EPILEPSY SURGERY FOR FOCAL CORTICAL DYSPLASIA: CLINICOPATHOLOGICAL CORRELATES AND ADVANCES IN NEUROIMAGING

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Epilepsy Surgery for Focal Cortical Dysplasia: Clinicopathological Correlates and Advances in Neuroimaging

Epilepsiechirurgie voor focale corticale dysplasie:
clinicopathologische correlaten en ontwikkelingen in
neurologische beeldvorming
(met een samenvatting in het Nederlands)

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INTRODUCTION

An epileptic seizure is defined as 'a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain'. The manifestation is dependent on location in the brain where this abnormal activity originates and the propagation to other brain regions and various other factors. They can manifest as varying combinations of alterations in sensory, motor and autonomic functioning, in cognitive functioning or behaviour. Epilepsy is a brain disorder characterised by a persistent predisposition to generate epileptic seizures and the consequences one experiences¹. A patient is said to suffer from epilepsy when an epileptic seizure has occurred and the brain demonstrates a enduring tendency to have recurrent seizures, in absence of a temporary or reversible factor that could trigger a seizure (such as seizure directly after a concussion or induced by medication or substances)². Epilepsy can result from a great variety of underlying brain pathologies that fall under six broad categories: genetic, structural, metabolic, infectious, immune or unknown³. In most cases when a patient presents with epilepsy that warrants treatment, initial therapy will be with anti-epileptic medication^{4,5}. However, despite of optimal pharmacotherapeutic treatment circa 20 -30% of all patients with epilepsy have persistent seizures⁶⁻⁸; these patients are said to have drug resistant or refractory epilepsy⁹. Epilepsy is classified as focal, generalized or combined. This refers to whether the onset of seizures originate from a circumscribed brain region or multiple regions in focal epilepsy or conversely that seizures can arise from anywhere in the brain with generalized abnormal electrophysiology³. In many patients with focal epilepsy the underlying cause is a developmental abnormality of the brain (falls in structural category), 20% of all patients who eventually undergo epilepsy surgery¹⁰. Focal developmental abnormalities can be classified as Focal Cortical Dysplasia (FCD) or mild Malformation of Cortical Development (mMCD)¹¹. These are the most common diagnosed pathologies – found in 27% – among children who undergo epilepsy surgery¹⁰. The affected area in the cerebral cortex functions abnormally and can generate epileptic activity. Structurally there is disruption of the architecture of the cortex, which normally consists of six layers of neurons, and where the position of the neurons is associated with specific functioning and connections with other neurons. Deep of these six layers is the white matter in which myelinated fibres lie that form the connections with neurons at greater distance within the nervous system. In addition to abnormal layering there can be presence of immature and abnormal neurons.

To harmonize definitions and histopathological assessment the International League Against Epilepsy established a three-tier classification system in 2011¹². Type I is characterized by abnormal layering of the cerebral cortex, either by persistent radial micro-columnar architecture (Ia) or by a disruption of the horizontal lamination (Ib) or both (Ic). Type II is characterized by complete disorganization of the cortex and dysmorphic neurons (IIa), and balloon cells in addition (IIb). FCD type III refers to FCD in combination with other pathologies, such as hippocampal sclerosis (FCD type IIIa), glial or glio-neuronal tumours (FCD type IIIb), vascular malformations (FCD type IIIc) or any other principal lesion acquired during early

life (FCD type IIId). The most subtle architectural abnormalities in the FCD spectrum are classified as mMCD, with intact cortical architecture and absence of aberrant cells, but with excessive number of neurons in the molecular layer (type 1) or white matter (type 2)¹³. This system built on the previous definitions by Palmini¹⁴ and Taylor¹⁵. While definitions for ILAE FCD IIb are identical to the previous schemes, more subtle lesions cannot be translated into the new classification system without actual histopathological re-evaluation of tissue samples¹⁶. See figure 1 for examples of histopathological findings in mMCD/FCD subtypes.

The exact pathogenesis of all FCD subtypes is yet to be completely revealed, but isolated FCD lesions are thought to arise from disruption during early neuronal development, when neurons from the subventricular zone migrate outwards to their position in the cortical ribbon and mature into pyramidal neurons. Depending on the timing of the disruptive events, different aberrant cortical patterns are formed¹⁷, corresponding with ILAE types. There are indications that increased mammalian target of rapamycin (mTOR) activity plays a role in pathogenesis. mTOR signalling pathways integrate intracellular and extracellular signals and have important regulating functions in cell metabolism, growth, proliferation and survival¹⁸. Especially for the FCD IIb subtype, evidence is mounting for these mutations as underlying mechanism with often activation of downstream proteins, and associations with mutations in mTOR-regulatory genes. Similar mTOR dysregulation is found in patients with epilepsy associated with tubers in tuberous sclerosis complex and Hemimegalencephaly^{17,19}. Pathogenesis of FCD I and mMCD is more elusive. A limited number of neurons in white matter is considered normal in mature cortex. Excess white matter neurons could represent failure of neuronal migration or de novo generation and it is unclear whether this is de result or consequence of epilepsy¹⁷.

Patients with FCD typically experience first seizures very early in life, before age of 10. Seizures are often severe and refractory²⁰. Several mechanisms for epileptogenesis of FCD have been suggested. Overexpression of subunits of the glutamate receptors NMDA and AMPA have been found in dysmorphic and dysplastic neurons in FCD, which lead to overexcited states²¹. Other studies found decreased synaptic inhibitory circuits with decreased number of GABAergic neurons and diminished expression of GABA_A receptor subunits²¹. Also glial dysfunction has been identified in FCD; balloon cells possibly have a hyper-excitability-modifying role through presence of glutamate transporters and the glutamate-metabolizing enzyme glutamine synthase²¹. The close future will see further elucidation of the pathobiology, molecular and electrical characterization of FCDs resulting from the intensive research that is currently carried out.

In patients with epilepsy the burden of disease is not only attributable to epileptic seizures but also to various comorbidities. A specific underlying pathology can be the cause of both epilepsy and the comorbid condition, both also direct causal relations are possible in which

Figure 1. Examples of histological findings in mMCD and FCD.

A: NeuN/mMCD type 2. Normal cytoarchitecture of cortical layers II-VI, but excess neurons in molecular layer (top of figure) and in white matter (bottom of figure).

B: NeuN/FCD IA. abnormal radial cortical lamination, with typical microcolumnar organisation.

C: NeuN/FCD IIB. In FCD II subtypes there is often complete disruption of cortical layering, and loss of grey/white matter border.

D: SMI32/FCD IIA. Immature, dysmorphic neurons with abnormal orientation and positive staining by SMI32 characterise FCD IIA.

E: Vimentin/FCD IIB. Typical balloon cells, large undifferentiated cells lacking Nissl substance, and positive Vimentin staining.

epileptic activity and/or its treatment are responsible for the comorbidity or vice versa epilepsy is the result of a condition. Not always the mechanisms can be identified, and there can be reciprocity and complex interplay of factors²². A wide range of numerous medical comorbidities are known, for instance migraine, cerebrovascular disease and traumatic injury²³. Considering only epilepsy due to focal developmental abnormalities, the most clinically relevant

comorbidities are psychiatric and cognitive in nature. Roughly half of the patients with focal epilepsy has at least one psychiatric comorbidity in pre-surgical evaluation. Mood disorders are most common, and also anxiety, personality disorders and psychoses are associated^{24–26}. Cognitive disability is common in patients with epilepsy, also in patients with focal aetiology. Around 60% in paediatric FCD cohorts have cognitive impairment (defined as an intelligence quotient below 70)^{27,28}. Moreover, a portion of patients present with epileptic encephalopathy. On top of the disturbances related to the structural anatomy, epileptic activity causes memory consolidation disruption²⁹ and in epileptic brain there is a deviation from the optimal network organisation^{30,31}. Cognitive disability is not only caused by epileptic activity or the underlying pathology, but also the use of anti-epileptic drugs has negative effects, due to general dampening of neuronal signalling and consequent detrimental effects on cognitive development³².

For patients with refractory focal epilepsy, surgery can be a treatment option with prospect of reduction of seizure load or even becoming completely seizure free³³. On top of this, the possibility of discontinuation of AEDs can be an important argument for surgery, because of inherent and common drug side effects and aforementioned detrimental cognitive effects.

Depending on the location and extent of the lesion in combination with the clinical picture surgery can entail focal resection, anatomical lobectomy, and which may include hippocampectomy. Disconnection of larger epileptogenic zones from normal functioning brain is performed to stop seizure propagation and its damaging effects to areas distant from the ictal onset zone³⁴.

Following resective epilepsy surgery in patients with mMCD/FCD, long term seizure freedom rates of 53%–77% were reported in recent literature^{35–42}. This corresponds with Engel 1 scores. A stricter definition is complete and continuous seizure freedom ever since surgery (Engel 1A), which was achieved in 49%³⁷.

Completeness of resection is consistently the most important predictor of seizure freedom. Other factors that have been reported to positively influence chances of seizure freedom are: MRI-visible lesion, temporal lesion, unilobar lesions which facilitate complete resection^{37,43}. Shorter epilepsy duration and surgery before adulthood are also associated with seizure freedom³⁷. In some studies mMCD⁴⁰ or FCD I^{28,44} histology has been related to unfavourable outcome, but in others there is no relation with histological subtype^{35–37,39,41}. Moreover, there is a paucity of studies that relate outcome to the revised FCD classification. New studies on possible relations between histological subtype and clinical characteristics and outcome after surgery are warranted to validate the new classification scheme. Available surgical studies have limited statistical power due to small sample sizes. The largest long-term outcome study to date included 211 patients with FCD³⁷, most studies were much smaller.

Blümcke et al¹⁰ conducted a multicentre study across 36 centres from 25 European countries, with the primary goal to accurately determine underlying pathologies in epilepsy surgery patients. This is by far the largest epilepsy surgery cohort, but there was limited outcome data, and from a total of 9523 patients, for 5248 (55%) seizure outcome at 1 year could be used for post hoc analyses to study associations between histology and seizure outcome. No further determinants of seizure outcome were studied.

