegaki et al., 2005; Shinnar et al., 2001b; Sillanpaa and Shinnar, 2002; Stroink et al., 2007; Verity et al., 1993). The risk of recurrence in children with pre-existing neurological abnormalities is 3 to 23 times that of children without the abnormalities (Chin et al., 2006; Shinnar et al., 1992). In addition, a history of previous CSE significantly increases the risk of recurrence during follow-up (Berg et al., 2004a; Stroink et al., 2007; Barnard and Wirrell, 1999).

The recurrence risk following PFS/FSE was reported to be 3.5% at 29 months follow-up and 9% at 35.2 months follow-up in two hospital-based studies (Maytal and Shinnar, 1990; Shinnar et al., 1992). While a higher recurrence rate (17% in 30 children, 95% CI 7-34) within a year of PFS was reported in a prospective population-based study from north London, no recurrences were observed in 15 children (95% CI 0-20%) with PFS at 5 to 10 years follow-up in another UK population-based study (Chin et al., 2006; Verity et al., 1993). Although both studies were population-based, in the study by Verity et al., seizure details were collected retrospectively from parental questionnaires and medical records, and therefore potential recall bias as well as a smaller sample size with wide 95% CI could be the reasons for low point estimate of recurrence after PFS in that study. The recurrence risk is highest during the first year after the episode of CSE, but the risk continues beyond this period (Raspall-Chaure et al., 2007; Hesdorffer et al., 2007; Berg et al., 2004a; Chin et al., 2006; Maytal and Shinnar, 1990; Shinnar et al., 1992; Sillanpaa and Shinnar, 2002; Stroink et al., 2007).

2.3.4 Motor, cognitive, and behavioural outcomes

In addition to epilepsy, motor deficits, cognitive impairment, and behavioural problems are associated with childhood CSE, but current knowledge on the incidence of and specific risk factors for each of these adverse outcomes is limited. Part of this may reflect the challenges in determining outcomes without developmental data prior to the

episode of CSE. For example, subtle pre-existing neurodevelopmental problems in young children may go unnoticed by their parents and underreported at CSE presentation. Consequently, it would be difficult to determine whether any subsequently recognized developmental problems are sequelae or predate CSE episode. To address this, an ideal study in humans would require formal neuropsychological testing in a large population of children under the age of 5 (when CSE is most common) and compare the subsequent development in those who experience CSE against those do not. However, this would be extremely expensive since it would require large numbers taking attrition into account. In addition, given the young ages at onset and the different types of neuropsychological measures that would be appropriate at different ages, even this ideal approach may not provide a definitive answer.

In a major retrospective hospital-based review of childhood CSE, Aicardi and Chevrie reported a generally poor outlook, with morbidity in excess of 50% in 239 children presenting with an episode of CSE between 1961 and 1968 (Aicardi and Chevrie, 1970). However, only children with seizures lasting >60 minutes were included in that review and most were assessed retrospectively several years after CSE, thus skewing their population towards bad outcomes. Data from recent studies suggest more favourable outcomes (Table 2.3 & Table 2.4).

Most studies do not report motor and cognitive impairments separately and it is often difficult to determine whether all reported impairments are incident or predate the CSE episode (Raspall-Chaure et al., 2006). The risk of neurological sequelae within 5 to 10 years after CSE due to all causes ranges between 14% and 37%, with most high quality studies reporting rates of less than 15% (Table 2.3) (Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Kang et al., 2005; Kwong et al., 1995; Lacroix et al., 1994; Maegaki et al., 2005; Maytal et al., 1989; Prins et al., 2014; Tabarki et al., 2001; Verity et al., 1993). However, estimates outside this range were reported in a few studies. In a retrospective hospital-based study from the UK, although 6/70 (9%) previously neurologically normal children demonstrated neurological sequelae on discharge, the symptoms resolved in 5 and persistent sequelae were seen in only 1 (1.4%) child at follow-up 1.2 years after CSE (Hussain et al., 2007). On the contrary, a prospective hospital-based study on a selective population of infants presenting with seizures in first year of life reported neurological sequelae in 41/64 (64%) after 5-10 years follow-up (Cavazzuti and Lalla, 1984). However, the proportion of children with neurological

abnormalities before CSE is not stated and therefore it is possible that the true incidence may be lower than reported.

Prospective studies have shown negligible morbidity following PFS/FSE (Table 2.4) (Maytal and Shinnar, 1990; Shinnar et al., 2001b; Verity et al., 1993; Maytal et al., 1989; Ellenberg and Nelson, 1978). A relatively high (9% to 21%) incidence of cognitive impairment after FSE is reported in a few studies, but the impairments were mild in most children (Eriksson and Koivikko, 1997; Esch et al., 1996; Lewis et al., 2014; Tabarki et al., 2001). The neurological morbidity following unprovoked CSE is less than 10% (Raspall-Chaure et al., 2006; Maytal et al., 1989; Eriksson and Koivikko, 1997; Awaya et al., 1992). The poorest outcome is reported following acute symptomatic CSE, particularly for CNS infections, with neurological sequelae in more than 20% (Maytal et al., 1989; Eriksson and Koivikko, 1997; Sadarangani et al., 2008; Barnard and Wirrell, 1999; Maegaki et al., 2005; Aicardi and Chevrie, 1970; Kwong et al., 1995; Scholtes et al., 1996; Tabarki et al., 2001).

Reference	Study type/ design	Follow-up	Motor deficit	Cognitive impairment	All neurological sequelae
Prins et al., 2014	Population/ Pros	3-4y	11/33 (33%)	12/33 (36%)	12/33 (36%)
Barnard and Wirrell, 1999	Population/ Retro	4.4y	5/12 (42%)	6/17 (35%)	NR
Verity et al., 1993	Population/ Pros	5-10y	NR	2/10 (20%)	2/10 (20%)
Lacroix et al., 1994	Hospital/ Retro	12m	NR	NR	23/103 (23%)
Hussain et al., 2007	Hospital/ Retro	14.4m	1/70 (1%)	0/70 (0%)	1/70 (1%)
Kang et al., 2005	Hospital/ Retro	17m	NR	NR	62/189 (33%)
Kwong et al., 1995	Hospital/ Retro*	22.7m	5/28 (18%)	8/28 (29%)	9/28 (32%)
Maytal et al., 1989	Hospital/ Retro & Pros	27.5m 8.1m	14/113 (12%)	10/113 (9%)	17/113 (15%)
Kravljanac et al., 2011	Hospital/Pros	3y	NR	NR	39/274 (14%)
Eriksson and Koivikko, 1997	Hospital/ Retro	3.6y	5/46 (11%)	7/46 (15%)	9/46 (20%)
Tabarki et al., 2001	Hospital/ Retro$	4y	NR	43/117 (37%)	43/117 (37%)
Maegaki et al., 2005	Hospital/ Retro	5.35y	NR	NR	36/171 (21%)
Cavazzuti and Lalla, 1984	Hospital/ Pros@	5-10y	5/64 (8%)	41/64 (64%)	41/64 (64%)
Awaya et al., 1992	Hospital/ Pros£,+	13y	2/54 (4%)	4/54 (7%)	6/54 (11%)
Aicardi and Chevrie, 1970	Hospital/ Retro£	Unclear	47/198 (24%)	78/203 (38%)	~50%

NR Outcomes not separately reported, *60% were acute symptomatic CSE, £CSE defined as seizure duration of >60 minutes, + Symptomatic CSE not included, $Only children under 2y included, @Only infants under 1y included

Table 2.3 Incidence of neurological sequelae, other than epilepsy alone, after childhood CSE arranged according to study type/design

Reference	Study type/ design	Follow-up	Motor deficit	Cognitive impairment	All neurological sequelae
Verity et al., 1993	Population/ Pros	5-10y	NR	1/16 (6%)	1/16 (6%)
Shinnar et al., 2001b	Hospital/ Pros	1m	(0%)	(0%)	0/142 (0%)
Scott et al., 2003	Hospital/ Pros	5.5m	0/14 (0%)	1/14 (7%)	1/14 (7%)
Lewis et al., 2014	Hospital/ Pros	12m	NR	31/226 (14%)	31/226 (14%)
Hussain et al., 2007	Hospital/ Retro	15.6m	1/47 (2%)	0/47 (0%)	1/47 (2%)
Esch et al., 1996	Hospital/ Retro	21.8m	5/57 (9%)§	12/57 (21%)§	12/57 (21%)§
Maytal et al., 1989	Hospital/ Retro & Pros	27.5m 8.6m	0%	0%	0/36 (0%)
Maytal and Shinnar, 1990	Hospital/ Retro & Pros	3.4y 2.7y	0%	0%	0/35 (0%)
Eriksson and Koivikko, 1997	Hospital/ Retro	3.6y	1/24 (4%)	2/23 (9%)	3/24 (12.5%)
Tabarki et al., 2001	Hospital/ Retro*	4y	NR	10/56 (18%)	10/56 (18%)

NR Outcomes not separately reported, *Only children under 2y included, §53% received prophylactic phenobarbital therapy for a mean of 18 months after CSE

Table 2.4 Incidence of neurological sequelae, other than epilepsy alone, after PFS/FSE arranged according to study type/design

In almost all studies, cognitive outcome is determined either by no formal neurocognitive testing, or only global measures such as full-scale intelligence quotient (FSIQ) are used. While such assessments test for global neurocognitive difficulties, they are not designed to identify specific problems such as memory, executive function, language development etc. As previously discussed, the hippocampal formation seems to be particularly susceptible to CSE-induced neuronal damage, and therefore it is possible that prolonged seizures may selectively affect hippocampal-dependent functions such as memory. Long-term memory impairments have been reported following hyperthermia-induced prolonged seizures in previously normal animal models (Dube et al. 2009). Similarly, in a longitudinal study investigating hippocampal-dependent memory in children following PFS, Martinos et al. observed recognition memory impairments that persisted one year after PFS (Martinos et al., 2012). The memory impairment was independent of the age at PFS, seizure duration and the cognitive ability, but correlated with hippocampal volumes. Interestingly, in a separate study, the same group also observed that although the global neurocognitive scores for the PFS group were within the normal range for age, their performance was significantly worse compared to healthy controls (Martinos et al., 2013). Similar findings were reported by Roy et al. who noted that the global, performance, and eye-hand coordination scores were within the normal range for children with a single PFS, but their performance was significantly worse compared to healthy controls (Roy et al., 2011). Seizure-related factors such as CSE duration had no effect on outcomes in both studies. These data suggest that prolonged seizures may be associated with cognitive and memory impairments in previously healthy children, and that the impairments may not be transient. However, as previously discussed, it is difficult to determine whether these impairments are a direct consequence of CSE, or were pre-existing but only recognized after CSE.

Very few studies report behavioural and psychiatric morbidity following childhood CSE separate from other neurological outcomes. Behavioural problems have been reported in less than 5% of children following CSE (Eriksson and Koivikko, 1997; Esch et al., 1996; Scott et al., 2003). Although Barnard & Wirrell reported behavioural problems in 15 of 37 (41%) children on whom information was available, it is not clear how many of them had the problems prior to CSE episode (Barnard and Wirrell, 1999). In addition, the authors do not report the severity of behavioural symptoms and the prevalence of psychiatric diagnoses.

As with epilepsy, current data on the risk factors associated with neurological sequelae after CSE is conflicting. While an increased risk of neurological sequelae with longer seizure duration (particularly >120 minutes) was observed in some retrospective hospital-based studies, a significant association was not seen in most prospective and population-based studies (Dunn, 1988; Eriksson and Koivikko, 1997; Esch et al., 1996; Hayakawa et al., 1979; Hussain et al., 2007; Kang et al., 2005; Kravljanac et al., 2011; Lacroix et al., 1994; Maegaki et al., 2005; Maegaki et al., 2015; Maytal et al., 1989; Nelson and Ellenberg, 1978; Viani et al., 1987; Barnard and Wirrell, 1999; Verity, 1998; Verity et al., 1993). However, as seizure duration of >60-120 minutes is often seen in children with acute symptomatic CSE, it is difficult to separate the effect of seizure duration from that due to the acute insult (Eriksson and Koivikko, 1997; Kim et al., 2001b; Kwong et al., 1995; Sahin et al., 2001; Singhi et al., 2002; Tabarki et al., 2001). Similarly, young age at CSE (<1 year), presence of neurological abnormalities before CSE, and CSE characteristics such as focal-onset and continuous CSE were found to influence outcome in some studies, but not in others (Dunn, 1988; Eriksson and Koivikko, 1997; Esch et al., 1996; Hayakawa et al., 1979; Hussain et al., 2007; Kang et al., 2005; Kravljanac et al., 2011; Lacroix et al., 1994; Maegaki et al., 2005; Maegaki et al., 2015; Maytal et al., 1989; Nelson and Ellenberg, 1978; Viani et al., 1987; Barnard and Wirrell, 1999; Tabarki et al., 2001). Thus, the heterogeneity of current data makes it difficult to determine the additional risk attributable to CSE characteristics. In order to generate comparable data, it is essential that future studies use similar protocols with clear outcome definitions and standardised assessment for neurologic sequelae (e.g. formal cognitive testing).

2.3.5 Educational outcomes

Educational outcomes following CSE have been reported in only a few studies. While 43% of 35 school-aged children had special educational needs at a mean follow-up of 53 months after CSE in a retrospective population-based study from Canada, 24% of 29 children had special educational needs 5-10 years after CSE in a prospective population-based study from the UK (Barnard and Wirrell, 1999; Verity et al., 1993). Between 12 and 13% had special educational needs after PFS in two prospective long-term follow-up studies (Tarkka et al., 2003; Verity et al., 1993). Prospective cohort studies in children with new onset seizures and uncomplicated epilepsy report similar educational outcomes in those with and without CSE, thus suggesting CSE may not itself have additional adverse impact on learning (Sillanpaa and Shinnar, 2002; Camfield and Camfield, 2012; Sogawa et al., 2010).

2.4 Neuroimaging findings after childhood CSE

As discussed in chapter 1, current evidence suggests that children may acutely develop hippocampal injury following CSE, but whether or not the hippocampal changes evolve over a longer-term period and progress to MTS is uncertain. In addition to conventional qualitative imaging, quantitative methods such as hippocampal volumetry and diffusion imaging are being increasingly used to detect subtle changes after CSE, and particularly to investigate the relationship between PFS/FSE and MTS.

2.4.1 Structural imaging

Structural neuroimaging abnormalities are reported in about 30% of children presenting with an episode of CSE (Singh et al., 2010; Yoong et al., 2012). In a prospective population-based study from north London, clinically significant MRI abnormalities were found in 16.2% of 80 children within 6 weeks of CSE episode (Yoong et al., 2012). All major abnormalities were seen in children with acute and remote symptomatic CSE. Presence of neurological abnormalities, non-PFS aetiology and continuous CSE were identified as predictors of abnormalities on neuroimaging. Radiological features of MTS were reported in 0-4% on initial imaging after CSE, but only in children with remote symptomatic aetiology (Singh et al., 2010; Yoong et al., 2012; Scott et al., 2002).

Clinically significant neuroimaging abnormalities are not frequently seen in children with PFS. Clinically significant MRI abnormalities were not seen in any child within 5 days (0/21) or within 6 weeks (0/33) of PFS episode in two studies from north London (Scott et al., 2002; Yoong et al., 2012). In contrast, definite hippocampal abnormalities were reported in 15.7% of 191 children within a week of FSE episode in The Consequences of Prolonged Febrile Seizures in Childhood (FEBSTAT) study (Shinnar et al., 2012). However, the reported hippocampal abnormality was either an isolated T2 signal increase or a developmental abnormality in most, and therefore the abnormalities are of unclear clinical significance. MRI evidence of MTS was seen in 2 (1%) children; a 5-year-old with autism but no previous seizures and an 18-month-old normal child with a history of prior simple febrile seizure. Thus, less than 1% of children with PFS/FSE show MRI features of MTS on acute imaging (Scott et al., 2002; Yoong et al., 2012; Shinnar et al., 2012).

The FEBSTAT study also reported a higher prevalence of developmental abnormalities in the hippocampus in children with FSE compared with simple febrile seizures (p=0.0097) (Shinnar et al., 2012). Hippocampal malrotation (HIMAL) was the most common abnormality and was present in 7.9% of FSE and 2.1% of controls (p=0.06). Interestingly, HIMAL was left-sided in the majority (87%), and there were no cases of exclusively right-sided HIMAL. Of 33 children with PFS, 1 (3%) child met full criteria for unilateral HIMAL in the STEPIN study (Yoong et al., 2012). The clinical significance of HIMAL or incomplete hippocampal inversion (IHI) is however still debated (Bajic et al., 2009; Barsi et al., 2000; Gamss et al., 2009; Shinnar et al., 2012). While some authors report HIMAL prevalence of up to 19% in non-epileptic population and question its clinical significance, this view is challenged by others reporting a low prevalence of HIMAL in control population and a higher prevalence in those with seizures/epilepsy and FSE (Bajic et al., 2009; Barsi et al., 2000; Gamss et al., 2009; Raininko and Bajic, 2010; Shinnar et al., 2012). Differences in the criteria used for HIMAL diagnosis appears to be an important reason for the reported inconsistency in its prevalence. Whether HIMAL/IHI predisposes the brain to seizures, or increases the susceptibility to seizure-induced brain injury, or is just an incidental finding is yet uncertain.

There is limited data on prevalence of MTS beyond 6 weeks after PFS/FSE. In the two studies from north London, none of the children with PFS developed MTS at follow-up 6 to 12 months later (Scott et al., 2003; Yoong et al., 2013a). In contrast, 9/226 (4%) children developed MTS between the FSE episode and follow-up imaging after about 12 months in the FEBSTAT study (Lewis et al., 2014). However, of these 9 children with MTS, 2 had major extra-hippocampal structural abnormalities and developmental delay at baseline, and 3 others had subtle pre-existing hippocampal abnormalities. Thus only 4 (1.8%) children without any previous hippocampal abnormalities developed MTS within a year after PFS. Interestingly, development of MTS during follow-up was seen only in children with abnormal hippocampal T2 signal on initial imaging, indicating abnormal hippocampal signal on initial MRI as a potential risk factor for future development of MTS. Similar correlation between the presence of abnormal hippocampal signal within 72 hours of FSE and subsequent development of MTS was also observed in a previous study (Provenzale et al., 2008). In contrast to the FEBSTAT results, none of 24 children with PFS demonstrated radiological features of MTS in a prospective study at 12.5 years follow-up (Tarkka et al., 2003). Thus, current data suggest that the risk of developing MTS after PFS/FSE is generally low, but the risk may be higher in a subgroup showing hippocampal T2 signal abnormalities on acute imaging.

2.4.2 Quantitative imaging

Quantitative MRI methods such as volumetry and diffusion imaging are useful in investigating subtle hippocampal abnormalities and to study longitudinal evolution of changes following CSE.

2.4.2.1 Acute hippocampal changes

Several studies have consistently reported acute hippocampal abnormalities, including increased hippocampal volumes and right-left volume asymmetry, suggestive of transient oedema within 5-7 days of an episode of PFS/FSE (VanLandingham et al., 1998; Shinnar et al., 2012; Scott et al., 2002; Scott et al., 2006; Natsume et al., 2007). In addition to hippocampal volume increase, Scott et al., also observed prolongation of hippocampal T2 relaxation times in children investigated within 48 hours of an episode of PFS but normal T2 relaxation time in those investigated after 48 hours (Scott et al., 2002). In the same study they also demonstrated a reduction in apparent diffusion coefficient (ADC) in the hippocampus between the acute and follow-up time points, but only in children initially investigated within 48 hours of PFS and not in those investigated between 3 and 5 days (Scott et al., 2006). Together the findings are consistent with the presence of acute hippocampal vasogenic oedema after PFS/FSE that resolves within the time frame of 3 to 5 days.

2.4.2.2 Longitudinal hippocampal changes

Almost all longitudinal studies reporting the evolution of hippocampal changes are limited either by small sample size and short follow-up duration, or selection bias towards more severe cases.

Longitudinal studies report increased right-left hippocampal volume asymmetry 6 months to 6 years after PFS and FSE (Scott et al., 2003; Yoong et al., 2013a; Yoong, 2012; Provenzale et al., 2008; Farrow et al., 2006; Lewis et al., 2014). While increased hippocampal volume asymmetry was accompanied by radiological features of MTS in a minority, increased asymmetry was the only finding in the majority of subjects in all studies. Thus, the significance of isolated finding of increased hippocampal volume asymmetry is uncertain. Longer-term follow-up of these subjects could determine whether or not increased hippocampal volume asymmetry is an early marker of evolving MTS. In the only reported long-term follow-up study, Tarkka et al. found no significant difference in the average hippocampal volumes between the PFS and simple

febrile seizure group, but the PFS group had a lower mean right-left volume difference at 12.5 years follow-up (Tarkka et al., 2003). Although 3 of 24 (12.5%) subjects with PFS had a significantly smaller right hippocampus, none had radiological features of MTS and all were neurologically normal. Interestingly, the FEBSTAT study also reported significantly lower right hippocampal volume in the FSE group compared with controls, both at acute and follow-up MRI 12 months later (Lewis et al., 2014). The finding of reduced right hippocampal volume in subjects with PFS/FSE at baseline and at follow-up, and the predominance of hippocampal T2 hyperintensity on the right side reported in the FEBSTAT study may reflect pre-existing developmental abnormality of the right hippocampus that predispose to FSE and subsequent development of MTS (Shinnar et al., 2012; Roper et al., 2011; Janszky et al., 2003; Lewis et al., 2014).

Limited data on hippocampal volume changes following non-PFS CSE suggest that similar to those with PFS, children with non-PFS CSE also demonstrate abnormal hippocampal volume asymmetry during follow-up. Yoong et al. found hippocampal volume loss in 10/34 (29%) children 6 to 12 months after non-PFS CSE (Yoong et al., 2013a). Similarly, Grunewald and colleagues reported progressive hippocampal volume asymmetry in over half of subjects 6.5 years after first febrile or afebrile CSE (Grunewald et al., 2001; Farrow et al., 2006). However, the results of this study could potentially be biased due to (1) a relatively small sample size, (2) high prevalence of extra-hippocampal imaging abnormalities, and (3) considerable attrition with follow-up imaging performed in only 8 out of 17 subjects. Thus, there is very limited published data on hippocampal volume changes following non-PFS CSE.

2.4.2.3 Extra-hippocampal imaging changes

Although the hippocampus has been the primary focus in the majority of studies investigating the consequences of prolonged seizures, acute seizure-related injury has also been reported in extra-hippocampal structures such as the white matter (WM) (Kim et al., 2001a; Okamoto et al., 2006). However, there is hardly any published data on the outcome of extra-hippocampal imaging changes after CSE. In the only reported study, Yoong et al. used diffusion tensor imaging (DTI) to investigate longitudinal WM microstructural changes in the first year after PFS, and found widespread changes suggestive of reduced WM integrity at 1 and 6 months, but with apparent normalization 12 months post-PFS (Yoong et al., 2013b). While it is possible that the WM changes seen in this study may represent normalisation of WM microstructure 12 months after PFS-

induced injury, it is equally likely that the apparent normalisation could be a phase in the long-term evolution and not the end point. Longer-term follow-up studies are necessary for further clarification of evolution of WM microstructural changes after PFS.

2.5 Summary and rationale for the doctoral project

It is evident from this review that despite considerable interest and substantial basic science and clinical research on the topic, the data are still insufficient, and even confusing at times. There is wide variability between studies in the reported rates of mortality and morbidity associated with childhood CSE, and data on risk factors for adverse outcomes is inconsistent. This is primarily due to heterogeneity in study methodology (design/type), study population, definition of CSE and adverse outcomes, and the length of follow-up. This review also highlights important methodological deficiencies that can affect reported outcomes such as unclear definitions for outcome variables, lack of standardised testing to determine cognitive impairment, potential selection bias, and lack of dealing with missing data due to attrition.

Understanding the natural history and prognosis after childhood CSE requires reliable data on estimates of adverse outcomes from an unselected population after sufficiently long follow-up period. Longer duration of follow-up is particularly relevant in the context of childhood CSE as retrospective data suggest that some adverse outcomes such as MTS and TLE may manifest after a latency period of several years and therefore could potentially be missed in studies with follow-up shorter than the latency period. Although the long-term clinical prognosis after childhood CSE is reported in a few studies, the results are focussed on the risk of epilepsy after PFS and lack details on neurological sequelae other than epilepsy, especially for CSE groups other than PFS, and the analyses limited due to small sample size. In addition, while the immediate structural consequences after childhood CSE are well described, data on the longer-term evolution of these changes is limited. The above highlighted gaps in current knowledge and methodological limitations of published studies need to be addressed in future studies investigating outcomes after childhood CSE.

Our group conducted the largest reported epidemiological study addressing CSE in a wholly paediatric population, the North London Convulsive Status Epilepticus in Childhood Surveillance Study (NLSTEPSS), between May 2002 and April 2004 (Chin et al., 2006). This prospective population-based study had high ascertainment and

collected detailed clinical and socio-demographic data. We therefore are in a unique position to investigate this cohort and provide population-based estimates of longer-term outcomes after childhood CSE.

The overall aim of this doctoral project, the Status Epilepticus in Childhood Outcomes study (STEPSOUT), is to estimate the risk of mortality and morbidity within 10 years after childhood CSE and identify predictors of adverse outcomes. Aside from estimating the mortality risk associated with childhood CSE, STEPSOUT endeavours to provide a comprehensive long-term clinical outlook for children by describing prevalence of epilepsy, MTS, CSE recurrence, motor disability, intellectual disability, behavioural problems, and educational difficulties within 10 years after CSE. In STEPSOUT, we will attempt to address current gaps in knowledge on outcomes after childhood CSE and methodological limitations of published studies, towards ensuring completeness and quality of our data. To avoid potential subjective bias, validated instruments will be used for testing memory and cognitive function, and standardized questionnaires to screen for behavioural problems. Further, to minimize potential selection bias due to attrition, missing data will be systematically addressed using appropriate statistical methods. Although this study is not designed to confirm or reject the PFS-MTS hypothesis, we will be able to determine the prevalence of neuroimaging abnormalities within 10 years after childhood CSE in an unselected population and provide novel data to inform the on-going debate. And finally, quantitative MRI analyses used in this study could potentially further our understanding of the microstructural changes underlying the development of MTS.

All neurocognitive and behavioural assessments undertaken in this project were performed by Dr Marina Martinos, clinical research psychologist, who will be analysing and reporting neurocognitive and behavioural outcome results separately. Hence, these data will not be dealt with in this thesis. The candidate will however use global intelligence scores to determine intellectual functioning for intellectual disability definition.

Specific aims of the studies reported in this thesis are:

1. To estimate the risk of death within 10 years after childhood CSE and investigate the risk factors associated with mortality

2. To describe morbidity associated with childhood CSE by estimating the prevalence of epilepsy, recurrent CSE, motor and cognitive disability, psychiatric disorders, and educational difficulties at follow-up

3. To estimate the incidence and types of epilepsy, motor and cognitive disability within 10 years after childhood CSE

4. To examine the association between clinical and sociodemographic factors at CSE presentation and incident adverse outcomes

5. To estimate the prevalence and characterize the spectrum of structural brain abnormalities, MTS in particular, within 10 years after childhood CSE

6. To investigate whether hippocampal volumes and diffusion properties in CSE subjects are different from age-appropriate healthy controls

7. To investigate white matter microstructural properties within 10 years after PFS using diffusion MR imaging compared with age-appropriate healthy controls

CHAPTER 3 METHODS

3.1 Background to STEPSOUT

3.1.1 NLSTEPSS

STEPSOUT entails follow-up of the inception cohort identified from the North London Convulsive Status Epilepticus in Childhood Surveillance Study (NLSTEPSS), a prospective population-based study (Chin et al., 2006). Between May 1, 2002, and April 30, 2004, children aged between 29 days and 15 years with episodes of CSE and who lived in north London were enrolled into the study through a clinical network that covered the target population. Multiple source reporting was employed to maximise ascertainment. The clinical and demographic data were obtained from anonymised copies of a standardised admission proforma. Additional information was obtained from accident and emergency, ambulance and intensive care records, and from direct interview with the involved medical professionals and parents. The study described the incidence, aetiology, clinical characteristics, acute treatment, and mortality within 28 days of an episode of CSE (Chin et al., 2006; Chin et al., 2008; Chin et al., 2009).

3.1.2 Brief summary of NLSTEPSS results

226 children were enrolled into NLSTEPSS, of whom 176 had first-ever episodes of CSE and 50 had at least one previous episode. The crude incidence of CSE was 14.5 per 100,000 per year (95% CI 11.5-17.6), and ascertainment-adjusted incidence was 17-23 per 100,000 per year (Chin et al., 2006). The incidence of CSE was highest in children younger than 1 year compared with all the other age groups, and this was particularly notable in the acute symptomatic group. 56% of children with first-ever episodes of CSE were previously neurologically healthy (normal neurodevelopment, no history of epilepsy, and no neurological deficits). The incidence, aetiological classification, and the characteristics of first-ever episodes of CSE are presented in Table 3.1.

	Age (years)				Age-adjusted incidence* (95% CI)
	<1 number (incidence)*	1–4 number (incidence)*	5–15 number (incidence)*	0–15 number (incidence, 95% CI)*	
Cause					
PFS	15 (18·1)·	41 (12·7)	0 (0·0)	56 (4·6, 2·8 to 6·4)	4·1 (3·7 to 4·8)
Acute symptomatic	14 (16·9)	8 (2·5)	7 (0·9)	30 (2·5, 1·3 to 3·6)	2·2 (1·9 to 2·5)
Remote symptomatic	4 (4·8)	12 (3·7)	13 (1·6)	29 (2·4, 1·2 to 3·6)	2·3 (2·0 to 2·6)
Acute on remote	5 (6·0)	17 (5·3)	6 (0·7)	28 (2·3, 1·1 to 3·5)	2·1 (1·8 to 2·4)
Idiopathic	1 (1·2)	8 (2·5)	9 (1·1)	18 (1·5, 0·5 to 2·5)	1·4 (1·2 to 1·7)
Cryptogenic	2 (2·4)	0 (0·0)	1 (0·1)	3 (0·2, –0·1 to 0·6)	0·2 (0·1 to 0·3)
Unclassified	1 (1·2)	8 (2·5)	4 (0·5)	12 (1·1, 0·2 to 1·9)	1·0 (0·8 to 1·2)
Epilepsy					
No epilepsy	39 (47·1)	86 (26·7)	30 (3·7)	155 (12·8, 10·0 to 15·7)	11·7 (11·0 to 12·3)
Before CSE	3 (3·6)	2 (0·6)	8 (1·0)	13 (1·1, 0·2 to 1·9)	1·1 (0·9 to 1·2)
At CSE	0 (0·0)	6 (1·9)	0 (0·0)	6 (0·5, –0·1 to 1·1)	0·4 (0·3 to 0·6)
After CSE	0 (0·0)	0 (0·0)	2 (0·2)	2 (0·2, –0·2 to 0·5)	0·2 (0·1 to 0·3)
Sex					
Male	24 (56·6)	51 (31·2)	20 (4·9)	95 (15·4, 11·0 to 19·8)	14·0 (13·0 to 15·0)
Female	18 (44·5)	43 (27·1)	20 (5·1)	81 (13·6, 9·4 to 17·8)	12·5 (11·5 to 13·4)
Character					
Continuous	19 (22·9)	47 (14·6)	18 (2·2)	84 (6·9, 4·8 to 9·0)	6·3 (5·8 to 6·8)
Intermittent	23 (27·8)	47 (14·6)	22 (2·7)	92 (7·6, 5·4 to 9·8)	6·9 (6·4 to 7·4)
Final seizure type					
Focal	2 (2·4)	5 (1·6)	2 (0·2)	9 (0·7, 0·1 to 1·4)	0·7 (0·5 to 0·8)
Secondary generalised	14 (16·9)	26 (8·1)	12 (1·5)	52 (4·3, 2·6 to 5·9)	3·9 (3·6 to 4·3)
Primary generalised	26 (31·4)	63 (19·6)	26 (3·2)	115 (9·5, 7·0 to 12·0)	8·7 (8·2 to 9·3)
Motor type					
Tonic	8 (9·7)	10 (3·1)	5 (0·6)	23 (2·0, 0·8 to3·2)	1·6 (1·2 to 1·8)
Clonic	0 (0·0)	2 (0·6)	0 (0·0)	2 (0·2, –0·2 to 0·5)	0·1 (0·1 to 0·2)
Tonic-clonic	34 (41·1)	82 (25·5)	35 (4·3)	151 (12·5, 9·7 to 15·3)	11·5 (10·8 to 12·1)
Duration (min)					
30–60	17 (20·5)	36 (11·2)	17 (2·2)	71 (5·9, 3·9 to 7·8)	5·4 (4·9 to 5·8)
>60	25 (30·2)	58 (18·0)	22 (2·7)	105 (8·7, 6·3 to11·0)	7·3 (6·9 to 7·7)
All	42 (50·7)	94 (29·2)	31 (5·0)	176 (14·5, 11·5 to 17·6)	13·3 (12·6 to 14·0)

Table 3.1 Incidence, aetiological classification, and characteristics of first-ever episodes of childhood CSE in NLSTEPSS (Chin et al., 2006)

23 children with first-ever CSE (13%, 95% CI 9-19) had at least one recurrence during the follow-up with a median interval to first recurrence of 25 days (range 0-463). 18 children had their first recurrence within a year (1-year recurrence 16%, 95% CI 10-24). Children with a pre-existing neurological abnormality were 2.9 times (95% CI 1.01-8.45) more likely to have a recurrence within a year of their first CSE than previously neurologically healthy children. Of the 30 children with first-ever PFS, 5 (17%, 95% CI 7-34) had a recurrence within a year.

Seven children (3%, 95% CI 2-7) died within 28 days of the acute episode of CSE. Six had their first-ever episode of CSE (median age 1.1 years, 3 boys) and another boy aged 13.4 years had had a previous episode of CSE. Three children had acute bacterial meningitis, one had glutaric aciduria type 1, and three had undiagnosed progressive neurodegenerative conditions.

3.2 Definitions

3.2.1 CSE definition

As STEPSOUT study involves follow-up of NLSTEPSS cohort, this thesis is restricted to CSE.

Thus, for the purpose of this thesis, CSE is defined as:

"A tonic, clonic, or tonic-clonic seizure (continuous CSE), or two or more such seizures between which consciousness was not regained (intermittent CSE), which lasted for at least 30 minutes".

3.2.2 Aetiological classification of CSE

As the primary objective of the current thesis is to generate data on the prognosis and predictors of adverse outcomes after CSE, we opted to retain the original CSE classification used in NLSTEPSS (Chin et al., 2006; Chin et al., 2004).

CSE category	Definition
Prolonged febrile seizure	CSE in a previously neurologically normal child aged between 6 months and 5 years during a febrile (temperature >38˚C) illness, and in the absence of defined central nervous system (CNS) infection.
Acute symptomatic	CSE in a previously neurologically normal child, within a week of an identified acute neurological insult including CNS infection, head trauma, metabolic derangements, drug-related effects, hypoxia or anoxia, cerebrovascular disease.
Remote symptomatic	CSE in the absence of an identified acute insult but with a history of a pre-existing CNS abnormality more than 1 week previously.
Acute on remote symptomatic	CSE that occurred within a week of an acute neurological insult or febrile illness and occurred in a child with a history of previous neurological abnormality, including epilepsy. This category included children with cerebral palsy with a febrile illness not of CNS origin, and children with obstructed ventriculoperitoneal shunts for hydrocephalus. *Those previously classified as acute on remote symptomatic CSE are included in remote symptomatic CSE group for this thesis.*
Idiopathic epilepsy related	CSE that is not symptomatic (see above) and occurred in children with a previous diagnosis of idiopathic epilepsy or when the episode of CSE is the second unprovoked seizure that has led to a diagnosis of idiopathic epilepsy.
Cryptogenic epilepsy related	CSE that is not symptomatic (see above) and occurred in children with a previous diagnosis of cryptogenic epilepsy or when the episode of CSE is the second unprovoked seizure that has led to a diagnosis of cryptogenic epilepsy.
Unclassified	CSE that cannot be classified into any other group.

Table 3.2 Aetiological classification of childhood CSE (Adapted from Chin et al., 2006)

3.3 Recruitment procedures

3.3.1 Patient identification and recruitment

A flowchart depicting the recruitment process is presented in Appendix A.

STEPSOUT is a patient-specific cohort follow-up study of NLSTEPSS. We aimed to recruit all survivors (n=219) of the original 226 children ascertained during NLSTEPSS.

NLSTEPSS was a surveillance study, and the study subjects were recruited through local paediatricians from 21 hospitals in north London. Information on subject demographics, clinical details about CSE episode, medical history and developmental status prior to the CSE episode, clinical examination findings, results of investigations, and clinical outcome were collected for each child using a standardized proforma. All patient data were anonymised and shared with the central research team using a linked-anonymisation system and therefore no patient identifiable information was stored with the NLSTEPSS central research team. Each child was given a unique NLSTEPSS identification number that was shared between the central research team and local paediatricians to recall patient identifiable information. To facilitate possible follow up studies on the cohort of patients identified in NLSTEPSS, collaborators were asked to keep a record of study identification numbers of patients along with their demographic details.

Since its inception in 2002, the NLSTEPSS Collaborative Group has evolved and still remains active as the North Thames Paediatric Epilepsy Network (previously the North Central London Epilepsy Network), a collaborative network of paediatric neurologists, paediatricians, and other health professionals who have a responsibility for providing comprehensive care for children and young people with epilepsy in the North Thames region. The network remains active by providing a forum for discussion and collaborative work in paediatric epilepsy through regular clinical and educational meetings. Members of the network continue to collaborate in research studies and their support acknowledged in all resulting publications. In addition to NLSTEPSS, the network has successfully been used in subsequent epidemiological studies of childhood CSE and epilepsy in infancy (Eltze et al., 2013; Yoong, 2012).

As the central research team had no patient identifiable information and no prior contact with potential study subjects, recruitment for STEPSOUT was coordinated with the help of paediatricians from the local hospitals. The unique NLSTEPSS identification numbers shared between the central research team and local paediatricians were used to recall patient identifiable information stored at local hospitals. We envisaged the possibility that some children may have died during the period between the initial surveillance study and current recruitment. To ensure that deaths were accurately ascertained and to avoid contacting the families of children who have died, local

paediatricians were asked to review the hospital records of each child to check for deaths and inform the central research team. In addition, as the information on hospital records may not always be up-to-date, the collaborators confirmed the survival status for each child by checking demographic details on the national database (NHS Care Records Service, CRS) using their NHS numbers, a unique national ID for all persons utilizing the NHS. CRS is a national electronic record keeping service where patients' up-to-date demographic details and their general practitioner (GP) contact details are recorded. After confirming the survival status and before approaching the subjects, the local paediatricians sent a letter on behalf of the research team to each surviving child's GP asking if there was any reason (medical/social/other) for not contacting the family and also to confirm their up-to-date contact details. The local paediatricians then sent a letter of invitation to potential study subjects or their parents (if the subject was still a minor) on behalf of the research team, with written information about the study and consent forms, asking them to give written consent to participate in the study. A pre-paid, self-addressed envelope was included in the information pack for the participants to return the consent form to the central research team. All subjects and their parents were given the option to contact the research team and discuss their participation in the study, prior to consenting. Non-responders were sent reminders before labelling them as lost to follow-up (See flowchart in Appendix A).

The central research team was based at UCL Institute of Child Health (ICH) and Great Ormond Street Hospital for Children (GOSH), in London. Upon receiving the consent form, the candidate contacted the subjects and/or their parents to introduce the research team and carry out a brief telephone interview to establish the clinical status of the subject and suitability for performing assessments for the study (Appendix B). Following the telephone interview, the candidate sent a composite behaviour screening questionnaire for the parents to complete and return to the research team, and arranged an appointment for neurology and neurocognitive assessments and brain MRI at GOSH/ICH. If the participants were unwilling to undergo MRI scan, neurology and neurocognitive assessments were performed either at GOSH/ICH or, if preferred by participants, as a home visit. In addition, subjects identified to have potential behavioural problems on screening questionnaire were invited for an appointment with Professor Christopher Gillberg, consultant child and adolescent psychiatrist, for further assessment.

3.3.2 Tracing children lost-to-follow-up

Updating the patient contact details on CRS is dependent on GP practices regularly providing relevant information on the system. In situations where a response to the invitation letter was not received from the families, local paediatricians contacted the GPs named on CRS to confirm the most recent mailing address for the family. If this was different to the one to which the study invitation pack was sent, the invitation pack was resent. If contact details were not available through CRS or the GP, or if it was learnt that the child has moved overseas, the child was assigned lost-to-follow-up status.

3.3.3 Clinical information from hospital records

As part of the interview during neurology and neurocognitive assessment meeting, where appropriate, participants were asked whether they would consent for the research team to access their previous medical records, including investigation results such as neuroimaging data, from local hospitals. After obtaining consent, the candidate was able to collaborate with local paediatricians and, where available, gather additional clinical information to supplement data collected during the study and characterise the outcomes. Also, if a child had previously undergone neuroimaging for clinical indications, we obtained participant's consent to use the imaging data for our study.

3.4 Recruitment of controls

Age-appropriate healthy children with no known neurological or developmental problems were recruited as controls from various sources including, siblings of study subjects, personal contacts, and relatives of hospital and university employees (invited through e-mail lists and in-house advertisements) (Yoong, 2012; Weese-Mayer et al., 2001). In addition to the controls recruited directly for STEPSOUT, anonymised neurocognitive and brain MR imaging data from 40 age-appropriate controls recruited for *hypoxia/ischemia in children study* at ICH (Hypoxia-ischaemia in children: patterns of neuropathology and associated memory impairment GOSH/ICH. Ethics ref: 05/Q0502/88, R&D:03CN01) were used for analyses, as they are of similar age group and have had same neurocognitive assessments and MR imaging as in our study. The researchers for *hypoxia/ischemia in children study* had obtained consent from controls for sharing anonymised data with other research studies within the institute.

Written informed consent was obtained from all controls or their parents (if control was minor) recruited for STEPSOUT. All controls underwent same assessments as patients according to the study protocol.

All MRI scans performed were reviewed by an experienced neuroradiologist and any identified clinically significant structural abnormalities were immediately notified either to that child's paediatrician (if known) or to the GP (for children who did not have a named paediatrician). The results of neurocognitive testing were also sent to the child's GP to pass onto the parents.