Most studies reported overall cognitive improvement following epilepsy surgery^{45–48}. In general, postoperative cognitive outcome has been shown to correlate with seizure freedom^{28,48–51}, parental education⁵², preoperative seizure frequency²⁷, and with aetiology^{53,54}. Importantly, earlier onset^{27,46,55–62} and longer duration^{27,63} of epilepsy predicts more severe cognitive disability. AED policies also affect cognitive outcome^{51,53}. Significant differences in cognitive outcome between patients with different FCD subtypes have not been reported yet^{27,42}.

Good post-surgical outcome in combination with low morbidity and mortality and ongoing advance in surgical techniques subsequently has meant that surgical treatment has an increasingly important role in epilepsy therapy⁶⁴. Currently, surgery is the only treatment option for patients with medically intractable epilepsy to potentially become seizure free. We should therefore strive to be able to offer surgery to as many patients as possible and minimise delay to surgery. Localization of a target is paramount for successful surgery and the diagnostic process for determining surgical candidacy is often extensive, and always includes electroencephalograph (EEG) and structural magnetic resonance imaging (MRI) as first-line investigations. EEG visualises electrophysiological brain activity which are recorded by electrodes placed on the scalp. Epileptic foci express specific electrophysiological patterns during (ictal) and between epileptic seizures (interictal). In short, MRI provides structural imaging through magnetic excitation of nuclei and registration of differences in relaxation between tissues and substances⁶⁵. On indication also magnetoencephalography (MEG) or nuclear radiologic imaging such as positron emission tomography (PET-CT) and Single Photon Emission Computed Tomography (SPECT) may be performed. If imaging and non-invasive EEG registrations are unable to exactly localize a seizure focus, invasive EEG recordings can be considered; either needles are placed through the skull into the brain tissue (invasive EEG or iEEG with depth electrodes) or the skull is opened to place a mat of electrodes directly on top of the cortical surface (Electrocorticography or ECOG).

MRI plays an increasingly important role in the pre-surgical trajectory. A structural abnormality is a strong and reliable marker for the presence of epileptogenic tissue, even though not always there is perfect co-localisation between a MRI-visible lesion and the epileptogenic zone³⁴. A clear and well demarcated lesion obviates more additional

tests, and provides a target for surgery or invasive registrations. On the other hand, when a lesion is absent, it might be not possible to formulate a hypothesis to base a surgical plan on. Still, successful surgery is possible in MRI-negative patients, but chances of achieving seizure freedom are often found to be smaller^{33,66–69}. In recent literature—depending on subtype—roughly 15% to 40% of patients with FCD had a lesion that escaped identification on MRI prior to surgery^{37,70}. For mMCD this can be over 50%⁴². There are number of specific MRI findings associated with mMCD/FCD: most notably blurring of the boundary between grey and white matter and ‘transmantle sign’ – cone shaped T2 hyperintensity from cortex running towards the ventricle. Other findings are T1 or T2 signal changes of cortex or white matter. Various macro-architectural changes can be seen like thickening of the cortex, abnormal gyral patterns, local cortical atrophy. See figure 2 for examples of MRI findings in FCD. The ‘transmantle sign’ appears reserved for the ILAE FCD IIb subtype¹², whereas FCD IIa, FCD I and mMCD subtypes are harder to discriminate solely based on MRI features^{71–73}. There is still uncertainty how specific histological characteristics translate to identifiable MRI features and how the border with normal brain tissue can be identified accurately. Ideally, pre-surgical investigations would already reliably predict the eventual pathohistological diagnosis, as this could influence surgical strategy and improve pre-surgical patient counselling regarding therapeutic outcome. Furthermore, pathological diagnoses and subtyping of mMCD/FCD requires adequate positioning and tangential sectioning of the cortex and therefore benefit from accurate pre-surgical imaging diagnoses.

MRI and image analysis techniques have seen vast advancement, and further improvement is thought to be of great value in improvement of surgical therapies for epilepsy. In the last decade 7 tesla MRI systems for human use arrived. Compared to the current clinical standard – 1.5 and 3 tesla MRI with dedicated epilepsy scanning protocols – the higher field strength brings an increase in signal to noise ratio which can be utilized to scan with greater contrast, higher resolution or shorter acquisition. With regards to the pre-surgical work up in patients with epilepsy especially improving contrast and resolution have a theoretical benefit for detection of subtle abnormalities. However, higher field strength also brings more artefacts and technical difficulties in producing images of diagnostic quality. Furthermore, the price of purchase of an MRI system is roughly linearly related to magnet field strength. Scientific research is still needed to confirm the clinical benefit of 7T MRI in epilepsy diagnostics.

Since the advent of epilepsy surgery in the end of the 19th century, tremendous changes have taken place in the care for patients with refractory epilepsy. Our understanding of how epileptic seizures are produced, the relation with structural abnormalities and functional anatomy of the brain and how to monitor these has grown exponentially. Surgical techniques have been greatly refined and new methods have been developed. Malformations of Cortical Development make up an increasingly large share of the patient population, and it

Figure 2. Typical MRI findings in FCD. Coronal reconstructions of 3D T_2 images at 7T MRI, two patients with FCD IIb. A: bottom of sulcus dysplasia right parieto-occipital with cortical thickening, blurring of grey-white matter interface and signal changes in cortex and white matter. B: Pronounced FCD lesion in posterior temporal with also typical transmantle sign.

is currently the focus of intensive research to further knowledge and improve quality of care. There remain considerable challenges; in determining the cause of epilepsy, especially in absence of gross structural abnormalities; in identifying the borders between epileptogenic surrounding healthy tissues, when different modalities often provide information that does not perfectly align. Therapeutic decision-making requires a solid a-priori diagnosis and understanding of its repercussion on efficacy and risks of the available treatment options. We aim for this thesis to be a step in this direction.

Rationale and outline of this thesis

We describe clinical characteristics and predictors of seizure freedom and cognitive functioning in **chapters 2 and 3** respectively. Although in literature several factors have been brought in association, uncertainty exist concerning determinants in cohorts of only mMCD/FCD pathology, and what the influence is of histological subtypes as categorized according to the 2011 ILAE classification.

In **chapter 4** we studied the yet to determined clinical value of using 7 tesla MRI in the diagnostic work-up for determining candidacy for epilepsy surgery.

In **chapter 5** we present the observation of an imaging characteristic of FCD lesions on 7 tesla MRI images, which appears to represent vascular changes co-localizing with FCD lesions and has not previously been described in literature. Expanding on this chapter, and to find a histological substrate, we studied vascular density and relations with blood brain barrier dysfunction, and measures of neuroinflammation in FCD and tubers in TSC in **chapter 6**.

We conducted a prospective comparative in-vivo 7T MRI – ex-vivo 7T MRI – histology study with as goal to further improve detection, characterisation and delineation of subtle mMCD/FCD on MRI. The development of the methodological pipeline and results of image co-registration are described in **chapter 7**. Full analyses of the MR and histological will be published in the future.

Chapter 8 provides a summary of the work collected in this thesis, and a discussion and integration of our research findings. We discuss the implications for patients with mMCD and FCD, policies concerning epilepsy surgery, and future perspectives.

Aims of this thesis

- To analyse a surgical cohort of patients with mMCD/FCD and describe clinical characteristics and seizure and cognitive outcome and in relation to the 2011 FCD classification.
- To determine the added value of 7T MRI in the diagnostic work-up for candidates for epilepsy surgery.
- To explore possible new MRI imaging features to improve identification, characterisation and delineation of mMCD/FCD lesions.

LONG-TERM SEIZURE OUTCOME AFTER EPILEPSY SURGERY IN PATIENTS WITH MILD MALFORMATION OF CORTICAL DEVELOPMENT AND FOCAL CORTICAL DYSPLASIA

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Focal Cortical Dysplasia (FCD) and mild malformation of cortical development (mMCD) are frequent histopathological diagnoses in patients who undergo surgery for refractory epilepsy. Literature concerning surgical outcome in patients with mMCD, as well as its contrast with FCD, has been scarce.

We studied 88 patients with a histopathological diagnosis of isolated FCD (n=57) or mMCD (n=31), revised according to the latest ILAE guidelines, who underwent resective or disconnective surgery

Our findings suggest differences between mMCD and FCD in clinical presentation and surgical outcome after surgery. Patients with mMCD developed seizures later in life, and their lesions had a predilection for location in the temporal lobe, and remained undetected by MRI more frequently. A diagnosis of mMCD has a less favorable surgical outcome. Still, 32% of these patients reached continuous seizure freedom (Engel 1A) at a latest median follow-up duration of 8 years, compared to 59% in FCD. A histopathological diagnosis of mMCD, extra-temporal surgery and indication of an incomplete resection each were independent predictors of poor outcome.

Introduction

Focal cortical dysplasia (FCD) and mild malformation of cortical development (mMCD) are frequent histopathological diagnoses in patients who undergo epilepsy surgery¹⁰. The 2011 ILAE classification system differentiates FCD type I and II as isolated lesions from type III, which is associated with adjacent other pathology¹². The most subtle architectural abnormalities in the FCD spectrum are classified as mMCD, with intact cortical architecture and absence of aberrant cells, but with excessive number of neurons in the molecular layer (type 1) or white matter (type 2)¹⁴.

Several factors have been reported to predict postoperative seizure freedom in mMCD/FCD, of which complete resection is most consistently identified^{37,43}. In the few surgical outcome studies that also included mMCD worse outcome was suggested for mMCD and FCD type I, compared to type II FCD^{39,40}.

Although mMCD is relatively under-exposed in recent literature, it is a common diagnosis with 2.9% of all epilepsy surgery cases, compared to 2.8% and 9% for FCD I and II respectively¹⁰. We aimed to elucidate differences in clinical characteristics and seizure outcomes between mMCD and FCD subtypes, and identify determinants of postoperative seizure freedom.

Methods

The pathological diagnosis of all 634 patients who underwent epilepsy surgery between 2000 and 2012 in our center and had a histopathological report compatible with FCD or mMCD (e.g., malformation of cortical development [MCD], microdysgenesis, dysplasia) were reviewed according to the 2011 ILAE classification¹³. To avoid effects of associated pathology on outcome, patients with complex MCD, neurocutaneous syndromes, and FCD III subtypes were not included.

A lesionectomy – preferably en-bloc – was performed for distinct lesions. Intra-operative electrocorticography (ECOG) was used on indication to tailor resection. Disconnective surgery (functional hemispherectomy or frontal or posterior disconnection) was indicated in more widespread or multilobar seizure onset zones. Patients were only included when a tissue sample of the disconnected area was sent for pathological investigation. The revised diagnosis was based on the following immunohistochemical stainings: hematoxylin & eosin (H&E), neuronal nuclei, antibody neurofilament H non-phosphorylated, Vimentin, glial fibrillary acidic protein, and microtubule-associated protein 2. All samples were revised (AM and TV) and – if necessary – reclassified according to the 2011 ILAE classification system. In case of non-consensus the senior pathologist (EA) was consulted.

Age at epilepsy onset and surgery, seizure duration, generalized tonic-clonic seizures (GTCS) ever to have occurred, seizure frequency (daily or less frequent), location of surgery (frontal, temporal, parietal, occipital, multilobar), magnetic resonance imaging (MRI) lesion identified (visual assessment only), and surgery type (lesionectomy, lobectomy, (sub)lobar disconnection, functional hemispherectomy) were collected from patients' files. Indication for incomplete resection was based on intra-operative assessment by the surgeon, ECOG, or on post-operative MRI (if performed).

Information on post-surgical seizure and medication status was collected by telephone interview, 2-14 years following surgery.

Seizure outcome was classified as seizure freedom (completely seizure-free ever since surgery [Engel class 1a]) or not (all other Engel classes) at last follow-up. Acute postoperative seizures during the first month were not taken into consideration. In addition, seizure- and medication-freedom at last follow-up was noted ("Engel 1a AED-"). In case of a reoperation within 2 years, seizure outcomes were measured after the second surgery. The tissue sample with most obvious pathological features was considered for the definitive histopathological diagnosis.

Analysis

Ages at onset and surgery, and duration of epilepsy and follow-up failed tests for normality, therefore Mann-Whitney U was used to study differences between pathologies and test influence on outcome. Fisher exact tests were used to examine differences in MRI visibility, age at surgery (dichotomized at $<$ or $\geq$ 18 years), presence of GTCS, site of lesion, indication for incomplete resection, seizure freedom and cure, between FCD subtypes. Logistic regression was used for multivariate analysis of seizure outcome. To reduce multicollinearity several determinants were not analyzed in multivariate logistic regression and location was dichotomized into extra-temporal (including multilobar) and temporal surgery. By only including the following variables, variance inflation factors are kept below 2.5 and condition index below 15: sex, MRI negative, only focal versus also GTCS, daily seizures, duration of epilepsy, lateralization, extra-temporal surgery, indication for incomplete resection, and histopathological diagnosis mMCD versus FCD.

Results

Eighty-eight consecutive patients were included, of whom 11 (13%) underwent two intracranial surgical procedures, seven (8%) within 2 years after first surgery. Revised histology was mMCD type 1 (n=6), mMCD type 2 (n=25), FCD Ia (n=3), FCD Ib (n=4), FCD Ic (n=1), FCD IIa (n=20), FCD IIb (n=29).

Clinical characteristics of pathology categories are listed in Table 1. Seizure onset was significantly later in mMCD (median 10 years) compared to FCD (median 3 years). Age at surgery was older in mMCD (median 21.8 years) than in FCD (median 13.7 years).

FCD was more often localized in the frontal lobes (60%) compared to mMCD (23%), although FCD I subtypes (25%) had a similar distribution to mMCD. mMCD was more frequently located in the temporal lobe (55%) compared to FCD (18%).

A significantly larger portion of mMCD (45%) lesions was not detected on MRI compared to FCD (16%). See figure 1 for an example of MRI lesions in mMCD.

Seizure outcome

Outcome data was missing for one patient. For the remaining 87, follow-up ranged 2-14 years (median 7 years). Follow-up duration was similar between histological subtypes. Forty-three patients (49%) reached continuous complete seizure freedom at last follow-up, and 31 (36%) were also free of anti-epileptic medication (Supplementary table 1).

Figure 1. Patient diagnosed with mMCD type 2. Onset at 16 years, with complex partial seizures and secondarily generalized tonic-clonic seizures. Coronal 3T MRI FLAIR images revealed blurring of gray-white matter interface in left temporal lobe. No indication for hippocampal sclerosis. Patient underwent anterior temporal lobe resection at age 19 years. Engel score 1C at ten years follow-up. Recurrence at seven years, and again achieving seizure freedom with medication since.

Table 1. Clinical characteristics and seizure outcome in relationship to histological subtype.

	Total n=88	mMCD n=31 (35%)	FCD total 57 (65%)	FCD I 8 (9%)	FCD IIa 20 (23%)	FCD IIb 29 (33%)	Statistics mMCD vs FCD
Baseline characteristics							
Sex, female	43(49%)	17 (55%)	26 (46%)	5 (63%)	6 (30%)	15 (52%)	p=0.504
Age at epilepsy onset, median and range (years)	6 (0-38)	10 (0-38)	3 (0-26)	4.5 (0-17)	3.5 (0-22)	3 (0-26)	p=0.001
Age at surgery, median and range (years)	15.8 (0.23-48.1)	21.8 (1.0- 48.1)	13.7 (0.2- 45.0)	9.7 (0.55- 40.3)	13.0 (0.23- 44.9)	14.5 (0.74- 45.0)	p=0.022
Duration of epilepsy, median and range (years)	8 (0-37)	9 (1-33)	8 (0-37)	4.5 (1-23)	5 (0-37)	10 (1-36)	p=0.500
Generalized tonic- clonic seizures	61/88 (69%)	19 (61%)	42 (74%)	6 (75%)	15 (75%)	21 (72%)	p=0.238
Daily seizures	52/88 (59%)	13 (42%)	39 (68%)	6 (75%)	10 (50%)	23 (79%)	p=0.023
Site of surgery							p<0.001
Frontal surgery	41 (47%)	7 (23%)	34 (60%)	2 (25%)	12 (60%)	20 (69%)	p=0.002
Temporal surgery	27 (31%)	17 (55%)	10 (18%)	3 (38%)	4 (20%)	3 (10%)	p=0.001
Parietal surgery	6 (7%)	0	6 (11%)	0	2 (10%)	4 (14%)	p=0.086
Occipital surgery	2 (2%)	1 (3%)	1 (2%)	0	0	1 (3%)	p=1.000
multilobar surgery	12 (14%)	6 (19%)	6 (11%)	3 (38%)	2 (10%)	1 (3.4%)	p=0.331
Lateralization, left sided	39/88 (44%)	15 (48%)	24 (42%)	3 (38%)	9 (45%)	12 (41%)	p=0.655
MRI-negative lesion	23/88 (26%)	14 (45%)	9 (16%)	4 (50%)	4 (20%)	1 (3.4%)	p=0.005
Resective surgery (vs disconnective surgery)	76/88 (86%)	26 (84%)	50 (88%)	4 (50%)	18 (90%)	28 (97%)	p=0.747
long-term invasive monitoring	41/88 (47%)	14 (45%)	27 (47%)	1 (13%)	9 (45%)	17 (59%)	p=1.000
Indication for incomplete resection (1 st surgery)	16/76 (18%)	4 (13%)	12 (21%)	2 (25%)	2 (10%)	8 (28%)	p=0.400
Re-operations (intra- cranial surgery)	11/88 (12.5%)	3 (25%)	8 (14%)	2 (25%)	1 (5%)	5 (17%)	p=0.740
Outcome							
Engel 1A	43/87 (49%)	10 (32%)	33 (59%)	4 (50%)	10 (53%)	19 (66%)	p=0.025
Engel 1A AED-	31/87 (36%)	7 (23%)	24 (43%)	3 (38%)	7 (36.8%)	14 (48%)	p=0.066
Follow-up duration, median and range (years)	7 (2-14)	6 (2-12)	7 (0-14)	7.5 (3-13)	7 (3-14)	5 (2-14)	p=0.682

Clinical features and postoperative outcome in relation to FCD subtypes. Fisher's exact for categorical variables and Mann-Whitney U test for continuous non-parametric variables.

Seizure freedom was significantly more common after surgery for FCD (59%) than for mMCD (32%). Patients with mMCD tended to be less likely free of seizures and medication (23%) compared to FCD (43%). Results of logistic regression analysis are displayed in table 2. Indication of incomplete surgery and mMCD diagnosis (versus FCD) both univariately negatively predicted continuous seizure freedom at last follow-up. In multivariate logistic regression analyses, both mMCD and incomplete resection remained significant predictors, as did extratemporal resection. In multivariate analysis only the presence of daily seizures predicted seizure- and medication freedom at last follow up. Older age at surgery and presence of daily seizures were negative predictors in univariate analysis.

Table 2. Determinants of favorable seizure outcome.

Univariate Odds ratio of determinant for favorable outcome				
	Engel 1A	95% CI	Engel 1A AED-	95% CI
Age at surgery (years)	NS	NS	0.96*	0.92-1.00
Daily seizures	NS	NS	3.43*	1.27-9.24
Indication for incomplete resection, (only resective surgery)	0.18*	0.04-0.92	NS	NS
mMCD (vs FCD)	0.33*	0.13-0.84	0.39 [†]	0.14-1.05
Multivariate Odds ratio of determinant for favorable outcome				
	Engel 1A	95% CI	Engel 1A AED-	95% CI
Daily seizures	NS	NS	4.06*	1.13-14.60
Extra-temporal surgery	0.20*	0.05-0.88	NS	NS
Indication for Incomplete resection	0.20*	0.04-0.99	NS	NS
mMCD (vs FCD)	0.12**	0.03-0.44	0.35 [†]	0.11-1.14

*: $p \leq 0.05$, **: $p \leq 0.05$, [†]: trend, $p \leq 0.1$, NS: not significant, $p > 0.1$

Univariate logistic regression, odds ratios and 95% confidence intervals. Determinants with $p > 0.1$ omitted from table: female sex, Age at epilepsy onset (years), Duration of epilepsy (years), generalized tonic-clonic seizures, right-sided, extra-temporal, frontal, temporal, parietal, occipital, multilobar surgery, MRI negative lesion, disconnective surgery, follow-up duration. multivariate logistic regression: entry of determinants for seizure outcome with constraints by multicollinearity. Determinants without significant relation with any outcome measure are omitted from table: sex, epilepsy duration, MRI-negative lesion, and bilateral seizures.