3.5 Ethics and R&D

STEPSOUT was approved by the Institute of Child Health/ Great Ormond Street Hospital Research Ethics Committee, and the approval applied to all the sites involved in the research (REC reference: 08/H0713/88). The Institute of Child Health/ Great Ormond Street Hospital Research and Development department acted as the sponsor (ref: 07NR01), and the study was registered with local Research and Development departments at all participating hospitals.

No financial incentives were offered for participation in the study, except reimbursement of travel expenses as compensation for the cost of travelling to GOSH for assessments.

3.6 Clinical neurological assessment

The candidate performed all clinical neurological assessment on all participants under the supervision of either of two consultant paediatric neurologists (Richard Chin and Rod Scott), who were also his supervisors. All subjects and their parents were interviewed to obtain the following details using a structured proforma to ensure systematic and consistent enquiry: presence or absence of seizures/epilepsy (before/after CSE and at follow-up), description of seizure semiology and the clinical course of epilepsy, CSE recurrence, cognitive development, behavioural problems/psychiatric diagnoses, education and schooling, results of investigations, and any drug treatment or interventions (Appendix C). In addition, a structured neurological examination was performed and all normal and abnormal findings clearly documented.

In children with recurrent seizures, epilepsy diagnosis and classification of epilepsy syndromes was done using information collated from clinical assessment and available results of investigations. Investigations such as EEG and genetic testing were not included in the study protocol. However, any available results of investigations performed for clinical reasons were used to supplement data collected for the study.

3.7 Neurocognitive and behavioural assessment

Neurocognitive assessments and analysis of screening questionnaires data were performed by Dr Marina Martinos, clinical research psychologist, under the supervision of Dr Michelle de Haan and Professor Christopher Gillberg. Results from these assessments will be reported separately by Dr Martinos and therefore will not be presented in this thesis.

3.7.1 Screening questionnaires

The following questionnaires were used in the study to comprehensively screen for any behavioural problems, cognitive difficulties and memory impairment:

3.7.1.1 Behaviour screening questionnaire

The behaviour screening questionnaire used in this study is an adaptation of the questionnaire used in the Bergen Child study (Stormark et al., 2008). The questionnaire is made up of three main standardised, validated instruments; **1)** ***the Strengths and Difficulties Questionnaire (SDQ)***- to screen for emotional symptoms, conduct problems, hyperactivity/inattention, and peer relationship problems, **2)** ***the Autism Spectrum Screening Questionnaire (ASSQ)***- for assessing symptoms of autism spectrum disorder, and **3)** ***the Swanson, Nolan and Pelham (SNAP-IV) rating scale***- for assessing symptoms of Oppositional Defiant Disorder (ODD) and Attention-Deficit/Hyperactivity Disorder (ADHD) (Goodman, 2001; Swanson et al., 2001; Ehlers et al., 1999). Additionally, the questionnaire also includes scores for Obsessive Compulsive Disorder (OCD) and tic disorder.

3.7.1.2 Behaviour Rating Inventory of Executive Function (BRIEF)

The BRIEF is a standardised, validated screening instrument designed to assess executive functioning in children and adolescents (Gioia et al., 2000).

3.7.1.3 The Everyday Memory Questionnaire (EMQ)

EMQ has been shown to have acceptable correlation with formal memory tests, especially verbal memory (Sunderland et al., 1983; Drysdale et al., 2004). Our colleagues in Cognitive Neuroscience and Neuropsychiatry use a modified version of the questionnaire suitable for use in paediatric population and have collected normative data.

3.7.2 Neurocognitive assessments

3.7.2.1 The 4-subtest Wechsler Abbreviated Scale of Intelligence (WASI)

WASI (Wechsler, 1999) is used to obtain a brief measure of intelligence and provides scores for full-scale IQ as well as performance IQ and verbal IQ.

3.7.2.2 Children's Memory Scale (CMS)

CMS (Cohen, 1997) comprehensively assesses the integrity of memory functions in children and provides scores for immediate verbal memory, delayed verbal memory, general memory, immediate visual memory, and delayed visual memory.

3.7.3 Neuropsychiatry assessment

All subjects identified to have behavioural difficulties on the screening questionnaire were offered an appointment with Professor Christopher Gillberg, an experienced child and adolescent psychiatrist, for a neuropsychiatry assessment. The Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) classification was used for making psychiatric diagnoses in children with symptoms meeting diagnostic criteria.

3.8 Magnetic Resonance Imaging

3.8.1 Scanning protocol for patients and controls

MRI was performed with the subject awake or in a natural sleep and lying still in the scanner in almost all, with only three patients (two boys and one girl, age between 7.5 and 7.8 years, all with cognitive impairment) needing sedation. Sedation for MRI was performed by the GOSH clinical radiology sedation nursing team, using their sedation protocol, after assessing the child for safety and with parental written informed consent.

If the parents or the subject were unwilling to undergo neuroimaging for the study, or consented for an unsedated MRI scan but the child was uncooperative, we used their previous brain imaging data, if the scan was performed at least 5 years post-CSE and the images were of adequate quality and available for review by the research team. As GOSH is the referral children's hospital for north London, the majority of clinical scans were performed at GOSH. Scans performed in other hospitals were electronically transferred securely using the Picture Archiving and Communications System (PACS). For subjects who had had more than one MRI scan, data from the most recent scan was used.

3.8.2 MR image acquisition

MR imaging was carried out on an Avanto 1.5 Tesla whole body scanner (Siemens, Erlangen, Germany) for all children. A copy of the protocol used for image acquisition is presented in Appendix D.

An imaging protocol used for the evaluation of children with epilepsy and optimised for the visualisation of mesial temporal lobe structures was carried out. In addition to conventional MR sequences such as T1–weighted, T2–weighted, and fluid attenuated inversion recovery (FLAIR) in the axial and coronal planes, we acquired three-dimensional fast low angle shot (3D-FLASH) and diffusion-weighted sequences for quantitative techniques such as hippocampal volumetry and diffusion tensor imaging (DTI) to investigate abnormalities of hippocampal and extra-hippocampal tissue.

Echo-planar diffusion-weighted images were acquired for an isotropic set of 20 non-collinear directions, using a weighting factor of b=1000 s mm^{-2}, along with a T_2-weighted (b=0) volume. This protocol was repeated 3 times in a single scan session, and the data merged together without averaging. Forty-five contiguous axial slices of width 2.5 mm were imaged, using a field of view of 240 x 240 mm and 96 x 96 voxel acquisition matrix, for a final image resolution of 2.5 x 2.5 x 2.5 mm.

3.8.3 MRI processing pipeline

Raw DICOM files were downloaded from the MRI scanner, anonymised, and archived to CD. Files were transferred to an encrypted hard disc and stored using a unique random number generated by the scanner.

All MR imaging processing and quantitative analysis was performed on the Macintosh platform (Mac OS X v10.6.8). *TractoR* scripts (www.tractor-mri.org.uk) were used to sort the DICOM files into directories for each MRI sequence, convert the DICOM files into

NifTI and ANALYZE image formats for further analyses, and to change the orientation of 3D sequences for hippocampal volumetry.

Further processing of the diffusion datasets was performed using *TractoR* to generate individual measures of anisotropy and diffusivity (Clayden et al., 2011). Diffusion data were pre-processed to correct for eddy current-induced distortions using tools in the FSL package v5.0.2.1 (http://www.fmrib.ox.ac.uk/fsl). The brain was segmented using FSL's brain extraction tool, and diffusion tensors were calculated using least squares fitting. Individual measures of anisotropy (fractional anisotropy) and diffusivity (mean diffusivity, axial diffusivity, and radial diffusivity) were calculated and used to investigate hippocampal DTI changes and for tract-based spatial statistics (TBSS).

Further methodological details on MR imaging processing and analysis will be presented in relevant chapters.

3.9 Statistical analysis

Except where otherwise stated, all statistical analysis was performed using SPSS (IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY:IBM Corp.). All statistical tests were performed as two-tailed and $p<0.05$ was set as the cut-off for significance.

Missing data for clinical outcomes were dealt with using multiple imputation. Further methodological details missing data analysis are presented in chapter 5.

Having described the general methods used for the studies set forth in this thesis, the following chapters will advance the findings of each study comprising this overall project, with further explanation of the methods used for each individual section as required.

CHAPTER 4 MORTALITY WITHIN 10 YEARS AFTER CHILDHOOD CSE

4.1 Introduction

In chapter 2 we described how childhood CSE is associated with significant short-term mortality i.e., death during hospital admission or within first 30-60 days of an episode of CSE. However, prior to the current study, knowledge about the risk of, and factors associated with long-term mortality was limited. Reduction of mortality requires identification of such factors that may predict death so that strategies that minimize the risk of death can be developed.

High quality studies from resource-rich countries report short-term mortality of 2.7-5.2% after childhood CSE, whereas studies from resource-poor countries report much higher rates (7.1-23.2%) (Chin et al., 2006; DeLorenzo et al., 1996; Maegaki et al., 2005; Mah and Mah, 1999; Maytal et al., 1989; Siddiqui et al., 2008; Verity et al., 1993; Waterhouse et al., 1999; Molinero et al., 2009; Raspall-Chaure et al., 2006; Sadarangani et al., 2008; Asadi-Pooya and Poordast, 2005; Kravljanac et al., 2011). Limited data, mostly from retrospective studies, estimate longer-term mortality risk ranging between 5.4% and 23% within 5 to 10 years after childhood CSE (Barnard and Wirrell, 1999; Kwong et al., 1995; Lacroix et al., 1994; Tabarki et al., 2001; Prins et al., 2014; Logroscino et al., 1997; Logroscino et al., 2002; Verity et al., 1993). While demographic and CSE characteristics such as younger age at CSE, symptomatic aetiology, longer seizure duration, focal seizure onset, and continuous CSE have been associated with short-term mortality, whether any of these factors influence longer-term mortality risk after childhood CSE is yet to be determined (Raspall-Chaure et al., 2006; Sadarangani et al., 2008; Dunn, 1988; Kravljanac et al., 2011; Logroscino et al., 1997; Maegaki et al., 2005; Maytal et al., 1989; Scholtes et al., 1996; Siddiqui et al., 2008; Towne et al., 1994; Gulati et al., 2005; Hussain et al., 2007; Cavazzuti and Lalla, 1984; Chin et al., 2006; Tabarki et al., 2001).

The objectives of the study reported in this chapter are to (1) estimate the risk of death within 10 years after childhood CSE, (2) provide information on the immediate causes of death associated with childhood CSE, and (3) investigate clinical and sociodemographic factors associated with long-term mortality following childhood CSE.

4.2 Methods

4.2.1 Patient cohort and determination of survival status

Details about the general methods for subject identification for STEPSOUT and determination of their survival status were presented in chapter 3.

4.2.2 Determination of the cause of death

For the deceased children, clinical background data were obtained from the NLSTEPSS database, and, where available, additional clinical details gathered from their hospital medical records. Death certificates of all deceased children were obtained from the UK General Register Office (www.gro.gov.uk), with date of death and cause of death defined as stated on the certificates.

4.2.3 Statistical analysis

Case fatality was determined as the total number of deaths within the cohort following the CSE episode divided by the total number of children in the cohort on whom the survival information was available. The number of person-years (P-Y) of follow-up was calculated for all participants in the cohort. Mortality rate was estimated as the death rate per 1000 P-Y of follow-up for study subjects. Standardised mortality ratio (SMR) was calculated as the observed number of deaths in the study cohort divided by the expected number of deaths during the same time period in the age-matched population of north London. The 95% confidence interval (95% CI) of the SMR was calculated using a standard Poisson-based method. The data for calculating mortality in the reference population (population estimates from 2002 to 2009) was obtained from the UK Office for National Statistics (www.ons.gov.uk).

All statistical analyses for the study presented in this chapter were performed on SPSS version 19.0 (Chicago, Illinois) and StatXact version 4.0, using a complete case analysis approach. After initial descriptive analysis, Cox regression analysis was used to estimate survival following CSE and to investigate the predictors of mortality. Difference in proportions and Mann-Whitney U test were used for comparing the characteristics of follow-up cohort with those lost to follow-up.

To investigate the clinical and demographic factors associated with increased mortality, the subjects were grouped on the basis of presence or absence of clinically significant neurological impairment (motor and/or cognitive) prior to CSE. Children with pre-existing clinically significant neurological impairments, with or without epilepsy, at the time of CSE (i.e. those with remote symptomatic and cryptogenic CSE) were incorporated into group 1. Those who had no prior neurological impairments or had epilepsy without additional neurological impairments (i.e. those with PFS, acute symptomatic, idiopathic, and unclassified CSE) were incorporated into group 2.

Cox regression analyses were performed to identify any factors significantly associated with death as the outcome and clinical and sociodemographic variables as risk factors. Factors significantly associated with the outcome on univariable analysis were then entered in the multivariable models to test their independent association. Any factors not reaching a significance level of 10% ($p \geq 0.1$) on univariable analysis were subsequently included in the final multivariable model to identify any factors that were associated with the outcome only after adjustment for other factors. The sociodemographic factors examined were, age at CSE, gender, ethnicity, and Index of Multiple Deprivation 2004 (IMD 2004) scores as indicator of socioeconomic status (Chin et al., 2009). The higher the IMD score, the more socioeconomically deprived are the subjects. The clinical factors investigated were, first-ever CSE (incident) versus a past history of CSE, duration of CSE (30-60 minutes vs. >60 minutes), intermittent vs. continuous CSE, type of CSE (focal- vs. generalised-onset), and presence or absence of clinically significant neurological impairment prior to CSE (group 1 vs. group 2).

4.3 Results

4.3.1 Patient demographics

Of the 226 children ascertained during NLSTEPSS, 20 were lost to follow-up, and therefore information on the survival status was available for 206 (91%, 95% CI 87-94). The clinical and demographic characteristics of the 20 children lost to follow-up were similar to those on whom survival data were available with the exception that those lost to follow-up were less socioeconomically deprived (median IMD 2004 score of 35.66 in follow-up and 28.72 in lost to follow-up, p=0.048) (Table 4.1).

Of the 206 subjects on whom survival information was available, 52% were male and 57% had no clinically significant neurological impairment prior to CSE. In most, the episode of CSE at inclusion in NLSTEPSS was their first-ever (incident) CSE (78%), was more than 60 minutes in duration (61%), and was of generalised seizure type (63%) (Table 4.1). The median duration of follow-up was 93.7 months (range 0.03-104.3).

4.3.2 Mortality rates

23 children (12 male) died within 10 years of their episode of CSE (case fatality=11%, 95% CI 7.5-16.2; mortality rate=15.4/1000 PY, 95% CI 10.3-23.0). 7 children died during the hospital stay for their episode of CSE (within 30 days) (short-term case fatality=3.1%, 95% CI 1.5-6.2), and the remaining 16 during follow-up. For the 30-day survivors, the overall median time to death following an episode of CSE was 52.8 months (range 1.0-88.8). 3 of the 7 children who died during the acute period had acute symptomatic CSE (acute bacterial meningitis, ABM) and the remaining 4 had remote symptomatic CSE (progressive neurological disorders). The aetiology of CSE in those who died during follow-up were remote symptomatic (n=12), cryptogenic (n=3) and acute symptomatic (n=1). There were no deaths in children who had PFS or idiopathic CSE.

The SMR was 46.1 (95% CI 28.6-70.5) for the whole cohort compared with the reference population of north London, and was 30.8 (95% CI 16.8-51.6) for 30-day survivors (Table 4.2). In children with pre-existing clinically significant neurological impairments (group 1), the SMR was 91.4 (95% CI 53.2-146.3), and in those with no pre-existing impairments (group 2), it was 14.8 (95% CI 4.0-37.9). The mortality in 30-day survivors with no pre-existing impairments was not significantly different from the reference population (SMR 3.7, 95% CI 0.1-20.6).

Characteristic	Followed-up (%) *n* = 206	Lost to follow-up (%) *n*= 20	Difference in proportion (*95% CI*)
Gender			
Male	107 (52)	10 (50)	0.02 (-0.19, 0.23)
Female	99 (48)	10 (50)	
Median age at CSE, months (range)	32.3 (1-192)	52 (6-177)	0.076+
Neurological impairment prior to CSE			
Present (Group 1)	89 (43)	8 (40)	0.03 (-0.19, 0.22)
Absent (Group 2)	117 (57)	12 (60)	
CSE category			
Prolonged febrile seizures	45 (22)	5 (25)	-0.03 (-0.25, 0.12)
Acute symptomatic	35 (17)	1 (5)	0.12 (-0.07, 0.19)
Idiopathic	23 (11)	5 (25)	-0.14 (-0.36, 0.01)
Cryptogenic	9 (4.5)	0 (0)	0.04 (-0.2, 0.08)
Remote symptomatic	80 (39)	8 (40)	-0.01 (-0.23, 0.18)
Unclassified	14 (6.5)	1 (5)	0.02 (-0.17, 0.07)
CSE occurrence			
First-ever (Incident)	160 (78)	15 (75)	0.03 (-0.12, 0.25)
History of CSE (Recurrence)	46 (22)	5 (25)	
Seizure duration			
30-60 min	81 (39)	12 (60)	-0.2 (-0.4, 0.02)
>60 min	125 (61)	8 (40)	
Seizure character			
Continuous	102 (49.5)	9 (45)	0.05 (-0.17, 0.25)
Intermittent	104 (50.5)	11 (55)	
Seizure type			
Focal-onset	76 (37)	7 (35)	0.02 (-0.21, 0.20)
Generalized-onset	130 (63)	13 (65)	-0.02 (-0.20, 0.20)
Ethnicity			
White	76 (37)	9 (45)	-0.08 (-0.30, 0.12)
Black	36 (17)	6 (30)	-0.119 (-0.35, 0.04)
Asian	64 (31)	5 (25)	0.06 (-0.16, 0.21)
Other	30 (15)	0 (0)	0.15 (-0.02, 0.2)
Socioeconomic status (Median IMD 2004 scores)	35.66	28.72	0.048+

+ *p* value on Mann-Whitney U test

Table 4.1 Clinical and sociodemographic characteristics of the follow-up cohort and those lost to follow-up

	Follow-up (person-years)	Mortality for the whole cohort			Mortality for 30-day survivors		
		Observed*	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)
Children with neurological impairment prior to CSE	610.9	17	0.186	91.4 (53.2- 146.3)	13	0.186	69.9 (37.2-119.5)
Children without neurological impairment prior to CSE	884.9	4	0.27	14.8 (4.0- 37.9)	1	0.27	3.7 (0.1- 20.6)
Overall	1495.8	21	0.455	46.1 (28.6-70.5)	14	0.455	30.8 (16.8-51.6)

* 2 deaths that occurred in 2010 were not included in SMR calculation as the vital statistics for the reference population for the year 2010-11 were not available at the time of analysis

Table 4.2 Mortality following childhood CSE

4.3.3 Factors associated with mortality

On Cox regression, factors that significantly predicted mortality on univariable analysis were presence of clinically significant neurological impairment prior to CSE (Hazard Ratio, HR, 6.7 95% CI 2.3-19.7, *p* 0.001) and previous history of CSE at the time of presentation (HR 2.7, 95% CI 1.18-6.2, *p* 0.018). On multivariable analysis, the only statistically significant predictor of subsequent mortality was presence of clinically significant neurological impairment prior to CSE (Table 4.3).

Variable	**Univariable analysis**		**Multivariable analysis**	
	Hazard ratio (95% CI)	***p* value**	**Hazard ratio (95% CI)**	***p* value**
Pre-existing clinically significant neurological impairment at CSE	6.7 (2.3, 19.7)	**0.001***	6.7 (2.3, 19.7)	**0.001***
Gender (male v female)	0.99 (0.43, 2.24)	0.98	-	-
Ethnicity (white, black, Asian, other)	1.05 (0.43-2.6)	0.51	-	-
Socioeconomic status (IMD 2004)+	1.0 (0.98, 1.04)	0.62	-	-
Age at CSE (months)	1.02 (0.92, 1.14)	0.69	-	-
Previous CSE at presentation (recurrence v incident)	2.7 (1.18, 6.2)	**0.018***	1.8 (0.77, 4.3)	0.17
CSE Duration (30-60 min v >60min)	1.91 (0.75, 4.8)	0.17	-	-
CSE Character (continuous v intermittent)	1.08 (0.47, 2.44)	0.86	-	-
CSE Type (focal- v generalized-onset)	1.36 (0.56, 3.3)	0.50	-	-

+IMD- Index of multiple deprivation

Table 4.3 Regression analysis for predictors of death within 10 years after childhood CSE

4.3.3 Interval between CSE episode and death

Among the 30-day survivors, deaths were observed from 1 month after the CSE episode in those with pre-existing neurological impairments, and occurred at regular intervals throughout the follow-up. In contrast, the only death in the child with acute symptomatic CSE occurred 5 years following the CSE episode (Figure 4.1). The survival estimates for individual CSE categories show that the life expectancy is most severely affected in children with an underlying progressive neurological disorder with almost 80% of them not surviving for 20 months after the episode of CSE (Figure 4.2). This is followed by cryptogenic CSE and remote symptomatic CSE. Most deaths in acute symptomatic CSE occurred within 30 days of an episode of CSE.

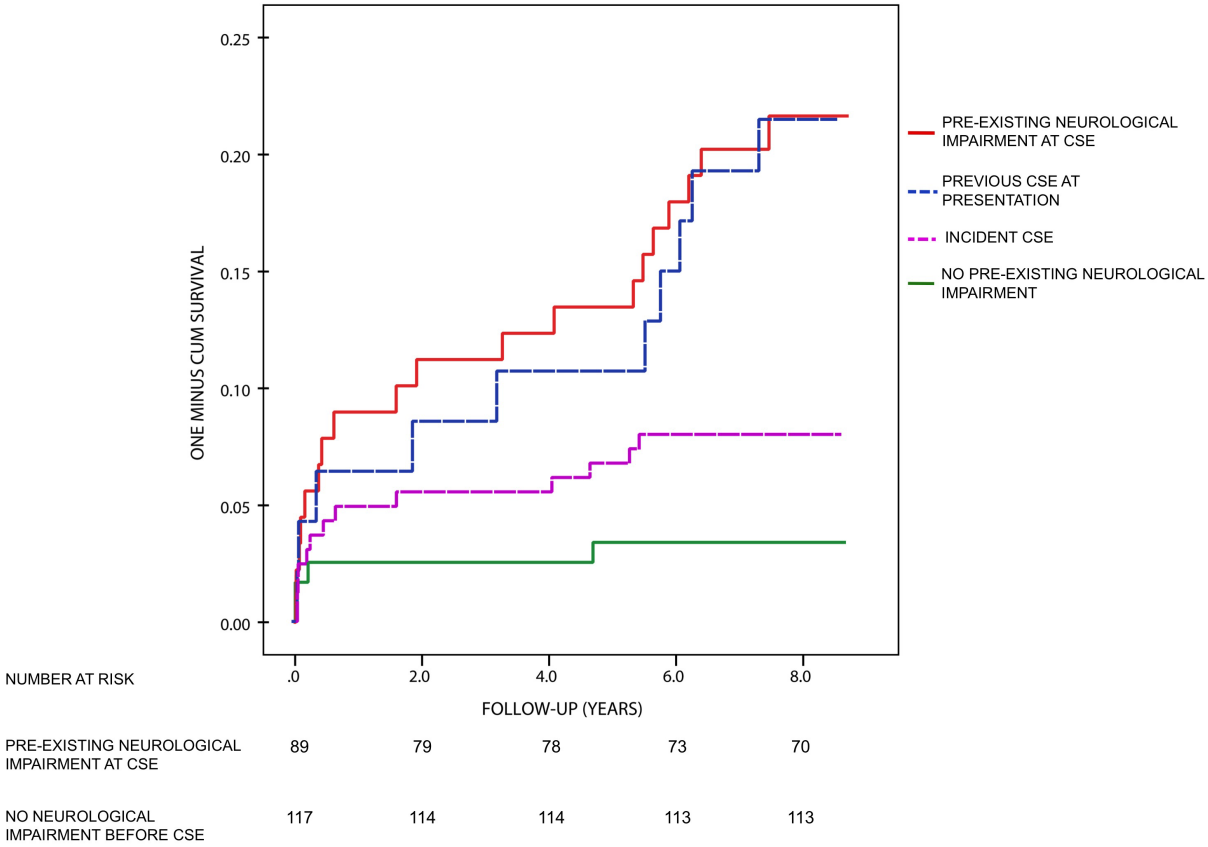


Figure 4.1 Cumulative risk of death following CSE according to presence of pre-existing neurological impairment at CSE presentation (survival curves according to presence or absence of previous CSE are superimposed for comparison)

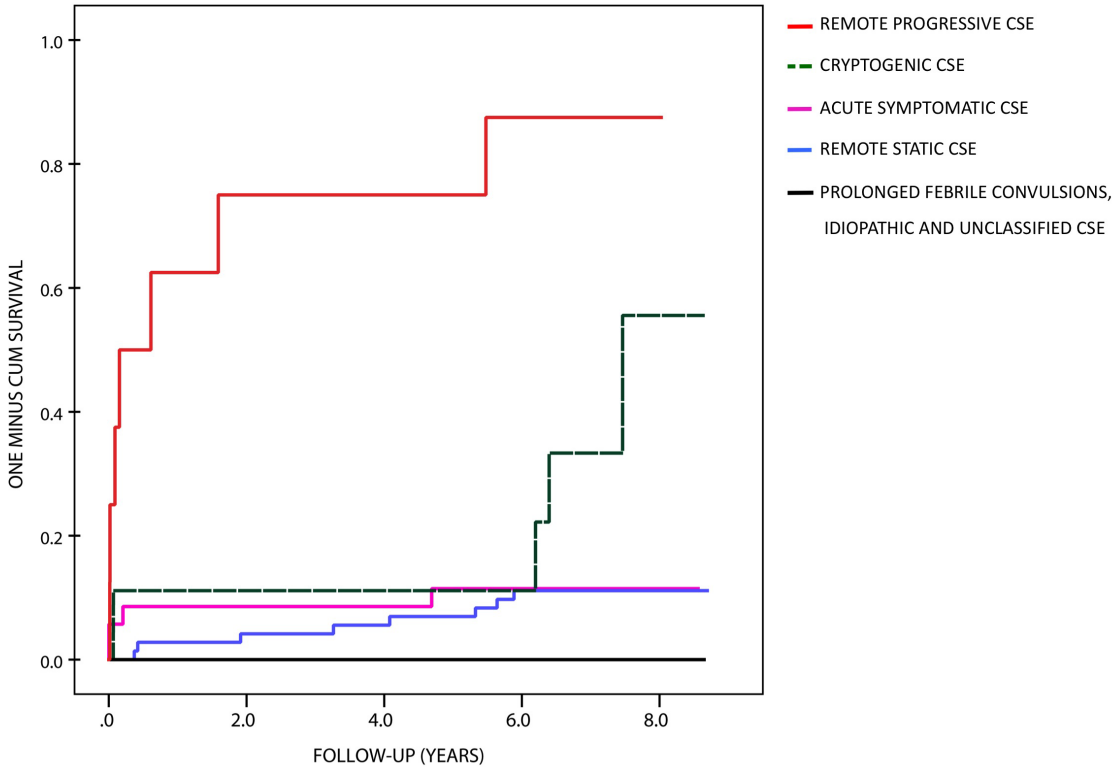


Figure 4.2 Cumulative risk of death following CSE episode according to CSE category

4.3.4 Causes of death

The causes of death as stated on their death certificates and the clinical characteristics in the deceased are listed in Table 4.4. A post mortem examination was performed on 3 children (13%). All 3 deaths within 30 days in children with acute symptomatic CSE were due to complications of the underlying cause (ABM). Only 1 child who had acute symptomatic CSE died during follow-up (5 years after the index episode of CSE), and the certified primary cause of death was status epilepticus. Amongst those with remote symptomatic CSE, the primary certified cause of death was respiratory complications in 5, small bowel complications in 2, intractable seizures/ status epilepticus in 2, acute complications of underlying medical condition in 2, and the underlying medical condition/diagnosis in 5. In those who had cryptogenic CSE, one each were certified to have died due to status epilepticus, severe sepsis and sudden cardiac death with myocarditis.

Case	Age at CSE (years)	Age at death (years)	CSE category	Cause of death (as stated on death certificate)
1*	1.2	1.2	Acute symptomatic	I (a) Brainstem herniation (b) Cerebral oedema (c) Pneumococcal meningitis
2*	2.1	2.1	Acute symptomatic	I (a) Increased intracranial pressure, coning (b) Meningococcal meningitis
3*	1.0	1.0	Acute symptomatic	I (a) Respiratory arrest (b) Pneumococcal meningitis II Cerebral palsy, ventricular peritoneal shunt for hydrocephalus, intraventricular haemorrhage at birth
4*	5.6	5.6	Remote symptomatic	I (a) Bronchopneumonia (b) Intractable epilepsy (c) West syndrome II Global developmental delay
5*	5.1	5.1	Remote symptomatic	I (a) Acute encephalopathy (b) Glutaric aciduria type I
6*	0.4	0.4	Remote symptomatic	I (a) Brain infarction (b) Congenital lactic acidosis
7*	0.5	0.5	Remote symptomatic	I (a) Progressive neurodegenerative disorder (b) Congenital mitochondrial disorder
8+	3·7	8·4	Acute symptomatic	I (a) Acute brain swelling/Haemorrhagic infarction (b) Status epilepticus II Influenza A infection
9+	3·0	9·2	Cryptogenic	I (a) Sudden cardiac death (b) Acute lymphocytic myocarditis II Epilepsy
10	9·5	17·0	Cryptogenic	I (a) Septic shock with multiple organ failure (b) Community acquired pneumonia II Epilepsy
11	3·2	9·6	Cryptogenic	I (a) Acute cardiorespiratory failure (b) Status epilepticus (c) Dravet syndrome
12	5·2	8·5	Remote symptomatic	I (a) Lower respiratory tract infection (b) Intractable seizure disorder II Gastro oesophageal reflux

13	12·2	16·3	Remote symptomatic	I (a) Pneumonia (b) Cerebral palsy, Spastic quadriplegia, Epilepsy II Gastro oesophageal reflux with gastrostomy
14+	7·8	13·1	Remote symptomatic	I (a) Small bowel infarction (b) Volvulus II Cerebral palsy
15	1·5	7·0	Remote symptomatic	I (a) Cytochrome C Oxidase deficiency
16	2·7	4·2	Remote symptomatic	I (a) Neurodegenerative disorder of unknown aetiology
17	13·4	13·5	Remote symptomatic	I (a) Status epilepticus (b) Degenerative brain disease (c) Mitochondrial encephalopathy
18	0·6	1·2	Remote symptomatic	I (a) Respiratory failure II Neurodegenerative disorder
19	0·7	1·1	Remote symptomatic	I (a) Congenital denervating neurological condition
20	11·2	16·8	Remote symptomatic	I (a) Bronchopneumonia II Cerebral palsy
21	2·6	3·0	Remote symptomatic	I (a) Severe pyridoxine deficiency (b) Severe epilepsy
22	1·0	6·9	Remote symptomatic	I (a) Ileus (b) Severe complex neurological disability
23	2·3	4·2	Remote symptomatic	I (a) Intractable seizures II Global developmental delay

* Deaths within 30 days of CSE

+ Had post-mortem examination

Table 4.4 Clinical characteristics and cause of death in the deceased

4.4 Discussion

The main findings of the study in this chapter are that (1) children with CSE are 46 times more likely to die within 10 years after their CSE compared to the reference population, (2) a history of pre-existing neurological impairment, with or without accompanying epilepsy, at the time of CSE (remote or cryptogenic CSE) is a major risk factor for death with no evidence that other clinical or demographic factors increase risk, and (3) cardio-respiratory complications and/or intractable seizures are the most commonly reported causes of death.

Although the overall mortality in our cohort was 46 times higher than expected in the reference population, this effect was predominantly seen in children with pre-existing clinically significant neurological impairments at the time of CSE (remote symptomatic and cryptogenic CSE). Almost all deaths in previously neurologically normal children occurred within 30 days of their CSE episode, and those who survive beyond this period were not at a significantly increased risk of death compared to the reference population.

The overall risk of death following CSE was almost seven times higher in children with pre-existing neurological impairments compared to those who were previously neurologically normal; and this risk increased to 19 times in those who survived beyond 30 days of their CSE episode. All deaths in previously neurologically normal children occurred in those with acute symptomatic CSE; three children with ABM died during the acute episode, and one with viral encephalitis died 5 years later. Although the numbers are small, the case fatality rate for CSE due to ABM (most caused by *Streptococcus pneumoniae*) in our study is comparable with that for pneumococcal meningitis in the developed world (27.3%, 95% CI 9.7-56.6 vs. 15.3%, CIs not provided) (Baraff et al., 1993). There were no deaths in children following PFS, an observation similar to previous reports of very low risk of mortality following PFS, and comparable with the mortality in simple febrile seizures (Logroscino et al., 2002; Vestergaard et al., 2008; Verity et al., 1993; Maytal et al., 1989; Shinnar et al., 2001b; Nelson and Ellenberg, 1978).

We found that none of the clinical and demographic factors at the time of CSE were independent predictors of subsequent mortality except pre-existing neurological impairment. Previous studies have reported higher long-term mortality in individuals with prolonged SE (>60 minutes), continuous SE (compared to intermittent) and older

age, but this observation was valid only in adults and not for the paediatric population (Towne et al., 1994; Waterhouse et al., 1999; DeLorenzo et al., 1999). Also, none of the previously reported predictors of short-term mortality following CSE in children, such as, acute symptomatic cause of CSE, young age at onset, longer duration of CSE, and focal-onset of CSE, were independently associated with mortality beyond 30 days in our cohort (Raspall-Chaure et al., 2006; Sadarangani et al., 2008; Dunn, 1988; Kravljanac et al., 2011; Logroscino et al., 1997; Maegaki et al., 2005; Maytal et al., 1989; Scholtes et al., 1996; Siddiqui et al., 2008; Towne et al., 1994; Gulati et al., 2005; Hussain et al., 2007; Cavazzuti and Lalla, 1984; Chin et al., 2006; Tabarki et al., 2001). The observed differences in our study and previously reported hospital–based studies may at least in part be related to the inclusion of more severe cases in hospital-based studies. It is possible that recurrent CSE and medication non-compliance could potentially increase overall mortality. But, as details on medication compliance, epilepsy control and frequency of CSE during follow-up were not available for the deceased, we were unable to adjust for these factors in our modelling.

Most deaths within 30 days of an episode of CSE were as a consequence of complications of the underlying cause, such as bacterial meningitis or a progressive neurological disorder. Even amongst the 30-day survivors, the majority of deaths seem to have been associated with complications of their underlying brain disorder, rather than as a direct consequence of CSE. Only four deaths were seizure related; three as a result of CSE and one as a result of intractable seizures. There were no sudden unexpected deaths in epilepsy (SUDEP) or accidental deaths. This is in contrast to the observation by Aicardi and Chevrie, who reported that about half the deaths in their cohort with CSE were seizure related (Aicardi and Chevrie, 1970). There are several possible explanations for this observed difference. The first was that Aicardi study was hospital-based, and CSE was defined as seizure activity lasting >60 minutes rather than the 30-minute definition used in our study. Thus, children in Aicardi study may reflect children with more severe conditions compared with our cohort. It is also possible that improvements in acute management of CSE in recent years may explain reduced risk of death as a direct consequence of CSE. Another explanation may be inaccuracies in the cause of death as stated on the death certificates as only 3 out of 23 in our study had autopsies, and the stated cause of death on certificates may not reflect circumstances surrounding death (Sillanpaa and Shinnar, 2010). It is possible that the actual cause of death may be due to seizure activity although not listed as such, e.g. unobserved seizure activity may lead to aspiration, and subsequent respiratory failure.

Our results suggest that CSE duration does not have additional impact on subsequent mortality above that related to pre-existing neurological impairment at the time of CSE, Ideally, a direct comparison of the mortality in children with a pre-existing significant neurological disorder and no CSE, with a similar population of children with CSE; or, mortality between idiopathic epilepsy without CSE and with CSE may help to determine the additional impact of CSE on mortality. Even such approach may not provide a satisfactory answer to this important question as the outcome may still be confounded by the severity of the underlying brain disorder and CSE itself could be a marker of the severity. One such study done in adult population showed a non-significant 2.4-fold increased risk of death at 10 years in incident idiopathic/cryptogenic CSE compared with short seizures (95% CI 0.9-6.3) (Logroscino et al., 2008). However, the study was limited by a small sample size, and the increased risk was restricted to the elderly and those who later developed epilepsy. Similar studies have not been reported in children with first unprovoked seizures. While comparing mortality in children with simple febrile seizures against that with PFS could potentially be a good approach to investigate the additional impact of CSE on mortality, negligible long-term mortality associated with both brief and prolonged seizures precludes its utility (MacDonald et al., 1999; Nelson and Ellenberg, 1978; Verity and Golding, 1991). Data from childhood-onset epilepsy cohort studies suggest that CSE has no additional impact on long-term mortality above that related to the underlying diagnosis (Sillanpaa and Shinnar, 2002; Sillanpaa and Shinnar, 2010; Camfield et al., 2002; Berg et al., 2004b; Callenbach et al., 2001; Berg et al., 2004a; Stroink et al., 2007).

An indirect way of analysing the attributive role of CSE on mortality could be by comparing the mortality in idiopathic CSE in our cohort with the reported mortality in children with idiopathic epilepsy, and mortality in children with neurological impairments in our cohort with that in children with cerebral palsy, a cohort likely to have similar neurological impairments. There were no deaths in children who had idiopathic CSE in our cohort (SMR 0.0, 95% CI 0.0-3.68), an observation similar to the low mortality reported in children with idiopathic epilepsy (Berg et al., 2004b; Callenbach et al., 2001; Camfield et al., 2002; Geerts et al., 2010; Harvey et al., 1993; Sillanpaa and Shinnar, 2010). The SMR for children with significant neurological impairment prior to their episode of CSE in our study was 91.4 (95% CI 53.2-146.3), which is comparable with that reported in children with cerebral palsy (SMR 36.4 for mild and 102.8 for severe CP, 95% CI not provided) (Strauss et al., 1999). However, it is probable that a significant proportion of children with severe cerebral palsy also had

epilepsy and some even experienced CSE. Therefore, although useful, these comparisons need to be interpreted with caution considering the heterogeneity of the study populations.

This study has some important limitations. Although our study population is the largest prospective population-based childhood CSE cohort, it is possible that lack of statistical significance for some of the clinical and demographic factors could be due to modest sample size. As is inevitable in follow-up studies, there will be some degree of attrition and our 9% attrition is within the acceptable range for follow up cohort studies (Fewtrell et al., 2008). Furthermore, the children who were lost to follow-up were similar to those in which we had follow-up data suggesting that our results are representative of the entire CSE cohort. Finally, this study from a resource-rich country may well not reflect the longer-term mortality in resource-poor setting where it is already clear that the mortality within 3-4 years after CSE is substantially higher (Sadarangani et al., 2008; Prins et al., 2014).

Our study provides novel information that will be useful for prognostication. The results suggest that there is a substantially increased risk for death within 10 years after childhood CSE, but mostly in children with pre-existing significant neurological abnormalities at CSE presentation. Most deaths during follow-up occur as a complication of underlying brain disorder rather than seizures themselves. In addition, the study has generated the hypothesis that there is minimal additional impact of CSE itself on mortality. Case-control studies specifically designed to investigate this hypothesis are now required.

In this chapter, mortality, causes and risk factors associated with death within 10 years after childhood CSE were presented. The next chapter will address neurological morbidity associated with childhood CSE, during the same time period, among survivors.

CHAPTER 5 NEUROLOGICAL, COGNITIVE, BEHAVIOUR AND EDUCATIONAL OUTCOMES

5.1 Introduction

As discussed in chapter 2, while it is widely accepted that childhood CSE is associated with increased mortality and morbidity, there is considerable variability in reported adverse outcome rates (Raspall-Chaure et al., 2006). In particular, there is conflicting data on the causative role of PFS in the later development of TLE and MTS (Lewis et al., 2014; Patterson et al., 2014; Raspall-Chaure et al., 2006; Shukla and Prasad, 2012; Tarkka et al., 2003). We established in chapter 4 that the mortality within 10 years is significantly higher in children with CSE compared to the reference population, but seen predominantly in children with pre-existing clinically significant neurological impairments. Apart from mortality, the reported adverse outcomes following childhood CSE include development of subsequent epilepsy, MTS, CSE recurrence, permanent motor deficits, intellectual impairment and behavioural problems (Raspall-Chaure et al., 2006). However the incidence, prevalence, spectrum and risk factors for these adverse outcomes are uncertain. Such data are needed to help in prognostication and development of interventional strategies to alleviate potentially modifiable risk factors. Most published studies on childhood CSE outcomes are constrained by methodological limitations such as hospital-based and/or retrospective design, unclear definition of outcomes, lack of formal neurocognitive assessment and neuroimaging, small sample size and short follow-up period (Raspall-Chaure et al., 2006). Thus, there is a need for research, such as the current study, on the long-term neurological outcomes in a large unselected childhood CSE cohort.

The overall objectives of the study reported in this chapter are to (1) describe the prevalence and spectrum of epilepsy, recurrent CSE, motor disability, intellectual disability, behavioural problems and educational difficulties, (2) estimate the incidence of epilepsy, motor disability and intellectual disability, and (3) investigate clinical and sociodemographic factors associated with new-onset epilepsy, motor and intellectual disability, within 10 years after PFS and other forms of childhood CSE.

5.2 Methods

5.2.1 Subject recruitment

The details of methods for recruitment, follow-up assessments, and general definitions used in this thesis are described in chapter 3. A brief description of the assessments and definitions relevant to the study reported in this chapter are presented in the following sections.

5.2.2 Assessments and definitions

5.2.2.1 Baseline characteristics

For all subjects consenting to participate in this follow-up study, background sociodemographic, medical and developmental data prior to CSE, and clinical details about the episode of their initial CSE were obtained from the inception NLSTEPSS cohort database. As the current study aimed to generate useful data on the prognosis and predictors of adverse outcomes following CSE, we opted to retain the original CSE classification used in NLSTEPSS (Table 3.2) (Chin et al., 2006; Logroscino et al., 2002; Verity et al., 1993). However, we opted to combine idiopathic and cryptogenic epilepsy related CSE into a single group because there were very few (n = 4) cryptogenic CSE subjects and the baseline characteristics of the subjects in the two groups were comparable.