Discussion

For 31 of 88 (35%) of all patients with isolated malformations of cortical development – those with complex and neurocutaneous disorders excluded – mMCD was a common diagnosis, while FCD I was relatively rare with only 9%. FCD II was found in over half of the patients (FCD IIb 33%, IIA 23%). In a recent multicenter study which included 1612 patients with

mMCD and FCD, FCD II was equally frequent (53%) but mMCD was not more common than FCD I (both 17%)¹⁰. However, 13% of patients with MCD had lesions reported as “FCD not otherwise specified”. Moreover, difference in FCD subtyping was thought to have arisen due to the lack of international classification system before the ILAE consensus of 2011. Recent consecutive cohort studies including adult patients, in which tissue was (re)classified according to ILAE histopathological classification, did not include mMCD diagnoses, and the ratio between FCD I and FCD II varied from 26%/74% to 44%/56%^{35–37}. In our cohort the proportion of FCD I was relatively low. There are several explanations for these differences in presented histology. Observer variability possibly remains despite efforts to harmonize histopathological classification, and with current definitions especially the milder pathologies can be debatable. No formal inclusion of mMCD in the ILAE classification system might compel to classify very subtle dysplastic lesions as FCD I, when mMCD would be more suitable. Discrepancy in utilization of immunohistochemical methods may also play a role in variability in subclassification. Although ILAE Type II FCD is an established entity, there is still debate concerning the definitive identifying histological and contrasting clinical features of mMCD versus FCD I⁷⁴.

Our findings suggest that clinical characteristics help differentiating patients with mMCD from those with other FCD subtypes. They had onset of epilepsy later in life and consequently were operated at older age, the majority (61%) even at adult age, whereas only 32% of patients with FCD were operated during adulthood. Epilepsy duration was not significantly different between histological subtypes.

Younger age at onset and surgery in patients with FCD II compared to FCD I has been reported previously³⁵. Patients with mMCD were less likely to have highly frequent (daily) seizures. As reported in previous studies^{35,75,76} we observed a predilection of FCD II types for the frontal lobe, whereas mMCD and FCD I were most often localized in the temporal lobes. In case of multilobar lesions, mMCD and FCD I were most often diagnosed.

In our series, epilepsy surgery resulted in a continuing seizure freedom rate of 49% at a median follow-up of 7 years; 36% had also discontinued anti-epileptic medication. Patients with mMCD were less likely to reach seizure freedom. Patients with FCD IIb diagnoses had distinctively higher chance of favorable outcome. A number of studies did not show significant differences in seizure outcome between different histological subtypes^{35–37,41}. It must be noted that no, or very few, mMCD cases were included in these studies. Pathology was neither a predictor of outcome in a study that did include 29 mMCD cases³⁹. In a study by Kim et al. more patients with FCD (85/145, 59%) were seizure free at last follow up compared to mMCD (9/21, 43%)⁴⁰. Others studies, not including mMCD, showed less favorable outcome in patients with FCD I histology^{28,44}. The inferior outcome after surgery in mMCD is likely due to difficulties in determining location and margins on imaging and during surgery, complicating accurate identification and complete removal of the epileptogenic zone. Even though we

did not see indication for incomplete resection more often in patients with mMCD, residual lesions are most likely the best explanation for the difference in seizure outcome, albeit that these might not be suspected based on intra-operative observations by the surgeon, ECOG or post-surgical imaging. Complete resection of the lesion is the most reported predictor of favorable outcome, along with factors that facilitate this: temporal focus, severe pathological features, and a MRI-defined lesion⁴³. Accordingly, in our multivariate analyses, mMCD, extra-temporal surgery and indication for incomplete resection were predictors of unfavorable seizure outcome. We could not reproduce a relation between absence of an MRI lesion and surgical outcome, neither when analyzing histological subgroups separately. Patients who suffered from highly frequent, daily seizures, were more likely to discontinue medication after complete seizure freedom. An explanation might be that in these patients the positive effect on daily life after successful surgery is more outspoken and is an encouragement for earlier discontinuation of medication.

Conclusion

Our results suggest mMCD has a differentiating clinical presentation, which may be relevant in predicting seizure outcome after epilepsy surgery. Patients with mMCD develop seizures later in life, compared to FCD ILAE type I and II. mMCD has a predilection for temporal lobes and remains undetected by MRI more frequently. A diagnosis of mMCD has a less favorable surgical outcome. Nonetheless, one-third of these patients are expected to reach complete and ongoing seizure freedom, thus making the consideration of surgical treatment worthwhile.

Supplementary table 1. Engel scores at 2-year intervals.

	years of follow up							
	2	4	6	8	10	12	14	latest
Engel 1a	52 (60%)	41 (55%)	30 (60%)	19 (56%)	8 (47%)	5 (50%)	1 (50%)	43 (49%)
Engel 1	56 (64%)	46 (62%)	33 (66%)	21 (62%)	12 (71%)	6 (60%)	1 (50%)	54 (62%)
Engel 2	5 (6%)	4 (5%)	3 (6%)	4 (12%)	1 (6%)	2 (20%)	0	6 (7%)
Engel 3	17 (20%)	14 (19%)	9 (18%)	6 (18%)	4 (24%)	2 (20%)	0	15 (17%)
Engel 4	9 (10%)	10 (14%)	5 (10%)	3 (9%)	0	0	1 (50%)	12 (14%)
Total	87	74	50	34	17	10	2	87

Engel scores at 2-year intervals after surgery

COGNITIVE FUNCTIONING AFTER EPILEPSY SURGERY IN CHILDREN WITH MILD MALFORMATION OF CORTICAL DEVELOPMENT AND FOCAL CORTICAL DYSPLASIA

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Mild malformation of cortical development (mMCD) and focal cortical dysplasia (FCD) subtypes combined are by far the most common histological diagnoses in children who undergo surgery as treatment for refractory epilepsy. In patients with refractory epilepsy a substantial burden of disease is due to cognitive impairment. We studied IQ or developmental quotient (DQ) values, and their change after epilepsy surgery in a consecutive series of 42 children (median age at surgery 4.5, range 0-17.0 years) with refractory epilepsy due to mMCD/FCD. Cognitive impairment, defined as IQ/DQ below 70, was present in 51% prior to surgery. Cognitive impairment was associated with earlier onset of epilepsy, longer epilepsy duration and FCD type I histology. Clinically relevant improvement of ≥ 10 IQ/DQ points was found in 24% of children and was related to the presence of presurgical epileptic encephalopathy. At time of post-surgical cognitive testing 59% children were completely seizure free (Engel 1A). We found no association between cognitive outcome and seizure or medication status at two years follow-up. Epilepsy surgery in children with mMCD or FCD not only is likely to result in complete and continuous seizure freedom, but also improves cognitive function in many.

Introduction

Focal cortical dysplasia (FCD) subtypes, including minimal malformation of cortical development (mMCD), are by far the most common histological diagnoses in children who undergo epilepsy surgery¹⁰. In FCD focal cortical abnormalities are characterized by aberrant organization of cortical layers (type I), in which also abnormal neurons can occur (type II)¹³. The most subtle focal architectural abnormalities in the malformation of cortical development spectrum are classified as mMCD, with intact cortical architecture and absence of aberrant cells, but with excessive number of neurons in the molecular layer (type 1) or white matter (type 2)¹⁴.

An important comorbidity in children and adults with refractory epilepsy is developmental delay. The underlying pathology, epileptic activity, and use of anti-epileptic medication all contribute to cognitive disturbances³². In children with refractory epilepsy due to FCD, 53–68% have cognitive impairment (IQ<70)^{27,28}. In children with so-called epileptic encephalopathy, in which the epileptic activity further decreases the child's cognitive developmental abilities, early treatment is warranted to improve cognitive outcome⁷⁷.

Most studies reported overall cognitive improvement following epilepsy surgery^{45–48}. In children with severe epilepsy who undergo surgery at young age and who often had preoperative developmental arrest or regression, clinically relevant increases in IQ scores of e.g. 7–10 or more points are often not achieved, although the child's cognitive age clearly starts to increase after surgery^{77,78}.

In general, postoperative cognitive outcome has been shown to correlate with seizure freedom^{28,48–51}, parental education⁵², anti-epileptic drug (AED) policies^{51,53}, preoperative seizure frequency²⁷, and with aetiology^{53,54}. Importantly, earlier onset^{27,46,55–62} and longer duration^{27,63} of epilepsy predicts more severe cognitive impairment. Significant differences in cognitive outcome between patients with different FCD subtypes have not yet been reported^{27,42}.

The aim of this study is to assess pre- and postoperative cognitive functioning in children with mMCD/FCD, and to identify its predictors. In particular, we wanted to quantify the level of cognitive functioning – expressed as full-scale IQ-scores – in these children, both before and after epilepsy surgery. Furthermore, we aimed to assess whether or not the following clinical factors determined a change in IQ-scores after surgery: histopathological diagnosis, age of onset and at surgery, duration of epilepsy, preoperative high seizure frequency and epileptic encephalopathy, surgery type, and post-operative seizure- and AED status.

Methods

All children were included who underwent epilepsy surgery between 2000 and 2012 at the University Medical Center Utrecht, had a histopathological diagnosis of an isolated focal cortical dysplasia or mMCD, and underwent neuropsychological testing.

We reviewed the surgical database for all patients with a reported histological diagnosis that could be compatible with focal cortical dysplasia (e.g. malformation of cortical development, microdysgenesis, dysplasia) and revised the histology according the most recent ILAE classification¹³. Patients with dysplastic lesions in the context of other epileptogenic pathologies (FCD III types e.g. with mesiotemporal sclerosis or tumours) and those with complex or multifocal malformations of cortical development (e.g. polymicrogyria, hemimegalencephaly, tuberous sclerosis complex) were excluded.

Children were neuropsychologically assessed shortly before, one or two years after surgery. A range of tests was used, according to the age and cognitive level of the children, to determine their (full-scale) Intelligence Quotient (IQ). Most frequently used was the Wechsler Intelligence Scale for Children-III (WISC-III) (pre/post n=17), other tests were Wechsler Intelligence Scale for Children Revised (WISC-R) (pre/post n=5), Wechsler Adult Intelligence Scale (WAIS) (pre/post n=3/4), Snijders-Oomen non-verbal intelligence tests (SON) (pre/post n=3), Bayley Scales of Infant and Toddler Development – II (BSID-II) (pre/post n=6/8), Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III) (pre/post n=3/2) and Kaufman Adolescent and Adult Intelligence Test (KAIT) (pre/post n=1). For children who scored below the lower end of the norms' range, or for whom the age-appropriate tests were too difficult to perform, a developmental quotient (DQ) was calculated, using the ratio mental age / calendar age x 100.

Change in IQ/DQ was defined as post-surgical (one or two years after surgery) minus pre-surgical IQ/DQ. An IQ or DQ change of 10 points was considered clinically meaningful. Ten IQ points is two-thirds of a standard deviation and three times the standard error of the most used test in this cohort, the WISC-III, and an IQ change of 10 is considered unlikely to be the result of retesting or related to errors inherent to the performed test. Ratio DQ is known to be less stable than normed IQ. For the main analysis, children with IQ and DQ scores were pooled. Separate analyses were performed for patients in whom normed IQ scores were obtained, to address possible influence of incorporating DQ in the analyses. Determinants for IQ/DQ change were additionally studied excluding patients with hemispheric surgery, to rule out this subgroup determines possible associations in the total cohort. We categorized results of neuropsychological testing as normal (IQ > 85), mild delay (IQ 70–85), moderate delay (IQ 55–70) and severe delay (IQ < 55). In the latter group with severe

delay were many children in whom norm IQ scores could not be determined. Children with IQ/DQ < 70 are considered to have cognitive impairment.

The following clinical information was collected from the electronic patient file: presence of an MRI lesion compatible with FCD or mMCD, presence of bilateral epileptic encephalopathy in the period prior to surgery, occurrence of generalized tonic clonic seizures, seizure frequency, age at onset of epilepsy, location of seizure focus, location of surgery (multilobar, frontal, temporal, parietal, occipital), age at surgery, type of surgery (hemispheric versus focal), post-surgical seizure freedom (defined as Engel Ia; completely seizure-free since surgery) and the use of anti-epileptic drugs at latest follow-up and at time of latest neuropsychological assessment. We additionally used the dichotomized seizure outcome "Worthwhile seizure reduction" or not, corresponding with Engel classes 1-3, and defined as more than 50% reduction in seizure frequency. Information on post-surgical seizure and medication status was completed by means of telephone interview, the time of which was noted as latest follow-up.

Acute postoperative seizures during the first month were not taken into consideration when classifying seizure outcome.

Statistical analysis

Statistical analysis was performed in SPSS Statistics version 25.

For testing for normality we used Shapiro-Wilk test, and histogram and Q-Q plot. TIQ was normally distributed and T-test (including Levene's test for testing equal variances) was used to compare groups, or ANOVA for the three pathology groups, with Bonferroni correction for post-hoc tests. Change in IQ/DQ, age at surgery, epilepsy onset and duration had non-normal distribution for which we used appropriate non-parametric tests, either Kruskal-Wallis or Mann-Whitney U. Fisher's exact was used test to analyse association between baseline characteristics, cognitive functioning categories and meaningful IQ/DQ change (≥ 7 points), and histology. Kendall Tau-C was used for the association between cognitive functioning categories and meaningful IQ/DQ change and presence of daily seizures, epileptic encephalopathy, type of surgery (focal versus hemispheric), seizure- or AED-freedom in order to account for ordinal ties. Pearson's R or Spearman's rank were used for analysis of correlation. For comparing IQ/DQ before and after surgery related samples paired T-test was used and for categories of cognitive functioning Wilcoxon signed rank test.

Results

Between 2000 and 2012, 88 patients underwent epilepsy surgery and had a diagnosis of isolated FCD or mMCD according to the ILAE guidelines. Fifty-two were under the age of 18 years at time of surgery. The 42 who underwent cognitive testing at any time, either

before surgery, after surgery, or both, were included. Of these, the revised pathological diagnosis was mMCD in 10, FCD I in 6, and FCD II in 26 children. For 30 patients normed IQ scores could be calculated with age-appropriate tests both before and after surgery. In five children, only developmental quotient scores could be determined pre- and post-surgery. One child had a normed IQ score pre- and DQ post-surgery. Three patients had no neuropsychological examination prior to surgery, and one not after surgery. Two patients had pre-surgical IQ/DQ scores < 55, that could not be further quantified, of which one with a post-surgical IQ and one with a DQ score.

Therefore, in 36 patients IQ/DQ scores could be compared between pre and post-surgery tests. In a total of 38 children, categories of functioning could be determined both before and after surgery.

The latest cognitive test was performed two years after surgery in 35 patients, and at one year in 6 patients.

In three patients re-resection was performed within one year and thus before having done their first post-operative neuropsychological evaluation. For these patients post-operative seizure outcome and cognitive function was reported after the second surgery. Baseline functioning was tested prior to first surgery in all cases.

Clinical characteristics

Clinical characteristics of patients are presented in table 1. Median age at onset and surgery were 2.3 and 10.0 years respectively, with no statistical difference between histological diagnoses. Children with FCD I and mMCD were more likely to undergo hemispheric surgery ($p=0.004$) and presented more often with epileptic encephalopathy ($p=0.005$).

Prior to surgery, 20/39 (51%) of all tested children had IQ/DQ values < 70, with a mean score of 66 (distribution of IQ/DQ scores displayed in table 2).

At time of the latest post-operative cognitive assessment, 24 (59%) were seizure free and 16 (39%) were also already free of medication. Thirty-five (85%) children achieved at least worthwhile seizure reduction (Engel 1-3). There was a trend towards less patients with mMCD diagnoses to be seizure- and medication-free at time of latest post-surgical cognitive testing ($p=0.055$).

Determinants of pre-surgical cognitive functioning

All studied determinants of pre-surgical cognitive functioning are shown in table 3a.

There were significant differences in pre-surgical IQ between the three pathology groups ($p=0.004$). Patients with FCD I had significantly lower DQ/IQ (post-hoc testing FCD I versus mMCD: $p=0.030$, versus FCD II: $p=0.003$). The difference between FCD II and mMCD was not significant and there was no statistical difference when only analysing the children with normed IQ scores. There was no significant correlation between pathology groups and

Table 1. Baseline characteristics

	Total n=42	mMCD 10 (24%)	FCD total 32 (76%)	FCD I 6 (14%)	FCD II 26 (62%)	p mMCD vs FCD I vs FCD II
Sex, male	24 (57%)	4 (40%)	20 (63%)	3 (50%)	17 (65%)	0.370
Age at epilepsy onset, median and range (years)	2.3 (0-12.0)	5.9 (0.0-10.0)	2 (0.0-12.0)	1.5 (0.0-8.0)	2.0 (0.0-12.0)	0.424
Age at surgery, median and range (years)	10.0 (0.2-18.5)	11.9 (1.0-16.3)	9.6 (0.2-18.5)	4.3 (0.6-15.8)	10.0 (0.2-18.5)	0.445
Duration of epilepsy, median and range (years)	4.5 (0-17.0)	4 (1-15)	5.5 (0-17)	2.5 (1-10)	6 (0-17)	0.239
generalized seizures (ever)	30 (71%)	4 (60%)	24 (75%)	5 (83%)	19 (73%)	0.620
generalized seizures (last yr.)	29 (69%)	5 (50%)	24 (75%)	5 (83%)	19 (73%)	0.387
Daily seizures	31 (74%)	7 (70%)	24 (75%)	5 (83%)	19 (73%)	1.000
Presence of epileptic encephalopathy pre-surgery	10 (24%)	4 (50%)	5 (24%)	3 (50%)	2 (8%)	0.005
Type of surgery						
Hemispheric	8 (19%)	2 (20%)	6 (19%)	4 (67%)	2 (8%)	0.004
Focal	32 (76%)	8 (80%)	28 (88%)	2 (33%)	26 (100%)	
Frontal	21 (62%)	3 (38%)	18 (56%)	1 (50%)	17 (71%)	0.074
Temporal	7 (21%)	3 (38%)	4 (13%)	1 (50%)	3 (13%)	
Parietal	4 (12%)	0	4 (13%)	0	4 (17%)	
Occipital	2 (6%)	2 (25%)	2 (6%)	0	2 (6%)	
Lateralisation, left sided	20 (48%)	6 (60%)	14 (44%)	2 (33%)	12 (46%)	0.681
MRI-negative lesion	6 (14%)	2 (20%)	4 (13%)	2 (33%)	2 (8%)	0.101
Re-operations (intra-cranial surgery)	7 (17%)	2 (20%)	5 (16%)	2 (33%)	3 (12%)	0.290
Engel 1A, year of latest cog test	24/41 (59%)	4 (40%)	20 (65%)	5 (83%)	15 (60%)	0.263
Engel 1A drug-free, year of latest cog test	16/41 (39%)	1 (10%)	15 (48%)	4 (67%)	11 (44%)	0.055
Engel 1-3 (worthwhile seizure reduction)	35/41 (85%)	9 (90%)	26 (84%)	6 (100%)	20 (80%)	0.566

categories of cognitive functioning ($p=0.097$), although it is striking that all children with FCD I had cognitive impairment. Higher pre-surgical cognitive functioning was strongly related to older age at epilepsy onset ($p=0.001$). Patients with severe cognitive delay were operated earlier in life, but age at surgery was similar for the other categories of cognitive ability. Therefore, in the total group, age at surgery and IQ/DQ score were positively correlated ($p=0.021$), while there was no association when excluding those with DQ scores, since 6 of 7 (86%) of them had severe delay. In patients with normed IQ scores, lower pre-surgical IQ was related to a longer duration of epilepsy ($p=0.022$). Almost all patients with DQ scores had severe delay, and these children had shorter epilepsy duration, although differences

Table 2. cognitive functioning pre and post-surgery

	Before surgery	after surgery	p
Categories cognitive functioning			
severe delay	8 (22%)	8 (22%)	0.248
moderate delay	10 (22%)	11 (31%)	
mild delay	14 (39%)	8 (22%)	
normal	4 (11%)	9 (25%)	
Mean IQ/DQ (std, range)	66 (± 18 ; 17 – 96)	70 (± 20 ; 31 – 103)	0.010
Mean IQ, only formal IQ scores (std, range)	71 (± 13 ; 46-96)	75 (± 17 ; 49-103)	0.029

Numbers in this table are based on the 36 patients in whom both pre and post-surgical cognition had been assessed. For 30 of these patients normed IQ scores were available both pre and post-operatively.

in epilepsy duration between the four categories of pre-surgical cognitive functioning did not reach significance ($p=0.112$)

Patients with daily seizures tended to be more often in the lowest categories of cognitive functioning ($p=0.057$) and there was a trend for lower IQ/DQ in these children ($p=0.052$). in patients with a clinical presentation compatible with epileptic encephalopathy, there was an overrepresentation of patients with severe cognitive delay ($p=0.017$) and IQ/DQ was lower, but not significantly so ($p=0.079$). IQ/DQ was significantly lower in patients who were to undergo hemispheric surgery ($p=0.031$).