The baseline motor functioning was determined from the neurological examination findings documented at CSE presentation. For pragmatic reasons, a formal cognitive assessment was not performed at initial CSE presentation; but, parent-reported developmental information before the CSE episode was documented on the NLSTEPSS database for all subjects. Therefore, we used documented prior developmental information to determine baseline developmental status and classified baseline development as "abnormal" if there was a formal diagnosis of intellectual disability, or if delay was documented in two or more of the following developmental domains: motor, speech/language, cognition, and social/personal skills (Shevell, 2006; Shevell et al., 2003; Chung et al., 2010).

5.2.2.2 Follow-up assessments

Data collated from interviews, medical records, neurology and neurocognitive assessments were used to determine outcomes at follow-up. We adopted the International League Against Epilepsy (ILAE) Commission on Epidemiology guidelines for epilepsy definitions and classification (Thurman et al., 2011). The definitions for motor and cognitive outcomes were adapted from the 2001 WHO Global Burden of Disease Project (Edmond et al., 2010). To determine behavioural outcomes, parents were asked if their child had any behavioural problems, and had ever been diagnosed by a qualified professional to have a behavioural/psychiatric disorder. We used parental information on provision of special educational support at school to determine educational outcomes.

5.2.2.3 Definitions for clinical outcomes at follow-up

The definitions of clinical outcomes at follow-up are presented in Table 5.1. Subjects were considered to have adverse neurological outcomes if one or more of the following were present at follow-up: (1) epilepsy (active or in remission), (2) motor disability, (3) intellectual disability, (4) psychiatric disorder, or (5) a statement of special educational needs.

Seizures/Epilepsy	
Epilepsy	Two or more unprovoked seizures occurring at least 24 hours apart
Terminal remission	Seizure-free off drug treatment in the preceding 2 years
Active epilepsy	Seizures within the past 2 years or currently on drug treatment for epilepsy
CSE recurrence	Recurrence of one or more episodes of CSE during FU (i.e. between entry into NLSTEPSS and end of follow up in the current study)
Motor functioning	
Minor impairment	Isolated hypotonia, gait or coordination difficulties
Motor disability	Spasticity or paresis of one or more limbs (e.g., cerebral palsy, hemiparesis)
Intellectual functioning	
Normal	Full-scale IQ above 85 on WASI
Borderline intellectual functioning	Full-scale IQ between 71 and 84 on WASI
Intellectual disability	Full-scale IQ below 70 on WASI, or untestable due to profound impairment
Behavioural problems	
Psychiatric diagnosis	Diagnosis of a psychiatric disorder as recorded in parental interviews and medical records (e.g. Attention Deficit Hyperactivity Disorder, Autism Spectrum Disorder)
Educational difficulties	
Additional support in mainstream class	Special educational needs met effectively within mainstream settings through additional support (e.g. School Action/School Action Plus)
Statement of special educational needs	Children with a statutory statement of special educational needs requiring substantial additional support in school because of learning, medical or behavioural difficulties

Table 5.1 Definitions for clinical outcomes at follow-up

5.2.3 Attrition and addressing missing data

Missing data are inevitable in epidemiological and clinical research (Sterne et al., 2009). High rates of attrition are particularly common in long-term follow-up cohort studies that require clinic visit and investigations/assessments (Fewtrell et al., 2008). While attrition of <5% could generally be considered "ignorable", higher rates of attrition could result in potential bias, reduce statistical power and precision of the study, and affect conclusion and generalisability of the results to the wider population (Fewtrell et al., 2008; Sterne et al., 2009; Kristman et al., 2004). Therefore, as part of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Initiative, it is recommended that all epidemiological studies report the numbers and reasons for missing data and details of analysis used to address missing data (Sterne et al., 2009; von Elm et al., 2007).

The mechanism of missing data are commonly classified using Little and Rubin's framework as being (1) missing completely at random (MCAR- the probability of data being missing does not depend on the observed or unobserved data, i.e., missingness is completely by chance), (2) missing at random (MAR- the probability of data being missing does not depend on the unobserved data, conditional on the observed data), or (3) missing not at random (MNAR- the probability of data being missing does depend on the unobserved data, conditional on the observed data (Little and Rubin, 2002). Unless the data are MCAR, which is unlikely in clinical situations, complete-case analysis is deemed inappropriate and it is recommended that sophisticated statistical techniques such as multiple imputation are used to improve validity of analysis and minimise bias (Fielding et al., 2009; Groenwold et al., 2011; Sterne et al., 2009; White et al., 2011).

Multiple imputation (MI) is the statistical method of choice for complex missing data analysis (Rubin, 1987; Sterne et al., 2009; White et al., 2011). MI allows for the uncertainty about the missing data by creating several different plausible imputed datasets and appropriately combining results obtained from each of them. Briefly, the stages of MI include:

1. *Generating imputed datasets:* The unknown missing data are replaced by *m* independent simulated sets of values drawn from the posterior predictive distribution of the missing data conditional on the observed data. Multivariate imputation by chained equations (MICE) is a common method to generating imputations based on a set of imputation models, one for each variable with

missing values (Buuren and Groothuis-Oudshoorn, 2011). This process incorporates all sources of variability and uncertainty in the imputed values.

2. *Analysing imputed datasets:* Each imputed dataset is analysed separately, and the statistical parameters of interest (e.g., regression coefficients) are estimated from each imputed dataset, together with their variance-covariance matrices. Thus step 2 will generate *m* different estimates because of the variation introduced in the imputation of the missing values.
3. *Combining estimates from imputed datasets:* The m estimates are pooled into an overall estimate and standard errors using Rubin's rules (Rubin, 1987). The combined standard error incorporates both within-imputation variability (uncertainty about the results from one imputed data set) and between-imputation variability (reflecting the uncertainty due to the missing information).

5.2.4 Statistical analysis

All analyses were carried out using SPSS (IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY:IBM Corp.), the R software environment for statistical computing (Version 3.0.1), and StatXact (Version 4, Cytel Software, Cambridge, MA, USA). We used exact difference in proportions and Mann-Whitney U tests to compare the sociodemographic and clinical characteristics between the cohort members participating in the follow-up study and those lost to follow-up.

5.2.4.1 Prevalence and incidence of adverse neurological outcomes

Prevalence for each adverse neurological outcome was calculated as the proportion of CSE cohort who had adverse neurological outcomes at follow-up.

Incidence for each adverse neurological outcome was calculated by dividing the number of incident (new-onset) cases during follow-up by the total number without adverse outcome at baseline and available for follow-up (cumulative incidence), and by the number of person-years accumulated in the population without adverse outcome at baseline and available for follow-up (incidence rate). The 95% CI of the incidence rates were calculated based on the Poisson distribution.

Given the majority of children in our cohort were of preschool age at CSE presentation, and that the major symptoms of a psychiatric disorder may not manifest unequivocally and difficult to diagnose in very young children, it was deemed unfeasible to accurately

determine the true prevalence of significant behavioural problems and educational difficulties at baseline in our cohort (Chin et al., 2006; Campbell, 1995). Thus, we were not confident in determining the prevalence of behavioural disorders and educational difficulties at baseline, as defined in the current study. Therefore, we did not calculate incidence for adverse behavioural and educational outcomes.

5.2.4.2 Multiple imputation for missing data

To investigate the predictors of adverse neurological outcomes following CSE, we first used a complete-case approach that includes all subjects with complete baseline and follow-up data i.e. 134 (66%) of 203 survivors from NLSTEPSS cohort. Complete-case approach excluded 34% of cohort due to missing follow-up data. We therefore performed multivariate imputation by chained equations (MICE) in R (v2.17), and incorporated 19 key predictor and 4 outcome variables (with missing data) to generate 35 complete datasets for 203 subjects (Buuren and Groothuis-Oudshoorn, 2011). The outcome variables included were any epilepsy (active or in remission), active epilepsy, full-scale IQ, and motor disability. All sociodemographic and clinical characteristics previously reported to be associated with outcomes or could potentially influence outcomes were included in multiple imputation model (Raspall-Chaure et al., 2006). The following predictor variables were included: age at CSE, gender, prematurity (gestation <37 weeks), ethnicity, socioeconomic status (IMD 2004 scores), family history of epilepsy, previous febrile seizures, previous CSE, epilepsy diagnosis at baseline, motor deficit at baseline, abnormal cognition at baseline, pre-hospital treatment given or not, interval between seizure onset and first treatment given, febrile or afebrile at CSE presentation, CSE duration, intermittent or continuous CSE, focal or generalised CSE, CSE recurrence, and aetiological classification of CSE. The imputed datasets generated in R were exported to SPSS for further statistical analysis. The pooled overall estimates and standard errors for the imputed datasets were obtained using Rubin's rules (Rubin, 1987).

5.2.4.3 Regression analysis

We performed logistic regression analyses to investigate the predictors of adverse neurological outcomes on both complete-case and imputed datasets. We first carried out univariable analyses to identify the clinical and sociodemographic factors reaching a statistical significance level of 10% ($p \leq 0.1$). These factors were then assessed for significance using multivariable analyses ($p < 0.05$). Any factors not significant on univariable analyses were subsequently included in the final model to identify any

factors that were associated with adverse outcomes only after adjustment for other factors. The sociodemographic variables included were: age at CSE (as continuous and categorical variable), gender, prematurity (gestation <37 weeks), family history of epilepsy, and Index of Multiple Deprivation 2004 (IMD 2004) scores as the indicator of socioeconomic status. The clinical variables investigated were: previous febrile seizures, first-ever (incident) versus recurrent CSE, febrile (temperature >38^{0}C) versus afebrile CSE, whether pre-hospital treatment was given or not, interval between seizure onset and first treatment, CSE duration (both as continuous and categorical variable), intermittent versus continuous CSE, CSE onset (focal versus generalised), and presence of epilepsy, motor disability and abnormal cognition at baseline.

5.3 Results

5.3.1 Characteristics of study cohort

At follow-up, 23 (10%) of the 226 children ascertained during NLSTEPSS had died (Pujar et al., 2011). Amongst 203 survivors, 134 (66%) were recruited for follow-up, 20 (10%) could not be contacted and 49 (24%) declined consent (Figure 5.1). There was no significant difference in the clinical or demographic characteristics between subjects participating in STEPSOUT and dropouts (Table 5.2). The CSE classification in the follow-up cohort was PFS in 26%, acute symptomatic (AS) in 11%, idiopathic & cryptogenic in 14%, remote symptomatic (RS) in 40% and unclassified in 9%. Of the participants, 67 (50%) were male, and the median age was 2.7 years (interquartile range, IQR 1.1-5.1) at CSE presentation and 11.6 years (IQR 9.8-14.3) at follow-up. The median follow-up duration was 8.9 years (IQR 8.2-9.5).

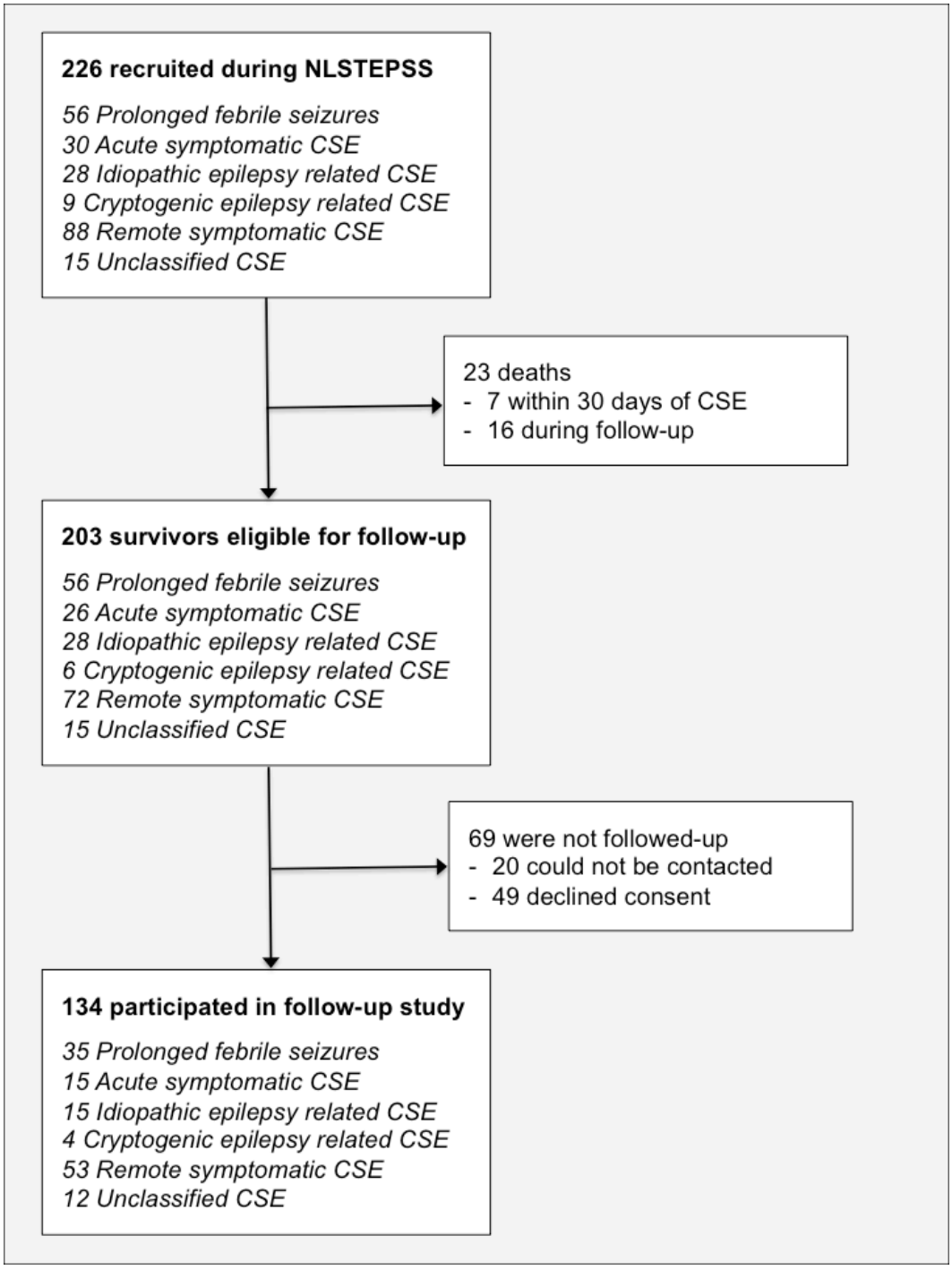


Figure 5.1 Flowchart showing recruitment and follow-up of CSE cohort for STEPSOUT

Sociodemographic/ Clinical Characteristics	Followed-up (Total = 134) N (%)	No follow-up (Total = 69) N (%)	Difference in percentages (95% CI) /p value for difference in medians
Gender			
Male	67 (50)	35 (50.7)	-0.7 (-14.9, 13.5)
Female	67 (50)	34 (49.3)	
Median age at CSE, months (range)	32.7 (1-192)	31.8 (1.4-186)	0.80+
Neurological impairment before the CSE episode			
Absent	59 (44)	33 (47.8)	-3.8 (-17.9, 10.4)
Present	75 (56)	36 (52.2)	
CSE category			
Prolonged febrile seizures	35 (26.1)	21 (30.4)	-4.3 (-17.8, 8.2)
Acute symptomatic	15 (11.2)	11 (15.9)	-4.7 (-16.0, 4.6)
Idiopathic epilepsy related	15 (11.2)	13 (18.8)	-7.6 (-19.2, 2.2)
Cryptogenic epilepsy related	4 (3)	2 (2.9)	0.1 (-7.2, 5.0)
Remote symptomatic	53 (39.6)	19 (27.5)	12 (-1.9, 24.5)
Unclassified	12 (9)	3 (4.3)	4.6 (-3.9, 11.3)
CSE occurrence			
First-ever (Incident)	106 (79.1)	57 (82.6)	-3.5 (-14.0, 8.7)
Previous CSE (Recurrence)	28 (20.9)	12 (17.4)	
Seizure duration			
30-60 min	56 (41.8)	29 (42)	-0.2 (-14.5, 13.6)
>60 min	78 (58.2)	40 (58)	
Seizure character			
Continuous	70 (52.2)	29 (42)	10.2 (-4.2, 23.9)
Intermittent	64 (47.8)	40 (58)	
Seizure type			
Generalised-onset	86 (64.2)	38 (55.1)	9.1 (-4.9, 23.1)
Focal-onset	48 (35.8)	31 (44.9)	
Ethnicity			
White	54 (40.3)	20 (29)	11.3 (-2.7, 23.9)
Black	22 (16.4)	18 (26.1)	-9.7 (-22.3, 1.8)
Asian	41 (30.6)	21 (30.4)	0.2 (-13.5, 12.8)
Other	17 (12.7)	10 (14.5)	-1.8 (-13.0, 7.5)
Socioeconomic status (Median IMD 2004 scores)	33.8	36.5	0.22+

+ *p* value on Mann-Whitney U test

Table 5.2 Clinical and sociodemographic characteristics at CSE presentation of the follow-up cohort and those not followed-up

5.3.2 Seizures/Epilepsy

5.3.2.1 Prevalence, and predictors of active epilepsy at follow-up

Of 134 subjects followed-up, 79 (59%, 95% CI 50.5-66.9) had either active epilepsy (41%, 95% CI 33.1-49.5) or epilepsy in remission (17.9%, 95% CI 12.3-25.3) (Table 5.3). The prevalence of epilepsy was low in the PFS (14.3%, 95% CI 6.3-29.4) and AS CSE (13.3%, 95% CI 3.7-37.9) groups; none in the PFS group had active epilepsy at follow-up. No one in the PFS group had a subsequent diagnosis of Dravet syndrome.

In contrast, the majority in the idiopathic & cryptogenic (79%, 95% CI 56.7-91.5) and RS (67.9%, 95% CI 54.5-78.9) CSE groups had active epilepsy at follow-up. Having epilepsy at CSE presentation was the only independent predictor of active epilepsy at follow-up (OR 8.5, 95% CI 2.1-34.6) (Table 5.4).

5.3.2.2 Incidence and predictive factors for epilepsy

The cumulative incidence of epilepsy was 24.7% (95% CI 16.2-35.6) (Table 5.5). The frequencies of epilepsy at CSE presentation and incident cases during follow-up are presented according to aetiology in Figure 5.2.

About 14% in the PFS and AS CSE groups, and nearly 50% in the other CSE groups developed incident epilepsy ($p = 0.03$). All incident epilepsies emerged within 5 years post-CSE, the majority within 6 months (Figure 5.2). Absence of fever at CSE presentation (afebrile vs. febrile CSE) was the only significant predictor of incident epilepsy (OR 7.5, 95% CI 2.5-22.0) on multivariable analysis (Table 5.6, multivariable model not shown).

CSE category	Epilepsy			Motor functioning			Intellectual functioning*		
		N	% (95% CI)		N	% (95% CI)		N	% (95% CI)
Prolonged febrile seizures (N=35)	None	30	85.7 (70.6, 93.7)	Normal	30	85.7 (70.6, 93.7)	Normal	28	80 (64.1, 90)
	TR	5	14.3 (6.3, 29.4)	Minor impairment	5	14.3 (6.3, 29.4)	Borderline	6	17.1 (8.1, 32.7)
	Active	0	0 (0, 9.9)	Motor disability	0	0 (0, 9.9)	Intellectual disability	1	2.9 (0.5, 14.5)
Acute symptomatic (N=15)	None	13	86.7 (62.1, 96.3)	Normal	14	93.3 (70.2, 98.8)	Normal	14	93.3 (70.2, 98.8)
	TR	1	6.7 (1.2, 29.8)	Minor impairment	1	6.7 (1.2, 29.8)	Borderline	1	6.7 (1.2, 29.8)
	Active	1	6.7 (1.2, 29.8)	Motor disability	0	0 (0, 20.4)	Intellectual disability	0	0 (0, 20.4)
Idiopathic & cryptogenic (N=19)	None	0	0 (0, 16.8)	Normal	3	15.8 (5.5, 37.6)	Normal	2	10.5 (2.9, 31.4)
	TR	4	21 (8.5, 43.3)	Minor impairment	9	47.4 (27.3, 68.3)	Borderline	1	5.3 (0.9, 24.6)
	Active	15	79 (56.7, 91.5)	Motor disability	7	36.8 (19.1, 59)	Intellectual disability	16	84.2 (62.4, 94.5)
Remote symptomatic (N=53)	None	6	11.3 (5.3, 22.6)	Normal	9	17 (9.2, 29.2)	Normal	6	11.3 (5.3, 22.6)
	TR	11	20.8 (12, 33.5)	Minor impairment	11	20.8 (12, 33.5)	Borderline	7	13.2 (6.5, 24.8)
	Active	36	67.9 (54.5, 78.9)	Motor disability	33	62.3 (48.8, 74.1)	Intellectual disability	40	75.5 (62.4, 85.1)
Unclassified (N=12)	None	6	50 (25.4, 74.6)	Normal	9	75 (46.8, 91.1)	Normal	8	66.7 (39.1, 86.2)
	TR	3	25 (8.9, 53.2)	Minor impairment	2	16.7 (4.7, 44.8)	Borderline	0	0 (0, 24.2)
	Active	3	25 (8.9, 53.2)	Motor disability	1	8.3 (1.5, 35.4)	Intellectual disability	4	33.3 (13.8, 60.9)
All CSE (N=134)	None	55	41 (33.1, 49.5)	Normal	65	48.5 (40.2, 56.9)	Normal	58	43.3 (35.2, 51.7)
	TR	24	17.9 (12.3, 25.3)	Minor impairment	28	20.9 (14.9, 28.5)	Borderline	15	11.2 (6.9, 17.6)
	Active	55	41 (33.1, 49.5)	Motor disability	41	30.6 (23.4, 38.8)	Intellectual disability	61	45.5 (37.3, 54)

TR Terminal remission

* Intellectual functioning- Normal IQ≥85, Borderline IQ 71-84, Intellectual disability IQ≤70

Table 5.3 Outcomes for epilepsy, motor and intellectual functioning following childhood CSE

Clinical and demographic characteristics	Complete case (N=134)				Imputed dataset (N=203)			
	Univariable		Multivariable		Univariable		Multivariable	
	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age at CSE (months)	1.02 (1.01, 1.03)	**0.001**	1.01 (0.99, 1.02)	0.48	1.015 (1.006, 1.02)	**0.001**	1.0 (0.99, 1.02)	0.69
Previous CSE	5.8 (2.4, 14.3)	**<0.001**	1.0 (0.3, 3.2)	0.96	5.7 (2.4, 13.2)	**<0.001**	1.0 (0.3, 2.9)	0.99
Afebrile at CSE	4.8 (2.1, 10.9)	**<0.001**	1.8 (0.6, 5.4)	0.32	3.9 (1.8, 8.5)	**<0.001**	1.7 (0.6, 5.0)	0.32
Epilepsy at baseline	34.6 (11.0, 108.5)	**<0.001**	8.4 (1.8, 39.0)	**0.006**	22.4 (8.4, 59.4)	**<0.001**	8.5 (2.1, 34.6)	**0.003**
Motor disability at baseline	17.7 (7.1, 44.1)	**<0.001**	1.8 (0.4, 7.7)	0.40	12.5 (5.2, 30.0)	**<0.001**	2.5 (0.7, 9.4)	0.17
Abnormal cognition at baseline	27.2 (8.8, 84.2)	**<0.001**	3.0 (0.5, 17.7)	0.22	13.0 (4.6, 36.5)	**<0.001**	1.5 (0.3, 8.6)	0.66

OR Odds Ratio

Table 5.4 Predictors of active epilepsy at follow-up

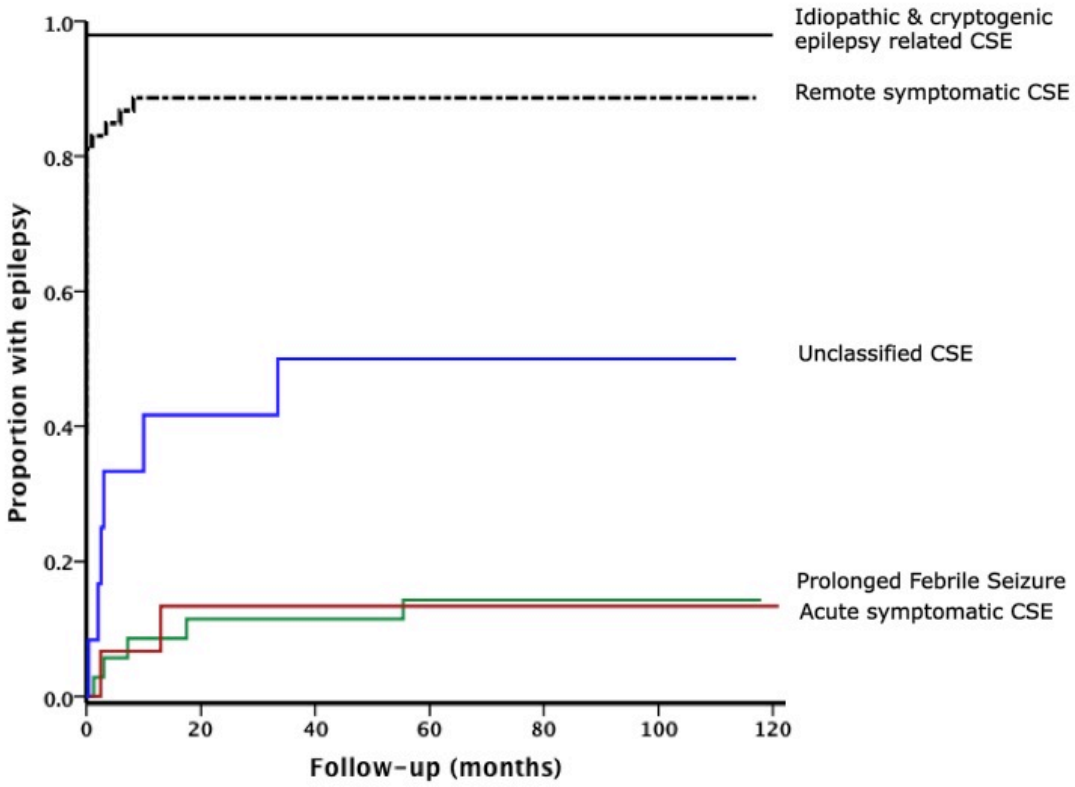


Figure 5.2 Graph illustrating prevalence of epilepsy at CSE presentation (baseline) and incident epilepsy during follow-up according to CSE category

CSE category	Epilepsy				
	Epilepsy diagnosis at CSE presentation	Incident cases	Cumulative incidence % (95% CI)	Person-years	Incidence rate* (95% CI)
Prolonged febrile seizures (N=35)	0/35	5/35	14.3 (6.3, 29.4)	296	16.9 (5.5, 39.4)
Acute symptomatic (N=15)	0/15	2/15	13.3 (3.7, 37.9)	127	15.7 (1.9, 56.9)
Idiopathic & cryptogenic (N=19)	19/19	NA	NA	NA	NA
Remote symptomatic (N=53)	42/53	5/11	45.5 (21.3, 72)	98	51 (16.6, 119)
Unclassified (N=12)	0/12	6/12	50 (25.4, 74.6)	103	58.3 (21.4, 126.8)
All CSE (N=134)	61/134	18/73	24.7 (16.2, 35.6)	624	28.8 (17.1, 45.6)

*Incidence per 1000 person-years

NA - Not applicable as all subjects in the group, by definition, had epilepsy at CSE presentation

Table 5.5 Incidence of epilepsy during follow-up after childhood CSE according to CSE category

Clinical and sociodemographic characteristics	Epilepsy			
	Complete case (N = 134)		Imputed dataset (N = 203)	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age < 1 year at CSE	2.1 (0.6, 7.2)	0.23	1.3 (0.5, 3.3)	0.69
Male gender	0.7 (0.3, 2.0)	0.55	0.7 (0.3, 1.6)	0.37
Gestation <37 weeks	1.4 (0.3, 6.4)	0.63	1.8 (0.5, 6.8)	0.38
Socioeconomic status (IMD 2004 scores)	1.004 (0.97, 1.037)	0.83	1.002 (0.970, 1.035)	0.92
Previous CSE*	-	-	-	-
Previous febrile seizures	1.4 (0.4, 4.6)	0.60	1.0 (0.3, 3.1)	0.96
Family history of epilepsy	6.0 (0.5, 70.1)	0.153	7.3 (0.6, 86.4)	0.115
Pre-hospital treatment given	0.5 (0.2, 1.4)	0.20	0.6 (0.3, 1.6)	0.32
Interval between seizure onset and first treatment given	0.99 (0.96, 1.04)	0.89	1.00 (0.97, 1.04)	0.82
Afebrile at CSE	8.4 (2.6, 26.4)	**<0.001**	5.3 (1.8, 15.3)	**0.002**
CSE duration (min)	0.99 (0.98, 1.01)	0.41	0.99 (0.98, 1.01)	0.49
Intermittent v continuous CSE	0.8 (0.3, 2.2)	0.65	1.1 (0.4, 3.1)	0.89
CSE onset (focal v generalised)	1.2 (0.4, 3.5)	0.74	1.3 (0.5, 3.6)	0.64
Motor disability at baseline	2.9 (0.4, 22.4)	0.30	2.2 (0.4, 12.7)	0.36
Abnormal cognition at baseline	2.5 (0.6, 10.4)	0.21	2.1 (0.5, 8.3)	0.30

*Zero cells

Table 5.6 Predictors of new-onset epilepsy following childhood CSE

5.3.2.3 Types of incident epilepsy

Seizure/epilepsy types were classified as recommended in the International League Against Epilepsy (ILAE) Commission on Epidemiology guidelines (Thurman et al., 2011). Out of five children with incident epilepsy in the PFS group, only one developed TLE 4.5 years after their PFS and also had MTS on MRI (Figure 5.3 & Table 5.7). In addition, two children with unclassified CSE (one with MTS and one who declined MRI) and one with RS CSE (and found to have MTS) developed incident TLE. MTS was on the left side in all three children. Thus, 2.9% (95% CI 0.5-14.5) with PFS and 5.5% (95% CI 2.2-13.3) of the entire follow-up cohort developed incident TLE following CSE, and amongst those with TLE that were scanned, MTS left sided in all. Amongst the four children with TLE, three were in terminal remission at follow-up and one had active epilepsy (See Table 5.7 for further details epilepsy classification, neuroimaging findings, and seizure outcomes at follow-up in subjects with incident epilepsy).

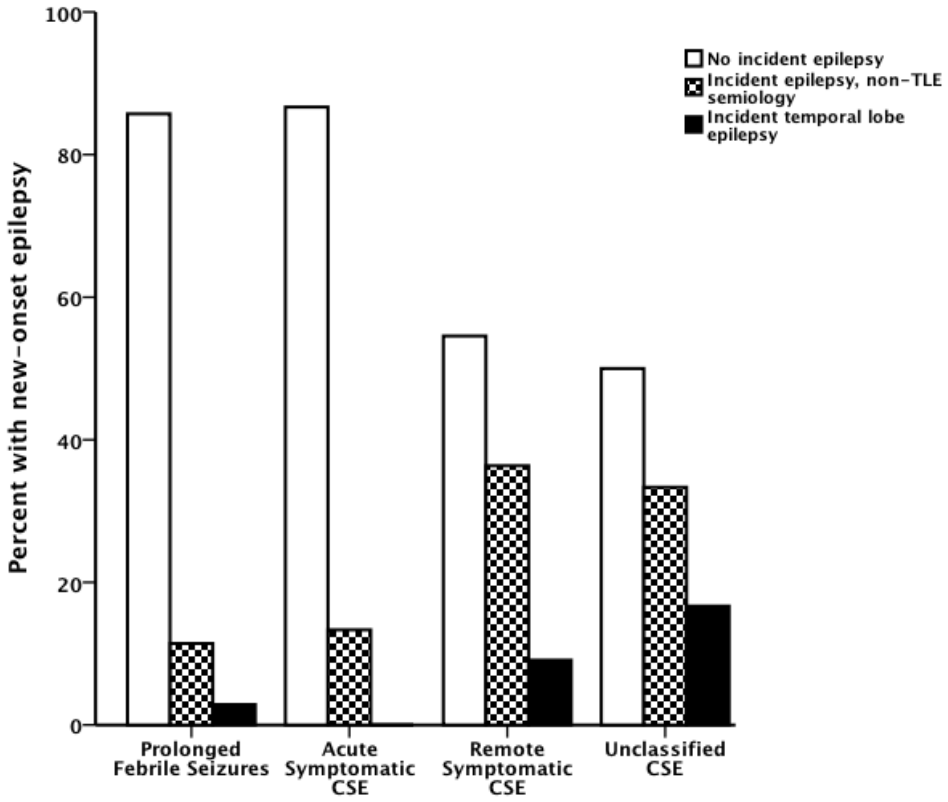


Figure 5.3 Incidence of temporal lobe epilepsy (TLE) and epilepsy of non-TLE semiology following childhood CSE

CSE category	Age at CSE	Interval between CSE & epilepsy onset	Seizure/epilepsy type	Direct aetiology	MRI findings	Epilepsy outcome at follow-up
Prolonged febrile seizures (N=35)	10m	17m	Generalised convulsive	Presumed genetic	Normal	Terminal remission
	4y 8m	7m	Generalised convulsive	Unknown	Normal	Terminal remission
	1y	55m	Dyscognitive focal seizures	Structural/metabolic	Left MTS	Terminal remission
	3y 4m	3m	Focal onset with secondary generalization	Presumed genetic	Normal	Terminal remission
	2y 3m	2m	Focal onset with secondary generalization	Undetermined	Normal	Terminal remission
Acute symptomatic (N=15)	1y 6m	13m	Focal onset with secondary generalization Generalized other motor	Structural/metabolic	Normal	Terminal remission
	7y 6m	2m	Focal onset with secondary generalization	Structural/metabolic	Normal	Active
Remote symptomatic (N=11)	4y 6m	8m	Dyscognitive focal seizures	Structural/metabolic	Left MTS	Terminal remission
	12y 8m	6m	Focal onset with secondary generalization	Structural/metabolic	Focal cortical dysplasia	Terminal remission
	2y 3m	1m	Focal onset with secondary generalization	Structural/metabolic	Prominent ventricles	Terminal remission
	2y 1m	3m	Focal autonomic	Structural/metabolic	Periventricular leucomalacia	Terminal remission
	2y 10m	0m	Generalised convulsive	Undetermined	Uncooperative	Active
Unclassified (N=12)	2y 8m	2m	Convulsive undetermined	Undetermined	Normal	Terminal remission
	6y 4m	1m	Dyscognitive focal seizures	Structural/metabolic	Left MTS	Active
	3y 10m	2m	Dyscognitive focal seizures	Undetermined	Declined MRI	Terminal remission
	2y 3m	10m	Focal onset with secondary generalization	Undetermined	Normal	Active
	4y 5m	3m	Focal onset with secondary generalization	Undetermined	Normal	Terminal remission
	3m	33m	Focal onset with secondary generalization	Unknown	Delayed myelination	Active

Table 5.7 Epilepsy classification, neuroimaging findings, and seizure outcomes at follow-up in subjects with incident epilepsy

5.3.2.4 CSE recurrence during follow-up

Over the follow-up period, 36% (95% CI 27-45) of subjects who had incident CSE and 71% (95% CI 53-85) with previous CSE at baseline had one or more recurrences (Table 5.8). 15 of 106 (14%, 95% CI 9-22) followed-up children with incident CSE had CSE recurrence within 6 months (i.e. during NLSTEPSS). A further 23 (22%, 17-27) had CSE recurrence later during the follow-up. Similar to findings of NLSTEPSS, children with pre-existing neurological abnormality were 3.8 times (95% CI 1.8-8.0, $p < 0.001$) more likely to have a recurrence during follow-up. In the PFS group, 10 (29%, 95% CI 16-45) had CSE recurrence, and of these, 4 had epilepsy diagnosis during follow-up.

CSE category	CSE recurrence during follow-up				
	Previous CSE	Number at risk	During NLSTEPSS N (%, 95% CI)	After NLSTEPSS N (%, 95% CI)	All recurrences N (%, 95% CI)
Prolonged febrile seizures (N= 35)	Incident cases	35	4 (11, 5-26)	6 (17, 8-33)	10 (29, 16-45)
	Previous CSE	0	-	-	-
	All cases	35	4 (11, 5-26)	6 (17, 8-33)	10 (29, 16-45)
Acute symptomatic (N=15)	Incident cases	15	1 (7, 1-30)	0 (0, 0-20)	1 (7, 1-30)
	Previous CSE	0	-	-	-
	All cases	15	1 (7, 1-30)	0 (0, 0-20)	1 (7, 1-30)
Idiopathic & cryptogenic (N= 19)	Incident cases	11	2 (18, 5-48)	4 (36, 15-65)	6 (55, 28-79)
	Previous CSE	8	1 (12.5, 2-47)	5 (62.5, 40-80)	6 (75, 41-93)
	All cases	19	3 (16, 6-38)	9 (47, 27-68)	12 (63, 41-81)
Remote symptomatic (N= 53)	Incident cases	35	7 (20, 10-36)	9 (26, 14-42)	16 (46, 31-62)
	Previous CSE	18	6 (33, 16-56)	8 (44, 25-66)	14 (78, 55-91)
	All cases	53	13 (25, 15-38)	17 (32, 21-45)	30 (57, 43-69)
Unclassified (N= 12)	Incident cases	10	1 (10, 2-40)	4 (40, 17-69)	5 (50, 24-76)
	Previous CSE	2	0 (0, 0-66)	0 (0, 0-66)	0 (0, 0-66)
	All cases	12	1 (8, 1-35)	4 (33, 14-61)	5 (42, 19-68)
All CSE (N= 134)	Incident cases	106	15 (14, 9-22)	23 (22, 17-27)	38 (36, 27-45)
	Previous CSE	28	7 (25, 13-43)	13 (46, 30-64)	20 (71, 53-85)
	All cases	134	22 (16, 11-24)	36 (27, 20-35)	58 (43, 35-52)

Table 5.8 CSE recurrence during follow-up according to CSE category

5.3.3 Motor and intellectual disability

5.3.3.1 Prevalence of motor and intellectual disability

The prevalence of motor and intellectual disability at follow-up in our CSE cohort was 30.6% (95% CI 23.4-38.8) and 45.5% (95% CI 37.3-54) respectively (Table 5.3). Although the prevalence of motor and intellectual disability was high in the idiopathic & cryptogenic (37% and 84% respectively) and RS CSE groups (62% and 75.5% respectively), this only reflected their pre-existing disabilities at CSE presentation in most (Table 5.3). In addition, the majority with motor and/or intellectual disability also had epilepsy (Figure 5.4).

5.3.3.2 Incidence of motor and intellectual disability

Incidence rates for motor and intellectual disability are presented in Table 5.9. The cumulative incidence of motor disability was 2.1% (95% CI 0.6-7.4) and intellectual disability 8.8% (95% CI 4.3-17) for the whole CSE cohort. No one in the PFS and AS CSE groups developed motor disability, and only one child with PFS had intellectual disability and autism. The incidence of intellectual disability was low, but 17.1% (95% CI 8.1-32.7) in the PFS group and 6.7% (95% CI 1.2-29.8) in the AS CSE group had borderline intellectual functioning (FSIQ 71-84) (Table 5.3).

As there were only two incident cases of motor disability, regression analysis for predictive factors was not performed for this outcome. Presence of epilepsy at baseline was the only marginally significant predictor of intellectual disability (OR 6.6, 95% CI 1.0-43.0, p = 0.05) (Table 5.10).

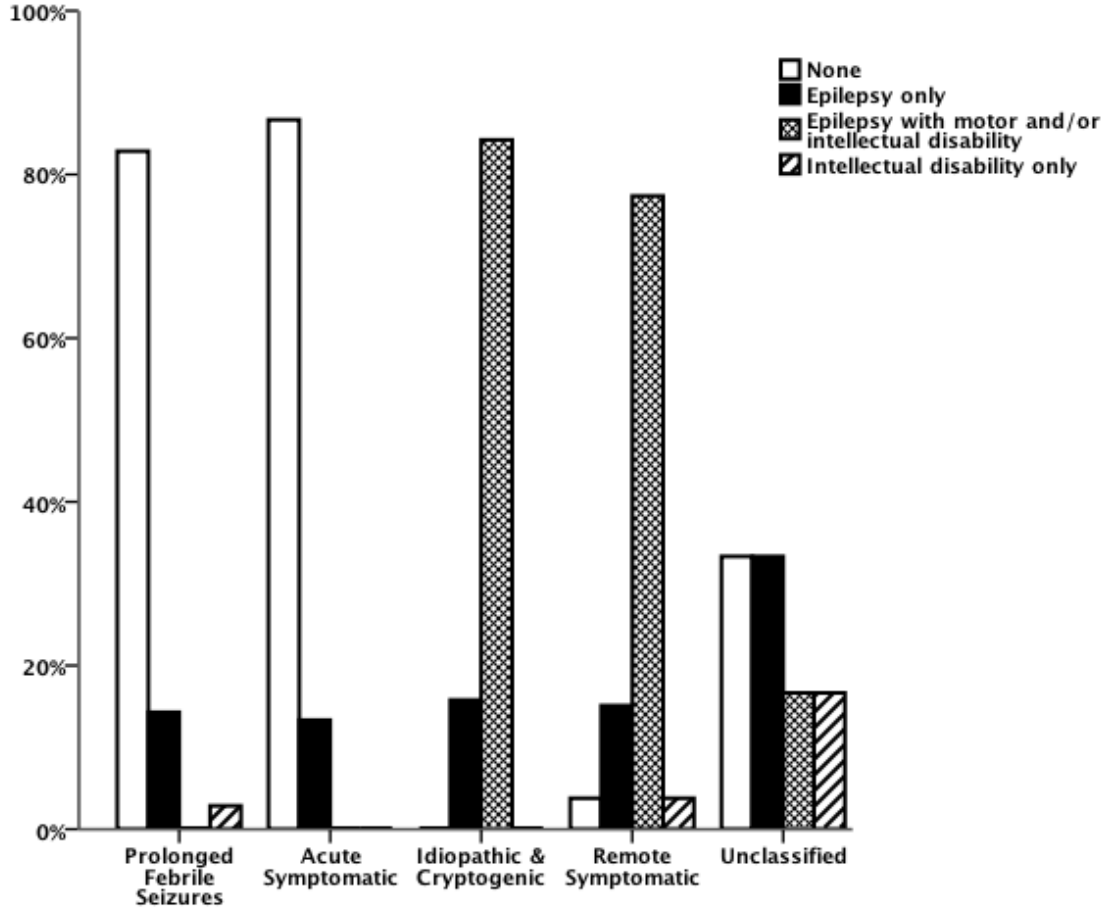


Figure 5.4 Prevalence of epilepsy, motor and intellectual disability at follow-up in childhood CSE cohort

CSE category	Motor disability					Intellectual disability				
	Abnormal neurology at baseline	Incident cases	Cumulative incidence % (95% CI)	Person -years	Incidence rate* (95% CI)	Abnormal cognition at baseline	Incident cases	Cumulative incidence % (95% CI)	Person -years	Incidence rate* (95% CI)
Prolonged febrile seizures (N=35)	0/35	0/35	0 (0, 9.9)	296	0 (0, 12.5)	0/35	1/35	2.9 (0.5, 14.5)	296	3.4 (0.1, 18.8)
Acute symptomatic (N=15)	0/15	0/15	0 (0, 20.4)	127	0 (0, 29)	0/15	0/15	0 (0, 20.4)	127	0 (0, 29)
Idiopathic & cryptogenic (N=19)	7/19	0/12	0 (0, 24.2)	101	0 (0, 36.5)	13/19	3/6	50 (18.8, 81.2)	47	63.8 (13.2, 186.5)
Remote symptomatic (N=53)	32/53	1/21	4.8 (0.8, 22.7)	189	5.3 (0.1, 29.5)	38/53	2/15	13.3 (3.7, 37.9)	135	14.8 (1.8, 53.5)
Unclassified (N=12)	0/12	1/12	8.3 (1.5, 35.4)	103	9.7 (0.2, 54)	3/12	1/9	11.1 (2, 43.5)	79	12.7 (0.3, 70.5)
All CSE (N=134)	39/134	2/95	2.1 (0.6, 7.4)	816	2.5 (0.3, 9)	54/134	7/80	8.8 (4.3, 17)	684	10.2 (4.1, 21.1)

*Incidence per 1000 person-years

Table 5.9 Incidence of motor and intellectual disability following childhood CSE

Clinical and sociodemographic characteristics	Intellectual disability			
	Complete case (N = 134)		Imputed dataset (N = 203)	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age < 1 year at CSE	0.8 (0.1, 4.5)	0.80	0.8 (0.2, 4.3)	0.84
Male gender	2.58 (0.46, 14.3)	0.278	1.37 (0.29, 6.46)	0.688
Gestation <37 weeks*	-	-	-	-
Socioeconomic status (IMD 2004 scores)	1.00 (0.95, 1.053)	0.99	1.01 (0.96, 1.062)	0.725
Previous CSE	5.1 (0.4, 64.6)	0.21	3.9 (0.3, 44.3)	0.28
Pre-hospital treatment given	4.2 (0.5, 37.1)	0.195	2.9 (0.4, 23.7)	0.315
Interval between seizure onset and first treatment given	0.937 (0.876, 1.002)	**0.059**	0.973 (0.906, 1.044)	0.44
Afebrile at CSE	2.9 (0.6, 14.3)	0.18	2.5 (0.5, 12.2)	0.25
CSE duration (min)	1.00 (0.99, 1.01)	0.72	1.00 (0.99, 1.01)	0.68
Intermittent v continuous CSE	1.5 (0.3, 7.0)	0.63	1.4 (0.3, 5.6)	0.67
CSE onset (focal v generalised)	3.6 (0.7, 17.8)	0.12	2.3 (0.5, 11.4)	0.29
Motor disability at baseline*	-	-	-	-
Epilepsy at baseline	8.0 (1.1, 59.6)	**0.042**	6.6 (1.0, 43.0)	**0.049**

*Zero cells

Table 5.10 Predictors of incident intellectual disability following childhood CSE

5.3.4 Behavioural problems

Nearly 40% of subjects in the idiopathic & cryptogenic and RS CSE group had a psychiatric diagnosis (Table 5.11). In contrast, less than 7% in the PFS and AS CSE group had psychiatric disorders. Nearly all with a psychiatric diagnosis also had epilepsy and/or intellectual disability (Table 5.12).

Autism spectrum disorder (78%, 95% CI 59-89) was the most frequent psychiatric diagnosis, followed by ADHD (22%, 95% CI 11-41). Both children in the PFS group with a psychiatric diagnosis had autism spectrum disorder (one isolated and the other with epilepsy).

5.3.5 Educational difficulties

60% (95% CI 51-68) reported educational difficulties requiring additional support at school, and 51% (95% CI 43-60) had received a statement of special educational needs (Table 5.11). However, there was considerable variability in the level of educational needs in different CSE groups. While almost all children in the PFS and AS CSE groups required no support or additional support in mainstream class, the majority in idiopathic & cryptogenic (89%, 95% CI 69-97) and RS CSE (87%, 95% CI 75-93) groups were placed in a special class/school. Only 2 (6%, 95% CI 2-19) children who initially presented as PFS and diagnosed with ASD during follow-up, and 4 (33%, 95% CI 14-61) in the unclassified CSE group required placement in special class/school.

Significant intellectual and/or behavioural impairment was the primary reason for special educational provision in all. A diagnosis of epilepsy, motor and intellectual disability, and psychiatric disorder were all significantly associated with a need for special educational provision (Table 5.13).

	Prolonged febrile seizures n (%, 95% CI)	Acute symptomatic n (%, 95% CI)	Idiopathic & cryptogenic n (%, 95% CI)	Remote symptomatic n (%, 95% CI)	Unclassified n (%, 95% CI)
Behavioural problems					
No psychiatric diagnosis	33 (94, 81-98)	14 (93, 70-99)	11 (58, 36-77)	34 (64, 51-76)	10 (83, 55-95)
Psychiatric diagnosis	2 (6, 2-19)	1 (7, 1-30)	8 (42, 23-64)	19 (36, 28-45)	2 (17, 5-45)
Special educational provision					
None	28 (80, 64-90)	13 (87, 62-96)	1 (7, 1-30)	4 (8, 3-18)	8 (67, 39-86)
Additional support in mainstream class	5 (14, 6-29)	2 (13, 4-38)	1 (7, 1-30)	3 (6, 2-15)	0 (0, 0-24)
Statement of special educational needs	2 (6, 2-19)	0 (0, 0-20)	17 (89, 69-97)	46 (87, 75-93)	4 (33, 14-61)
Total	35	15	19	53	12

Table 5.11 Behavioural problems and educational difficulties in childhood CSE cohort

	N	Psychiatric diagnosis		P value
		No (%)	Yes (%)	
Epilepsy				
No	55	50 (90.9)	5 (9.1)	0.001
Yes	79	52 (65.8)	27 (34.2)	
Intellectual disability				
No	73	67 (91.8)	6 (8.2)	<0.001
Yes	61	35 (57.4)	26 (42.6)	
Motor disability				
No	93	74 (79.6)	19 (20.4)	0.16
Yes	41	28 (68.3)	13 (31.7)	

Table 5.12 Associations between psychiatric disorders and epilepsy, motor and intellectual disability in childhood CSE cohort

	N	Statement of special educational needs		P value
		No (%)	Yes (%)	
Epilepsy				
No	55	49 (89.1)	6 (10.9)	<0.001
Yes	79	16 (20.3)	63 (79.7)	
Intellectual disability				
No	73	64 (87.7)	9 (12.3)	<0.001
Yes	61	1 (1.6)	60 (98.4)	
Motor disability				
No	93	65 (69.9)	28 (30.1)	<0.001
Yes	41	0 (0)	41 (100)	
Psychiatric diagnosis				
No	102	62 (60.8)	40 (39.2)	<0.001
Yes	32	3 (9.4)	29 (90.6)	

Table 5.13 Associations between need for special educational provision and epilepsy, motor and intellectual disability, and psychiatric diagnosis in childhood CSE cohort

5.4 Discussion

The major findings of our prospective study investigating neurological, cognitive, behavioural, and education outcomes at median follow-up of 8.9 years in a population-based childhood CSE cohort are: (1) most children with no prior neurological abnormality have a favourable outcome following CSE, and do not develop epilepsy, motor or intellectual disability; (2) subsequent TLE can be found following all forms of CSE, not just PFS, but are uncommon; (3) children with prior epilepsy and/or neurological impairment at CSE presentation often have active epilepsy and substantial motor, cognitive, and behavioural comorbidity at follow-up; and (4) aetiology is the main determinant of adverse neurological outcomes.

Despite a longer median follow-up of 8.9 years, the cumulative incidence of epilepsy in our cohort (25%) is comparable with 15-36% incidence reported within 3 to 5 years after childhood CSE (Raspall-Chaure et al., 2006; Aicardi and Chevrie, 1970; Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Maegaki et al., 2005; Maytal et al., 1989; Tabarki et al., 2001; Prins et al., 2014). With longer follow up one may expect more cases emerging but 90% of CSE cohort who developed epilepsy in our study did so within 18 months after CSE suggesting that most of those going to develop epilepsy after CSE will do so within that period. About 10% of children with new-onset epilepsy can present with CSE as their first unprovoked seizure, and 80-90% of recurrences after a first unprovoked seizure occur within 2 years (Berg, 2008; Berg et al., 1999b; Sillanpaa and Shinnar, 2002). This implies that unprovoked CSE, like brief seizures, could be the presentation that leads to the diagnosis of epilepsy within 2 years in the majority, with low risk of epilepsy thereafter.

We found a high prevalence of active epilepsy but this may be related to continued medication despite adequate seizure control, especially in RS CSE group. There was a marked difference in the proportion of those with active epilepsy who were previously normal compared those who were not (5% vs. 55%, $p<0.001$). When taken with data about the higher risk of recurrent CSE and poor seizure outcome in children with remote symptomatic epilepsy and/or abnormal neurology reported in other studies, questions are raised about whether CSE is the cause or merely a marker of poor seizure outcomes in children with pre-exiting neurological problems (Berg et al., 2004a; Camfield and Camfield, 2012; Sillanpää et al., 2014; Sillanpaa and Shinnar, 2002; Stroink et al., 2007).

Our findings provide further reassuring prognostic information of generally favourable neurological and cognitive outcomes for children with PFS (Nelson and Ellenberg 1978, Maytal, Shinnar et al. 1989, Maytal and Shinnar 1990, Verity, Ross et al. 1993, Verity, Greenwood et al. 1998, Shinnar, Pellock et al. 2001, Raspall-Chaure, Chin et al. 2006, Chungath and Shorvon 2008). Our study also provides compelling evidence to add to the long-standing debate over the causative relationship between PFS and later development of MTS and TLE (Verity, 1998; Raspall-Chaure et al., 2006; Patterson et al., 2014; Shukla and Prasad, 2012; Tarkka et al., 2003). In our cohort, TLE was not common in those who developed epilepsy within 8.9 years after PFS and was found in children with other forms of CSE, but this was also uncommon. Data in chapters 7 and 8 of this doctoral thesis will demonstrate little MRI evidence suggestive of progressive hippocampal changes leading to MTS. In a recent longitudinal study with MRI at 3 spaced intervals one year post-CSE, Yoong et al. found similar lack of evidence for long-lasting and progressive hippocampal changes leading to MTS (Yoong, 2012; Yoong et al., 2013a). When these data are considered with observations from earlier prospective studies reporting low incidence of TLE following PFS (Chungath and Shorvon, 2008; Nelson and Ellenberg, 1978; Lewis et al., 2014; Verity, 1998), distinct possibilities emerge: (1) PFS does not directly lead to MTS/TLE in the majority of patients and the debate should be laid to rest; (2) the causative relationship between PFS and MTS/TLE is not specific to that type of CSE only; (3) a longer interval than 8.9 years from CSE may be needed for the emergence of MTS/TLE although it would seem unlikely in view of the data discussed above; or (4) there is a small subset of patients that may develop MTS following PFS/CSE and the characteristics of such patients remain uncertain. The FEBSTAT study group have reported pre-existing subtle hippocampal abnormalities in a subgroup of children that may predispose them to having FSE, but it is unclear whether this predisposes them to later development of MTS (Lewis et al., 2014). In our PFS cohort we found MRI features of HIMAL in 27%, but these were not associated with MTS. The neuroimaging findings will be addressed in detail in chapter 6.

Although nearly a third to a half of our cohort had intellectual and/or motor disability at follow-up, the disabilities were seen mostly in idiopathic & cryptogenic and RS CSE groups. In addition, the disabilities were present before the CSE episode in the majority. Prevalence of motor and/or intellectual disability was very low in children with no pre-existing neurological abnormality at CSE presentation (e.g. PFS and AS CSE groups). Low risk of permanent neurological sequelae in previously neurologically normal children seen in our study is broadly consistent with favourable prognosis reported for this

group in previous prospective studies (Raspall-Chaure et al., 2006; Awaya et al., 1992; Maytal and Shinnar, 1990; Maytal et al., 1989; Nelson and Ellenberg, 1978; Shinnar et al., 2001b; Verity et al., 1993; Lewis et al., 2014; Scott et al., 2003). Acute symptomatic CSE, particularly due to CNS infections, has generally been reported to be associated with increased risk of neurological sequelae (Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Raspall-Chaure et al., 2006; Aicardi and Chevrie, 1970; Kwong et al., 1995; Maegaki et al., 2005; Tabarki et al., 2001; Scholtes et al., 1996). Our findings match the results of a few previous studies suggesting that although acute symptomatic CSE is associated with increased short-term mortality, the long-term neurological outcome among survivors is favourable (Hussain et al., 2007; Kravljanac et al., 2011; Maytal et al., 1989). However, consistent with previous reports, we observed poor prognosis for CSE due to CNS infections (nearly 50% with known outcomes either died or developed epilepsy) (Eriksson and Koivikko, 1997; Kwong et al., 1995; Raspall-Chaure et al., 2006; Tabarki et al., 2001; Scholtes et al., 1996).

Data from population-based studies suggest that in addition to seizure frequency and motor/cognitive disability, presence of behavioural problems and educational difficulties also can have an adverse impact on health-related quality of life in childhood-onset epilepsy and CSE (Reilly et al., 2015; Baca et al., 2011; Ferro et al., 2014). While there has been much research focused on seizure outcomes and neurocognitive morbidity associated with childhood CSE, few studies report behavioural and psychiatric comorbidity, separate from other neurological sequelae (Raspall-Chaure et al., 2006). 24% of the follow-up CSE cohort had a psychiatric diagnosis at follow-up. Nearly all with a psychiatric diagnosis also had epilepsy and/or intellectual disability. The prevalence of psychiatric disorders in 34% of our cohort with epilepsy is similar to the reported rates in children with complicated epilepsy (Reilly et al., 2014; Barnard and Wirrell, 1999; Berg et al., 2011b; Davies et al., 2003; Rutter et al., 1970). Given the high prevalence of psychiatric comorbidity in our study, it seems that screening for behavioural disorders should be considered, particularly in children who have epilepsy and/or intellectual disability. Early recognition and management of behavioural disorders may potentially improve long-term psychosocial outcomes.

In our study, nearly 90% in idiopathic & cryptogenic and RS CSE groups had special educational needs. While the populations may not be directly comparable, similar high rates of special educational needs are reported in a population-based cohort with complicated epilepsy (Berg et al., 2005; Berg et al., 2011c). Only 6% in the PFS group in

our study had special educational needs, which is comparable with 12-13% prevalence reported in two long-term follow-up studies (Tarkka et al., 2003; Verity et al., 1993). Prospective cohort studies in children with new onset seizures and uncomplicated epilepsy report similar educational outcomes in those with and without CSE, thus suggesting that CSE may not itself have additional adverse impact on learning (Camfield and Camfield, 2012; Sillanpää, 2004; Sillanpaa and Shinnar, 2002; Sogawa et al., 2010). Although only one child in the PFS group had intellectual disability, another 17% had borderline intellectual functioning (FSIQ 71-84) in our study. Similar finding of subtle developmental and memory impairments within a few months after PFS have recently been reported (Martinos et al., 2012; Martinos et al., 2013; Roy et al., 2011). As these children still perform within the reference range, it is possible that subtle difficulties potentially affecting learning may be under recognised in children with PFS and CSE in general.

Our study provides strong population-based data confirming the mounting evidence that aetiology is the main determinant of outcome following CSE in childhood (Raspall-Chaure et al., 2006; Verity, 1998; Maegaki et al., 2005; Maytal et al., 1989). Neurological, cognitive, and behavioural comorbidity was much higher in those with symptomatic CSE and pre-existing neurological abnormalities, and low in previously neurologically normal children. In addition to aetiology, CSE-related characteristics such as younger age at CSE, absence of fever, focal seizure onset, previous history of seizures/CSE, and seizure duration have previously been associated with poor neurological outcomes, but none of these, except absence of fever at CSE presentation, were independently significant for increased risk of incident epilepsy in our study (Raspall-Chaure et al., 2006),

Our finding of seizure duration not being a predictor for risk of subsequent epilepsy is similar to that reported in previous studies (Barnard and Wirrell, 1999; Lewis et al., 2014; Maytal et al., 1989; Nelson and Ellenberg, 1978; MacDonald et al., 1999; Pavlidou and Panteliadis, 2013; Verity and Golding, 1991; Viani et al., 1987) but we recognise that it is also in contrast to others (Annegers et al., 1987; Eriksson and Koivikko, 1997; Maegaki et al., 2005; Scholtes et al., 1996). There are several possible explanations for this finding. Poor outcomes with longer seizure duration have been reported primarily in hospital-based studies, which generally report worse outcomes for refractory CSE (seizures lasting >90 minutes) (Eriksson and Koivikko, 1997; Kim et al., 2001b; Maegaki et al., 2005; Raspall-Chaure et al., 2006; Scholtes et al., 1996), and in studies comparing

outcomes between brief seizures (<30 minutes) and CSE (seizure duration >30 minutes) (Annegers et al., 1987; Chungath and Shorvon, 2008; MacDonald et al., 1999; Metsäranta et al., 2004; Verity et al., 1993). Our study included only children with seizures ≥30 minutes, with seizure duration less than 90 minutes in the majority. Therefore, our results do not exclude the possibility of a critical threshold under 30 minutes or over 90 minutes where seizure duration may significantly influence outcomes. Another possibility is that the lack of statistical significance for clinical features, including duration, as predictors may be because our study is underpowered. However, our results suggest that any effect size that they may contribute is not large, and therefore are unlikely to be major predictors in the general population. Further support for our finding of seizure duration in CSE not being a major predictor is comparison with the risk of epilepsy following a single unprovoked seizure. The risk of epilepsy 8 to 10 years after a brief first unprovoked seizure is 46-52%, which is similar to the 48% incidence of epilepsy after unprovoked CSE in our study (Shinnar et al., 2000; Berg, 2008). Therefore, we could conclude that CSE by itself or longer seizure duration does not "cause" subsequent epilepsy.

While our study has the advantage of a prospective, long-term follow-up of a large population-based cohort, it has some limitations. Loss to follow-up is inevitable in long-term cohort studies, but 34% dropout rate in our study is within the acceptable range (Fewtrell et al., 2008). In addition, we used multiple imputation to systematically address missing data and minimise potential bias from a complete-case analysis and found our results to be consistent using both approaches (White et al., 2011). The latency to the development of TLE and MTS after PFS/CSE could be longer than our follow-up duration, and therefore their incidence could eventually be higher than our estimates (Patterson et al., 2014). Also, although detailed clinical and developmental data were collected at CSE presentation, formal cognitive testing was not performed due to inherent methodological limitations and therefore we were unable to determine the presence of cognitive impairment at baseline by this method. Hence, we cannot say with confidence whether intellectual disability was pre-existing or developed after the CSE episode. While this factor should not affect prevalence, it should be borne in mind when considering our estimates for the incidence of intellectual disability.

Thus, results of the study presented in this chapter show that childhood CSE is associated with substantial long-term neurological, neurocognitive, and behavioural comorbidity, but this is seen primarily in children who have pre-existing epilepsy

and/or significant neurological abnormalities at CSE presentation. The outcome is favourable for children with PFS and survivors of acute symptomatic CSE, with a low risk of developing long-term sequelae. Collectively, the current data further support the concept that aetiology is the main determinant of long-term neurological outcomes following childhood CSE and suggest that CSE is probably a marker of severity of underlying aetiology rather than the cause of adverse outcomes. While these data are reassuring, whether prolonged seizures can influence outcomes and their causal association, especially with subtle neurocognitive dysfunction, still remain uncertain. Therefore, considering the high mortality and morbidity associated with childhood CSE, pending clarification of the direct effect of seizure duration on prognosis, CSE should still be regarded as a medical emergency and treated promptly.

In chapters 4 and 5 we discussed long-term mortality and morbidity associated with childhood CSE. While there is growing evidence that aetiology is the main determinant of long-term outcomes after childhood CSE, the relationship between the outcomes and structural brain abnormalities have not been established. Also, the causal role of prolonged seizures in childhood, particularly PFS, in hippocampal injury and the development of MTS has long been debated and yet unresolved. In the following chapters the candidate will use qualitative and quantitative MR Imaging methods to determine the prevalence of structural brain abnormalities, and investigate the long-term consequences of PFS and non-PFS CSE on hippocampal and extra-hippocampal structures.

CHAPTER 6 CLINICAL MRI FINDINGS

6.1 Introduction

The long-standing debate on the causative role of childhood CSE, PFS in particular, in the development of mesial temporal sclerosis (MTS) and temporal lobe epilepsy (TLE) is yet to be resolved (Shukla and Prasad, 2012; Raspall-Chaure et al., 2006). Several studies have demonstrated MRI evidence of acute hippocampal injury and abnormal hippocampal structure and growth several months following PFS and also other forms of CSE in a substantial minority of children (Raspall-Chaure et al., 2006; Yoong et al., 2013a; Shinnar et al., 2012; Lewis et al., 2014; Scott et al., 2003). However, there is limited data on longer-term evolution of these changes and the risk of development of MTS. In addition, while there is growing evidence that the outcome of CSE in childhood is largely dependent upon aetiology, the relationship between the outcomes following CSE and structural brain abnormalities have not been established (Raspall-Chaure et al., 2006). Determination of the underlying structural brain abnormalities following childhood CSE in children could potentially be beneficial in facilitating more targeted treatment (e.g. epilepsy surgery) and help in prognostication.

Recent reports have suggested a possible association between PFS and developmental abnormalities of the hippocampus, particularly hippocampal malrotation (HIMAL) (Shinnar et al., 2012; Yoong et al., 2012). In addition, HIMAL, or incomplete hippocampal inversion (IHI), is increasingly reported in people with epilepsy as well as in healthy individuals, and therefore the clinical significance of this finding is unclear and still debated (Bajic et al., 2009; Barsi et al., 2000; Connolly, 2013; Gamss et al., 2009; Raininko and Bajic, 2010). Between 14-15 weeks and 18-20 weeks of fetal development, the hippocampal formation undergoes progressive folding into the temporal lobe, and the overall structure resembles that of the adult by 21 weeks of gestation (Ge et al., 2015; Bajic et al., 2008). In HIMAL or IHI, the folding/inversion is incomplete and the hippocampus has a vertically oriented, rounded or pyramidal appearance in coronal view. While the imaging features of HIMAL/IHI are well described, there is no consensus on its definition, and therefore there is considerable variability in the criteria used to define HIMAL/IHI between different studies (McLean, 2012; Cury, 2015). For example, HIMAL/IHI was defined as presence of at least one of the described criteria by Hamad et al., at least three of the criteria by Bernasconi et al., and presence of all of the criteria by Gamss et al. (Bernasconi et al., 2005; Gamss et al., 2009; Hamad et al., 2013).

Furthermore, several studies define HIMAL/IHI as incomplete rotation of the hippocampus with normal size and signal intensity but abnormally rounded shape and blurred internal structure, without specifying the number of criteria each study subject had to meet for HIMAL/IHI definition (Bajic et al., 2009; Bajic et al., 2008; Barsi et al., 2000; Shinnar et al., 2012; Yoong et al., 2012; Depondt et al., 2002; Henry et al., 2011; McLean, 2012; Cury, 2015). It is therefore not surprising that there is considerable heterogeneity in reported prevalence of HIMAL/IHI in individuals with and without seizures/epilepsy (Raininko and Bajic, 2010).

The objectives of the study presented in this chapter are to (1) determine the prevalence of clinically significant MRI abnormalities, MTS in particular, within 10 years after childhood CSE, (2) investigate factors associated with clinically significant MRI abnormalities, and (3) compare the prevalence of HIMAL/IHI in children with PFS, non-PFS CSE, and age-appropriate healthy controls.

6.2 Methods

6.2.1 MR Image acquisition

The MRI methods used in this study have been described in chapter 3.

6.2.2 Patient cohort

The details of children undergoing MRI scan for STEPSOUT and the reasons for not having a scan are presented in Table 6.1. Of the 45 (33% of the total) children who either declined consent or consented for an unsedated scan but were uncooperative for research MRI, 17 (13% of the total, all had epilepsy) had an MRI scan performed at least 5 years post-CSE for clinical reasons and the MR images were available for review by the research team. They were therefore included in the current analysis.

CSE category	MRI during study n (%)	Declined consent for MRI n (%)	Uncooperative for MRI n (%)	No MRI during study but had recent MRI n (%)
Prolonged febrile seizures (n=35)	32 (91)	2 (6)	1 (3)	0
Acute symptomatic (n=15)	12 (80)	3 (20)	0	0
Idiopathic & Cryptogenic (n=19)	8 (42)	1 (5)	10 (53)	5 (26)
Remote symptomatic (n= 53)	28 (53)	3 (6)	22 (42)	12 (23)
Unclassified (n= 12)	9 (75)	2 (17)	1 (8)	0
Total (n= 134)	**89 (66)**	**11 (8)**	**34 (25)**	**17 (13)**

Table 6.1 Subjects included in clinical MRI analysis

6.2.3 Clinical MRI analysis and classification

All structural MRI scans were visually analysed and reported by experienced paediatric neuroradiologists, as they would do in standard clinical practice. All MRI scans were further reviewed by another paediatric neuroradiologist with extensive epilepsy imaging experience to characterize the type of MRI abnormality (blinded to clinical details) and assign each MRI scan to one of three diagnostic categories: (1) normal or normal variant, (2) abnormal, but of uncertain clinical significance, or (3) clinically significant abnormality. The radiologic criteria for MTS were presence of definite hippocampal atrophy, increased T2 signal in the hippocampus, and disturbed internal architecture (Malmgren and Thom, 2012).

6.2.4 MRI analysis for HIMAL/IHI

As discussed in previous section, the clinical significance of HIMAL/IHI is uncertain, with some neuroradiologists considering it as a common morphologic variant, and

therefore often this imaging finding is not reported as an abnormality in clinical MRI reports (Connolly, 2013; Raininko and Bajic, 2010). Likewise, the features of HIMAL/IHI were not specifically assessed and reported in clinical MRI reports by our neuroradiologists. Therefore, we performed a separate assessment for HIMAL/IHI.

Reliable assessment for HIMAL/IHI requires a comprehensive visualisation of the hippocampus, and therefore this analysis is limited to children with CSE and healthy controls in whom good quality 3D volume (3D FLASH) sequences were available. The following previously described criteria were used to evaluate the hippocampal head, body and tail: (1) round/pyramidal shape, (2) vertical orientation (craniocaudal diameter of hippocampus equal to or longer than the transverse diameter), (3) medial positioning with respect to temporal horn, (4) normal signal intensity and size, (5) blurred internal structure, (6) deep, vertical orientation of the collateral sulcus, (7) an abnormal size and position of the fornix, (8) empty, large appearing lateral part of temporal horn, (9) a temporal lobe of normal size, and (10) a normal corpus callosum (Bajic et al., 2008; Barsi et al., 2000; Bernasconi et al., 2005).

In the absence of consensus on HIMAL/IHI definition, we identified the features consistently reported in all previous studies and, in our study, classified scans as HIMAL/IHI if at least the following features were present in the same subject: (1) abnormally rounded/pyramidal shape of hippocampus, (2) vertical orientation and medial position of hippocampus, (3) vertical orientation of collateral sulcus (assessed at the hippocampus body level), and (4) normal signal intensity and size (Cury, 2015; McLean, 2012). While blurring of the internal structure is often included in the definition of HIMAL/IHI, this is rather non-specific and dependent on subjective interpretation. Also, it is difficult to reliably attribute “blurry” appearance to the underlying anatomy and not to poor image quality, especially in children in whom patient motion is quite common. Furthermore, results from recent higher resolution MR Imaging studies (using 3T and 7T scanner) show that the majority of subjects with HIMAL/IHI do not have blurring of the internal structure (Henry et al., 2011; McLean, 2012). Hence, we did not include blurring of the internal structure as an essential criterion for HIMAL/IHI definition.

Two trained paediatric neuroradiologists independently assessed all MRI scans for HIMAL/IHI using the above criteria, blinded to clinical details. Any discrepancies between the reviewers were discussed and a consensus reached.

6.2.5 Statistical analysis

All statistical analysis was performed using SPSS (IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY:IBM Corp.).

Logistic regression was used to investigate relevant clinical and demographic factors predictive of clinically significant MRI abnormality. Multivariable regression analysis was performed using the backward stepwise and forced entry models with a $p=0.05$ threshold for inclusion and $p=0.1$ for removal of a variable. The following previously reported clinical and demographic characteristics at CSE presentation which may increase the risk for structural brain lesions were investigated: age at CSE; gender; gestation <37 weeks; socioeconomic status (IMD 2004 score); presence or absence of fever at CSE; CSE duration (analysis performed both as a continuous and as a categorical variable >60 minutes vs. <60 minutes); continuous vs. intermittent CSE; focal vs. generalized onset; whether prehospital treatment was given or not; the time between CSE onset and when the first treatment was given; previous history of afebrile seizures; previous history of CSE; presence of motor disability (as defined in chapter 5); and history of developmental delay (Yoong et al., 2012; Raspall-Chaure et al., 2006; Singh et al., 2010). In addition, to identify the clinical characteristics at follow-up that predict the likelihood of clinically significant MRI abnormality, the following factors were investigated: CSE recurrence during follow-up; epilepsy diagnosis; presence of motor disability; and intellectual disability (FSIQ <70).

Inter-observer agreement for HIMAL/IHI between the two raters was assessed using Cohen's kappa (k) coefficient and a 95% confidence interval (CI) was constructed (McHugh, 2012). Values of k between 0.8 and 0.9 indicate strong agreement and above 0.9 indicate almost perfect agreement (McHugh, 2012). Comparison of the frequencies of HIMAL/IHI in healthy controls and CSE groups was performed using Pearson Chi-Square test and Fisher's Exact test.

6.3 Results

6.3.1 Clinical MRI analysis

6.3.1.1 Patient characteristics

MRI scans of 106 (52.2% of original cohort) subjects were suitable for analysis. There were no significant differences in the clinical and sociodemographic characteristics of the 106 subjects included in MRI analysis compared with the 97 not included (Table 6.2). The median interval between CSE and MR imaging was 7.8 years (range 5.1 to 10.2) for the whole CSE cohort, and 8.4 years (range 6.3 to 10) for the PFS and acute symptomatic CSE groups.

6.3.1.2 Clinical MRI findings

Overall, 37 out of 106 scans (34.9%, 95% CI 26.5-44.3) were classified as showing clinically significant abnormality (Table 6.3). The majority (77.5%, 95% CI 62.5-87.7) in the remote symptomatic CSE group and fewer than 10% in the other CSE groups had clinically significant abnormalities. Figure 6.1 shows the clinical profile of children with and without clinically significant abnormalities. Of 69 subjects without clinically significant MRI abnormality, nearly half had epilepsy with or without motor/cognitive/ behavioural disability (as defined in chapter 5) and the other half were neurologically normal. In contrast, only 10% with clinically significant MRI abnormality were neurologically normal and over 60% had complex epilepsy with motor/cognitive/ behavioural comorbidity.

Sociodemographic/ Clinical Characteristics	Had MRI (Total = 106) N (%)	No MRI (Total = 97) N (%)	Difference in percentages (95% CI)
Gender			
Male	49 (46.2)	53 (54.6)	-8.4 (-21.7, 5.3)
Female	57 (53.8)	43 (45.4)	
Median age at CSE, months (range)	31.0 (0.9-192)	34.6 (1.4-186.4)	0.24+
Neurological impairment before the CSE episode			
Absent	52 (49.1)	42 (43.3)	5.8 (-7.9, 19.1)
Present	54 (50.9)	55 (56.7)	
CSE category			
Prolonged febrile seizures	32 (30.2)	24 (24.7)	5.4 (-6.9, 17.4)
Acute symptomatic	12 (11.3)	14 (14.4)	-3.1 (-12.7, 6.2)
Remote symptomatic	40 (37.7)	32 (33.0)	4.7 (-8.4, 17.5)
Idiopathic & cryptogenic	13 (12.3)	21 (21.6)	-9.4 (-19.8, 1.0)
Unclassified	9 (8.5)	6 (6.2)	2.3 (-5.4, 9.9)
CSE occurrence			
First-ever (Incident)	86 (81.1)	77 (79.4)	1.8 (-9.2, 12.8)
Previous CSE (Recurrence)	20 (18.9)	20 (20.6)	
Seizure duration			
30-60 min	36 (34.0)	38 (39.2)	-5.2 (-18.2, 7.9)
>60 min	70 (66.0)	59 (60.8)	
Seizure character			
Continuous	54 (50.9)	45 (46.4)	4.6 (-9.1, 17.9)
Intermittent	52 (49.1)	52 (53.6)	
Seizure type			
Generalised-onset	66 (62.3)	58 (59.8)	2.5 (-10.8, 15.7)
Focal-onset	40 (37.7)	39 (40.2)	
Ethnicity			
White	43 (40.6)	31 (32.0)	8.6 (-4.6, 21.3)
Black	16 (15.1)	24 (24.7)	-9.6 (-20.6, 1.3)
Asian	33 (31.1)	29 (29.9)	1.2 (-11.4, 13.7)
Other	14 (13.2)	13 (13.4)	-0.2 (-9.9, 9.2)
Socioeconomic status			
(Median IMD 2004 scores)	34.3	36.2	0.32+

+ *p* value on Mann-Whitney U test

Table 6.2 Comparison of the sociodemographic and clinical characteristics of CSE cohort members with and without MRI scans

CSE category	Normal or normal variant n (%)		Abnormal, but of uncertain clinical significance, n (%)		Clinically significant abnormality n (%)	
PFS (n=32)	Normal	28 (87)	Non-specific WM abnormality	1 (3)	Left MTS	1 (3)
	Small left hippocampus, normal T2 signal	1 (3)			Features of neurofibromatosis type 1	1 (3)
Acute symptomatic (n=12)	Normal	6 (50)	Non-specific WM abnormality	1 (8)	Left MTS	1 (8)
	Small choroid fissure cyst	2 (17)	Bilateral cerebellar WM signal change	1 (8)	Focal left frontal lobe atrophy	1 (8)
Idiopathic & Cryptogenic (n=13)	Normal	8 (61)	Minimal cerebellar atrophy	2 (15)	Left MTS	1 (8)
			Delayed myelination	1 (8)		
			Non-specific WM abnormality	1 (8)		
Remote symptomatic (n=40)	Normal	3 (8)	Non-specific WM abnormality	2 (5)	Hypoxic ischemic insult	8 (20)
	Minor irregularity of left lateral ventricle	1 (2)	Prominent ventricles and sulci	1 (2)	Perinatal middle cerebral artery infarction	4 (10)
	Small left hippocampus, normal T2 signal	1 (2)			Treated hydrocephalus	6 (15)
	Incidental arachnoid cyst	1 (2)			Bilateral PVL	3 (8)
					Old germinal matrix haemorrhage	2 (5)
					Left MTS	2 (5)
					Bilateral hypoglycemic insult	1 (2)
					Tuberous sclerosis	1 (2)
					Focal cortical dysplasia, type 2	1 (2)
					Septo-optic dysplasia	1 (2)
					Bifrontal neuronal migration disorder	1 (2)
					Agenesis of corpus callosum	1 (2)
Unclassified (n=9)	Normal	6 (67)	Non-specific WM abnormality	1 (11)	Left MTS	1 (11)
			Delayed myelination	1 (11)		
Total (n=106)	**57 (54)**		**12 (11)**		**37 (35)**	

WM White matter, MTS Mesial temporal sclerosis, PVL Periventricular leucomalacia

Table 6.3 Clinical MRI findings for subjects in each CSE category

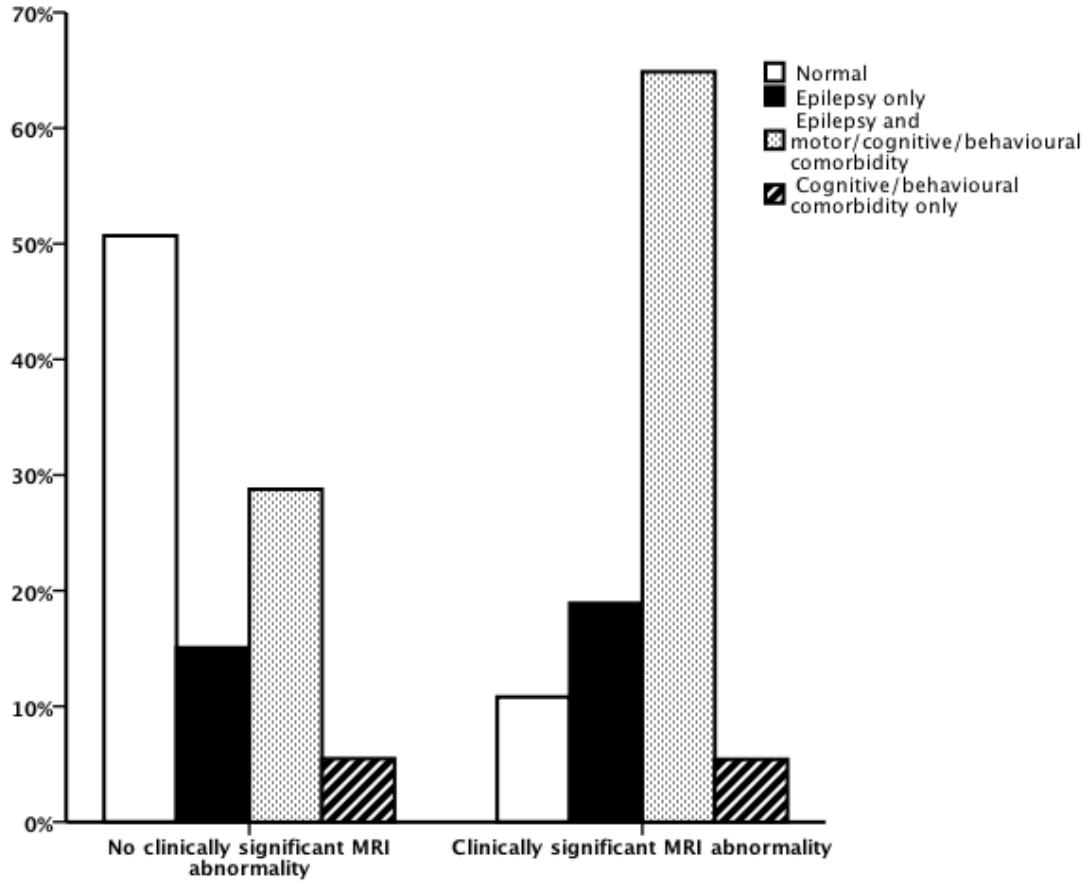


Figure 6.1 Clinical profile of children with and without significant MRI abnormalities

6.3.1.3 Predictors of clinically significant MRI abnormalities

Of the clinical and demographic variables at CSE presentation, only presence of motor disability at baseline (OR 9.9, 95% CI 1.9-52.0) was identified as a predictor of clinically significant MRI abnormalities on multivariable regression analysis (Table 6.4). Focal CSE onset (OR 2.7, 95% CI 0.99-7.3, p = 0.053) also showed a marginal association with clinically significant MRI abnormalities. Multivariable analysis of clinical characteristics at follow-up also identified motor disability as the independent predictor (OR 5.6, 95% CI 1.7-18.7) (Table 6.5). In addition, a diagnosis of epilepsy, either at baseline or during follow-up, was marginally significant (OR 3.6, 95% CI 0.95-13.3) on multivariable analysis.

Predictor	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age at CSE (months)	1.01 (1.00, 1.021)	**0.045**	1.001 (0.987, 1.016)	0.85
Male	1.5 (0.7, 3.3)	0.31	-	-
Gestation <37 weeks	3.4 (1.2, 9.5)	**0.017**	2.7 (0.7, 9.5)	0.13
Socioeconomic status (IMD 2004 score)	0.99 (0.97, 1.02)	0.65	-	-
Afebrile at CSE	2.5 (1.1, 5.6)	**0.028**	1.5 (0.5, 4.5)	0.47
CSE duration (min)	1.00 (0.997, 1.003)	0.99	-	-
Continuous v intermittent CSE	1.3 (0.6, 2.8)	0.54	-	-
Focal v generalised onset	2.7 (1.2, 6.2)	**0.016**	2.7 (0.99, 7.3)	**0.053**
Previous afebrile seizures	6.3 (2.6, 15.0)	**<0.001**	2.5 (0.7, 9.6)	0.17
Previous CSE	1.6 (0.6, 4.3)	0.32	-	-
Pre-hospital treatment for CSE	0.9 (0.4, 1.9)	0.73	-	-
Interval between CSE onset and first treatment (min)	1.02 (0.99, 1.048)	0.20	-	-
Motor disability	16.3 (4.9, 54.1)	**<0.001**	9.9 (1.9, 52.0)	**0.007**
Developmental delay	6.7 (2.7, 16.4)	**<0.001**	1.4 (0.3, 7.6)	0.68

Table 6.4 Characteristics at CSE presentation (baseline) predictive of clinically significant MRI abnormality at follow-up

Predictor	Univariable analyses		Multivariable analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
CSE recurrence during follow-up	2.1 (0.95, 4.7)	**0.069**	1.0 (0.3, 2.7)	0.87
Epilepsy at baseline or during follow-up	6.6 (2.5, 17.8)	**<0.001**	3.6 (0.95, 13.3)	**0.059**
Motor disability	9.6 (3.6, 25.4)	**<0.001**	5.6 (1.7, 18.7)	**0.006**
Intellectual disability (FSIQ <70)	4.4 (1.9, 10.1)	**0.001**	1.0 (0.3, 3.3)	0.98

Table 6.5 Characteristics at follow-up predictive of clinically significant MRI abnormality

6.3.2 Mesial temporal sclerosis

Radiologic features of unilateral MTS (left sided in all) were noted in 6 (5.7%, 95% CI 2.6-11.8) children. However, one child was known to have MTS before the CSE episode, and thus MTS was newly identified following CSE in 5 (4.7%, 95% CI 2.0-10.6) children. As previous imaging was not available for comparison, it is uncertain whether or not any hippocampal abnormalities were present before CSE in these children. The clinical and demographic characteristics of children with newly identified MTS following CSE are presented in Table 6.6. One child each in the PFS (3.1%, 95% CI 0.6-15.7) and unclassified CSE group (11.1%, 95% CI 2.0-43.5) had MTS. Of interest, one child with a febrile CSE due to pneumococcal meningitis at 8 months had unequivocal radiologic features of unilateral MTS. He had not had any seizures after CSE and had normal neurological examination and cognition at follow-up.