Change in cognitive functioning after surgery

After surgery, the proportion of children in each of the four cognitive functioning categories did not significantly change, although relatively more had normal IQ after surgery (Table 2). When pooling IQ and DQ scores for all patients with both discrete pre- and post-operative values available ($n=36$), mean quotients significantly increased from 66 to 70 ($p=0.010$). Considering only the children in whom normed IQ scores could be determined ($n=30$), mean IQ increased from 71 to 75 ($p=0.029$).

Whether or not there was a clinically relevant IQ change could be determined in 37 children. Nine of these (24%) showed an improvement of ≥ 10 points, 2 (5%) deteriorated ≥ 10 points, and 26 (70%) had post-surgical scores within 10 IQ points of their pre-surgical scores.

Determinants of post-surgical cognitive functioning

All studied possible determinants of post-surgical cognitive functioning are shown in table 3b. Post-surgical IQ/DQ was strongly correlated with pre-surgical IQ/DQ. No significant correlation was seen between histological subtype and post-operative cognition. The relation between post-surgical cognitive functioning and duration of epilepsy and age at onset and surgery,

Table 3a. determinants of pre-surgical cognitive functioning

Pathology	Category of cognitive functioning (n=39)				IQ/DQ, all (n=37)		IQ, only formal scores (n=32)	
	severe delay 10 (26%)	moderate delay 10 (26%)	mild delay 14 (36%)	normal 5 (13%)	P	Mean IQ (std) / Correlation coefficient	p	mean IQ (std) / Correlation coefficient
mMCD	3 (30%)	3 (30%)	2 (20%)	2 (20%)	0.097	69 (15)	0.004	71 (15)
FCD I	3 (50%)	3 (50%)	0	0		45 (24)		66 (2)
FCD II	4 (4%)	4 (30%)	12 (52%)	3 (13%)		72 (15)		72 (15)
Age at onset, median (range)	0.0 (0-7)	2 (0-8)	3 (0-12)	10 (6-12)	0.001	rs=0.636	<0.0005	rs=0.562
Age at surgery, median (range)	4.9 (0.6-14.5)	12.0 (4.3-18.5)	11.3 (1.8-17.7)	12.6 (10.1-14.6)	0.094	rs=0.378	0.021	rs=0.159
Duration of epilepsy median (range)	3.5 (1-14)	7 (2-17)	5.5 (2-14)	3 (1-5)	0.112	rs=-0.051	0.763	rs=-0.403
Daily seizures								
Yes	10 (36%)	7 (25%)	7 (25%)	4 (14%)	0.057	64 (20)	0.052	69 (15)
No	0	3 (27%)	7 (64%)	1 (9.1%)		74 (11)		74 (11)
Epileptic Encephalopathy								
Yes	6 (67%)	1 (11%)	1 (11%)	1 (11%)	0.017	49 (27)	0.079	73 (17)
No	4 (13%)	9 (30%)	13 (43%)	4 (13%)		71 (14)		71 (14)
Type of Surgery								
Focal	5 (16%)	8 (26%)	13 (42%)	5 (16%)	0.008	71 (15)	0.031	71 (15)
Hemispheric	5 (62%)	2 (25%)	1 (13%)	0		47 (23)		68 (4)

Determinants of pre-surgical cognitive functioning. 32 Patients with normed IQ scores, 5 with DQ. In two patients no further determination of IQ/DQ was possible other than <55. No pre-surgical testing in three children. Rs=Spearman correlation coefficient. P-values in bold are significant at <0.05 level.

Table 3b. Determinants of post-surgical cognitive functioning

	Category of cognitive functioning (n=41)				IQ, all (n=41)		IQ, only formal scores (n=32)	
	severe delay 11 (27%)	moderate delay 12 (29%)	mild delay 8 (20%)	normal 10 (24%)	P	Mean IQ (std) /Correlation coefficient	p	mean IQ (std) /Correlation coefficient
presurgical cog functioning						r=0.869	<0.0005	r=0.801
Severe delay	8 (80%)	2 (20%)	0	0	<0.0005			
moderate delay	1 (10%)	8 (80%)	0	1 (10%)				
mild delay	0	2 (14%)	8 (57%)	4 (29%)				
normal	0	0	0	4 (100%)				
Pathology								
mMCD	3 (30%)	4 (40%)	0	3 (30%)	0.199	65 (27)	0.209	74 (19)
FCD I	3 (50%)	2 (33%)	0	1 (17%)		56 (28)		76 (24)
FCD II	5 (20%)	6 (24%)	8 (32%)	6 (24%)		72 (17)		75 (15)
Age at onset, median (range)	0 (0-6)	2 (0-9)	2.5 (0-5)	7 (2-12)	<0.0005	rs=0.616	<0.0005	rs=0.457
Age at surgery, median (range)	1.6 (0.2-14.5)	11.5 (4.3-18.5)	9.6 (1.8-15.7)	12.8 (5.4-17.7)	0.009	rs=0.457	0.003	rs=0.051
Duration of epilepsy median (range)	2 (0-14)	5.5 (2-17)	6.5 (2-14)	4.5 (1-10)	0.123	rs=0.165	0.304	rs=-0.327
Daily seizures								
Yes	11 (36%)	9 (29%)	4 (13%)	7 (23%)	0.052	65 (22)	0.087	74 (17)
No	0	3 (30%)	4 (40%)	3 (30%)		78 (14)		78 (14)
Epileptic Encephalopathy								
Yes	6 (60%)	2 (20%)	0	2 (20%)	0.037	54 (28)	0.015	81 (19)
No	5 (16%)	10 (32%)	8 (26%)	8 (26%)		73 (17)		74 (16)

	Category of cognitive functioning (n=41)				P	IQ, all (n=41)		IQ, only formal scores (n=32)	
	severe delay 11 (27%)	moderate delay 12 (29%)	mild delay 8 (20%)	normal 10 (24%)		Mean IQ (std) /Correlation coefficient	p	mean IQ (std) /Correlation coefficient	p
Type of Surgery									
Focal	4 (13%)	10 (32%)	8 (26%)	9 (29%)	0.002	75 (17)	<0.0005	76 (17)	0.656
Hemispheric	7 (70%)	2 (20%)	0	1 (10%)		47 (21)		71 (17)	
Seizure Freedom									
Yes	7 (29%)	5 (21%)	5 (21%)	7 (29%)	0.646	71 (22)	0.377	78 (16)	0.261
No	4 (24%)	7 (41%)	3 (18%)	3 (18%)		65 (21)		71 (15)	
Freedom of AEDs									
Yes	5 (31%)	5 (31%)	4 (25%)	2 (13%)	0.342	63 (18)	0.265	71 (13)	0.262
No	6 (24%)	7 (28%)	4 (16%)	8 (32%)		71 (23)		77 (18)	
Worthwhile seizure reduction									
Yes	10 (29%)	10 (29%)	6 (17%)	9 (26%)	0.776	69 (21)	0.563	75 (17)	0.783
No	1 (17%)	2 (33%)	2 (33%)	1 (17%)		63 (27)		73 (14)	

Determinants of post-surgical cognitive functioning. 32 children with normed IQ scores and 9 with DQ. One child did not undergo post-surgical cognitive testing. Rs=Spearman correlation coefficient. P-values in bold are significant at <0.05 level.

was similar to that found before surgery; younger ages at onset and at surgery were correlated with lower IQ ($p < 0.0005$ and 0.003 respectively). Patients with severe cognitive delay were operated far earlier ($p = 0.009$). Again, after exclusion of the children with only DQ available, there was no association between age at surgery and IQ. Lower post-surgical cognitive scores tended to correlate with longer epilepsy duration ($p = 0.068$).

Patients who suffered from epileptic encephalopathy, and those who underwent hemispheric surgery, were more often in the lowest categories of post-surgical cognitive functioning ($p = 0.037$ and $p = 0.002$). IQ/DQ was also significantly lower in these patients ($p = 0.015$ and < 0.0005), but not when excluding patients with only DQ.

There was no relation between cognitive functioning and freedom of seizures or of anti-epileptic drugs.

Determinants of change in cognitive functioning

Studied determinants of change in cognitive functioning are shown in table 3c.

There was no relation between change in cognitive scores and presurgical cognition, epilepsy duration, nor with age at onset or surgery.

Five of eight (63%) patients with epileptic encephalopathy in whom IQ change could be assessed showed an improvement of ≥ 10 points, compared to only 4 of 29 (14%) patients without epileptic encephalopathy ($p = 0.017$). Change in IQ was significantly higher in patients with EE ($p = 0.049$). Children with FCD II diagnoses were more likely to have stable IQ/DQ after surgery and less likely to have an increase of 10 points or more, in comparison to children with mMCD and FCD I diagnoses ($p = 0.024$).

Change in IQ, and the proportion of patients with a clinically relevant change in IQ, were not different between patients who achieved seizure freedom and those with recurrent seizures, neither did AED discontinuation influence change in IQ.

Analyses were repeated with exclusion of patients who underwent hemispheric surgery; in this subgroup 6 (20%) had clinically relevant TIQ improvement, 24 (80%) had stable scores, and no patients deteriorated. In this subgroup of patients, however, there was no significant correlation any more between pre-surgical EE and IQ/DQ change ($p = 0.177$), or between an increase of 10 or more IQ points and presurgical EE ($p = 0.094$). In this subgroup of patients who did not undergo hemispheric surgery, those with IQ improvement of ≥ 10 points had experienced later age at onset compared to patients with stable IQ ($p = 0.005$). The results of this sub-analysis are shown in supplement table 1.