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
CSE category	Prolonged febrile seizure	Pneumococcal meningitis	Idiopathic	Remote symptomatic	Unclassified
Age at CSE	12m	8m	4.5y	4.5y	6.3y
Febrile at CSE	Yes	Yes	No	No	No
CSE duration	60 min	80 min	80 min	40 min	60 min
Continuous/ intermittent	Intermittent	Intermittent	Continuous	Continuous	Intermittent
CSE onset	Generalised	Focal	Generalised	Generalised	Focal
Previous seizures/CSE	No	No	Epilepsy & CSE	Febrile sz, no CSE	Febrile sz, no CSE
Perinatal risk factors	None	Prematurity (34 weeks)	None	Birth asphyxia	No
Abnormal neurology pre-CSE	No	No	No	No	No
Developmental delay pre-CSE	No	No	Yes	No	No
Age at epilepsy diagnosis	5.5y	No epilepsy	1.5y (before CSE)	5.1y	6.4y
Epilepsy type	TLE	-	TLE	TLE	TLE
MRI finding	Left MTS	Left MTS	Left MTS	Left MTS	Left MTS

Table 6.6 Characteristics of children with newly identified MTS after CSE

6.3.3 HIMAL/IHI

MRI scans of 73 children with CSE (median age 10.8y, 36 male) and 71 (median age 11.4y, 31 male) healthy controls were suitable for HIMAL/IHI analysis.

There was a strong inter-rater agreement for HIMAL/IHI (k=0.82, 95% CI 0.69-0.95; p<0.001).

CSE category	No HIMAL/IHI n (%, 95% CI)	HIMAL/IHI n (%, 95% CI)
Controls (n=71)	62 (87, 78-93)	9 (13, 7-22)
Prolonged febrile seizures (n=30)	22 (73, 56-86)	8 (27, 14-44)
Acute symptomatic (n=12)	10 (83, 66-93)	2 (17, 5-45)
Remote symptomatic (n= 19)	15 (79, 57-91)	4 (21, 9-43)
Idiopathic & unclassified (n= 12)	10 (83, 66-93)	2 (17, 5-45)
Total (n= 144)	119 (83, 76-88)	25 (17, 12-24)

Table 6.7 Prevalence of HIMAL/IHI in children with CSE and controls

16 (22%, 95% CI 14-33) children with CSE and 9 (13%, 95% CI 7-22) controls met HIMAL/IHI criteria (Table 6.7). HIMAL/IHI was left-sided in 20 (80%), right-sided in 3 (12%), and bilateral in 2 (8%). Blurring of internal structure was observed in 11 (44%) and the quality of images was felt to be inadequate for assessing internal structure in 14 (56%). HIMAL/IHI was the only finding in 20 (80%) and additional neuroimaging abnormalities were noted in 5 (20%) children with CSE (Table 6.8).

CSE category	Neuroimaging abnormalities seen with HIMAL/IHI, n (%)	
Controls (n=71)	-	0
Prolonged febrile seizures (n=30)	NF 1 with hamartoma in contralateral hippocampus	1 (3)
Acute symptomatic (n=12)	Focal frontal lobe atrophy	1 (8)
Remote symptomatic (n= 19)	Periventricular leucomalacia Hypoxic ischaemic insult	2 (10) 1 (5)
Idiopathic & unclassified (n= 12)	-	0
Total (n= 144)		5 (3)

Table 6.8 MRI abnormalities associated with HIMAL/IHI

There was no significant difference in the prevalence of HIMAL/IHI between controls and children with CSE (13% vs. 22%, $\chi^2 = 2.1$, df = 1, p = 0.14), or between the PFS group and non-PFS CSE (27% vs. 19%, $\chi^2 = 0.7$, df = 1, p = 0.41). Interestingly, when the analysis was limited to the PFS group and controls, the point prevalence of HIMAL/IHI was higher in the PFS group (27% vs. 13% in controls, $\chi^2 = 2.95$, df = 1, p = 0.086), although the difference was of weak statistical significance.

Within the PFS group, there were no significant differences in gender, handedness, prematurity, previous febrile seizures, age at CSE presentation, CSE characteristics (type, focality, duration), or CSE recurrence between those with and without HIMAL/IHI ($p > 0.05$ for all). Also, there was no significant association between HIMAL/IHI and the prevalence of epilepsy, intellectual disability, or behavioural problems. There was no significant association between HIMAL/IHI and family history of epilepsy (0% vs. 4.5%, Fisher's Exact p = 1.0), or of febrile seizures (62% vs. 27%, Fisher's Exact p = 0.10).

None of the controls with HIMAL/IHI had any history of seizures or neurological problems. Among the CSE subjects, 7/16 (44%) with, and 21/57 (37%) without HIMAL/IHI had epilepsy (Fisher's Exact p = 0.77) (Table 6.9). There was no significant association between HIMAL/IHI and epilepsy either in the PFS group or in non-PFS CSE groups (Fisher's Exact $p > 0.05$ for all). Similarly, no significant association was observed between HIMAL/IHI and intellectual disability or psychiatric disorder (as defined in chapter 5).

CSE category	Epilepsy			Intellectual disability			Psychiatric diagnosis		
	No HIMAL/IHI n (%)	HIMAL/IHI n (%)	P value	No HIMAL/IHI n (%)	HIMAL/IHI n (%)	P value	No HIMAL/IHI n (%)	HIMAL/IHI n (%)	P value
Prolonged febrile seizures (n=30)	2/22 (9)	1/8 (12)	1.0	0/22 (0)	0/8 (0)	-	3/22 (14)	0/8 (0)	0.55
Acute symptomatic (n=12)	2/10 (20)	0/2 (0)	1.0	0/10 (0)	0/2 (0)	-	1/10 (10)	0/2 (0)	1.0
Remote symptomatic (n= 19)	12/15 (80)*	4/4 (100)*	1.0	7/15 (47)	1/4 (25)	0.60	4/15 (27)	1/4 (25)	1.0
Idiopathic & unclassified (n= 12)	5/10 (50)⁺	2/2 (100)⁺	0.47	3/10 (30)	1/2 (50)	1.0	1/10 (10%)	1/2 (50)	0.32
Total (n= 73)	21/57 (37)	7/16 (44)	0.77	10/57 (17)	2/16 (12)	1.0	9/57 (16)	2/16 (12)	1.0

*3/4 with HIMAL and 10/12 without HIMAL had epilepsy at CSE presentation

⁺Both children with HIMAL and 4/5 without HIMAL had epilepsy at CSE presentation

Table 6.9 Prevalence of epilepsy, intellectual disability, and psychiatric diagnosis in CSE cohort with and without HIMAL/IHI

6.4 Discussion

Ours is the first study to describe the longer-term MR imaging findings and investigate predictors of clinically significant MRI abnormalities in a population-based childhood CSE cohort. The main findings are: (1) a third of children with CSE due to all causes have clinically significant MRI abnormalities at 5 to 10 years follow-up, (2) presence of neurological disability is the main predictor of clinically significant MRI abnormalities, (3) MTS is not frequent within 6.3 to 10 years following PFS and can also be associated with non-PFS CSE, and (4) the prevalence of HIMAL/IHI is marginally higher in children with PFS, but is not uncommon in healthy controls.

About two thirds of CSE subjects in our study had either normal brain MRI findings or abnormalities of uncertain clinical significance. Perhaps not surprisingly, the majority of clinically significant MRI abnormalities were seen in those with symptomatic aetiology; but significant abnormalities were also seen in the PFS group, albeit much less frequent. The prevalence of clinically significant MRI abnormalities in our study (35%, 95% CI 26.5-44) is comparable with 30% (95% CI 23-38) significant neuroimaging abnormalities reported in children with first presentation of seizures lasting >20 minutes (Singh et al., 2010). In contrast, clinically significant MRI abnormalities were seen in only 16% (95% CI 10-26) of children within 13 weeks after CSE in STEPIN study (Yoong et al., 2012). The observed difference in the prevalence of significant MRI abnormalities between our study and STEPIN study is probably due to a lower proportion of children with acute symptomatic (3.8% vs. 11.3% in our study) and remote symptomatic (26.2% vs. 37.7% in our study) CSE in the STEPIN study, and also due to longer follow-up duration in our study (Yoong et al., 2012).

Similar to the results of STEPIN study, we found motor disability at CSE presentation as the main predictor of MRI abnormalities and no significant effect of seizure duration (Yoong et al., 2012). However, in contrast to STEPIN results, focal CSE onset was marginally significant and continuous CSE not significant on multivariable analysis in our study. Children with both major and minor MRI abnormalities were included in regression analyses in STEPIN whilst our analyses were restricted to those with major abnormalities. Thus, differences in statistical analysis, together with lower proportion of symptomatic CSE subjects in STEPIN study could be the reason for the differences observed between STEPIN and our study. Of the clinical variables at follow-up, motor disability was again the main predictor of clinically significant MRI abnormality and a

diagnosis of epilepsy was of marginal significance. Although cognitive impairment was not statistically significant on multivariable analyses, the majority of children with motor disability also had cognitive impairment, both at baseline and at follow-up. Our findings would support the suggestion arising from previously published data that brain MRI does not need to be done routinely in all patients post-CSE, but should be considered in children presenting with a non-PFS CSE, a history of previous afebrile seizures, CSE of focal-onset and abnormal neurological examination (Yoong et al., 2012; Raspall-Chaure et al., 2006). Likewise, MRI during follow-up should be considered in those with motor/intellectual disability and/or epilepsy; routine imaging in all children post-CSE is probably not needed.

In our study, only 1 (3.1%, 95% CI 0.6-15.7) child in the PFS group had MTS and one other had hippocampal asymmetry but no T2 hyperintensity, at a median follow-up of 8.4 years. The FEBSTAT group, prospectively investigating the outcomes following febrile status epilepticus (FSE), found neuroimaging evidence of MTS in 2 out of 191 (1%, 95% CI 0.3-3.7) cases at initial presentation (Shinnar et al., 2012). On longitudinal follow-up, 9 children of 130 with technically adequate follow-up imaging (6.9%, 95% CI 3.7-12.6) developed MRI changes suggestive of MTS between the FSE episode and follow-up imaging about a year later (Shinnar et al., 2012; Lewis et al., 2014; French and Kuzniecky, 2014). However, of these 9 children with MTS, 2 had major extra-hippocampal structural abnormality and developmental delay at baseline, and 3 others had subtle pre-existing hippocampal abnormalities. Thus only 4 (3.1%, 95% CI 1.2-7.6) developed MTS within a year following FSE in the absence of previous structural brain abnormalities in FEBSTAT study (French and Kuzniecky, 2014; Lewis et al., 2014). In the only published long-term follow study of children with PFS, none of the 24 children with PFS (0%, 95% CI 0-13.8) developed MTS after a mean follow-up of 12.5 years (Tarkka et al., 2003). Together, the current data therefore suggest the risk of developing MTS within 8 to 12 years after PFS is low, and certainly not near the 44% of patients with TLE and MTS who had a history of febrile seizures reported in retrospective surgical series (Chungath and Shorvon, 2008). This implies that the causal link between PFS and MTS is not as strong as previously thought, and/or the latency period for the development of MTS following PFS is much longer. To address the latter issue, investigating long-term changes in hippocampal volumes and architecture may shed light on the possibility of subtle hippocampal changes in the absence of MR imaging features of overt MTS and will be addressed in chapters 7 and 8.

MRI features of MTS were not only seen in the PFS group, but also in other CSE groups in our study. All children, except one boy, had epilepsy of temporal lobe semiology at follow-up. This boy, born prematurely at 34 weeks gestation but developing normally and no neurologic problems, had a focal-onset, febrile CSE due to pneumococcal meningitis at 8 months of age. His neuroimaging 7.6 years after CSE shows features of left MTS. He however has not had further seizures and is neurologically intact. Whether his hippocampal injury is primarily due to meningitis/CSE or his hippocampi were susceptible to injury due to prematurity is not clear. Unilateral MTS following meningitis has previously been reported and epilepsy may develop several years after infection (Sellner and Trinka, 2013). Therefore, while he is currently asymptomatic, he may still be at risk of developing epilepsy and associated morbidity.

HIMAL/IHI was observed in 13% of healthy controls and 22% of CSE cohort in our study. As discussed earlier in this chapter, the prevalence of HIMAL/IHI and its clinical significance in individuals without seizures/epilepsy is still a matter of debate. While some studies report a frequency of less than 6% in controls and 14% to 43% in patients with epilepsy, thus suggesting a pathogenic potential of HIMAL/IHI, frequency as high as 37% have been observed in healthy controls in other studies (Table 6.10) (Bajic et al., 2009; Bajic et al., 2008; Bernasconi et al., 2005; Gamss et al., 2009; Hamad et al., 2013; McLean, 2012; Peltier et al., 2005). In fact, recently for her doctoral thesis, Claire Cury performed quantitative assessment on 2008 healthy young people aged between 13 and 17 years and observed total HIMAL/IHI in 17% (Cury, 2015). Our finding of HIMAL/IHI in 13% of healthy controls is therefore comparable with results of previous studies. In addition, as previously reported, HIMAL/IHI was left-sided in 80% in our study (Cury, 2015; McLean, 2012).

Author/Year	Subject age group	Study population	Number of participants	HIMAL/IHI prevalence
Control population				
Cury, 2015	12.9 to 17.2y	Healthy controls	2008	17%
Gamss et al., 2009	Mostly adult	No seizures	497	6 (1.2%)
Bajic et al., 2009	Children & adult	No epilepsy	150	28 (19%)
Bajic et al., 2008	Children & adult	No epilepsy	100	19 (19%)
McLean, 2012	Adult	Healthy controls	84	31 (37%)
Peltier et al., 2005	Adult	Healthy controls	50	3 (6%)
Bernasconi et al., 2005	Adult	Healthy controls	50	5 (10%)
Hamad et al., 2013	Adult	Healthy controls	30	0
Epilepsy				
Barsi et al., 2000	Children & adult	Suspected epilepsy	597	32 (6%)
Lehericy et al., 1995	Adult	TLE	222	2 (0.9%)
Bajic et al., 2009	Children & adult	Epilepsy	201	60 (30%)
Peltier et al., 2005	Adult	Refractory TLE	97	14 (14%)
Bernasconi et al., 2005	Adult	Refractory TLE	30	13 (43%)
Febrile seizures				
Shinnar et al., 2012	Children	Simple febrile seizures	99	2 (2.1%)
Shinnar et al., 2012	Children	Febrile status epilepticus	191	15 (7.9%)
Yoong et al., 2012	Children	PFS	33	4 (12%)

Table 6.10 Reported prevalence of HIMAL/IHI in patients with seizures and in control population

Another interesting finding in our study was a marginally higher prevalence of HIMAL/IHI in the PFS group compared with controls. The occurrence of HIMAL/IHI in children with PFS/FSE has only been reported in two studies previously. Both in FEBSTAT and in STEPIN the frequency of HIMAL/IHI was marginally higher in children with PFS/FSE, similar to findings in our study (Shinnar et al., 2012; Yoong et al., 2012). While the association between HIMAL and a family history of febrile seizures fell short of significance in the current study, previous reports of a high prevalence of HIMAL in individuals with and without seizures in families with familial febrile seizures and TLE suggest possible genetic predisposition for the development of HIMAL/IHI (Depondt et al., 2002; Fernández et al., 1998). These observations, together with reports of higher prevalence of HIMAL/IHI in individuals with malformations of cortical development and chromosomal syndromes, support the hypothesis that HIMAL/IHI results from a disruption of fetal brain development (Bernasconi et al., 2005; Depondt et al., 2002; Hamad et al., 2013; Kuchukhidze et al., 2010; Ge et al., 2015). While it is possible that HIMAL/IHI could potentially predispose to seizures, whether it is pathogenic on its own and whether it increases the risk of developing MTS following prolonged seizures is uncertain.

While our study has the advantage of a long-term follow-up of a population-based childhood CSE cohort, the main limitation is the lack of universal baseline neuroimaging at CSE presentation. However, previous studies from our group on comparable childhood CSE cohorts have shown that there is a low prevalence of clinically significant MRI abnormalities within 4-12 weeks after CSE in children with non-symptomatic aetiology (Scott et al., 2002; Yoong et al., 2012). None of the children with PFS and non-PFS CSE had radiological evidence on MTS on acute imaging in either study. Nonetheless, it is difficult to be certain whether the neuroimaging abnormalities, MTS in particular, found at median 8.4 years follow up in this study pre-date or developed after CSE. Also, neuroimaging could not be performed in about a third of follow-up cohort, primarily due to lack of subject cooperation because of cognitive/behavioural problems. Although we included suitable recent clinical neuroimaging data where available, it is possible that our estimate of prevalence of clinically significant MRI abnormalities could be conservative. Nonetheless, as nearly 90% of children with no neurological issues prior to CSE underwent imaging in the current study, our estimates for MTS are likely to be close to the true prevalence in the general population.

In this chapter, the candidate has discussed novel clinical MRI findings within 10 years after childhood CSE. Our results indicate that fewer than 10% of children with no prior neurological impairment at CSE presentation have clinically significant imaging abnormalities at follow-up. Our data further support the value of abnormal neurological examination at CSE presentation and also at follow-up in predicting clinically significant MRI abnormalities. Our data further support previous reports suggesting low risk of developing MTS within 8 to 12 years after PFS. Furthermore, for the first time, we compared the prevalence of HIMAL/IHI in children with PFS and non-PFS CSE against that in healthy controls and established that although the prevalence may be marginally higher in the PFS group, HIMAL/IHI is not uncommon in healthy controls. Whether childhood CSE could result in long-term hippocampal/extra-hippocampal structural changes that are not obvious on qualitative visual analysis will be addressed in the following chapters.

CHAPTER 7 HIPPOCAMPAL VOLUMETRY

7.1 Introduction

7.1.1 Rationale for hippocampal volumetry

We established in the previous chapter that, at a median follow-up of 8.4 years, the majority of children with PFS do not have radiological evidence of MTS on visual analysis, and that MTS can also be associated with CSE due to other causes. However, as MTS could develop 8 to 11 years after PFS, the typical radiological features of MTS may not be evident during the latency period (Patterson et al., 2014; French et al., 1993; Janszky et al., 2004; Mathern et al., 2002). Also, in view of our finding that MTS occurs in other forms of CSE and a recent study showing that hippocampal volume loss can occur in other forms of childhood CSE, it would be reasonable to hypothesise that MTS can also occur in other forms of CSE following a latency period (Yoong et al., 2013a). Comparative studies have established that a unilateral hippocampal volume loss of at least 15 to 30% is necessary even for skilled neuroradiologists to reliably diagnose hippocampal atrophy (Reutens et al., 1996; Bronen et al., 1991; Tsai et al., 2013). Therefore, while hippocampal volume loss may not be obvious on visual assessment, quantitative analysis such as hippocampal volumetry could potentially uncover volume loss during the latency period.

To our knowledge, all published studies on hippocampal volume changes following CSE are limited either by (1) small sample size, (2) short follow-up duration, (3) restriction to only PFS, or (4) a combination of these factors. Despite their limitations, taken together, data from these studies suggests that acute hippocampal injury can occur after PFS and other forms of CSE, and a substantial minority may demonstrate hippocampal volume abnormalities several months later (Grunewald et al., 2001; Lewis et al., 2014; Natsume et al., 2007; Scott et al., 2002; Scott et al., 2003; Scott et al., 2006; Shinnar et al., 2012; VanLandingham et al., 1998; Yoong et al., 2013a). Therefore, a study that investigates longer-term hippocampal volume characteristics after PFS and other forms of childhood CSE is needed and is the subject of this chapter.

7.1.2 Technical aspects of hippocampal volumetry

Reliable quantitative volumetric analysis requires accurate measurement of hippocampal volumes. While manual segmentation is still considered the "gold standard", it is time-consuming and labour-intensive and therefore not feasible for large datasets. Also, manual segmentation is observer-dependent and therefore needs validation to ensure accuracy and reproducibility. Finally, the protocols used for segmentation often vary between studies, and absolute values may not be comparable. Hence, automated methods for segmentation have attracted considerable interest in recent years and are being used increasingly (Morey et al., 2009; Mulder et al., 2014). The frequently used automated methods such as FreeSurfer and FIRST utilize templates derived using data from healthy adults, and therefore may not be suitable for use in children due to age-dependent differences in regional brain morphology and tissue contrast (Wilke et al., 2008; Sanchez et al., 2012; Hoeksma et al., 2005; Yoon et al., 2009). Consequently, despite limitations, manual segmentation continues to be the preferred method for hippocampal volumetry in the paediatric population.

7.1.3 Objectives of the study

The objectives of the study presented in this chapter are to (1) characterise hippocampal volume changes within 10 years after PFS and other forms of childhood CSE compared to age-appropriate healthy controls, and (2) investigate risk factors associated with hippocampal volume abnormalities.

7.2 Methods

7.2.1 Study subjects

Of the 89 CSE subjects who underwent MR Imaging during the study, sequences adequate for hippocampal volumetry were available for 74 (55% of follow-up cohort). Compared to the rest of the CSE cohort, those included in volumetric analysis were younger, neurologically normal at CSE presentation, had first ever CSE when enrolled into the original CSE cohort, had a higher proportion of PFS and a lower proportion of idiopathic/unclassified CSE, but were similar in other clinical and sociodemographic characteristics. Details of comparison between the 74 CSE cohort included in volumetry analysis with the 129 not included is presented in Table 7.1. Images of 71 age-appropriate healthy controls were available for comparison.

Sociodemographic/ Clinical Characteristics	Had hippocampal volumetry (Total = 74) N (%)	No hippocampal volumetry (Total = 129) N (%)	Difference in percentages (95% CI)
Gender			
Male	37 (50)	65 (50.4)	-0.4 (-14.4, 13.6)
Female	37 (50)	64 (49.6)	
Median age at CSE, months (range)	25.6 (1-171)	36.7 (1-192)	**0.033**+
Neurological impairment before the CSE episode			
Absent	48 (64.9)	46 (35.7)	**29.2 (15.0, 41.8)**
Present	26 (35.1)	83 (64.3)	
CSE category			
Prolonged febrile seizures	30 (40.5)	26 (20.2)	**20.3 (7.4, 33.3)**
Acute symptomatic	12 (16.2)	14 (10.9)	5.3 (-4.0, 16.3)
Remote symptomatic	20 (27.0)	52 (40.3)	-13.3 (-25.6, 0.4)
Idiopathic & Unclassified	12 (16.2)	37 (28.7)	**-12.5 (-23.2, -0.2)**
CSE occurrence			
First-ever (Incident)	67 (90.5)	96 (74.4)	**16.1 (5.0, 25.6)**
Previous CSE (Recurrence)	7 (9.5)	33 (25.6)	
Seizure duration			
30-60 min	34 (45.9)	51 (39.5)	-6.4 (-20.2, 7.5)
>60 min	40 (54.1)	78 (60.5)	
Seizure character			
Continuous	39 (52.7)	60 (46.5)	6.2 (-7.9, 20.0)
Intermittent	35 (47.3)	69 (53.5)	
Seizure type			
Generalised-onset	48 (64.9)	76 (58.9)	6.0 (-8.0, 19.1)
Focal-onset	26 (35.1)	53 (41.1)	
Ethnicity			
White	28 (37.8)	46 (35.7)	2.2 (-11.1, 16.0)
Black	10 (13.5)	30 (23.3)	-9.7 (-19.7, 1.8)
Asian	23 (31.1)	39 (30.2)	0.8 (-11.8, 14.2)
Other	13 (17.6)	14 (10.9)	6.7 (-2.9, 17.8)
Socioeconomic status (Median IMD 2004 scores)	33.4	36.0	0.44+

+ *p* value on Mann-Whitney U test

Significant differences highlighted in bold

Table 7.1 Comparison of the sociodemographic and clinical characteristics of subjects included in volumetry analysis with remainder of CSE cohort

7.2.2 Hippocampal volumetry

7.2.2.1 Image acquisition

The general MRI methods used in this study have been described in chapter 3.

Hippocampal volumetry was performed using the images obtained from three-dimensional Fast Low Angle Shot (3D-FLASH) sequence (echo time = 4.9 ms, repetition time = 11ms, acquisition matrix = 224 x 256, in-plane resolution = 1.0 x 1.0 mm, slice thickness = 1 mm) (Appendix D). This sequence provided T1-weighted images with isometric voxels at a resolution of 1 mm^3.

7.2.2.2 Image processing

Further processing of 3D-FLASH images and hippocampal volumetry were performed using the FMRIB Software Library (FSL 5.0.2.1) package (Jenkinson et al., 2012). To improve visualisation of the hippocampal formation and increase reliability of volume measurements, the 3D-FLASH images were reformatted perpendicular to the long axis of the hippocampus using FLIRT tool in FSL. For hippocampal volume measurement, binary region of interest (ROI) masks were hand-drawn to encompass the entire hippocampus using the FSLView module.

7.2.2.3 Hippocampal segmentation

Two trained observers (SP and MM) independently performed manual hippocampal ROI tracing, blind to all clinical details. Binary ROI masks were drawn separately for each hippocampus. We defined anatomic boundaries of the hippocampus using a modified protocol based on previously published methods (see Appendix E for detailed protocol) (Watson et al., 1997; Konrad et al., 2009; Geuze et al., 2004). The average time required for drawing ROI mask for each hippocampus was 45 to 60 minutes. An example of hippocampal ROI is shown in Figure 7.1.

The volume for each ROI was automatically calculated using *fslstats* tool in FSL.

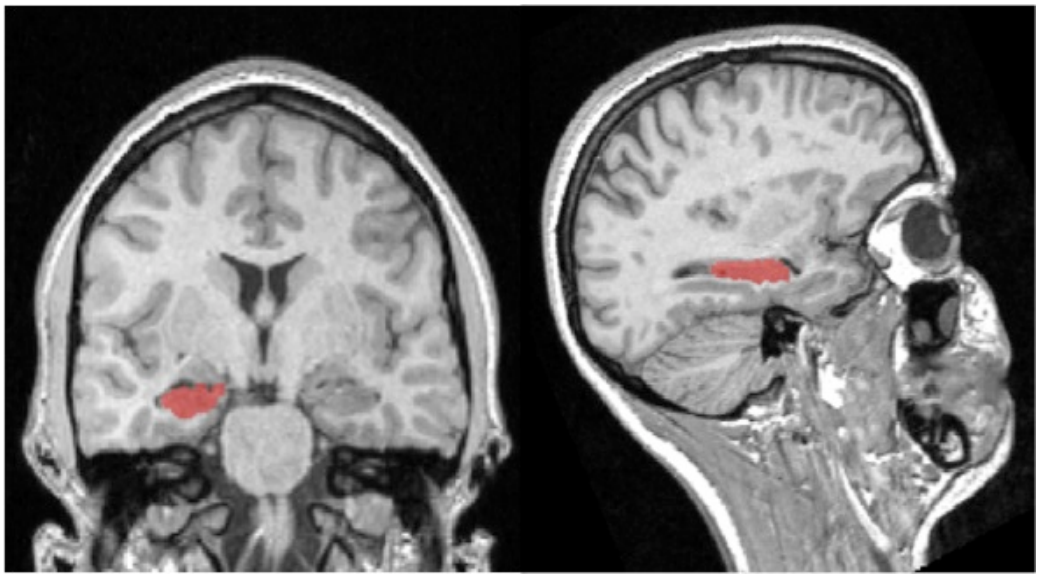


Figure 7.1 An example of right hippocampus ROI mask

7.2.2.4 Intracranial volume (ICV) estimation

Brain Extraction Tool (BET), provided as part of FSL package, was used to remove the skull and overlying tissues from the T1-wighted 3D-FLASH images (Smith, 2002). The initial command used for BET was:

Bet <input> <output> -R

The –R option runs more robust brain centre estimation, resulting in better segmentation of brain tissue.

The output image was visually inspected in FSLView to ensure correct segmentation. If the segmentation was inadequate, the parameters in BET command were adjusted to achieve an optimal result. Occasionally, for some children with significant structural abnormalities, the output image included considerable non-brain tissue despite adjusting the parameters. The output masks for such cases were manually edited in FSLView.

ICV was calculated using *fslstats* tool in FSL.

7.2.2.5 Normalisation of hippocampal volumes

Correction of hippocampal volume for brain volume is necessary to allow comparison between subjects of various ages (Obenaus et al., 2001). Correction of hippocampal volume for ICV was performed using a covariance method, commonly used for this purpose (Van Paesschen et al., 1997; Jack Jr et al., 1989). The equation used for deriving corrected hippocampal volume is as follows:

$$HippVol_{corrected} = HippVol_{measured} - gradient \times (ICV_{measured} - ICV_{mean})$$

where *gradient* is the slope of the regression fit of the left and right control hippocampal volumes to control ICV; and ICV_{mean} is the average ICV of the controls. The calculated gradient in our study was 1.339×10^{-3}, which is very similar to that reported in a paediatric study using the same approach (1.303×10^{-3}) (Berg et al., 2011a).

7.2.3 Statistical analysis

7.2.3.1 Inter- and intra-rater reliability

Consistency of volumetric measurements was assessed using the intraclass correlation coefficient (ICC) (Shrout and Fleiss, 1979). Inter-rater reliability was assessed by calculating ICC for ROI values between the two raters. In addition, repeat ROI tracing was performed by SP on 20 randomly selected subjects to assess intra-rater reliability.

The ICC values for inter-rater (0.94 for left & 0.95 for right) and intra-rater (0.93 for left & 0.95 for right) reliability for hippocampal tracing in our study are similar to the reported values in paediatric studies (0.87 to 0.97 and 0.9 to 0.97 respectively) (Kates et al., 1997; Thompson et al., 2014; Lewis et al., 2014). For each subject, the hippocampal volumes calculated from ROIs of the two raters were averaged and used for further analysis.

7.2.3.2 Hippocampal volume asymmetry

Hippocampal volume side-to-side asymmetry was determined by calculation of an asymmetry index (AI):

$$AI = \frac{2|Right\ HippVol - Left\ HippVol|}{Right\ HippVol + Left\ HippVol}$$

7.2.3.3 Correlations

Parametric and non-parametric correlations were performed to test the relationship between age, ICV and the hippocampal volumes in controls. As the results were similar, Pearson's correlation coefficients are reported.

7.2.3.4 Between-group analysis

Between-group analysis of hippocampal volumes were performed using the generalized estimating equations (GEE) model in SPSS (IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY:IBM Corp.). GEE enabled a single between-group analysis accounting for within-individual correlation (left and right side), thus minimizing the number of statistical tests used, and also allows using the most appropriate link function for the assumed distribution of the data. For the current analysis, GEE model with *Gamma* as distribution and *Log* as link function was used as this provided the best fit and age was included as a covariate in the model. Pairwise comparison of hippocampal volumes between controls and each of the CSE groups was performed with post-hoc Bonferroni correction for multiple comparisons. Wald test was used to report the *p*-values, which were considered significant for $p < 0.05$.

Between-group analysis of hippocampal volume asymmetry was performed by comparing hippocampal volume AIs using Krusal-Wallis 1-way ANOVA, with post-hoc Dunn's test for multiple comparisons.

7.2.3.5 Individual hippocampal volumes

For ease of interpretation, individual ICV-corrected right and left hippocampal volumes were transformed to z-scores based upon the control group's means and standard deviations (SD). Hippocampi with z-scores of -1.96 or lower were considered abnormally small.

Volumetric studies consistently report larger right than left hippocampus in healthy adults and children and therefore some rightward asymmetry is expected in healthy individuals (Lucarelli et al., 2013; Pfluger et al., 1999; Utsunomiya et al., 1999). Unilateral hippocampal volume loss may thus result in either increased or reduced hippocampal asymmetry depending on the side affected. Therefore, individual

hippocampal AI values below 2.5th centile and above 97.5th centile for control subjects were considered abnormal.

7.2.3.6 Regression analysis

Linear regression analyses were performed to explore the association between relevant clinical/demographic characteristics and hippocampal volume/ asymmetry. We first carried out univariable analyses to identify variables reaching a statistical significance level of 10% ($p \leq 0.1$). These were then assessed for significance using multivariable analysis ($p<0.05$). Factors not significant on univariable analyses were subsequently included in the final model to identify any factors that were associated with outcomes only after adjustment for other variables.

The factors investigated in regression models include: age at CSE (both as continuous and categorical), gender, handedness, prematurity (gestation <37 weeks), seizures prior to CSE (febrile/afebrile), first-ever (incident) versus recurrent CSE, febrile (temperature >38^{0}C) versus afebrile CSE, interval between seizure onset and first treatment (in minutes), duration of CSE (both as continuous and categorical), intermittent versus continuous CSE, type of CSE (focal- vs. generalised-onset), CSE recurrence during follow-up and new-onset (incident) epilepsy after CSE (any epilepsy and TLE investigated separately).

7.3 Results

7.3.1 Subject demographics

The demographic and clinical details of subjects included in hippocampal volumetry analysis are presented in Table 7.2. The demographic characteristics of subjects in CSE groups and controls were comparable. For those in the CSE group, the median interval between CSE episode and MR imaging was 8.6 years (range 6.3-10).

Clinical and demographic characteristics	Controls N = 71	Prolonged Febrile Seizures N = 30	Acute symptomatic N = 12	Remote symptomatic N = 20	Idiopathic & Unclassified N = 12
Gender, M/F	31 / 40	14 / 16	6 / 6	10 / 10	7 / 5
Age in years, Median (IQR)	11.4 (9.9-14.2)	9.7 (8.9-10.6)	10.2 (8.0-15.6)	12.7 (11.1-14.3)	12.5 (11.6-15.6)
Handedness, R/L/A	56 / 10 / 5	24 / 5 / 1	10 / 1 / 1	14 / 5 / 1	11 / 1 / 0
Age at CSE (months), Median (IQR)	-	12.6 (10.1-24.8)	21.3 (6.8-83.8)	51.1 (21.1-66.5)	46.0 (31.9-86.6)
Gestation <37 weeks	-	3 (10%)	1 (8%)	5 (25%)	1 (8%)
Previous seizures, Febrile / Afebrile	-	10 (33%) / 0 (0%)	2 (17%) / 0 (0%)	1 (5%) / 14 (70%)	1 (8%) / 5 (42%)
Incident CSE	-	30 (100%)	12 (100%)	16 (80%)	9 (75%)
Febrile at CSE	-	30 (100%)	9 (75%)	8 (40%)	1 (8%)
Continuous CSE	-	16 (53%)	6 (50%)	11 (55%)	6 (50%)
Focal CSE onset	-	6 (20%)	4 (33%)	10 (50%)	6 (50%)
Time to first treatment (min), Median (IQR)	-	35 (10-35)	22 (10-35)	35 (10-35)	22 (10-32.5)
CSE duration (min), Median (IQR)	-	55.5 (40-86)	67.5 (32.5-90)	60 (50-75)	80 (59-111)
CSE recurrence during FU	-	9 (30%)	1 (8%)	13 (65%)	4 (33%)
Incident epilepsy after CSE, TLE / non-TLE		0 (0%) / 3 (10%)	0 (0%) / 2 (17%)	1 (5%) / 2 (10%)	0 (0%) / 1 (8%)

Table 7.2 Clinical and demographic characteristics of study subjects

7.3.2 Normative results

7.3.2.1 Relationship between uncorrected hippocampal volumes, ICV and age in controls

There was no significant increase in ICV with age (r = 0.05, p = 0.67) (Figure 7.2). However, both left and right hippocampal volumes were significantly correlated with ICV (r = 0.49, p = <0.001 for left & r = 0.53, p = <0.001 for right) (Figure 7.3). There was also a significant correlation between the hippocampal volume and age on the right (r = 0.26, p = 0.03), and statistically insignificant trend towards a positive correlation on the left (r = 0.2, p = 0.09) (Figure 7.4).

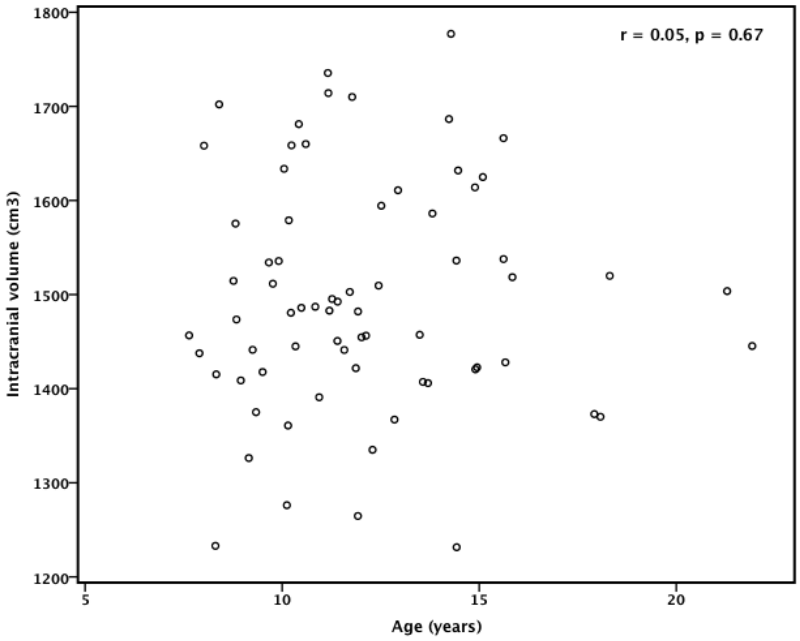


Figure 7.2 Relationship between intracranial volume (ICV) and age in controls

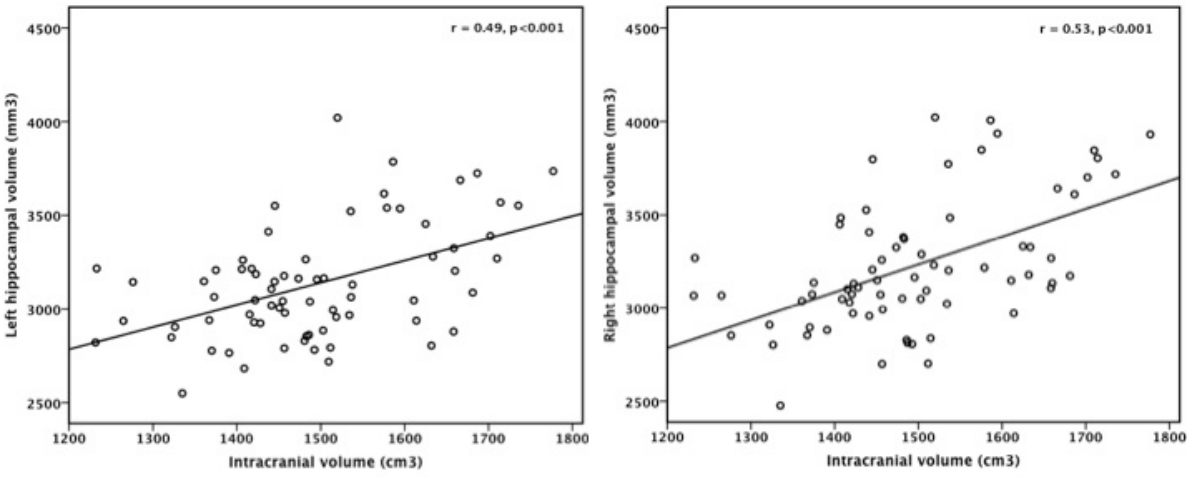


Figure 7.3 Correlation between hippocampal volume and intracranial volume in controls

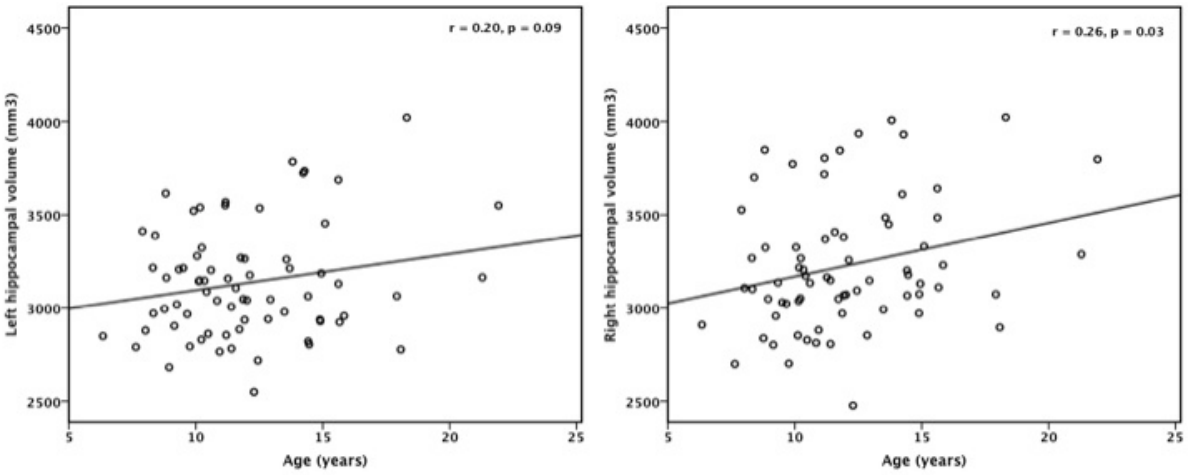


Figure 7.4 Correlation between hippocampal volume and age in controls

7.3.2.2 Hippocampal volume asymmetry

Right hippocampus was larger than the left in 48 (68%), with a mean difference of 92 mm^3 between the sides ($p<0.001$ on paired samples t test). The median AI was 0.042 (2.5^{th} centile 0.002 & 97.5^{th} centile 0.144).