Table 3c. Determinants of change in cognitive functioning

	relevant IQ change (≥10 IQ points) (n=37)			p	ΔIQ (n=35)	
	deterioration n=2 (5%)	stable n=26 (70%)	improvement n=9 (24%)		median (range) / Correlation coefficient	p
Pathology						
mMCD	1 (11%)	5 (56%)	3 (33%)	0.024	1 (-12 – +18)	0.326
FCD I	1 (17%)	2 (33%)	3 (50%)		11 (-11 – +39)	
FCD II	0 (0%)	19 (86%)	3 (14%)		3 (-9 – +16)	
presurgical cog functioning						
Severe delay	1 (11%)	5 (56%)	3 (33%)	0.451	6.5 (-12 – +14)	0.715
Moderate delay	1 (10%)	8 (80%)	1 (10%)		1 (-11 – +39)	
Mild delay	0	11 (79%)	3 (21%)		2.5 (-9 – +18)	
Normal	0	2 (50%)	2 (50%)		9 (-2 – +14)	
Presurgical IQ/DQ, mean (range)	61 (54-68)	66 (23-96)	65 (17-90)	0.908	rs=-0.046	0.789
Age at onset, median (range)	4 (0-8)	2 (0-12)	6 (0-12)	0.116	rs=0.018	0.919
Age at surgery, median (range)	7.7 (1.0-14.3)	10.0 (1.8- 18.5)	12.6 (0.6-17.7)	0.794	rs=-0.006	0.973
Duration of epilepsy median (range)	3.5 (1-6)	6 (2-17)	4 (1-10)	0.165	rs=—0.121	0.480
Daily seizures						
Yes	2 (7%)	18 (67%)	7 (26%)	1.000	4 (-12 – +16)	0.876
No	0	8 (80%)	2 (20%)		2.5 (-9 – +39)	
Epileptic Encephalopathy						
Yes	1 (13%)	2 (25%)	5 (63%)	0.017	13 (-12 – +18)	0.049
No	1 (3%)	24 (83%)	4 (14%)		2 (-11 – +39)	
Type of Surgery						
Focal	0	24 (80%)	6 (20%)	1.000	3 (-9 – +39)	0.611
Hemispheric	2 (29%)	2 (29%)	3 (43%)		8 (-12 – +18)	
Seizure Freedom						
Yes	2 (9%)	14 (61%)	7 (30%)	0.573	7 (-12 – +39)	0.626
No	0	12 (86%)	2 (14%)		2 (-9 – +14)	
Freedom of AEDs						
Yes	2 (13%)	11 (73%)	2 (13%)	0.103	-2 (-12 – +16)	0.125
No	0	15 (68%)	7 (32%)		5 (-9 – +39)	
Worthwhile seizure reduction						
Yes	2 (6%)	21 (66%)	9 (28%)	0.528	4 (-12 – +39)	0.723
No	0	5 (100%)	0		1 (-2 – +8)	

Rs=Spearman correlation coefficient. P-values in bold are significant at <0.05 level.

Discussion

In our study of cognitive functioning in 42 children who underwent surgery for refractory epilepsy due to mMCD or FCD, 51% had cognitive impairment prior to surgery. Cognitive functioning was poorer in patients with onset at younger age. Patients with severe cognitive impairment were often operated at very young age and consequently had the shortest epilepsy duration. In patients with IQ/DQ above 55, shorter epilepsy duration was related to better presurgical cognitive functioning. FCD I was infrequently diagnosed but these patients all had cognitive impairment and mean IQ/DQ was significantly lower than that in mMCD and FCD II.

We found an overall modest but statistically significant improvement of 4 mean IQ/DQ points, within 1 to 2 years after surgery. Relevant improvement of ≥ 10 IQ/DQ points was seen in 24% following surgery, while cognitive functioning was stable in 70% and deteriorated in 5%. Only a diagnosis of epileptic encephalopathy predicted cognitive improvement after surgery. Post-surgical cognitive functioning was strongly related to pre-surgical functioning and had similar associations with clinical characteristics as pre-surgical functioning. There was no association between postoperative cognitive scores and seizure or medication status.

Other mMCD/FCD studies have found a similar prevalence of cognitive impairment^{27,28}. Seizure activity and consequent network disturbances, the underlying pathology and use of anti-epileptic drugs are all likely to contribute to cognitive impairment.

In a rat model for FCD, in which developmental abnormalities were caused by injection of methyloaxymethanol acetate and consequently frequent seizures were induced in one group, researchers found no relation between cognition in adolescence and history of early-life seizures and concluded that the cortical malformations were the substrate for cognitive impairment. However, these mice with cortical malformations had significantly lower brain weights compared to a control group that received saline injections in-utero, suggesting methyloaxymethanol acetate injection causes more widespread abnormalities than those seen in human patients with FCD⁷⁹.

Despite the circumscribed nature of dysplastic lesions in most patients with FCD or mMCD, widespread network disruption can be observed, even when their seizures remain focal. There is evidence of loss of optimal, small-world, network configuration and abnormal diffusivity and decreased anisotropy in white matter distant from, and even contralateral to, the lesion^{30,31,80,81}. This results in cognitive deficits not directly associated with the location of a lesion. Following remission of epilepsy, both normalization of functional network organization and recovery of white matter bundle fractional anisotropy may be seen³¹. Also AED drug load is associated with disturbed network parameters⁸⁰. Drugs may have a generalized suppressive effect with alteration of neuronal excitability^{80,82}. Consequently,

many common AEDs are associated with cognitive problems. After diagnosis of epilepsy there is more often cognitive decline in patients with higher AED levels⁸³. In addition, in a randomized blinded placebo-controlled trial in seizure free patients cognitive function improved if AED treatment was discontinued⁸⁴. Likewise, a retrospective study in children who underwent epilepsy surgery showed improved post-surgical IQ and higher IQ change with reduction of AEDs⁵³.

In agreement with previous studies we found a relation between poorer cognition and younger age at onset^{27,46,55–62}. Pre-surgical impaired cognition was associated with longer duration of epilepsy, but only when excluding children with only DQ, of whom 6/7 (86%) had expedited surgical intervention due to catastrophic epilepsy syndromes. Kimura et al. found a similar relation between cognition and epilepsy duration when children were stratified by age of onset, but not when analysing the complete cohort²⁷. This is in agreement with another study that included only children that were operated before the age of three years⁶³. With impairment of brain function due to seizure activity, longer exposure to epileptic activity would reasonably increase the gap in cognitive function with normal developing peers.

A critical period in cognitive development during the first years of life has been suggested which lays the foundation for subsequent development of higher-order skills⁶⁰. Disruption during sensitive periods could result in more pronounced cognitive deficits later in life⁸⁵. On the other hand, both lower IQ and an earlier epilepsy onset could be the consequence of a more severe underlying epileptogenic pathology, rather than being causally related⁶¹.

Patients with severe cognitive delay were operated at younger age, but when excluding patients with only DQ—of whom 6/7 (86%) had severe delay—we did not observe a relation between age at surgery and pre or post-surgical cognitive ability. In a cohort consisting only of patients undergoing hemispherectomies, worse cognition was related to surgery at younger age and shorter epilepsy duration⁸⁶.

Presurgical cognition was lower in patients with FCD I compared to mMCD or FCD II. A possible explanation is that FCD I lesions are typically more widespread – with more frequent hemispheric surgery in our cohort – and these patients presented more often with epileptic encephalopathy. The latter could also explain why differences in cognition between histological subtypes were not significant after surgery; we observed greater improvement in cognition in patients with previous encephalopathy and more children with clinically relevant IQ improvement among those with mMCD and FCD I histological subtypes compared to FCD II. Previous studies did not find a relation between cognition and histological subtype^{27,42}, however, these groups used the 2002 Palmini histological classification scheme, what can lead to discrepancies in classification, especially in more subtle abnormalities.

A large share of patients in our cohort showed improvement in cognitive level after surgery. Studies showed full-scale IQ to have high stability and reliability with test-retest coefficients exceeding 0.9. Large studies, including around 600 children evaluated WISC-III (the most used test in our cohort) for special education eligibility, found full-scale IQ to decrease -0.4 in a mean interval just under three years^{87,88}. The average 4 point improvement in full-scale IQ on a group level we found is not likely due to retest effects, or retesting at older age since tests have been proven highly stable and are normed according to age. Although pre-surgical cognition has consistently been shown to be the strongest predictor of post-surgical functioning, as confirmed in our study, it does not necessarily relate to the change in IQ after surgery. For this outcome measure, the presence of pre-surgical epileptic encephalopathy was the only predictive factor in our study. This finding is in agreement with the concept of epileptic encephalopathy, in which epileptic activity by itself affects cognition more than what can be explained by the underlying pathology alone³.

We did not observe an association between postoperative cognitive outcome and seizure status, although several studies have reported cognitive improvement to be dependent of seizure freedom⁴⁹⁻⁵¹, with more pronounced improvement after discontinuation of medication as well⁵¹. These studies had a follow-up of a minimum of 4 years, up to 21 years after surgery. Although we observed overall improvement in cognition, it is likely that a longer follow-up period than two years is required to acknowledge the true effect of seizure freedom and medication policies on cognitive development following epilepsy surgery.

Our study has a number of limitations, besides those inherent to retrospective collection of clinical data and limited follow-up duration for cognition. Our moderate sample size precludes multivariate analyses and correction for possible interactions.

Environmental factors were not accounted for in this study. In a cohort of operated children with epilepsy of diverse aetiology, there was more improvement in cognition in children of parents with higher education, but only in those who became seizure free. Although this association was significant, the effect was small. In the same study there was no relation between parental education and pre-surgical IQ⁵². A large twin-study in a healthy British population showed the effect of genetics on intelligence to be similar among low and high socioeconomic status families⁸⁹.

Despite its limitations, the current study has enabled the assessment of clinically relevant determinants of cognitive outcome in a relatively homogeneous cohort of children with mMCD/FCD histopathological diagnoses revised following the most recent ILAE guidelines¹³.