There was no significant effect of gender and handedness either on hippocampal volume or on asymmetry.

7.3.3 Between-group comparisons

7.3.3.1 Hippocampal volumes

The right hippocampus was larger than the left across the groups with a mean difference of 125 mm^3 ($p<0.001$). There was a significant group effect for the hippocampal volumes after correcting for side and age ($p=0.001$). Subjects in the remote symptomatic CSE group had significantly smaller hippocampal volume compared to controls (mean difference 598 mm^3, $p=0.007$), the PFS group (mean difference 694 mm^3, $p=0.010$) and the idiopathic & unclassified CSE group (mean difference 518 mm^3, $p=0.031$) (Figure 7.5). The hippocampal volume in the remote symptomatic group was also smaller than acute symptomatic group with weak evidence of a statistical difference (mean difference 482 mm^3, $p=0.059$). There was no significant difference in the mean hippocampal volume between control, PFS, acute symptomatic, and idiopathic & unclassified CSE groups.

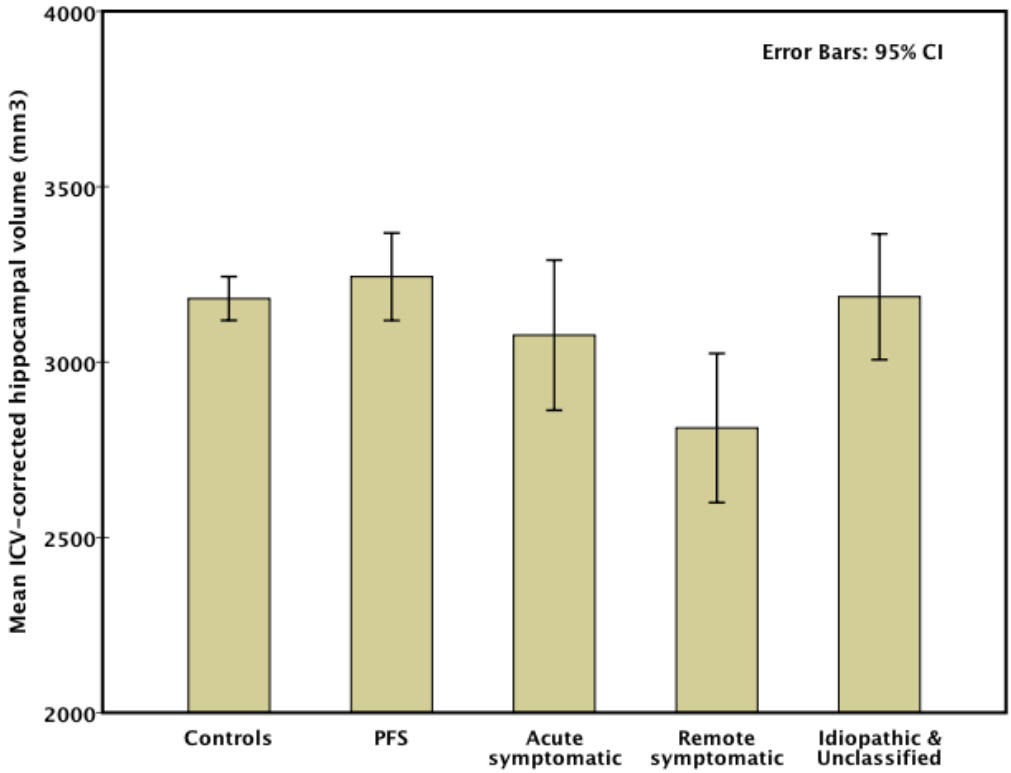


Figure 7.5 Mean ICV-corrected hippocampal volumes

7.3.3.2 Hippocampal volume asymmetry

Figure 7.6 shows the distribution of hippocampal volume AI for controls and each CSE group. The spread of AIs was similar in control, PFS and idiopathic & unclassified CSE groups. The acute symptomatic CSE group was also comparable, except for one extreme outlier (the child with unilateral MTS described in chapter 6). AI values in the remote symptomatic CSE group were more spread and upwardly skewed.

Group comparison using Krusal-Wallis ANOVA with post-hoc testing revealed that AI was significantly higher in the remote symptomatic CSE group compared to controls ($p<0.001$) and the PFS group ($p=0.029$). There was no significant difference in AI between the other groups.

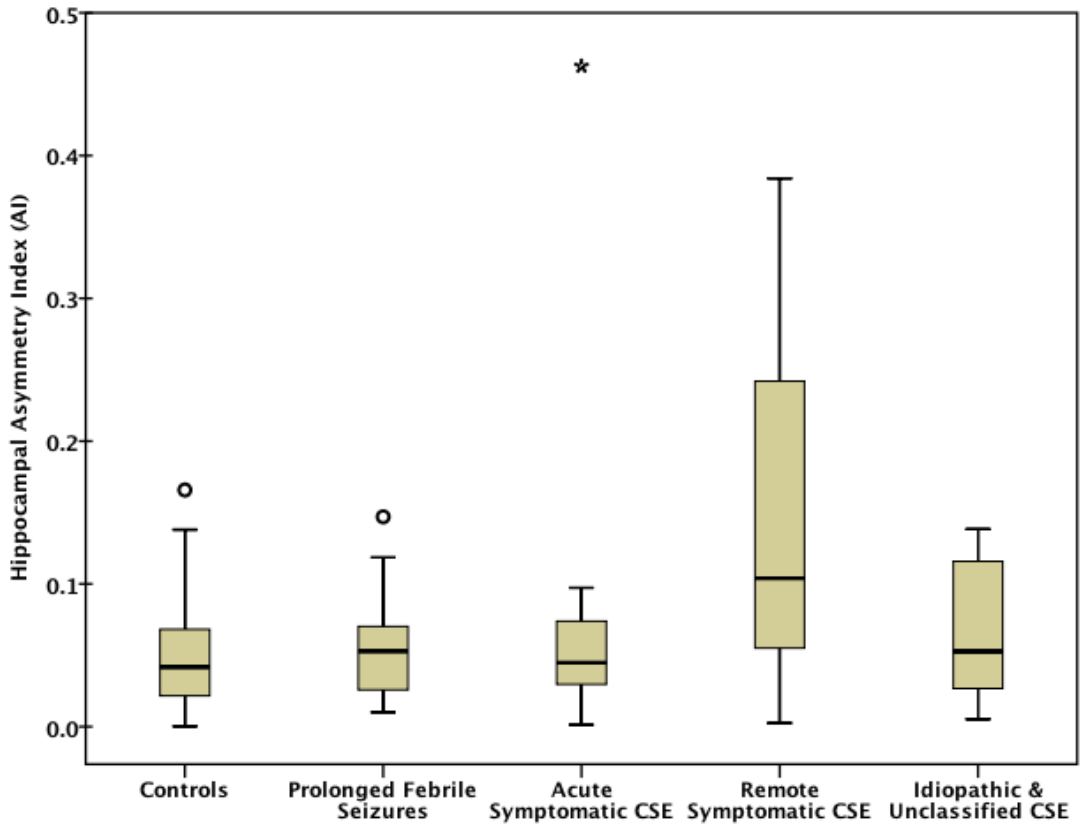


Figure 7.6 Boxplot showing distribution of hippocampal volume asymmetry

7.3.3.3 Individuals with abnormal hippocampal volumes

The types of individual abnormalities in hippocampal volume and asymmetry are summarised in Figure 7.7 & Table 7.3.

40% of subjects in the remote symptomatic group had either unilateral or bilateral hippocampal volume reduction with or without high AI. All of these had significant unilateral or bilateral structural imaging abnormalities on visual qualitative assessment. In addition, 3 children (2 with perinatal middle cerebral artery infarction and 1 mild periventricular leucomalacia) had high AI, but no significant volume reduction. One child in the acute symptomatic group, with neuroimaging features of unilateral MTS, had a high AI with unilateral hippocampal volume reduction. Interestingly, one girl in the PFS group had small hippocampi bilaterally with normal AI. She had a generalised CSE lasting 110 minutes at 2.5 years of age, but no further seizures and was completely healthy with normal neurological functioning. Another child with an initial diagnosis of PFS, found to have neuroimaging features of neurofibromatosis type 1 and a hamartoma in right hippocampus, had a high AI.

Some cohort members with features of unilateral MTS on visual MRI analysis did not have sequences suitable for hippocampal volumetry. Among the subjects on whom volumetry could be performed, all with unilateral MTS on visual analysis had unilateral volume reduction and high AI. Also, no one with normal results on qualitative visual assessment was found to have significant hippocampal volume reduction and high AI.

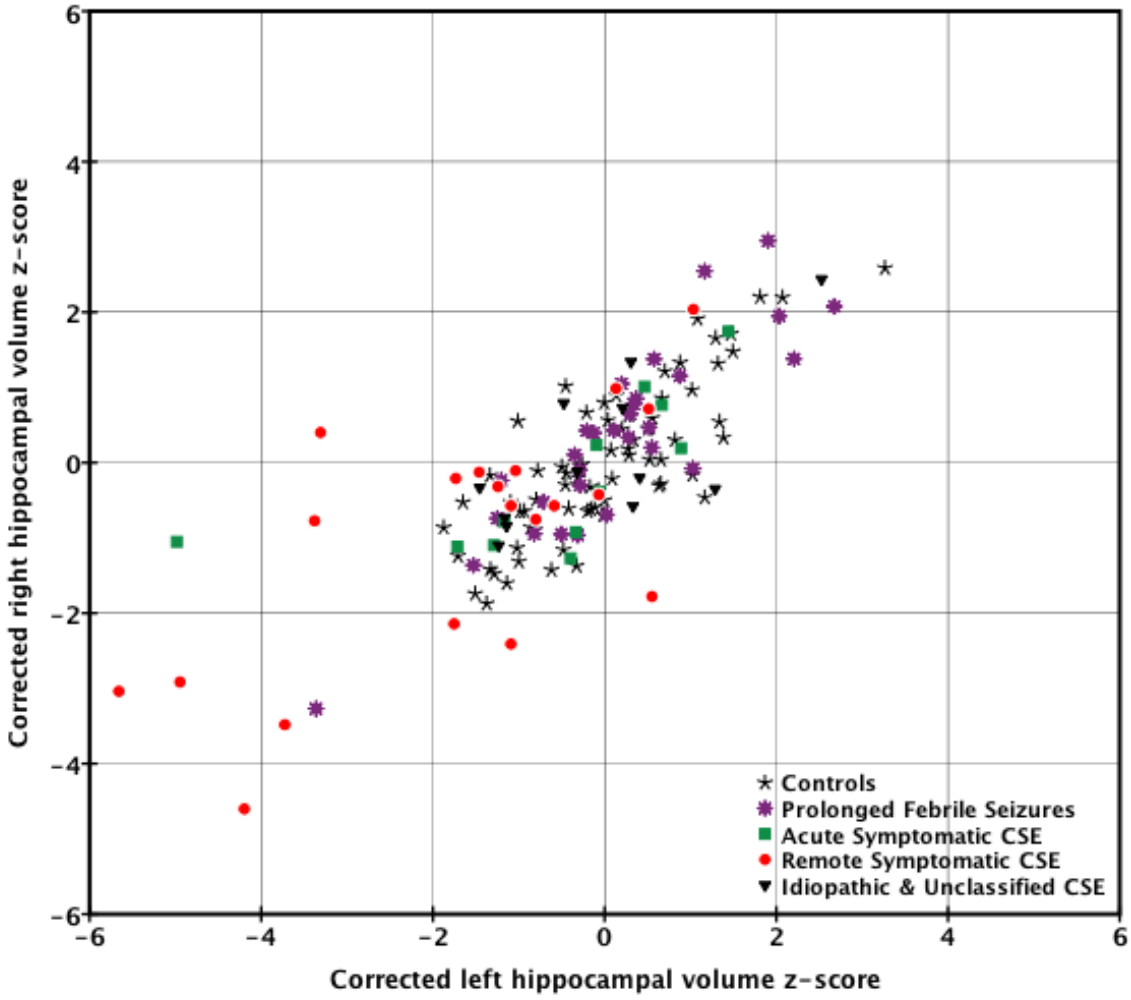


Figure 7.7 Distribution of left (x-axis) and right (y-axis) hippocampal volume z-scores in controls and CSE groups

	Controls (N = 71)	Prolonged Febrile Seizures (N = 30)	Acute symptomatic (N = 12)	Remote symptomatic (N = 20)	Idiopathic & Unclassified (N = 12)
Unilateral volume reduction only, N (%)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
Unilateral volume reduction and abnormal AI, N (%)*	0 (0)	0 (0)	1 (8)	3 (15)	0 (0)
Bilateral volume reduction only, N (%)	0 (0)	1 (3)	0 (0)	1 (5)	0 (0)
Bilateral volume reduction and abnormal AI, N (%)*	0 (0)	0 (0)	0 (0)	3 (15)	0 (0)
Abnormal AI only, no volume reduction, N (%)*	2 (3)	1 (3)	0 (0)	3 (15)	0 (0)
Total, N (%)	2 (3)	2 (7)	1 (8)	11 (55)	0 (0)

*AI above 97.5th centile for all except one control with AI below 2.5th centile

Table 7.3 Hippocampal volume abnormalities in CSE groups

7.3.3.4 Regression analysis

A history of previous afebrile seizures at CSE presentation (multivariable B = -367, p = 0.025) and focal CSE onset (multivariable B = -211, p = 0.043) had a negative association with hippocampal volume (Table 7.4).

Gestational age <37 weeks (multivariable B = 0.09, p<0.001) and previous afebrile seizures at CSE presentation (multivariable B = 0.07, p = 0.001) had a positive association with hippocampal AI (Table 7.5).

Clinical and demographic characteristics	**Univariable**		**Multivariable**	
	B (95% CI)	***p***	**B (95% CI)**	***p***
Age at CSE (months)	0.57 (-11.0, 12.2)	0.92	-	-
Male	48 (-115, 210)	0.56	-	-
Right handed	39 (-203, 280)	0.75	-	-
Gestation <37 weeks	-289 (-720, 141)	0.19	-	-
Socioeconomic status (IMD 2004 score)	2.7 (-3.3, 8.7)	0.38	-	-
Previous afebrile seizures	-507 (-801, -213)	**0.001**	-367 (-688, -46)	**0.025**
Incident CSE	189 (-36, 413)	**0.10**	125 (-190, 439)	0.44
Febrile at CSE	287 (-18, 591)	**0.065**	178 (-117, 475)	0.24
Intermittent CSE	-140 (-355, 74)	0.20	-	-
Focal CSE onset	-250 (-480, -19)	**0.034**	-211 (-416, -7)	**0.043**
CSE duration (min)	0.53 (-0.76, 1.8)	0.42	-	-
Pre-hospital treatment for CSE	51 (-114, 217)	0.54	-	-
Interval between CSE onset and first treatment (min)	-0.43 (-6.3, 5.5)	0.89	-	-
CSE recurrence during FU	-269 (-527, -10)	**0.042**	-137 (-341, 67)	0.19
Incident epilepsy during FU	-20 (-267, 227)	0.87	-	-

Table 7.4 Association between clinical and sociodemographic characteristics and hippocampal volumes

Clinical and demographic characteristics	Univariable		Multivariable	
	B (95% CI)*	*p*	B (95% CI)*	*p*
Age at CSE (months)	-0.05 (-0.1, 0.1)	0.86	-	-
Male	5.0 (-1.3, 11.0)	0.13	-	-
Right handed	3.0 (-4.4, 11.0)	0.41	-	-
Gestation <37 weeks	10.0 (4.3, 15.6)	**0.001**	9.2 (4.3, 14.2)	**<0.001**
Socioeconomic status (IMD 2004 score)	-0.1 (-0.2, 0.1)	0.26	-	-
Previous afebrile seizures	8.4 (4.1, 12.6)	**<0.001**	7.1 (2.9, 11.2)	**0.001**
Incident CSE	2.5 (-4.4, 9.4)	0.48	-	-
Febrile at CSE	-2.7 (-6.9, 1.5)	0.21	-	-
Intermittent CSE	1.4 (-2.6, 5.5)	0.49	-	-
Focal CSE onset	2.7 (-1.6, 6.9)	0.22	-	-
CSE duration (min)	-0.08 (-0.04, 0.02)	0.63	-	-
Pre-hospital treatment for CSE	-0.6 (-4.8, 3.6)	0.78	-	-
Interval between CSE onset and first treatment (min)	0.1 (-0.1, 0.2)	0.39	-	-
CSE recurrence during FU	5.6 (1.6, 9.6)	**0.007**	2.4 (-1.5, 6.2)	0.22
Incident epilepsy during FU	-0.01 (-6.4, 6.1)	0.965	-	-

* $x10^{-2}$

Table 7.5 Association between clinical and sociodemographic characteristics and hippocampal volume asymmetry

7.4 Discussion

This is to date the largest prospective long-term follow-up study investigating hippocampal volume changes following childhood CSE due to all causes. Using normative data from a large control population for comparison we have established that, at a median follow-up of 8.6 years after childhood CSE, (1) the prevalence of significant hippocampal volume abnormalities is low in children with PFS, acute symptomatic, and idiopathic & unclassified CSE; (2) almost all with small hippocampi on volumetric analysis had significant structural imaging abnormalities on visual

assessment; (3) presence of previous afebrile seizures at CSE presentation and focal CSE onset/ gestation age <37 weeks are associated with hippocampal volume reduction and/or increased asymmetry; (4) seizure duration does not seem have a significant negative effect on hippocampal volumes; and (5) perhaps the most important inference from the study in this chapter is that very few children demonstrate subtle abnormalities on hippocampal volumetry that are not detected on visual assessment.

Analysis of control data demonstrated a strong positive correlation between right and left hippocampal volumes and ICV. However, there was no significant correlation between ICV with age, and only a modest correlation between hippocampal volumes with age. This is consistent with the fact that most of the increase in global brain volume occurs by approximately age 5 years, and hippocampal volume by age 10 to 13 years (Brain Development Cooperative Group, 2012; Utsunomiya et al., 1999; Krogsrud et al., 2014; Uematsu et al., 2012). The right and left mean hippocampal volumes for our control group were 3227 mm^3 (SD 346) and 3134 mm^3 (SD 300) respectively, and the right hippocampus larger than the left with an average asymmetry index of 0.042. These results are consistent with published normative data for this age group (Jalaluddin et al., 2013; Krogsrud et al., 2014; Utsunomiya et al., 1999; Berg et al., 2011a). Thus, we are confident that data from our control group is representative of the general population.

On group analysis, statistically significant differences were observed only for the remote symptomatic CSE group, who were found to have lower hippocampal volumes and higher asymmetry compared to controls. Most children in this group had significant neurological impairments pre-dating the CSE episode, and obvious structural brain abnormalities both within and outside the temporal lobe. This observation is similar to the results of the STEPIN study reporting significantly lower hippocampal volumes and higher asymmetry in children with symptomatic CSE (Yoong et al., 2013a; Yoong, 2012).

With the PFS-MTS hypothesis in mind, we were particularly interested in investigating hippocampal changes in the PFS group. On group analysis, we found no significant difference between the PFS group and controls in hippocampal volumes, or in asymmetry. Our findings are broadly in keeping with previous results reporting lack of significant group differences in hippocampal volumes and asymmetry between the PFS group and controls 6 to 12 months after the episode (Scott et al., 2003; Yoong et al., 2013a). Of note, in both of these studies, similar to the current study, the PFS group did not include any children with pre-existing neurological/developmental problems. In contrast, the FEBSTAT study, recently reported significantly lower volume in the right

hippocampus and abnormal right/left volume ratio in children with FSE and normal structural MRI compared to those with simple febrile seizures, both on acute imaging and at follow-up 6 to 24 months later (Lewis et al., 2014). Similarly, in a longer-term follow-up study of 24 children with PFS, although no significant group differences were observed in the absolute right or left hippocampal volumes, children in the PFS group had a lower right-left volume difference compared to control children with simple febrile seizures at mean follow-up of 12.5 years (Tarkka et al., 2003). Three children in the PFS group (12.5%) and one control subject (3%) had small right-left hippocampal volume difference, but all were neurologically normal with no further seizures. Given that only a small minority of the patients are likely to develop lasting hippocampal injury following PFS, the results of the FEBSTAT study showing group difference for right hippocampal volume are particularly surprising (Lewis et al., 2014). Unlike studies from our group, which did not include children with pre-existing developmental problems in the PFS group and used healthy children as controls, 7% of children with FSE in the FEBSTAT study had abnormal development at presentation and children with simple febrile seizures were used as controls. In addition, the reported volumetry results were derived from 59 of 191 (31%) FSE and 38 of 96 (40%) simple febrile seizure patients who had MRI in the acute period, and therefore, although the authors only included subjects with normal acute MRI, the results may be confounded by selection bias (Lewis et al., 2014; Shinnar et al., 2012). While data from the FEBSTAT and the STEPIN study show some evidence of impaired hippocampal growth following FSE/PFS, the striking finding in the FEBSTAT study of reduced right hippocampal volume and the predominance of T2 signal hyperintensity on the right on baseline MRI suggest asymmetric hippocampal vulnerability due to subtle pre-existing abnormality.

Since our data and that of others suggest that a minority of children seem to develop lasting hippocampal injury following PFS, a statistical method of using cut-off limits derived from the distribution of control data (e.g. 2 or 3 SD, 95th centile), rather than comparison of group means/medians, to identify individuals with abnormal values is preferable (Scott et al., 2003; Tarkka et al., 2003; Yoong et al., 2013a). Using this approach, the reported rates of abnormal hippocampal asymmetry following PFS are: 14 to 21% within 7 days, 6% at 4 weeks, 14 to 36% at 6 months, 14% at 1 year and 12.5% at 12.5 years (Scott et al., 2002; Scott et al., 2003; Tarkka et al., 2003; VanLandingham et al., 1998; Yoong, 2012). In our study, only 1 (3%) child with PFS and unilateral hippocampal hamartoma had abnormal hippocampal asymmetry. Another child with PFS with findings of unilateral MTS on conventional imaging was not included in

volumetry analysis as suitable sequences were not available. Even if we consider her as having high AI, a liberal estimate of abnormal hippocampal volume asymmetry 8.6 years after PFS would be 6.5% (95% CI 1.8 to 20.7). A potential explanation for the lower rate of abnormal hippocampal volume asymmetry in our study compared to the reported rates at shorter follow-up is that PFS may induce unilateral hippocampal volume loss/altered growth with increased asymmetry in the short-term, and with recovery, the volume of the affected hippocampus subsequently increases resulting in reduced asymmetry between the sides (Lewis et al., 2014; Scott et al., 2003; Yoong et al., 2013a). Data from the STEPIN study seem to support this hypothesis. In that longitudinal study, there was a significant increase in the extremes of distribution of hippocampal AI in the PFS group than in controls at baseline and at 6 months, but not at 1 year (Yoong, 2012). In addition, abnormal increase in hippocampal AI was transient and normalised on subsequent imaging in 3/7 (43%) children with PFS. An alternative explanation could be that, with longer-term follow-up, growth of the contralateral hippocampus may also get affected resulting in reduced asymmetry. Longer-term follow-up of subjects in studies such as the STEPIN and the FEBSTAT studies may further our understanding of the evolution of hippocampal volume changes following PFS.

Of the clinical and demographic characteristics at CSE presentation, only presence of previous afebrile seizures and focal CSE onset were found to have a significant negative association with hippocampal volume in CSE subjects. Additionally, gestational age <37 weeks was associated with an increase in hippocampal AI. No clinically relevant associations with hippocampal volumes or AI were identified when analysis was restricted to the PFS group. We investigated the relationship between seizure duration and hippocampal volume with seizure duration separately as a continuous and also as a categorical variable and did not identify any significant association. This finding is consistent with previous studies reporting lack of a significant association between seizure duration and hippocampal volume (Gong et al., 2012; Grünewald et al., 2001; Lewis et al., 2014; Tarkka et al., 2003; Yoong et al., 2013a). A negative effect of focal CSE onset, previous afebrile seizures and prematurity on hippocampal volume and asymmetry have been reported previously (Thompson et al., 2014; VanLandingham et al., 1998; Yoong et al., 2013a; Giménez et al., 2004; Grunewald et al., 2001).

While it is difficult to categorically determine a cause-effect relationship, our findings of hippocampal volume abnormalities predominantly in those with pre-existing brain injury, and significant association with prematurity and previous afebrile seizures

suggest there may be increased vulnerability of an abnormal brain to CSE, i.e., multiple insults may cause increased hippocampal damage (Grünewald et al., 2001; Yoong et al., 2013a). These observations, together with recent reports of subtle pre-existing hippocampal abnormalities in children who developed MTS following FSE and experimental data from animal models, support the "multiple hit" hypothesis as an explanation for seizure-induced hippocampal damage (Lewis et al., 2014; Gibbs et al., 2011; Sanon et al., 2012; Mathern et al., 2002; Shinnar et al., 2012).

Although ours is the first long-term follow-up study on a population-based childhood CSE cohort, and we have data from a large age-appropriate healthy control population for comparison, there are limitations to the study. Due to methodological constraints, acute neuroimaging was not universally performed at CSE presentation, and therefore longitudinal volumetric analysis was not possible. In addition, a considerable proportion of the original cohort was not included in volumetric analysis either because of loss to follow-up, lack of subject cooperation for imaging, or unavailability of suitable MR sequences for volumetry, thus potentially introducing bias. As shown in Table 7.1, nearly 2/3rd of subjects excluded from volumetry analysis had neurological impairment predating the CSE episode and more than half with no prior neurological impairment were included in volumetry analysis. Thus, we are confident that our estimates are reliable at least in those with no prior neurological impairment, and reflect consequences of CSE on hippocampal volume in children without structural brain abnormality. Finally, it is possible that lack of statistical significance for some CSE characteristics in our study could be due to the sample size, but this is unlikely for seizure duration since findings similar to ours have been reported in other UK and US cohorts including the FEBSTAT study, which has a larger sample size (Gong et al., 2012; Grünewald et al., 2001; Lewis et al., 2014; Tarkka et al., 2003; Yoong et al., 2013a).

In this chapter, we have established that the prevalence of significant hippocampal volume abnormalities is low within 10 years after non-remote symptomatic CSE in children. Our data also supports the "multiple hit" hypothesis as an explanation for hippocampal damage following CSE and provides further evidence for a lack of negative effect of seizure duration on hippocampal volume. In chapter 8 the candidate will explore the use of diffusion tensor imaging to investigate the long-term consequences of CSE on hippocampal microstructure.

CHAPTER 8 HIPPOCAMPAL DIFFUSION TENSOR IMAGING

8.1 Introduction

In the previous chapter the candidate used hippocampal volumetry to assess whether we were able to detect subtle hippocampal volume abnormalities not identified on qualitative visual MRI assessment. We found that on group analysis, compared to controls, only children with remote symptomatic CSE had hippocampal volume reduction; all other forms of CSE and controls had similar hippocampal volumes. Almost all subjects with abnormal hippocampal volume also had abnormalities readily detectable on qualitative assessment and thus volumetric analysis provided only limited new information. However, there were individuals within each group that had hippocampal volume asymmetry; the significance of which is uncertain. Thus, our volumetry results suggest that the majority of subjects with structurally normal brain MRI do not demonstrate evidence of significant hippocampal neuronal loss at 8.6 years post-CSE. In addition to hippocampal neuronal loss, the other key feature of MTS is disturbed internal architecture (Malmgren and Thom, 2012). We established in chapter 6 that it is often difficult to reliably assess internal architecture of the hippocampus in children due to poor image resolution/motion artefacts and subjective interpretation of the assessors. Thus, an alternative imaging modality such as diffusion tensor imaging (DTI), which provides quantitative measures related to tissue microstructure, could be useful in investigating hippocampal microstructural changes following CSE and further our understanding of consequences of childhood CSE on hippocampal architecture.

8.1.1 Diffusion MR imaging

Diffusion imaging is a MRI technique based on the measurement of the Brownian motion of water molecules as they diffuse through the tissues and provides direct insight into their microscopic physical properties (Le Bihan, 2003). DTI uses tensor model to characterize and quantify effective diffusion in each voxel and allows non-invasive interrogation of neural tissue composition, integrity and orientation (Basser and Jones, 2002). Thus, DTI can reveal details about tissue micro-architecture in health and disease and is therefore increasingly being used in both clinical and experimental research (Mori and Zhang, 2006; Alexander et al., 2007). Also, as the technique is highly sensitive to changes at cellular and microstructural level, it can identify subtle brain abnormalities that are not apparent on conventional MRI sequences.

The most widely used DTI parameters are Mean Diffusivity (MD), a measure of the average diffusion of water molecules within a voxel, and Fractional Anisotropy (FA), which measures degree of diffusion anisotropy (Basser and Jones, 2002; Le Bihan, 2003; Mori and Zhang, 2006). MD is similar in both grey and white matter (WM) in adult human brain and is sensitive to cellularity, edema and necrosis (Alexander et al., 2007). FA reflects nerve fibre integrity and alignment, and is highly sensitive to microstructural changes (Alexander et al., 2007). FA ranges from nearly isotropic (FA closer to 0; e.g., CSF) to anisotropic (FA closer to 1; e.g., mature WM tracts).

8.1.2 Role of diffusion imaging in investigating the consequences of CSE

Transient changes on diffusion MR imaging suggestive of acute hippocampal oedema following CSE have been well described both in humans and animal models (Engelhorn et al., 2007a; Kato et al., 2012; Jansen et al., 2008; Yu and Tan, 2008; Scott et al., 2006; Natsume et al., 2007). We have previously reported longitudinal hippocampal diffusion changes within 5 days and then subsequently at a mean of 5.5 months after PFS in one study, and at 1, 6, and 12 months after PFS and non-PFS CSE in a more recent study, but the longer-term evolution of these changes is not known (Scott et al., 2006; Yoong, 2012).

As discussed in previous chapters, whether childhood CSE can cause irreversible hippocampal damage resulting in MTS is still debated (Patterson et al., 2014; Raspall-Chaure et al., 2006; Shukla and Prasad, 2012). While changes in hippocampal MD and FA have been reported in patients with established MTS, the time course of these changes is not well understood (Focke et al., 2008; Liacu et al., 2010; Müller et al., 2006; Kimiwada et al., 2006; Assaf et al., 2003; Salmenpera et al., 2006; Thivard et al., 2005). Experimental studies have demonstrated the utility of DTI as a potential non-invasive biomarker in understanding the longitudinal evolution of hippocampal microstructural changes following CSE (Ye et al., 2013; Yu and Tan, 2008; Jansen et al., 2008; Nehlig, 2011). It is therefore possible that DTI could be useful as an additional tool for investigating hippocampal microstructural changes post-CSE and to detect hippocampal abnormalities prior to the development of overt MTS.

Results of visual MRI analysis presented in chapter 6 suggested a statistical trend for higher prevalence of HIMAL/IHI in the PFS group than in controls. HIMAL/IHI is thought to be a developmental malformation of the hippocampus and demonstrates features of

abnormal orientation and morphology on MRI. As discussed in chapter 6, while blurring of the internal structure is often described as a MRI feature of HIMAL/IHI, this is rather non-specific, affected by image quality, and dependent on subjective interpretation. Investigating hippocampal tissue microarchitecture with DTI may uncover hippocampal DTI changes in individuals with HIMAL/IHI.

Previous brain diffusion imaging studies in normal populations have shown that the diffusion properties continue to evolve during development, with increases in FA and reductions in MD values with increasing age (Cascio et al., 2007; Neil et al., 2002; Yoshida et al., 2013; Mukherjee et al., 2001; Watanabe et al., 2013; Lebel et al., 2008). However, there is differential maturation of different brain structures, with considerable variability in the timing and magnitude of developmental changes (Lebel et al., 2008; Watanabe et al., 2013; Mukherjee et al., 2001; Kumar et al., 2012). While hippocampal DTI values in healthy adults have been reasonably well described, albeit with considerable heterogeneity, only two studies (with sample size of 14 and 25) report hippocampal FA and MD measures in healthy children (Kimiwada et al., 2006; Yoong, 2012). Therefore, to enable reliable comparison, it is necessary that we establish normative hippocampal DTI measures from a large healthy paediatric control population.

8.1.3 Objectives of the study

The objectives of the study presented in this chapter are to (1) describe normative hippocampal FA and MD measures in a large healthy paediatric control population, (2) characterise hippocampal FA and MD changes within 10 years after PFS and other forms of childhood CSE compared to age-appropriate healthy controls, and (3) investigate hippocampal FA and MD changes in subjects with and without features of HIMAL/IHI on qualitative MRI assessment.

8.2 Methods

8.2.1 MR imaging sequences

Details about MR image acquisition and processing of the diffusion datasets for DTI are described in chapter 3. FA and MD maps generated from processing the diffusion data were used for this study.

8.2.2 Defining hippocampal ROI

Yoong et al. compared four previously described methods for defining ROI for hippocampal DTI analysis and found manual placement of a fixed 3x2x2 ROI on MD map, and co-registration of manually defined ROI using T1 image onto FA map to be more reliable than outlining ROI on MD or b0 maps (Yoong, 2012). Of the two reliable methods, although fixed ROI enables more accurate placement within the hippocampus, the small ROI size covers only a portion of the hippocampus and is also more susceptible to noise. Therefore, in the current study, we used the co-registration method for defining hippocampal ROI. Hippocampal ROI masks manually drawn on the 3D-FLASH images as described in chapter 7 were used for this purpose. Co-registration was performed using image analysis tools in FSL (Smith et al., 2004).

To further minimize sampling errors due to partial volume effects on edge voxels and inclusion of non-hippocampal voxels in the ROI, we eroded one edge voxel from ROI mask in all 3 dimensions using *fslmaths*. For each subject, the FA and MD maps were then co-registered to the high-resolution 3D-FLASH images to map left and right hippocampal ROIs onto the FA and MD maps. The accuracy of co-registration of hippocampal ROIs with FA/MD maps for each subject was checked by visual inspection in FSLview. The mean FA and MD values within each ROI were calculated using *fslstats*.

8.2.3 Statistical analysis

8.2.3.1 Correlations

The relationship between hippocampal FA/MD and age in healthy controls were tested using parametric and non-parametric correlation analysis. As the results were similar, Pearson's correlation coefficients are reported.

8.2.3.2 Between-group analysis of hippocampal FA and MD

Similar to volumetry analysis, between-group analyses of hippocampal FA and MD were performed using GEE in SPSS (IBM SPSS Statistics for Macintosh, Version 21.0. Armonk, NY:IBM Corp.). A GEE model with *Normal* as distribution and *Identity* as link function provided the best fit for hippocampal FA, and model with *Gamma* as distribution and *Log* as link function provided the best fit for hippocampal MD. As the control data showed a significant correlation between age and hippocampal MD, age was included as a covariate. Pairwise comparison of hippocampal FA and MD between controls and each of the CSE groups was performed with post-hoc Bonferroni correction for multiple comparisons. Wald test was used to report the *p*-values, which were considered significant for $p<0.05$.

8.2.3.3 Comparison of hippocampal FA and MD asymmetry

Asymmetry index (AI) for FA and MD were calculated as:

$$AI = \frac{200|\text{Right mean ROI value} - \text{Left mean ROI value}|}{\text{Right mean ROI value} + \text{Left mean ROI value}}$$

AIs for FA and MD between the groups were compared using Krusal-Wallis 1-way ANOVA with post-hoc Dunn's test for multiple comparisons.

Similar to the method used for hippocampal volumetry, we considered individual hippocampal FA and MD AI values below 2.5th centile and above 97.5th centile for control subjects as abnormal.

8.2.3.4 Comparison of hippocampal FA and MD between subjects with and without HIMAL/IHI

Comparison of right and left hippocampal FA and MD values between subjects with and without HIMAL/IHI was performed using univariate ANOVA, with age as a cofactor for MD analysis. The distribution of right-left hippocampal FA and MD asymmetry between the two groups were investigated using the Mann-Whitney U test. All controls and CSE cohort with adequate DTI data were considered in the analysis.

8.3 Results

8.3.1 Subject demographics

Data suitable for hippocampal DTI analysis were available on 58 controls and 60 children with CSE. The demographic characteristics of subjects in CSE groups (median age 11.6y, IQR 9.5-13.3; 45% male) and controls (median age 11.5y, IQR 9.8-13.9; 47% male) were comparable. For subjects in the CSE group, the median interval between CSE episode and MR imaging was 8.6 years (IQR 7.9-9.3). CSE classification was PFS in 26 (43%), acute symptomatic in 9 (15%), remote symptomatic in 14 (23%) and idiopathic & unclassified in 11 (18%).

8.3.2 Normative results

8.3.2.1 Relationship between hippocampal FA and age in controls

There was no significant correlation between either the left or the right hippocampal FA and age ($p>0.05$) (Figure 8.1).

8.3.2.2 Relationship between hippocampal MD and age in controls

There was a negative correlation between hippocampal MD and age, but this was statistically significant only for the left hippocampus ($r = -0.36$, $p = 0.005$ for the left and $r = -0.18$, $p = 0.17$ for the right hippocampus) (Figure 8.2).

There was no significant effect of gender or handedness on hippocampal FA or MD correlations.

8.3.2.3 Hippocampal FA and MD values and right-left asymmetry

Mean FA was significantly higher in the right hippocampus (0.133, SD 0.016) than the left (0.128, SD 0.015), with a mean difference of 0.0058 ($p=0.002$ on paired samples t test). There was no significant difference in mean MD values between the right (9.41×10^{-4} mm^2/s, SD 0.35) and the left (9.38×10^{-4} mm^2/s, SD 0.36) hippocampus ($p=0.32$ on paired samples t test).

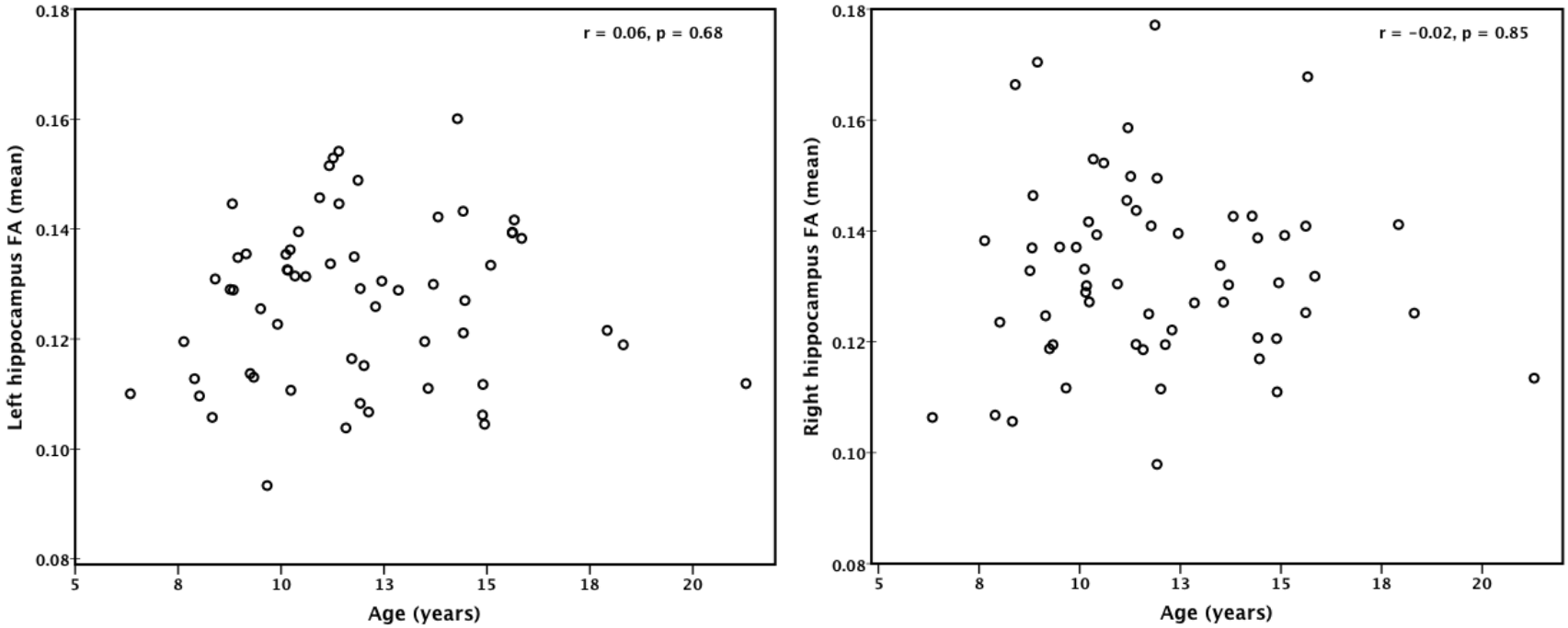
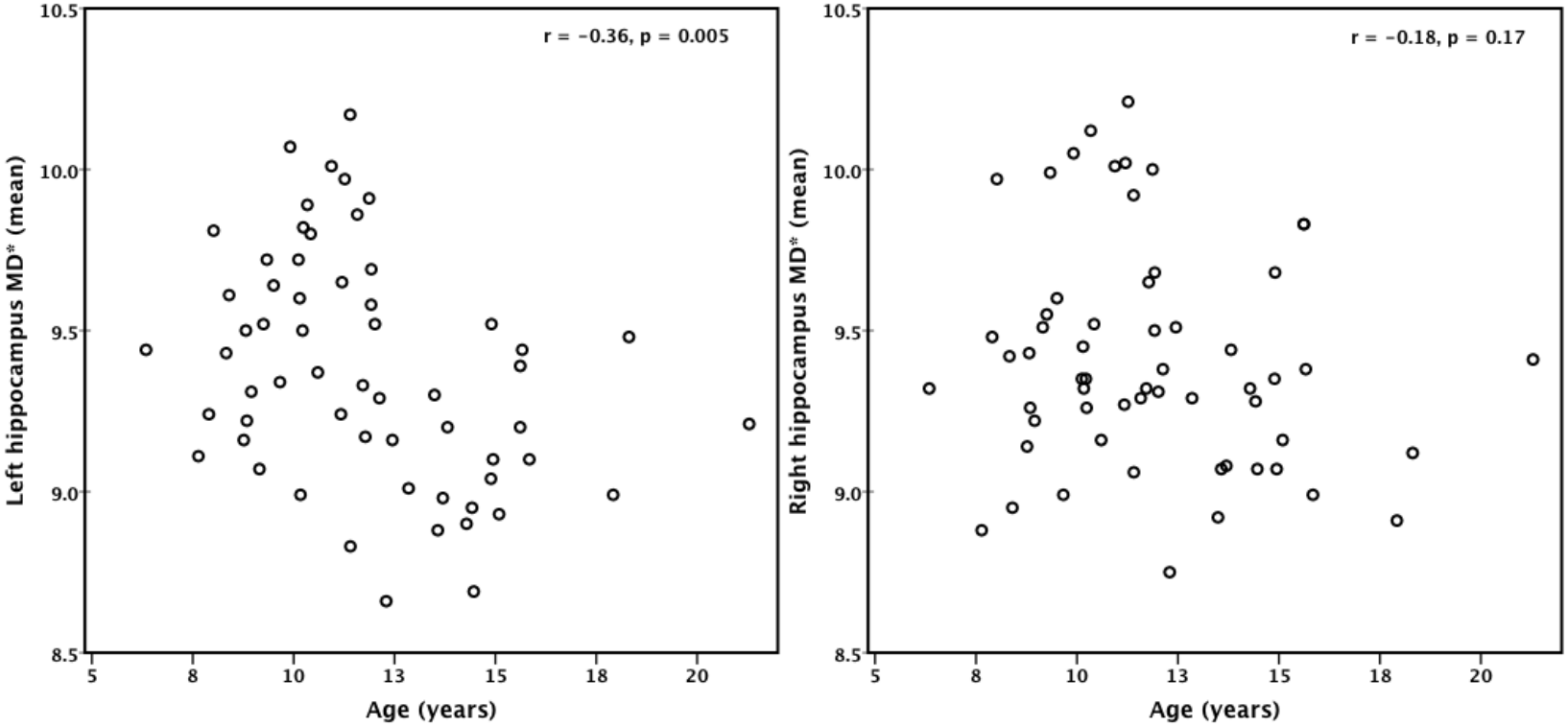


Figure 8.1 Correlation between hippocampal FA and age in controls



*(x 10^{-4} mm^2/s)

Figure 8.2 Correlation between hippocampal MD and age in controls

8.3.3 Between-group comparisons

8.3.3.1 Hippocampal FA

There was significant effect of side (right>left, p<0.001) and aetiological classification (p=0.014) on hippocampal FA. Compared to controls, the estimated mean hippocampal FA was lower in all CSE groups, but the difference was significant only for the PFS group, after Bonferroni correction for multiple comparisons (p=0.012) (Table 8.1). However, as the mean FA estimates for the other CSE groups were similar to that for the PFS group, the lack of statistical significance for these groups is likely to be due to small sample size.

Group	**Mean FA (95% CI)**	**P value⁺**	**Mean age-adjusted MD (x 10^{-4} mm^2/s)* (95% CI)**	**P value⁺**
Control	0.130 (0.127-0.134)	-	9.4 (8.1-10.9)	-
Prolonged febrile seizures	0.120 (0.116-0.125)	**0.012**	9.3 (8.0-11.0)	1.0
Acute symptomatic CSE	0.122 (0.110-0.133)	1.0	9.2 (7.9-10.6)	0.14
Remote symptomatic CSE	0.122 (0.113-0.130)	0.49	10.7 (7.7-14.8)	1.0
Idiopathic & unclassified CSE	0.125 (0.116-0.135)	1.0	9.2 (8.0-10.6)	0.22

* Values for corrected age of 11.97 years

⁺ P values for pairwise comparison with control mean after Bonferroni correction for multiple comparisons

Table 8.1 Mean hippocampal FA and MD values for controls and CSE groups

8.3.2.4 Hippocampal MD

There was no significant effect of age (p=0.31) and side (p=0.35) on hippocampal MD. Although there was a significant group difference in mean hippocampal MD values (p=0.037), none of the CSE groups had significantly different MD than controls on pairwise comparison after Bonferroni correction (Table 8.1).

8.3.2.5 Hippocampal FA and MD asymmetry

Results of Krusal-Wallis ANOVA identified a significant effect of group on hippocampal FA asymmetry (p=0.001) but not on MD asymmetry (p=0.417) (Figure 8.3 & Figure 8.4). Post-hoc analysis with correction for multiple comparisons showed that subjects in the remote symptomatic CSE group had significantly higher hippocampal FA asymmetry compared with controls (p=0.010) and the acute symptomatic CSE group (p=0.002). No statistically significant differences were observed between controls and the other CSE groups.

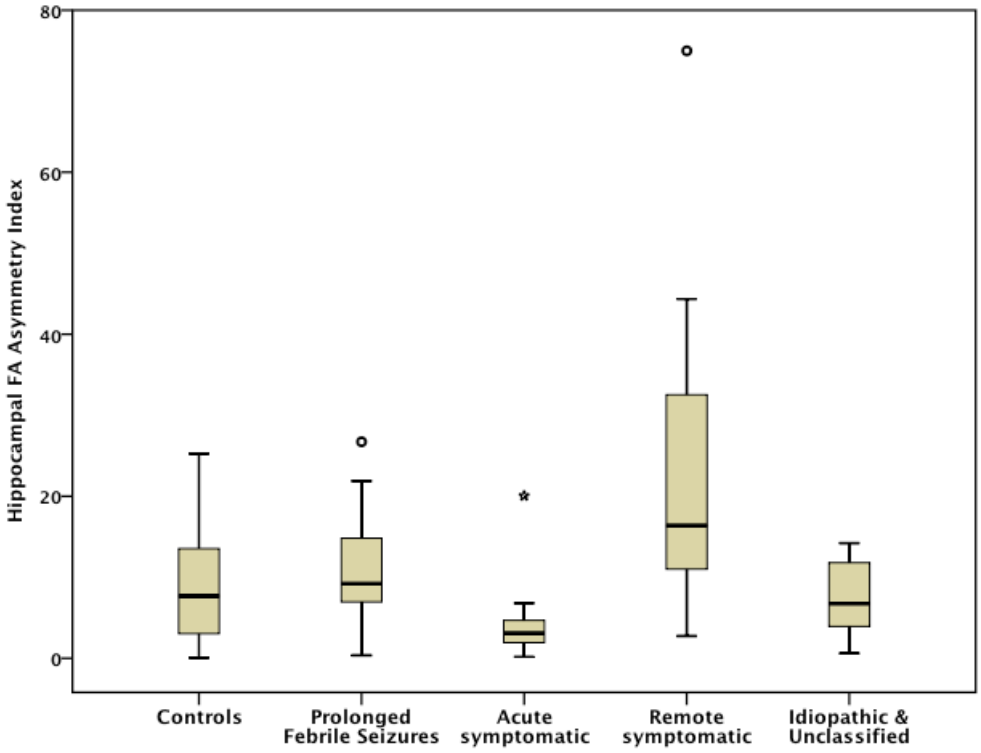


Figure 8.3 Distribution of hippocampal FA asymmetry indices

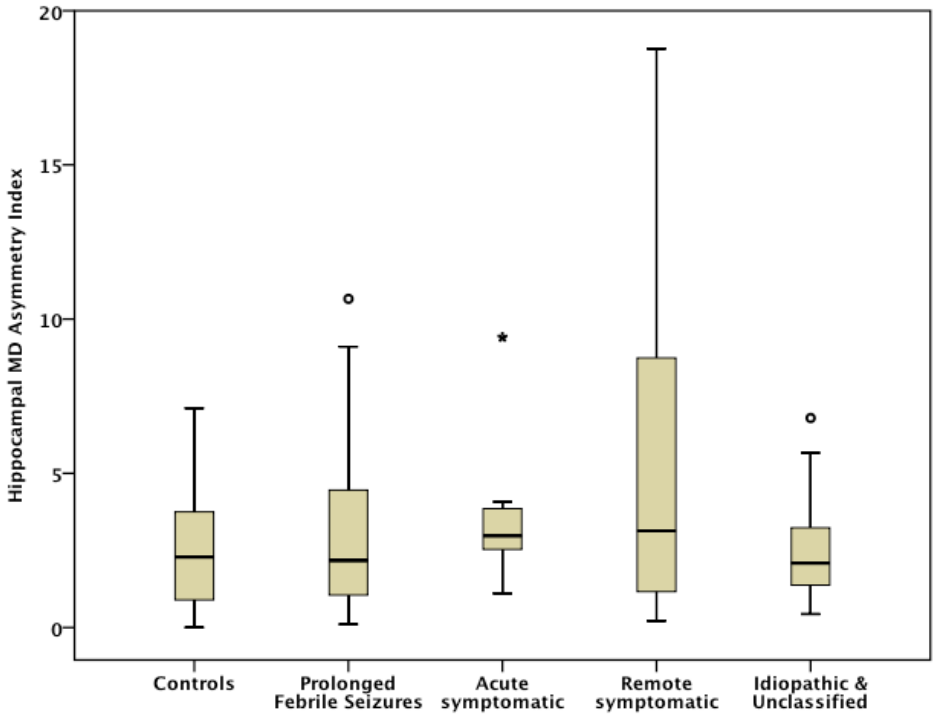


Figure 8.4 Distribution of hippocampal MD asymmetry indices

8.3.2.6 Relationship between abnormal hippocampal volume, FA, and MD asymmetry

9 out of 13 children with abnormal hippocampal volume asymmetry had images suitable for DTI analysis (Figure 8.5). Of these, 3 (33%) had abnormal AI for both FA and MD and one other child (11%) had abnormal AI for MD but not for FA. All four children were in the remote symptomatic CSE group and had significant structural pathology on visual MRI analysis. In addition, five children had abnormal FA asymmetry and six children abnormal MD asymmetry, but normal hippocampal volume AI. None with isolated abnormal hippocampal volume, FA, or MD asymmetry had obvious structural brain abnormalities.

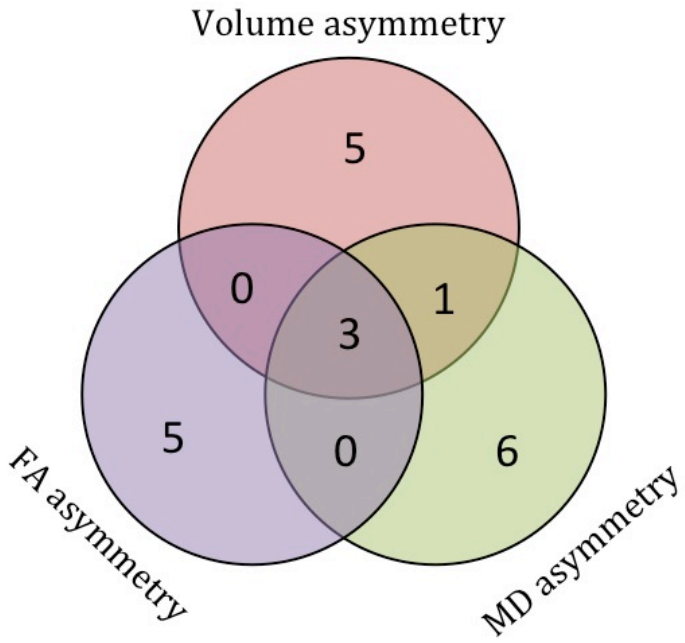


Figure 8.5 Venn diagram summarising the number of children with abnormal hippocampal volume, FA, and MD asymmetry

8.3.3 Comparison of hippocampal FA and MD between subjects with and without HIMAL/IHI

6 (24%) subjects with and 20 (17%) without HIMAL/IHI on visual analysis did not have suitable sequences for DTI analysis. In addition, six subjects (one with and five without HIMAL/IHI on visual analysis) with obvious hippocampal and/or severe global/hemispheric pathology (e.g. hypoxic ischaemic insult, MCA infarction) were subsequently excluded from the analysis as they could potentially have subtle hippocampal pathology that could confound the results. Thus, hippocampal DTI analysis was performed on 18 (72%) subjects with and 94 (79%) without HIMAL/IHI on visual analysis (Table 8.2).

Subject category	Included in visual analysis (n = 144)		Included in DTI analysis (n = 112)	
	No HIMAL/IHI n (%)	HIMAL/IHI n (%)	No HIMAL/IHI n (%)	HIMAL/IHI n (%)
Controls	62 (87)	9 (13)	51 (88)	7 (12)
Prolonged febrile seizures	22 (73)	8 (27)	21 (81)	5 (19)
Acute symptomatic	10 (83)	2 (17)	7 (78)	2 (22)
Remote symptomatic	15 (79)	4 (21)	6 (75)	2 (25)
Idiopathic & unclassified	10 (83)	2 (17)	9 (82)	2 (18)
Total	119 (83)	25 (17)	94 (84%)	18 (16)

Table 8.2 Subjects with and without HIMAL/IHI included in hipppocampal DTI analysis

Subjects with HIMAL/IHI had lower mean FA in the left hippocampus than those without, but the difference was only marginally significant (0.118 vs. 0.126, p = 0.05) (Table 8.3). There was no significant difference between the two groups for mean FA in the right hippocampus and for mean MD bilaterally. Similar results were seen when the 6 subjects with obvious hippocampal/extra-hippocampal pathology were included in the analysis. No statistically significant differences in mean FA or MD were observed on either side between those with and without HIMAL/IHI when the analysis was restricted to only the 26 subjects in the PFS group.

Subjects with HIMAL/IHI had significantly higher median FA asymmetry than those without (0.13 vs. 0.07, Mann-Whitney U p = 0.015) (Figure 8.6). There however was no significant difference in median MD asymmetry between the two groups (0.026 vs. 0.023, Mann-Whitney U p = 0.53) (Figure 8.7).

Group	Left hippocampus				Right hippocampus			
	Mean FA (95% CI)	**P**	**Mean age-adjusted MD* (95% CI)**	**P**	**Mean FA (95% CI)**	**P**	**Mean age-adjusted MD* (95% CI)**	**P**
No HIMAL	0.126 (0.123-0.129)	-	9.28 (9.21-9.35)	-	0.129 (0.125-0.132)	-	9.39 (9.32-9.47)	-
HIMAL	0.118 (0.111-0.125)	**0.05**	9.33 (9.18-9.49)	0.53	0.130 (0.124-0.139)	0.54	9.41 (9.24-9.57)	0.86

* MD (x 10^{-4} mm^2/s), adjusted for age 11.8y

Table 8.3 Comparison of hippocampal FA and MD values in subjects with and without HIMAL/IHI

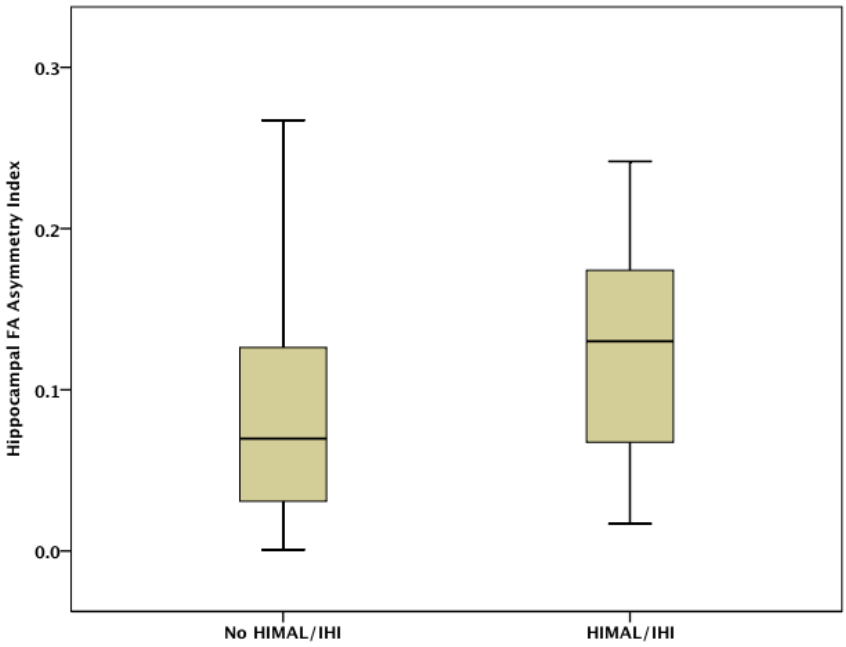


Figure 8.6 Hippocampal FA asymmetry in subjects with and without HIMAL/IHI

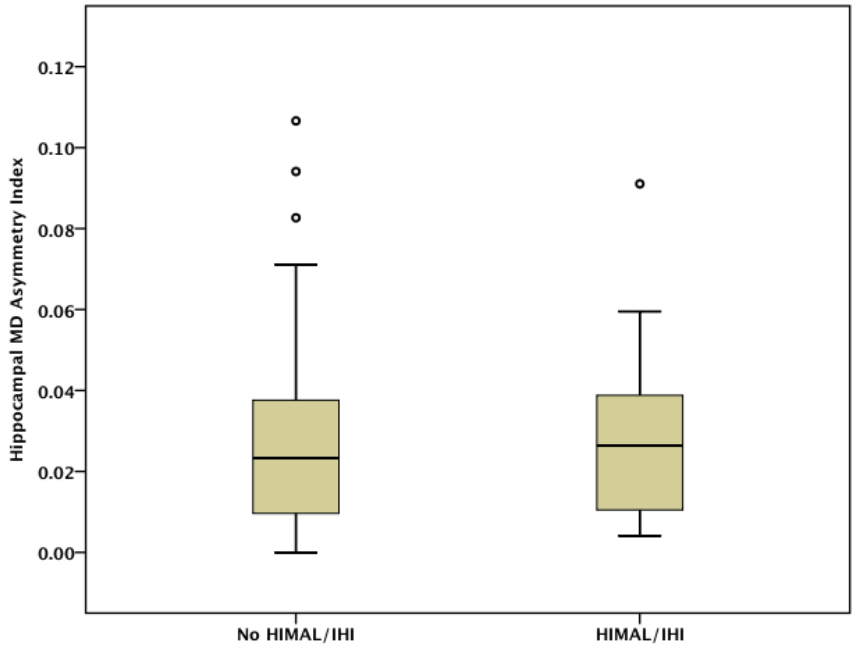


Figure 8.7 Hippocampal MD asymmetry in subjects with and without HIMAL/IHI

8.4 Discussion

In spite of growing interest in the utility of DTI in investigating subtle hippocampal pathology, there is limited normative data from paediatric population. In this study, we describe normal hippocampal FA and MD values from 58 healthy children between ages 7.7 and 21 years. Similar to findings of STEPIN study, we did not observe a significant relationship between age and hippocampal FA on either side in controls (Yoong, 2012). In contrast, while hippocampal MD showed a significant negative correlation with age bilaterally in healthy controls in STEPIN study, a negative correlation was statistically significant only for the left hippocampus in our study. As controls in our study were older (mean age 11.9y) than in STEPIN study (mean age 4.14y), the observed age dependency on the left but not on the right hippocampus in our study may reflect later maturation of left hippocampus (Hou et al., 2013; Njiokiktjien, 2006).

The mean hippocampal FA values in our controls (mean 0.13) are lower than those reported in healthy adults (0.15 to 0.23), and higher than mean FA of 0.12 reported in a younger population in the STEPIN study (Assaf et al., 2003; Liacu et al., 2010; Salmenpera et al., 2006; Thivard et al., 2005; Yoong, 2012). Similarly, mean hippocampal MD in our controls (9.4 x 10^{-4} mm^2/s) is higher than that typically reported in healthy adults (7.7 to 9.2 x 10^{-4} mm^2/s) and lower than mean MD of 10 x 10^{-4} mm^2/s reported in younger children (Assaf et al., 2003; Liacu et al., 2010; Salmenpera et al., 2006; Thivard

et al., 2005; Yoong, 2012; Müller et al., 2006). These findings are consistent with normal evolution of diffusion properties during development, with increases in FA and reductions in MD with age (Kumar et al., 2012; Lebel et al., 2008; Mukherjee et al., 2001; Watanabe et al., 2013). In contrast to our findings, Kimiwada et al., reported higher hippocampal FA (mean 0.21) and lower MD (mean 7.45 x 10^{-4} mm^2/s) in 14 controls with a mean age of 11 years (Kimiwada et al., 2006). The hippocampal mean FA (0.21) reported for children in Kimiwada study is in fact higher, and mean MD (7.45 x 10^{-4} mm^2/s) lower than the values reported in most adult studies (Assaf et al., 2003; Liacu et al., 2010; Müller et al., 2006; Salmenpera et al., 2006; Thivard et al., 2005). There are several possible explanations for the discrepancy between results in Kimiwada study and ours. In their study, Kimiwada et al., defined hippocampal ROI on low-resolution b0 images, but a recent study demonstrated that this method results in significantly higher variability and is therefore less reliable (Yoong, 2012; Kimiwada et al., 2006). In addition to methodological differences in ROI placement, a small sample size (n = 14) and a wide age range (3.7 to 17.2y) of controls in Kimiwada study could be the reason for the observed differences in control FA and MD values between our study and theirs. To maximize reliability of hippocampal DTI measurements we used the co-registration method to define ROI (Yoong, 2012). In addition, we eroded edge voxels from the ROI mask to minimize potential measurement errors due to partial volume effects due to inclusion of surrounding extra-hippocampal tissue such as CSF and white matter.

Children with PFS in our study had lower mean hippocampal FA compared to controls. This was not accompanied either by significant changes in MD or FA/MD asymmetry. Data from DTI studies in patients with MTS suggest MD increase to be a more consistent finding than FA reduction (Assaf et al., 2003; Focke et al., 2008; Kimiwada et al., 2006; Salmenpera et al., 2006; Thivard et al., 2005). Therefore, while hippocampal FA reduction in children with PFS could potentially be indicative of evolving MTS, absence of significant MD increase and FA/MD asymmetry makes this less likely. A second explanation could be that the hippocampal FA reduction in children with PFS is a consequence of prolonged seizure of any type, rather than being PFS specific. Although the hippocampal FA reduction was only statistically significant for the PFS group, the mean FA estimates for other CSE groups were similar to the PFS group, and the lack of significance could be a function of sample size. Therefore, it is possible that the observed hippocampal FA reduction could be a consequence of prolonged seizure of any type. Alternatively, isolated hippocampal FA reduction with no other changes of MTS could also represent an underlying subtle hippocampal developmental abnormality.

There is growing evidence to support the hypothesis that PFS may arise from an already predisposed brain due to subtle hippocampal developmental abnormality such as cortical dysplasia, HIMAL/IHI, and/or genetic alteration (Gibbs et al., 2011; Lewis et al., 2014; Park et al., 2010; Sanon et al., 2012; Scantlebury et al., 2005; Shinnar et al., 2012). As discussed in chapter 6, we found a higher prevalence of HIMAL/IHI in the PFS group than in controls, although the difference was only marginally significant ($p = 0.086$). To investigate whether the hippocampi with HIMAL/IHI have different diffusion properties we compared their hippocampal FA and MD values against those without HIMAL/IHI on visual analysis. We found a significantly lower mean FA in left hippocampus and higher median FA asymmetry index in children with HIMAL/IHI. These were not accompanied by any significant differences in hippocampal MD. Further analysis restricted to only the PFS group did not show significant differences between those with and without HIMAL/IHI. However, for the PFS group analysis, the sample size was reduced to 26, of which only 5 (3 left, 1 right, 1 bilateral) had HIMAL/IHI, and therefore the analysis could be insufficiently powered to identify significant differences. There is no published data on DTI findings seen in HIMAL/IHI to compare our results with. In addition, DTI cannot be used to reliably evaluate dysplastic grey matter in focal cortical dysplasia due to signal contamination by CSF and therefore we were unable to compare our DTI findings in HIMAL/IHI with other cortical malformations (Eriksson et al., 2001; Winston et al., 2014a). However, iontophoretic studies on surgically resected samples of focal cortical dysplasia demonstrate compromised diffusion caused primarily by reduced neuronal density and an increase in extracellular space tortuosity due to an increased number and branching of astrocytic processes, which could manifest as reduced FA on DTI studies (Zamecnik et al., 2012; Vargova et al., 2011; Winston et al., 2014a). Thus, based on our findings of a marginally higher prevalence of HIMAL/IHI (left sided in most) and a significant FA reduction in the left hippocampus in children with PFS, together with a lower FA in the left hippocampus and a higher FA asymmetry in children with HIMAL/IHI, it could be hypothesized that the DTI changes observed in children with PFS may reflect a subtle underlying developmental malformation of the hippocampus such as HIMAL/IHI. While this is plausible, our results are limited by modest statistical significance and sample size for the PFS group, and therefore further data on hippocampal DTI from larger studies such as FEBSTAT and from individuals with HIMAL/IHI are needed to support/refute this hypothesis.

Children with RS CSE had significantly higher hippocampal FA asymmetry than healthy controls. A combination of abnormal hippocampal volume, FA, and/or MD asymmetry

was seen only in children with major structural brain abnormalities. Of note, no individual with increased hippocampal volume asymmetry and normal structural imaging had abnormal FA or MD asymmetry and vice versa, suggesting the tissue properties underlying hippocampal volume and FA/MD are likely to be different. Our results corroborate the results of STEPIN study, in which longitudinal hippocampal volume loss, FA reduction, and MD increase post-CSE were often observed in different individuals, suggesting separate pathological processes such as neuronal loss and neuronal reorganisation may underlie these changes; a combination of neuronal loss and reorganisation may be necessary for the development of MTS (Yoong, 2012).

Whilst the diffusion tensor model provides useful quantitative measures, its limitations are increasingly being recognized. Hippocampal DTI measurement poses additional challenge owing to the difficulty in accurate ROI definition on low-resolution diffusion maps and partial volume effects by inclusion of surrounding non-hippocampal tissue. Also, as the large voxel size in diffusion imaging may contain a mixture of grey matter, white matter and CSF, the findings must be interpreted with caution, particularly in a complex structure such as the hippocampus. Recently proposed newer diffusion compartments models, referred to as "diffusion compartment imaging" (DCI) techniques, provide more accurate information about tissue microstructure by distinguishing diffusion MR signal arising from multiple compartments within a voxel (Scherrer et al., 2015; Winston et al., 2014a). Newer techniques such as DCI may overcome limitations of the DTI model and provide better insight into tissue microstructure, and therefore should be considered for use in future studies.

Owing to the PFS-MTS hypothesis, the hippocampus has been the primary focus in this chapter and the previous chapter. However, CSE-related injury has also been reported in extra-hippocampal grey matter and also in white matter (Kim et al., 2001a; Natsume et al., 2007; Okamoto et al., 2006; Yoong et al., 2013b). In chapter 9 the candidate will investigate long-term white matter diffusion properties following CSE. Ideally this investigation would have been in children with all forms of CSE, but as will be explained, this was restricted to only children who had PFS.

CHAPTER 9 TRACT-BASED SPATIAL STATISTICS

9.1 Introduction

9.1.1 Rationale for white matter diffusion analysis

As discussed in chapters 7 and 8, the hippocampus has been the primary focus of research interest in investigating the consequences of prolonged seizures due to the PFS-MTS hypothesis. However, acute seizure-related injury has also been reported in extra-hippocampal cortical and subcortical grey matter, and white matter (WM) (Natsume et al., 2007; Kim et al., 2001a; Okamoto et al., 2006). There is considerable data on the longer-term evolution of hippocampal changes following CSE, but the longer-term outcomes of WM changes have not yet been investigated (Provenzale et al., 2008; Scott et al., 2003; Tarkka et al., 2003).

Abnormal DTI findings such as reduced FA and increased MD have been reported in several WM tracts in the absence of a structural lesion on MRI in adults and children with epilepsy (Otte et al., 2012; Widjaja et al., 2013). Therefore, DTI analysis could provide insight into WM microstructure in children with CSE and normal structural MRI.

In a recent study, we performed longitudinal DTI analysis in the first year following PFS, and found widespread changes suggestive of reduced WM integrity at 1 and 6 months, with apparent normalisation 12 months post-PFS (Yoong et al., 2013b). The WM changes were seen in the absence of any hippocampal or extra-hippocampal structural abnormalities. Whether these WM changes persist or evolve after 1-year post-PFS is unknown, and therefore long-term follow-up is necessary to investigate the outcomes.

As the aim of this thesis is to determine the long-term outcomes of childhood CSE in general, ideally all investigations should be performed on the whole CSE cohort. However, we restricted the WM diffusion analysis to comparison between only children who had PFS and age-appropriate healthy controls. This was done for the following reasons: (1) Tract-Based Spatial Statistics (TBSS), the method used for analysis in this study, relies on accurate registration of multi-subject images onto a common template and inclusion of children with non-PFS CSE, who often have structural brain abnormalities, would increase the likelihood of registration errors. (2) We wanted to compare a homogeneous CSE cohort with age-appropriate controls to minimize

potential bias and allow for generalisability of findings. (3) Limiting the number of comparison groups would reduce the probability of type-1 error. (4) Hippocampal DTI analysis showed that the PFS group had significant FA reduction compared to the controls and a possible suggestion of an underlying subtle hippocampal developmental malformation, and therefore we were interested in investigating whether they also had extra-hippocampal diffusion abnormalities.

9.1.2 Tract-Based Spatial Statistics

Tract-Based Spatial Statistics (TBSS) is the most popular technique for operator-independent, automated voxel-wise analysis of multi-subject data to compare WM diffusion properties (Smith et al., 2006). TBSS offers the advantage of a hypothesis-free, automated whole-brain analysis, thus avoiding the limitations of ROI-based approach, and also solves the alignment and spatial smoothing issues inherent with voxel-based morphometry approach (Smith et al., 2006). It aligns all subjects' FA maps into a common space, creates a mean FA skeleton representing the centres of all tracts common to the group and then projects all subjects' FA data onto the mean FA skeleton, which is fed into voxel-wise analysis.

In addition to FA and MD, DTI also provides quantitative measures of diffusion parallel to WM tracts as axial diffusivity (AD) and diffusion perpendicular to WM tracts as radial diffusivity (RD). Extending TBSS analysis to other DTI parameters such as MD, AD, and RD could provide a better understanding of the WM microstructural properties underlying any DTI changes seen in the PFS group (Alexander et al., 2011; Hasan et al., 2012; Sadeghi et al., 2013).

9.1.3 Objective of the study

In the study presented in this chapter, we use TBSS to investigate WM diffusion properties within 10 years after PFS compared with age-appropriate healthy controls.

9.2 Methods

9.2.1 MR imaging sequences and processing

Details about MR image acquisition and processing of the diffusion datasets for DTI are described in chapter 3. FA, MD, AD, and RD maps generated from processing the diffusion data were used for TBSS analysis.

9.2.2 TBSS

Voxel-wise statistical analysis of the DTI data was carried out using TBSS, part of FSL (Smith et al., 2006). We performed TBSS using standard scripts provided within the FSL software package (FSL 5.0.2.1). We opted to generate a study-specific target image for nonlinear registration, as adult-derived standard-space FA image used in TBSS may be inappropriate for paediatric data. Accordingly, the identified target FA image from our study population was affine-aligned into 1x1x1mm MNI152 standard space, and every other FA image transformed into standard space by combining the nonlinear transform to the target image with the affine transform from the target image to MNI152 space. Next, the mean of all FA images was created and fed into the FA skeletonisation program to create a mean FA skeleton, which represents the centres of WM tracts. The mean FA skeleton was thresholded at an FA value of 0.2 to eliminate skeletal remnants at the outer WM tissue where higher inter-subject variability is likely to cause poor alignment. Finally, each subject's aligned FA data was projected onto the mean FA skeleton, which was then used for voxel-wise statistical analysis. Each subject's image was visually inspected in FSLView, part of FSL package, to verify accurate registration. Other DTI parameters (MD, AD, and RD) were also analysed in TBSS using the 'tbss_non_FA' script.

Voxel-wise analysis was performed using the 'randomise' tool in TBSS with 5000 permutations and DTI parameters compared between the PFS group and controls. As age, gender, and handedness can influence diffusion parameters, they were entered as covariates for voxel-wise analysis. In addition, although none of the subjects had radiological evidence of MTS, mean hippocampal volume (obtained from hippocampal volumetry as described in chapter 7) was also entered as a covariate for voxel-wise analysis to address the possibility that unrecognized subtle hippocampal damage could potentially influence WM diffusion properties. Threshold-Free Cluster Enhancement (TFCE) was performed in order to enhance cluster-like structures without prior

definition of a cluster-forming threshold or extensive data smoothing. In order to control for multiple voxel-wise comparisons, family-wise error (FWE) correction was performed and the resulting significance threshold was $p<0.05$. Results were visualised in FSLView as color-coded masks superimposed on the MNI152 brain template and the mean FA skeleton. The most probable anatomic localisation of each significant cluster was determined using John Hopkins University WM atlas tools in FSL (Wakana et al., 2004).

9.3 Results

9.3.1 Subject demographics

Of the 56 children with an initial diagnosis of PFS, 35 (62.5%) consented for follow-up. The reasons for loss to follow-up and exclusion from current analysis are presented in Figure 9.1. As our aim was to investigate WM microstructure in a homogeneous PFS cohort, we excluded three children who developed epilepsy during follow-up and one child with MRI features of neurofibromatosis type 1. In addition, five children had to be excluded from the analysis due to incomplete DTI data. There was no significant difference in the median age at PFS (13 months v 16 months, Mann-Whitney U $p=0.16$) or seizure duration (60 minutes v 60 minutes, Mann-Whitney U $p=0.73$) of children included in TBSS analysis and of those excluded.

26 children with PFS [11 male, mean age 9.9 years (standard deviation 1.72), 20 right-handed] and 27 age-matched controls [12 male, mean age 10.1 years (standard deviation 1.74), 22 right-handed] were included in the analysis. Conventional structural neuroimaging was normal in all, and none had neurological or cognitive problems. The mean interval between the episode of PFS and MR Imaging follow-up was 8.23 years (range 6.7 to 9.6).

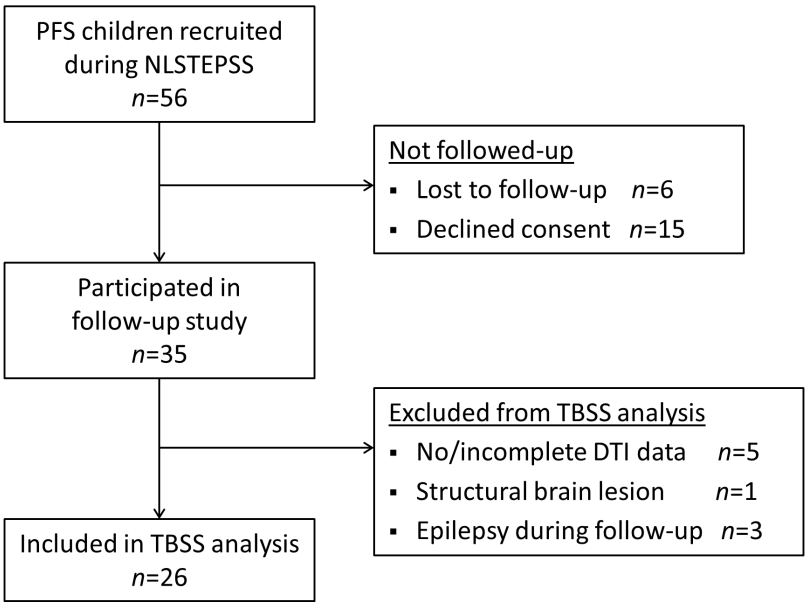


Figure 9.1 Flow diagram showing recruitment of subjects for TBSS analysis

9.3.2 TBSS analysis

No significant correlations were observed between mean values for FA, MD, AD, and RD over the entire WM skeleton and the hippocampal volumes, in either the PFS group or controls (p>0.05 for all).

On voxel-wise analysis, widespread differences in anisotropy and diffusivity measures were observed between the PFS group and controls (Figure 9.2). Compared to controls, the PFS group had FA increase in caudal central WM tracts, and MD, AD, and RD increase in peripheral WM tracts.

In the PFS group, bilateral FA increase was observed in the cerebral peduncles, corticospinal tracts, superior cerebellar peduncles, middle cerebellar peduncles and the pontine crossing tract of middle cerebellar peduncle (Figure 9.2, top row). FA was also increased in the left superior corona radiata, posterior limb of internal capsule and crus fornix.

There were widespread increases in MD and AD in the PFS group compared to controls (Figure 9.2, second & third row). These were predominantly bilateral involving the

corpus callosum, superior & inferior longitudinal fasciculi, inferior fronto-occipital fasciculi, corona radiata, posterior thalamic radiations, retrolenticular part of internal capsules, external capsules and cingulate gyri. MD increase was also seen in right anterior & posterior limbs of internal capsule. In addition, AD increase was also seen in the posterior limb of internal capsules, cerebral peduncles and cres fornices bilaterally, and in the right anterior limb of internal capsule.

Unlike MD and AD, regions of RD increase in the PFS group were less extensive, and limited to peripheral WM tracts such as superior & inferior longitudinal fasciculi, inferior fronto-occipital fasciculi, superior & posterior corona radiata and posterior thalamic radiations (Figure 9.2, bottom row).

None of the WM tracts had reduced FA or any of diffusivity indices in the PFS group.

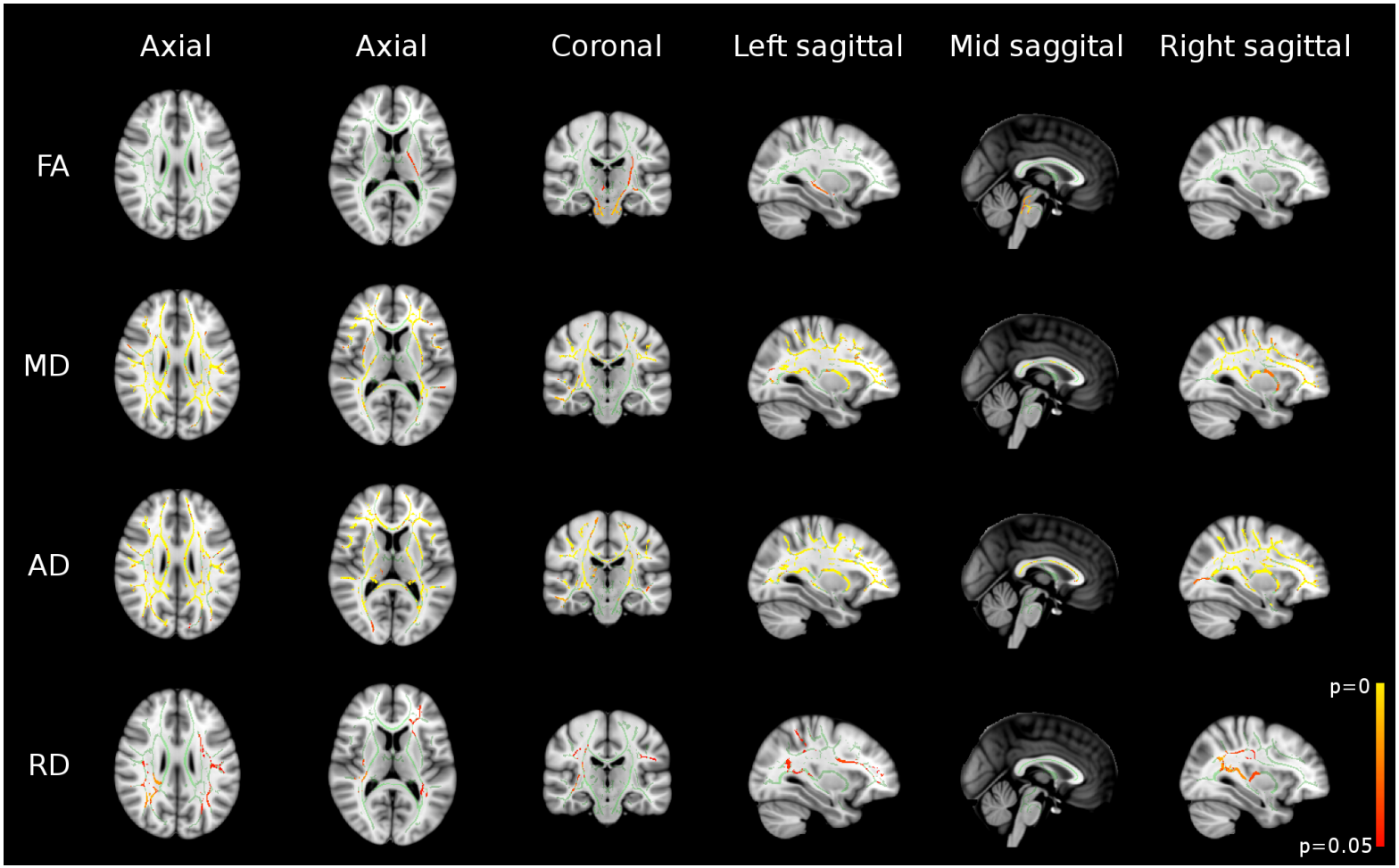


Figure 9.2 Voxel-wise analysis showing significant differences between the PFS group and controls. Regions in green represent the mean FA skeleton and therefore no significant difference between the two groups. Regions in red-yellow represent significant increases in the PFS group ($p<0.05$, FWE corrected).

9.4 Discussion

To our knowledge, this is the first study to report on WM microstructure at long-term follow-up in a population-based PFS cohort. The main findings from our study are that, compared to healthy controls, children who had PFS demonstrate; (1) FA increase primarily in caudal central WM tracts, (2) MD and AD increase in several peripheral WM tracts and rostral central WM tracts, and (3) RD increase limited to peripheral WM tracts, after a mean follow-up of 8.2 years. The WM diffusion abnormalities were observed in the absence of obvious hippocampal structural abnormalities.

Brain WM maturation in healthy children and adolescents follows predictable topographical and chronological sequences with myelination progressing from inferior to superior, from posterior to anterior, and from central to peripheral location within the brain (Geng et al., 2012; Gao et al., 2009; Dubois et al., 2014; Colby et al., 2011). Therefore, myelination occurs in brainstem WM before cerebral WM, in central WM before peripheral WM, and in projection and commissural pathways before association pathways. The rate of maturation of WM tracts however is not linear, with steep increase in the first year after birth and a slower rate in the following years (Gao et al., 2009; Geng et al., 2012; Chen et al., 2013; Hermoye et al., 2006; Dubois et al., 2008). In addition, WM tracts less mature at birth (e.g. peripheral WM) have a faster rate of maturation in early childhood years compared to those more mature at birth (e.g. corticospinal tracts), and therefore more vulnerable to insults during this period (Chen et al., 2013; Kinney et al., 1988; Lee et al., 2013; Geng et al., 2012; Hermoye et al., 2006).

The striking observation in our study is the widespread WM AD increase in the PFS group compared to controls. In addition, contrary to expectation, children with PFS had FA increase, albeit only in caudal central WM tracts. A possible mechanism for AD increase is greater coherence and alignment of the axons in WM tracts (Choe et al., 2012; Leergaard et al., 2010; Dubois et al., 2008; Dubois et al., 2014). Alongside this, increases in MD suggest disruption of WM microstructure perhaps as a result of decreased axonal and/or myelin content (Concha, 2014; Dubois et al., 2014; Winston, 2012). These two factors, axonal/myelin density and axonal coherence/alignment, contribute to FA in WM tracts. Figure 9.3 illustrates the balance between these two factors and shows how changes in FA can be explained in terms of them. For example, based on this model, an increase in axonal coherence (tending to increase FA) outweighs the more modest axonal loss (tending to reduce FA) in early-maturing WM tracts leading to an overall

increase in FA. However, in the late-maturing WM tracts the increase in axonal coherence is counter-balanced by a greater reduction in axonal density leading to no change in FA.

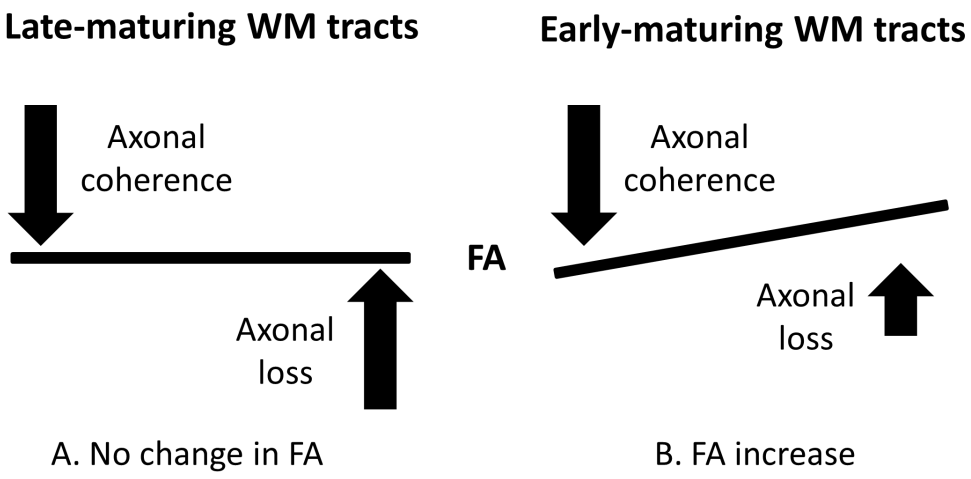


Figure 9.3 Illustration depicting the balance between axonal loss and axonal coherence, and their influence on FA. While increased axonal coherence with modest axonal loss in early-maturing WM tracts results in FA increase, the increased axonal coherence is counterbalanced by a greater axonal loss in late-maturing WM tracts with no change in FA.

Interestingly, the topographical pattern of diffusion changes seen in the PFS group in our study relates to normal WM maturation in childhood. Early-maturing WM tracts such as the corticospinal tracts, cerebral peduncles, and middle cerebellar peduncles, had increased FA in the PFS group, which we suggest is due to a combination of increased axonal coherence and modest axonal loss, compared to controls. In contrast, most late-maturing WM tracts had evidence of disrupted maturation with increases in MD, AD, and RD, without a significant change in FA. Therefore, it could be hypothesised that an insult during a "vulnerable period" for late-maturing WM tracts may be responsible for disrupted WM tract maturation in the PFS group. A recent report by Lee et al. of preferential susceptibility of late-maturing WM tracts to damage in patients with mesial temporal lobe epilepsy also supports this hypothesis (Lee et al., 2013).

In our previous study of longitudinal WM diffusion changes following PFS, Yoong et al. found widespread reductions in FA in several WM tracts at 1 and 6 months post-PFS, without a significant change in MD (Yoong et al., 2013b). The FA reductions were related predominantly to reductions in AD at 1 month, and increases in RD at 6 months post-PFS. While the reductions in AD almost disappeared, RD increases were more widespread at 6 months post-PFS. No significant differences between the PFS group and controls in any of the diffusion parameters were observed at 1-year post-PFS. Seizure-related temporary disruption of WM development was proposed as the most biologically plausible explanation for these findings. Data from the current study in a similar cohort suggests that despite apparent normalisation of WM DTI metrics at 1 year, alteration in WM microstructure compared to controls becomes evident about 8 years after PFS; and that this alteration is accompanied by increases in the coherence of the remaining WM structure. Similar findings of WM FA increase, often accompanied by AD increase, have also been reported several months after acquired brain insult in the form of brain surgery, stroke, and traumatic brain injury (Baek et al., 2013; Sidaros et al., 2008; Winston et al., 2014b; Yogarajah et al., 2010). It is tempting to suggest that this re-organization of the remaining WM structure is the brain's attempt to maintain efficient organization in face of an event disruptive to the normal trajectory of maturation, and one that has served to maintain neurological and cognitive function as observed in our cohort. However, it is important to recognize that gliosis can also show a high degree of directionality and result in similar DTI findings, and therefore it is possible that the observed changes could be due to post-injury reactive gliosis, and not a sign of recovery (Concha, 2014; Walhovd et al., 2014).

An alternative explanation for our findings is that the WM diffusion changes pre-exist the PFS and reflect aberrant WM development secondary to a subtle developmental malformation or genetic susceptibility, which also increase the predisposition to PFS. Our findings in chapter 6 and chapter 8 of hippocampal FA reduction and marginally higher HIMAL/IHI prevalence in the PFS group, and FA reduction in subjects with HIMAL/IHI, support the possibility of a pre-existing developmental malformation in the left hippocampus. It is plausible that the WM changes observed in children with PFS could reflect pre-existing aberrant WM development. However with the widespread distribution of WM changes observed in the current study and in the STEPIN study, plus the longitudinal evolution of WM DTI changes post-PFS suggestive of an acute axonal injury with subsequent long-term WM reorganization, this seems less likely (Yoong et al., 2013b).

The biophysical characteristics of WM affecting the diffusivity parameters on in vivo DTI are likely to be complex and yet to be fully understood (Winston, 2012; Concha, 2014; Groeschel et al., 2014; Jones et al., 2013). While FA (measure of intra-voxel directional coherence of water diffusion) and MD (measure of magnitude of diffusion) are sensitive to changes in underlying tissue diffusion properties, changes in these metrics are not specific to particular elements of the underlying microstructure. The assumption that AD (i.e. axial/longitudinal/parallel diffusivity) is primarily determined by the intrinsic characteristics of axons and axonal integrity, and RD (i.e. radial/transverse/ perpendicular diffusivity) by myelination, is overly simplistic as the relative contribution of WM tissue components to the diffusivity measures are yet to be accurately determined (Concha, 2014; Winston, 2012). The interpretation of DTI metrics is even more ambiguous in regions of crossing fibres (estimated to be as high as 75% to 90% of WM voxels), where their values are likely to be confounded by changes in the relative fractions of the multiply oriented fibre populations (Jones et al., 2013; Jeurissen et al., 2013; Groeschel et al., 2014). Thus, selective disruption to one of the fibre populations in regions of complex crossing fibres can influence eigenvalues and tensor shape, and as a consequence, DTI metrics. For example, selective disruption of fibres in one pathway crossing other relatively preserved pathways in a region such as centrum semiovale may result in either an increase or decrease in FA, AD, and/or RD, depending on the orientation of the principle eigenvectors. This could be one of the explanations for the inconsistent DTI findings reported in conditions such as autism, ADHD, and preterm birth injury (Aoki et al., 2013; Groeschel et al., 2014; Weyandt et al., 2013). Therefore, it is important that due caution is exercised when interpreting DTI results, and the limitations and non-specificity of the technique as a marker of WM integrity borne in mind. As discussed in chapter 8, newer techniques such as diffusion compartment imaging (DCI) enable measurement of compartment-specific diffusivity characteristics within a voxel, thus better characterising WM tissue microstructure and could overcome limitations of the DTI model, especially in regions of crossing fibres (Scherrer et al., 2015; Winston et al., 2014a).

Our study has some limitations. A longitudinal design could have provided a better understanding of the evolution of WM diffusion changes following PFS. We have referred to the results from our previous study on a comparable cohort, but with a shorter follow-up, to complement data from the current study and interpret the findings (Yoong et al., 2013b). A possible limitation of TBSS is misregistration of individual scans to the common template for group analysis, which may be an issue particularly for

structurally abnormal scans. However, all children in our study had structurally normal scans, and we used a study-specific target template instead of the adult-derived standard template for non-linear registration and also confirmed adequate registration by visual inspection. Finally, although our sample size is modest, we used an unselected, homogeneous PFS cohort and age-matched controls to minimise potential bias and allow for generalisability of our findings.

In this chapter, we investigated WM microstructural alterations 8.2 years post-PFS in a population-based cohort. We found FA increase in early-maturing central WM tracts and increases in AD and MD with/without increased RD in several late-maturing peripheral WM tracts. We propose temporary disruption in WM maturation secondary to PFS-induced axonal injury, and subsequent compensatory microstructural re-organisation as the probable explanation for our observations. An alternative plausible explanation is that the WM changes observed in children with PFS could reflect pre-existing aberrant WM development related to an underlying developmental malformation or genetic abnormality that also increases the predisposition to PFS, but this seems less likely. Longitudinal long-term follow-up studies will be required to further clarify the pathophysiological basis, and to determine the neurocognitive functional correlates associated with the evolution of WM diffusion changes observed in children post-PFS.

CHAPTER 10 DISCUSSION

The overall aims of the present thesis have been, through a prospective population-based follow-up study, (1) to estimate mortality and morbidity within 10 years after childhood CSE due to all causes and identify predictors of adverse outcomes, and (2) to investigate long-term consequences of childhood CSE on hippocampal and extra-hippocampal structures. In this concluding section the candidate will attempt to integrate the results presented in the preceding chapters towards addressing the aims of the thesis. In addition, the candidate will discuss the limitations of the study and advance some suggestions for future research on key questions related to outcomes after childhood CSE that could not be adequately addressed in the present study.

The review on the outcomes after childhood CSE reported in chapter 2 highlights the wide variability in the reported rates of mortality and morbidity associated with childhood CSE, and uncertainties about the risk factors associated with adverse outcomes. To address some of the methodological limitations of published studies and minimise potential bias, for the current study, we prospectively followed-up a large population-based childhood CSE cohort to determine outcomes using clear definitions of outcome variables, formal neurocognitive testing and appropriate statistical methods for addressing missing data. In addition, for quantitative neuroimaging analysis, we used data from a relatively large age-appropriate healthy control population to determine abnormalities in the CSE cohort.

10.1 Mortality and morbidity associated with childhood CSE

The first important observation from the current study was that the mortality within 10 years after childhood CSE was 46 times higher than expected in the reference population. However, this effect was predominantly seen in children with pre-existing neurological impairments at CSE presentation. Almost all deaths in previously neurologically normal children occurred within 30 days of their CSE episode, and those who survived beyond this period were not at a significantly increased risk of death compared to the reference population. The observation in the current study of no deaths in children following PFS is consistent with very low risk of long-term mortality associated with PFS/FSE reported in previous population-based studies (Chungath and Shorvon, 2008; Logroscino et al., 2002; Nelson and Ellenberg, 1978; Verity et al., 1993; Vestergaard et al., 2008). The majority of deaths in the current study were as a

consequence of complications of the underlying cause and only 4/23 deaths were seizure-related. This is in contrast to the findings of a previous French study in the 1960s and studies from resource-poor countries reporting that about 50% deaths observed in their series were seizure-related (Aicardi and Chevrie, 1970; Molinero et al., 2009; Prins et al., 2014; Sadarangani et al., 2008). Possible explanations for the observed difference between these and the current study will be discussed later in this section.

The long-term outcome was generally less favourable for children who had epilepsy and/or neurological impairments at CSE presentation, with the majority having active epilepsy and motor/intellectual disability at follow-up in the current study. In addition, the prevalence of psychiatric disorders and special educational needs were much higher in this group than in those who were previously neurologically normal. The observation in the current study that two or more morbidities often coexist in the same subject, usually in those with previous neurological abnormalities, is similar to that reported in previous population-based studies in childhood-onset epilepsy (Berg et al., 2011b; Berg et al., 2008; Berg et al., 1996; Berg et al., 2005; Camfield et al., 1993; Camfield and Camfield, 2007; Chin et al., 2011; Jalava et al., 1997; Kokkonen et al., 1997; Sillanpää, 2004; Sillanpää et al., 1998; Sillanpää et al., 2011; Wakamoto et al., 2000). This suggests that the co-morbidities associated with CSE are often seen in the subgroup with prior neurological abnormalities. This information will be useful for determining prognosis and counselling parents about the long-term outlook for their children following CSE.

Despite a longer median follow-up of 8.9 years, the cumulative incidence of epilepsy in the current study (25%) is comparable with 15-36% incidence reported within 3 to 5 years after childhood CSE (Raspall-Chaure et al., 2006; Aicardi and Chevrie, 1970; Barnard and Wirrell, 1999; Eriksson and Koivikko, 1997; Maegaki et al., 2005; Maytal et al., 1989; Tabarki et al., 2001; Prins et al., 2014). This is probably because 90% of CSE cohort who developed epilepsy in the current study did so within 18 months after CSE, similar to the observation that 80-90% of recurrences after a first unprovoked seizure (and therefore epilepsy diagnosis) occur within 2 years (Berg, 2008; Berg et al., 1999b; Sillanpaa and Shinnar, 2002; Shinnar et al., 1996; Shinnar et al., 2000). This implies that unprovoked CSE, like brief seizures, could be the presentation that leads to the diagnosis of epilepsy within 2 years in the majority, with low risk of epilepsy thereafter.

The results of the current study confirm generally favourable long-term outlook for children with PFS (Nelson and Ellenberg 1978, Maytal, Shinnar et al. 1989, Maytal and Shinnar 1990, Verity, Ross et al. 1993, Verity, Greenwood et al. 1998, Shinnar, Pellock et al. 2001, Raspall-Chaure, Chin et al. 2006, Chungath and Shorvon 2008). The study also provides compelling evidence to add to the long-standing debate over the causative relationship between PFS and later development of MTS and TLE (Verity, 1998; Raspall-Chaure et al., 2006; Patterson et al., 2014; Shukla and Prasad, 2012; Tarkka et al., 2003). Data from the current study corroborate those of previous epidemiological studies suggesting that only a small proportion of children develop TLE and/or MTS following PFS (Berg et al., 1999a; Camfield et al., 1994; Chungath and Shorvon, 2008; Nelson and Ellenberg, 1978; Raspall-Chaure et al., 2006; Tarkka et al., 2003; Verity and Golding, 1991; Verity et al., 1993; Yoong, 2012; Annegers et al., 1987). While it is possible that more subjects could potentially develop TLE and/or MTS if followed-up for a longer duration, given that the data from the current study demonstrate little MRI evidence suggestive of progressive hippocampal changes leading to MTS within 8 years after PFS, it would seem biologically implausible that many more subjects develop MTS upon further follow-up. Although a few subjects in the PFS group had reduced hippocampal volume and a few others reduced hippocampal FA, both changes were not seen together in any subject with PFS, and therefore the significance of these findings is uncertain.

The current study provides strong population-based data confirming the mounting evidence that aetiology is the main determinant of outcome following CSE in childhood (Awaya et al., 1992; Ellenberg and Nelson, 1978; Lewis et al., 2014; Maytal and Shinnar, 1990; Maytal et al., 1989; Nelson and Ellenberg, 1978; Scott et al., 2003; Shinnar et al., 2001b; Verity et al., 1993; Yoong, 2012; Raspall-Chaure et al., 2006). Neurological, cognitive, and behavioural comorbidity was much higher in those with symptomatic CSE and pre-existing neurological abnormalities, and low in previously neurologically normal children. Given that the comorbidities were present before the episode of CSE in the majority in the current study, and high prevalence of intractable epilepsy, recurrent CSE, and cognitive/behavioural problems in remote symptomatic epilepsy and/or abnormal neurology reported in other studies, the debate on whether CSE is the cause or merely a marker of poor outcomes in children with pre-exiting neurological problems is justified (Berg et al., 2004a; Camfield and Camfield, 2012; Sillanpää et al., 2014; Sillanpaa and Shinnar, 2002; Stroink et al., 2007; Reilly et al., 2014; Barnard and Wirrell, 1999; Berg et al., 2011b; Davies et al., 2003; Rutter et al., 1970; Berg et al., 2005; Berg et al., 2011c; Sillanpää 2004, Sogawa, Masur et al. 2010; Chin et al., 2011).

A causal association between long seizure duration and subsequent adverse outcomes has long been debated. Data from the current study are consistent with previous reports suggesting seizure duration does not have an independent adverse impact on mortality and morbidity following childhood CSE (Barnard and Wirrell, 1999; Lewis et al., 2014; Maytal et al., 1989; Nelson and Ellenberg, 1978; MacDonald et al., 1999; Pavlidou and Panteliadis, 2013; Verity and Golding, 1991; Viani et al., 1987; Hussain et al., 2007; Kang et al., 2005; Lacroix et al., 1994). However, as most of these studies only include children with CSE (i.e. seizure duration ≥30 minutes), the possibility that a critical threshold under 30 minutes where seizure duration may significantly influence outcomes cannot be excluded. Limited evidence from a few studies comparing outcomes between seizure duration <30 minutes and ≥30 minutes broadly suggest that seizure duration may not have additional impact on subsequent mortality and morbidity above that related to the aetiology (Nelson and Ellenberg, 1978; Verity and Golding, 1991; Vestergaard et al., 2008; Metsäranta et al., 2004; Fernández et al., 2014). Future studies comparing outcomes between seizure duration <30 minutes and ≥30 minutes, separately for each aetiology, could potentially help determine the direct impact of seizure duration on outcomes. However, even such approach may not provide a satisfactory answer to this important question as the outcome may still be confounded by the severity of the underlying brain disorder and seizure duration itself could be a marker of the severity.

10.2 Hippocampal and white-matter structural abnormalities

The MR Imaging work undertaken in the current study nearly 8 years after childhood CSE is an extension of previous research from our group investigating the short- and long-term consequences of childhood CSE, PFS in particular. The findings from our earlier acute imaging studies suggested the presence of acute hippocampal oedema within 2 days of after PFS that subsequently resolves within the time frame of 3 to 5 days (Scott et al., 2002; Scott et al., 2003; Scott et al., 2006). Longitudinal imaging data from our group and others also suggested that a significant proportion may demonstrate increased hippocampal volume asymmetry, often without other features of MTS, months to years after PFS/FSE (Farrow et al., 2006; Lewis et al., 2014; Scott et al., 2003; Tarkka et al., 2003; Yoong, 2012; Yoong et al., 2013a; Scott et al., 2006). The results of the current study are consistent with those of Tarkka et al. and suggest that the risk of developing MTS within 8 to 12 years after PFS/FSE is low, and certainly not near the 44% of patients with TLE and MTS who had a history of febrile seizures reported in retrospective surgical series (Chungath and Shorvon, 2008; Tarkka et al., 2003). Also,

MRI features of MTS were not only seen in the PFS group, but also in other CSE groups in the current study. This implies that MTS can also be associated with non-PFS CSE.

Neuroimaging findings suggestive of subtle hippocampal developmental abnormalities (e.g. HIMAL) have previously been reported in children with PFS/FSE (Lewis et al., 2014; Scott et al., 2006; Shinnar et al., 2012; Yoong, 2012; Yoong et al., 2012). In addition, other studies have reported subtle hippocampal malformations as predisposing to PFS and subsequent MTS ("dual pathology" and "two-hit hypothesis") (Fernández et al., 1998; Sloviter and Pedley, 1998; Bocti et al., 2003; Sanon et al., 2012). Thus the imaging findings in the current study of a higher, albeit only marginal, prevalence of HIMAL in the PFS group compared with controls may provide some support towards this hypothesis.

In a previous longitudinal diffusion imaging study, Yoong et al. observed changes suggestive of widespread disruption in WM microstructure at 1 and 6 months post-PFS but the changes were no longer apparent at 1 year post-PFS (Yoong et al., 2013b). Similar analysis in the current study 8 years post-PFS revealed differential microstructural disruption in early-maturing and late-maturing WM tracts. It seems that an insult in the form of PFS during a "vulnerable period" for late-maturing WM tracts may be responsible for disrupted WM maturation in the current study. A temporary disruption in WM maturation secondary to PFS-induced axonal injury, and subsequent compensatory microstructural re-organisation offers the most biologically plausible explanation for the observations in the two studies. Alternatively, the WM changes observed in children post-PFS could reflect pre-existing aberrant WM development related to an underlying developmental malformation or genetic abnormality that also increases the predisposition to PFS, but this seems less likely.

10.3 Limitations

While the current study has the advantage of a prospective, long-term follow-up of a large population-based childhood CSE cohort, it has some important limitations.

The most important limitation of the current study was the lack of baseline neuroimaging and formal cognitive testing at CSE presentation. The current study (STEPSOUT) entailed follow-up of the inception cohort identified from NLSTEPSS. Due to inherent methodological limitations of NLSTEPSS, a surveillance study, it was not

feasible to perform neuroimaging and formal cognitive testing at CSE presentation. Longitudinal imaging analysis would have been ideal in establishing the prevalence and types of structural abnormalities at baseline and characterise the evolution of new abnormalities post-CSE. Baseline neuroimaging data could potentially also have enabled longitudinal quantitative analysis and provide stronger evidence towards resolving the long-debated PFS-MTS hypothesis. Nonetheless, we were able to use data on neuroimaging findings within 5 days, and at 1, 6, and 12 months post-CSE from our previous studies on comparable patient population to interpret and contextualise the results of the current study. A detailed parent-reported developmental history was collected at CSE presentation for each subject, but we were unable to accurately determine the presence of intellectual disability at baseline due to lack of formal cognitive testing. Although developmental milestones are routinely used as a proxy for cognitive functioning in young children under the age of 5 years in clinical practice, an initial diagnosis of global developmental delay may not necessarily correlate with objective cognitive impairment on formal cognitive testing (Chung et al., 2010; Shevell, 2006; Riou et al., 2009). This important limitation needs to be borne in mind when considering the estimates of intellectual disability in the current study. However, every study design has its unique advantages and limitations. The above-discussed pragmatic difficulties are recognised inherent limitations of observational studies, and the results therefore should be interpreted taking these limitations into consideration.

Loss to follow-up is inevitable in long-term cohort studies, but 9% and 34% dropout rate, for mortality and clinical outcomes data analysis respectively, in the current study are within the acceptable range (Fewtrell et al., 2008). In addition, for clinical outcomes, we used multiple imputation to systematically address missing data and minimise potential bias from a complete-case analysis and found the results to be consistent using both approaches. Neuroimaging could not be performed in about a third of follow-up cohort in the current study; primarily due to lack of subject cooperation because of cognitive/behavioural problems, and therefore it is possible that the estimate of prevalence of clinically significant MRI abnormalities could be conservative. Still, as nearly 90% of children with no neurological issues prior to CSE underwent imaging, the estimates for MTS are likely to be close to the true prevalence in the general population.

Although the patient population in this study is the largest prospective population-based childhood CSE cohort, it is possible that lack of statistical significance for some of the clinical and demographic factors, such as seizure duration, in predicting adverse

outcomes could be due to modest sample size. In addition, the current study included only children with seizures ≥30 minutes, with seizure duration less than 90 minutes in the majority. Therefore, the results of this study do not exclude the possibility of a critical threshold under 30 minutes or over 90 minutes where seizure duration may significantly influence outcomes.

Finally, the results from this study, from a resource-rich country, may not be generalizable to resource-poor settings where the population have limited access to emergency medical care, suboptimal intensive care facilities and a different spectrum of CSE aetiology.

10.4 Future directions

While the length of follow-up in the current study should be sufficient to capture the majority of children who develop epilepsy after PFS, it could still be argued that this is shorter than the reported latency period of 8 to 11 years for the development of TLE (Patterson et al., 2014; French et al., 1993; Janszky et al., 2004; Mathern et al., 2002). Therefore, a further follow-up of the STEPSOUT cohort in about 5 years could be considered to make a definitive conclusion and finally settle the argument over the relationship between PFS and MTS/TLE.

Given the key role of the hippocampus in memory and learning, and previous reports of subtle cognitive and memory impairments in children within 12 months after PFS, it would be interesting to see whether some children have long-term memory impairment after PFS. In addition to cognition, memory assessments were also performed for the current study, and Dr Martinos will be reporting those results separately.

Data from the current study along with those from the STEPIN and the FEBSTAT study suggest that there is a much smaller proportion of children with PFS/FSE than previously believed who go on to develop MTS, and in fact MTS may also be seen after other forms of CSE rather than being unique to PFS (Lewis et al., 2014; Shinnar et al., 2012; Yoong, 2012; Yoong et al., 2012; Yoong et al., 2013a). Identification of risk factors for MTS in this subgroup of patients could help identify future remediable strategies. An approach to identifying such risk factors could include studies comparing genetic and/or structural predisposing factors between children with brief seizures, CSE, and healthy controls. It would also be of interest to see longer-term follow-up results of

prospective longitudinal studies, such as STEPIN and FEBSTAT, with neuroimaging and formal neurocognitive and memory testing at baseline and at discreet short, medium and long-term time intervals after childhood CSE to characterise the evolution of changes in hippocampal structure and function after PFS and non-PFS CSE.

Current data suggest higher prevalence of hippocampal developmental malformation such as HIMAL in children with PFS/FSE. However, as such developmental malformations have also been reported in individuals without seizures, their clinical significance is debated. The inconsistency in the reported rates of HIMAL seems to be primarily due to lack of consensus on definition, and subjective interpretation of defining criteria. Use of quantitative imaging techniques such as diffusion tensor imaging (DTI) and diffusion compartment imaging (DCI) could offer insight into tissue microstructure and identify subtle structural malformations. Applying such approaches in assessment of HIMAL would eliminate subjective bias and could also potentially differentiate structurally normal morphological variants from pathological malformations of the hippocampus.

10.5 Practical implications

The mortality and morbidity associated with childhood CSE have declined considerably in recent decades, at least in resource-rich countries. The potential reasons for the improved outcomes include: change in CSE definition from seizure duration >1 hour in the 1970s to the current 30-minute definition, increasing use of pre-hospital treatment for prolonged seizures, better out-of-hospital care and transport facilities, better intensive care facilities, availability of newer drugs for seizure management, protocols for management of seizures and secondary complications in intensive care, and a reduction in the incidence of acute symptomatic CSE due to universal immunisation and imposing strict regulations for prevention of traumatic brain injury and accidental poisoning. But, whether a similar trend towards more favourable outcomes following childhood CSE is observed in resource-poor countries is uncertain due to paucity of population-based data. A recent population-based study from Kenya reported the incidence of childhood CSE in Kenya to be 2 to 5 times that reported in London, and substantially higher seizure-related short- and long-term mortality (Prins et al., 2014; Sadarangani et al., 2008). In addition to the high incidence of CNS infections, outcomes after CSE in resource-poor countries may also be influenced by several unique challenges in the management of CSE. Delay in seeking medical care due to cultural

beliefs and/or poor access, poor healthcare infrastructure, the lack of intensive care facilities and limited availability of drugs for optimal management of CSE and its complications are at least some of the issues that could potentially influence the outcomes (Newton, 2009; Molinero et al., 2009; Prins et al., 2014; Murthy et al., 2007).

While further clarification of the direct effect of seizure duration on subsequent outcomes is no doubt important for prognostication and research in anti-epileptogenesis and neuroprotective strategies, such debate is extraneous with regards to treatment of prolonged seizures. Currently there is universal consensus for all convulsive seizures in children to be treated promptly and terminated at the earliest. Strategies for early prompt treatment of prolonged seizures, such as pre-hospital treatment by parents and/or paramedical crew, CSE management guidelines for emergency departments, and protocols for the management of refractory seizures and prevention of secondary complications in intensive care units, are already in practice in most resource-rich countries. Strategies for early identification and appropriate management of neurocognitive/behavioural difficulties after CSE may further improve long-term outcomes.

The situation in resource-poor countries offers a huge challenge as well as an opportunity in reducing mortality and morbidity associated with childhood CSE. The unique difficulties in the management of any acute illness in general, and seizures in particular, discussed earlier in this section are the main challenges for optimal management. Conversely, as a substantial proportion of childhood CSE cases in these countries are due to CNS infections, reducing incidence of CNS infections with primary preventive measures such as universal immunisation, improvement in nutritional status and sanitation could potentially lead to significant reduction in childhood CSE-related mortality and morbidity. In addition to strategies for prompt treatment of prolonged seizures discussed earlier, measures including development of health care infrastructure, ensuring availability of drugs, educating parents and primary health care professionals in emergency management of seizures are necessary. The on-going efforts by governmental and non-governmental organisations, and recent approval of a vaccine against malaria, raise the hope that we will soon see a reduction in the mortality and morbidity associated with childhood CSE in resource-poor countries.

10.6 Conclusion

The STEPSOUT study is a unique epidemiological study describing the natural history and long-term outlook after childhood CSE. This is the largest population-based study reporting mortality, neurological, cognitive, behavioural and neuroimaging outcomes beyond 5 years after childhood CSE due to all causes. This study provides useful data that will help clinicians in counselling parents about long-term prognosis after childhood CSE, which is surprisingly good if one is neurologically normal before CSE and survive beyond 30 days after CSE. The study also identifies key issues for future research. Data obtained from this study may also serve as the basis for the development of strategies for clinical surveillance of children at high risk of adverse outcomes after CSE. Early identification of difficulties after childhood CSE and appropriate intervention could potentially improve long-term outcomes and quality of life for children and their families.

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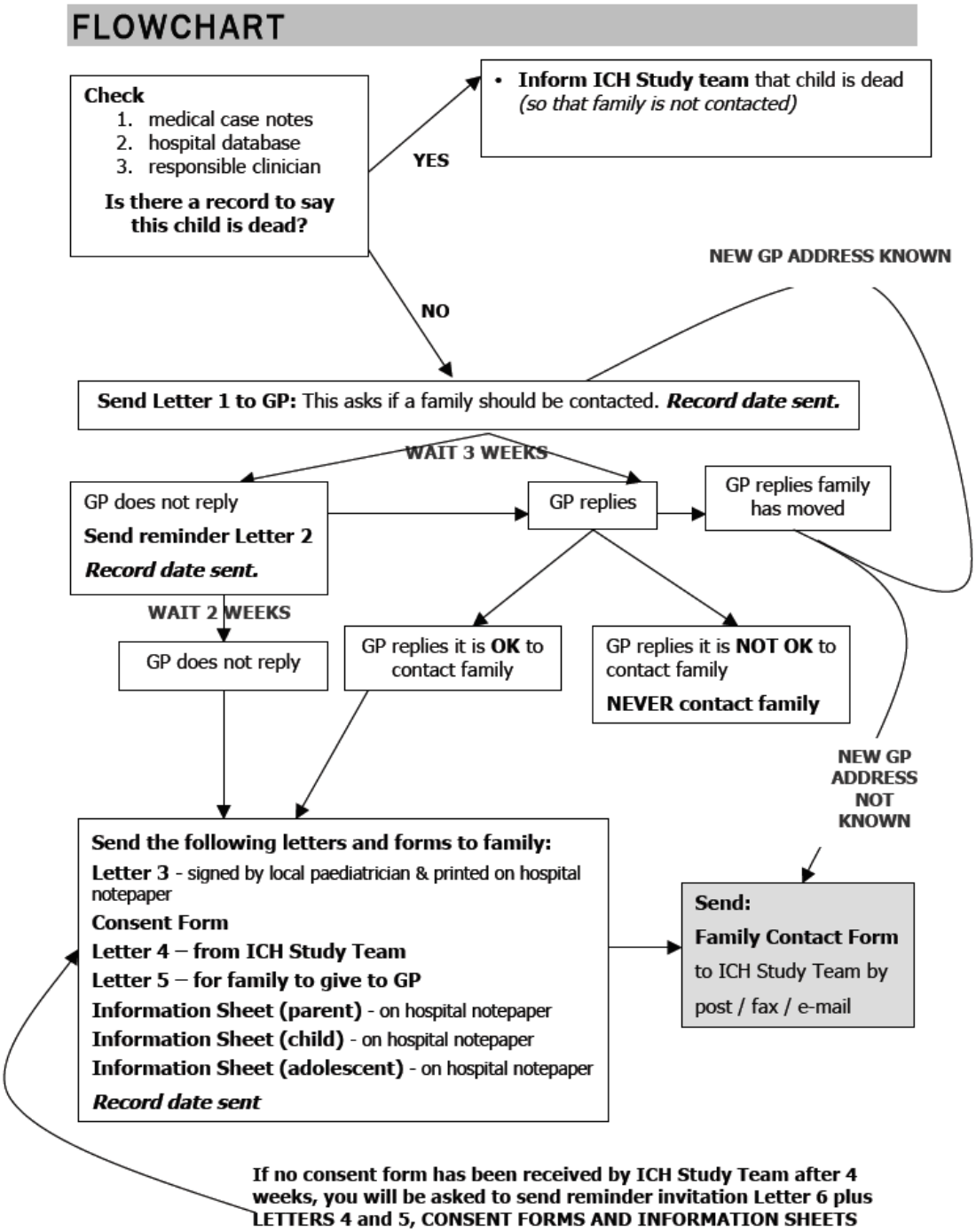
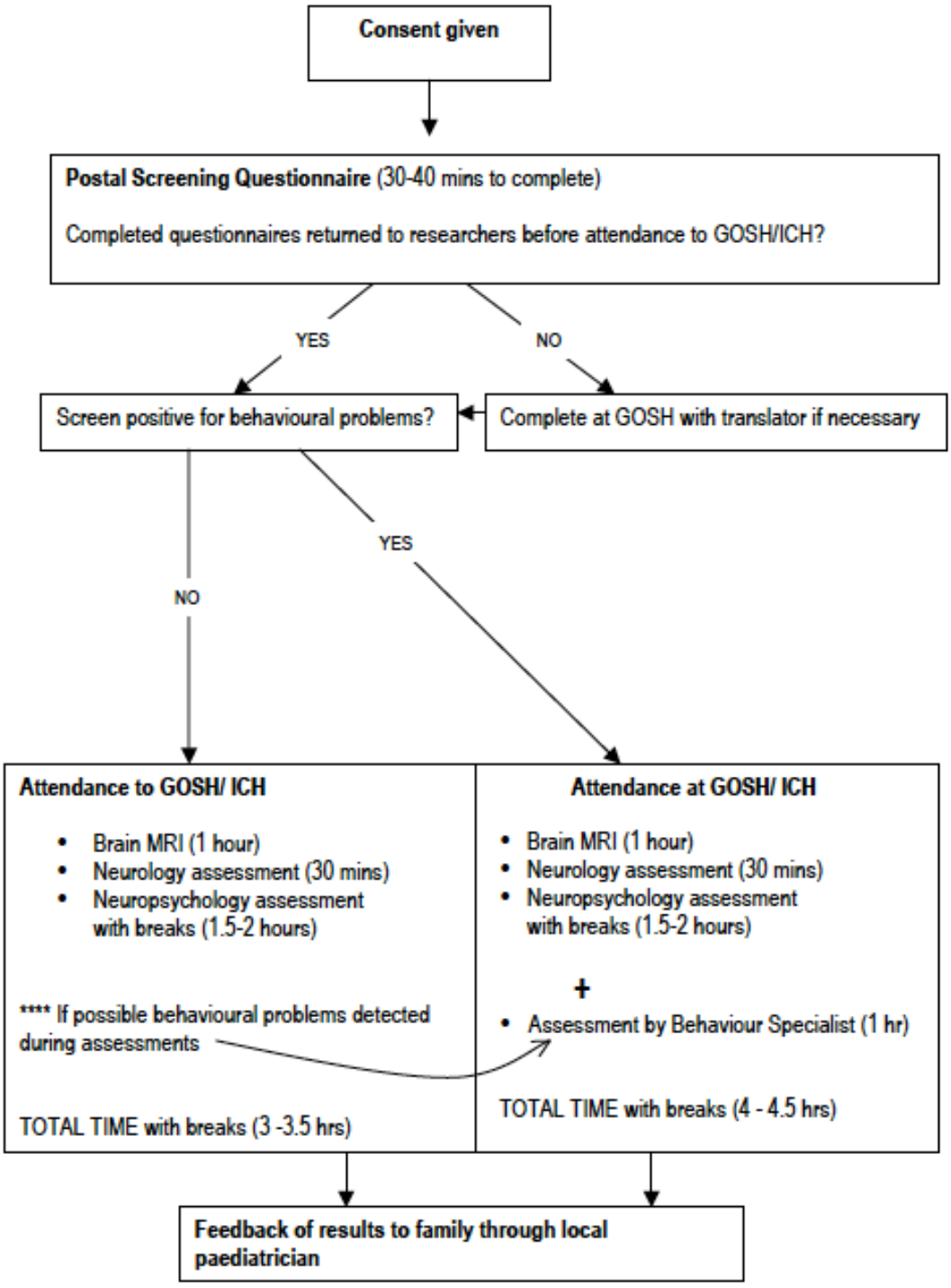
APPENDIX A

FLOWCHART OF THE RECRUITMENT PROCEDURE



APPENDIX B

FLOWCHART DEPICTING THE ASSESSMENT PROCEDURES PERFORMED FOR STEPSOUT



APPENDIX C

STEPSOUT CLINICAL NEUROLOGICAL ASSESSMENT PROFORMA

STEPSOUT

Childhood Status Epilepticus Outcome: Prevalence and Spectrum

Date:

Study ID:

Name:

DOB:

Sex:

Address:

GP details:

Paediatrician:

Hospital:

Date of CSE:

Final diagnosis:

Family history:

Birth history:

Development:

Behaviour:

Schooling:

CSE details:

Subsequent seizures/Epilepsy (onset/types/frequency, CSE):

Treatment (if any):

Any other medical problems:

Investigations (if any):

Examination:

APPENDIX D MRI IMAGE ACQUISITION PROTOCOL

Study title	Childhood Status Epilepticus Outcomes: Prevalence and Spectrum

Duration (minutes)	Sequence	No. of slices	Echo time (ms)	Repeat time (ms)	Slice thickness	Gap	Matrix	Field of view	Flip	Inversion time
05:34	3DFLASH	176	4.9	11	1 mm	20% (0.2 mm)	224x256	256x224	15	0
06:04	T2 MAPS Relaxometry	1	16 echos from 22 to 352 ms	2400	5 mm	-	150x256	210x157.5	180	-
03:13	Axial TSE T2	25	101	4920	4 mm	40% (1.6 mm)	245x384	220x175.3	150	-
03:13	Coronal TSE T2	25	101	4920	4 mm	40% (1.6 mm)	245x384	220x175.3	150	-
08:08	3DFLAIR	176	353	6000	1 mm	none	256x256	256	150	2200
14:15	3x20 DTI	45	89	6300	2.5 mm	none	96x96	240	N/A	-
01:04	DWI	19	96	2700	5 mm	30% (1.5 mm)	128x128	230	-	-

APPENDIX E

PROTOCOL FOR DRAWING HIPPOCAMPAL ROI MASKS

The following rules were observed while drawing hippocampal ROI masks to ensure consistency [representative approach adapted from previously published literature (Watson et al., 1997; Konrad et al., 2009; Geuze et al., 2004)]:

General rules

- The hippocampus was visualized in 3 orthogonal planes in FSLview.
- Binary ROI masks were drawn by tracing boundaries of the whole hippocampus in contiguous coronal slices and, if necessary, further refined in axial and sagittal views.
- Pixels with partial volume intensity at hippocampal boundaries (dark grey pixels adjacent to CSF or light grey pixels adjacent to white matter) were not included in the ROI mask.
- The subiculum, the CA regions, the dentate gyrus and the fimbria were included in the ROI, but the entorhinal cortex was excluded.

Anatomical boundaries of the hippocampus

Hippocampal Head (HH):

Uncal recess of the inferior horn of lateral ventricle (IHLV) was used as a landmark for identification of anterior, lateral and superior borders of HH. The alveus served as a useful landmark for defining the anterior and superior borders of HH.

- **Anterior:** The first slice where head of hippocampus is clearly visible.
- **Superior:** Defined by the uncal recess of the IHLV. The white matter of the alveus overlying the ventricular surface of the HH was used for defining the superior border when uncal recess was not visible. If neither uncal recess nor alveus were visible, a horizontal line drawn from the IHLV to the surface of uncus or the inferior margin of semilunar gyrus was used to define the superior border.
- **Inferior:** Defined by the white matter of the parahippocampal gyrus.
- **Medial:** The CSF in the ambient and uncal cisterns formed the medial boundary of HH. The boundary between the subicular complex and the parahippocampal

gyrus was defined by the angle formed by the most medial extents of these structures. The hippocampal sulcus was included.

- **Lateral:** The CSF in the IHLV or white matter.

Hippocampal Body (HB):

- **Superior:** The CSF in the choroidal fissure, and the fimbria, visible as white matter located superomedially, were used to define the superior border of HB. If gray matter was visible superior to fimbria, the first row of gray matter pixels was included in the measurement, as fimbria at this point is sometimes partially embedded in the CA2 or CA3 regions, and the row of gray matter above fimbria comprise of hippocampal cells.
- The medial, lateral and inferior borders of HB were defined as for HH.

Hippocampal Tail (HT):

Fornix was not included in the measurement of HT.

- **Posterior:** The most posterior part of HT was the slice where HT appears as an ovoid mass of gray matter inferiomedially to the trigone of the lateral ventricle.
- **Medial:** The CSF of the quadrigeminal cistern formed the medial border of HT in anterior slices, and the white matter surrounding HT in posterior slices. Care was taken to exclude the pulvinar of the thalamus, and the gray matter continuous with the splenium of corpus callosum, since it is considered part of subsplenial gyrus.
- **Lateral and superior:** The white matter of fornix defined the lateral and superior borders of HT anteriorly, and trigone of lateral ventricle posteriorly.
- **Inferior:** The white matter of parahippocampal gyrus.