Conclusion

In children with mMCD and FCD, pre-operative cognitive functioning is poorer when epilepsy onset is at younger age and duration of epilepsy is longer. Children with severe cognitive delay typically present with catastrophic epilepsy, that warrants an aggressive therapeutic strategy and surgical intervention at very young age.

Surgical therapy in children with refractory epilepsy due to mMCD or FCD not only is likely to result in complete and continuous seizure freedom, but also improves cognitive function in a substantial proportion of children, independent of histological subtype.

Supplement table 1. Determinants of change in cognitive functioning, excluding children with hemispheric surgery

	relevant IQ change (≥10 IQ points) (n=30)			p	ΔIQ (n=29)	
	deterioration n=0	stable n=24 (80%)	improvement n=6 (20%)		median (range) / Correlation coefficient	p
Pathology						
mMCD	0	4 (67%)	2 (33%)	0.187	0 (-9 – +13)	0.400
FCD I	0	1 (50%)	1 (50%)		21 (3 – +39)	
FCD II	0	19 (86%)	3 (14%)		3 (-9 – +16)	
presurgical cog functioning						
Severe delay	0	4 (80%)	1 (20%)	0.453	3.5 (-1 – +8)	0.720
Moderate delay	0	7 (88%)	1 (13%)		1.5 (-2 – +39)	
Mild delay	0	11 (85%)	2 (15%)		1 (-9 – +16)	
Normal	0	2 (50%)	2 (50%)		9 (-2 – +14)	
Presurgical IQ/DQ, mean (range)	-	68 (46-96)	79 (64-90)	0.101	rs=0.060	0.757
Age at onset, median (range)	-	2.25 (0-12)	7.5 (3-12)	0.005	rs=0.132	0.494
Age at surgery, median (range)	-	10.9 (1.8-18.5)	13.1 (11.4-17.7)	0.116	rs=-0.204	0.288
Duration of epilepsy median (range)	-	6 (2-17)	5.5 (1-10)	0.631	rs=—0.15	0.938
Daily seizures						
Yes	0	16 (76%)	5 (24%)	0.637	4 (-9 – +16)	0.764
No	0	8 (89%)	1 (11%)		1 (-9 – +39)	
Epileptic Encephalopathy						
Yes	0	1 (33%)	2 (67%)	0.094	10.5 (8 – +13)	0.177
No	0	23 (85%)	4 (15%)		2 (-9 – +39)	
Seizure Freedom						
Yes	0	13 (72%)	5 (28%)	0.358	5 (-9 – +39)	0.492
No	0	11 (92%)	1 (8%)		1 (-9 – +8)	
Freedom of AEDs						
Yes	0	10 (91%)	1 (9%)	0.372	-2 (-9 – +16)	0.238
No	0	14 (74%)	5 (26%)		4.5 (-9 – +39)	
Worthwhile seizure reduction						
Yes	0	19 (76%)	6 (24%)	0.553	3.5 (-9 – +39)	0.845
No	0	5 (100%)	0		1 (-2 – +8)	

Rs=Spearman correlation coefficient. P-values in bold are significant at <0.05 level.

SEVEN TESLA MRI IMPROVES DETECTION OF FOCAL CORTICAL DYSPLASIA IN PATIENTS WITH REFRACTORY FOCAL EPILEPSY

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Objective: The aim of this study is to determine if the use of 7 tesla (T) MRI in clinical practice leads to higher detection rates of focal cortical dysplasias in possible candidates for epilepsy surgery.

Methods: In our center patients are referred for 7T MRI if lesional focal epilepsy is suspected, but no abnormalities are detected at one or more previous, sufficient quality lower field MRI scans, acquired with a dedicated epilepsy protocol, or when concealed pathology is suspected in combination with MR-visible mesiotemporal sclerosis–dual pathology. We assessed 40 epilepsy patients who underwent 7T MRI for pre-surgical evaluation, and whose scans (both 7T and lower field) were discussed during multidisciplinary epilepsy surgery meetings that included a dedicated epilepsy neuroradiologist. We compared the conclusions of the multidisciplinary visual assessments of 7T and lower field MRI scans.

Results: In our series of 40 patients, multidisciplinary evaluation of 7TMRI identified additional lesions not seen on lower-field MRI in nine patients (23%). These findings were guiding in surgical planning. So far six of them underwent surgery, with histological confirmation of focal cortical dysplasia or mild malformation of cortical development.

Significance: 7T MRI improves detection of subtle focal cortical dysplasia and mild malformations of cortical development in patients with intractable epilepsy and may therefore contribute to identification of surgical candidates and complete resection of the epileptogenic lesion, and thus to postoperative seizure freedom.

Introduction

In patients with pharmacoresistant focal epilepsy, resection of the epileptogenic zone can be considered. Focal cortical dysplasia (FCD) is the most frequent etiology (42%) in patients under 18 years of age selected for surgery. In adults FCD is the third most common etiology (13%) after hippocampal sclerosis (43%) and tumors (30%)⁷⁰.

Focal cortical dysplasias are a subgroup of circumscribed malformations of cortical development (MCDs), and are characterized by abnormal migration, maturation, and differentiation of neurons¹³. Onset of epilepsy occurs typically before the age of twelve years and roughly 76% of patients have medically refractory seizures⁹⁰. Resective surgery can be highly effective, with reported seizure freedom rates ranging from 33 to 80% in mixed FCD type cohorts of patients operated in the last two decades^{28,36–38,40,70,72,75}. In a large study of 211 patients with FCD ILAE type I and II operated 1998–2010, 65% remained seizure free (Engel class I) up to latest follow-up (2–12 years)³⁷. Seizure freedom rate markedly increases in case of complete resection^{33,36,37,43,91}, averaging 77% in a literature review by Lerner and colleagues⁷⁰. Appropriate detection and delineation of a FCD on MRI contributes to identification of surgical candidates and complete resection of the epileptogenic lesion, and thus to postoperative seizure freedom^{66–68}.

However, the structural abnormalities in FCD are often very subtle and may escape detection on conventional structural neuroimaging. This makes FCD the most common pathology (45–51%) in patients who undergo epilepsy surgery for focal refractory epilepsy and have a “non-lesional MRI”^{70,92}. In a review of surgical series from 2000 to 2008, 37% of FCD type I lesions and 15% of type FCD type II lesions were not detected with 1.5 or 3 T MRI⁷⁰. Detection seems even more problematic for the most subtle architectural abnormalities in the FCD spectrum, classified as mild malformations of cortical development (mMCD), of which 58% were not visible on MRI in a series published in 2008⁴².

Studies comparing 1.5 and 3 tesla (T) MRI in epilepsy surgery candidates have shown that imaging at 3T can identify FCDs that escaped detection at 1.5T^{93–96}. In a cohort of patients with FCD type II, 3T MRI showed a lesion in 20% of patients with non-lesional 1.5T⁹⁶.

We hypothesize that a similar effect can be gained from 7T, by virtue of increased signal-to-noise ratio, allowing higher resolution and contrast⁹⁷. Dysplastic lesions previously detected using lower field MRI are reported to be detected with more confidence and visualized in greater detail at 7T⁹⁸. One previous study reported an improved detection rate of 7T MRI compared to lower field MRI, although half of the patients in whom a new lesion was detected at 7T underwent previous scanning only at 1.5T, and not at the currently considered optimal field strength of 3T in pre-surgical evaluation⁹⁹.

In our study we aim for verification of the clinical value in a larger group of patients, of whom most underwent previous 3T MRI, and using a full 3D scanning protocol with sub-millimeter resolutions in FLAIR, T_1 , T_2 and T_2^* -weighted, and white matter suppression

sequences. We wanted to determine if the use of 7T MRI in clinical practice led to higher detection rates of focal cortical dysplasia in candidates for epilepsy surgery, and how this influenced surgical decision making.

Materials and methods

Patients

The University Medical Center Utrecht is a tertiary referral center for patients with refractory epilepsy and for epilepsy surgery. A multidisciplinary team consisting of neurologists, neurophysiologists, neurosurgeons, neuroradiologists, nuclear medicine physicians, and neuropsychologists from different centers, evaluates surgical candidacy of each patient, determines the type and order of ancillary investigations, and decides on the selection or rejection for surgery and on surgical strategy during epilepsy surgery meetings (ESMs). Patients are referred for 7T MRI if lesional focal epilepsy is suspected but no abnormalities are detected at one or more previous, sufficient quality MRI scans, acquired with a dedicated epilepsy protocol (3T in most cases)¹⁰⁰, or when concealed pathology is suspected in combination with MR-visible mesiotemporal sclerosis (dual pathology).

For this series we consecutively included 40 patients who underwent 7T MRI for pre-surgical evaluation between March 2009 and February 2016, and whose scans (both 7T and lower field scans) were discussed in the ESMs. Two young patients, age 7 and 8 years, scanned in this period were excluded from analysis. In one the examination had to be stopped because of insoluble fear. In the other severe movement artefacts in all sequences made accurate assessment impossible. In the 40 included patients at least one sequence was of sufficient quality for reliable visible assessment, although some degree of movement artefacts in one or more sequences was common, especially with pediatric patients. For the review and analysis of patient data institutional ethical board approval was acquired. All patients have given informed consent for the 7T MRI examination.

MRI acquisition

7T MRI Scans were performed on a Phillips Achieva 7T MRI system (Philips Healthcare, Best, the Netherlands) with a 16 channel receive coil or, after May 2011, a 32-channel receive head coil combined with dual channel transmit coil (Nova Medical, Wilmington, MA, USA). From mid-2015 on, dielectric pads, containing calcium titanate (Leiden University Medical Center, Leiden, the Netherlands) were routinely used to reduce artefacts and signal loss in the temporal regions¹⁰¹. Pads were placed to the sides of the subjects' head. Patients all used ear plugs to protect from acoustical noise.

In our center 3T MRI scans were performed with 3T Philips Achieva with eight-channel sensitivity encoding (SENSE) head coil. The 3T is the default machine for epilepsy patients;

only on specific indication (e.g., vagal nerve stimulator in situ), and in the past, 1.5T was performed, on a Phillips Achieva 8ch or Philips Ingenia dStream.

Scan protocols

The 7T scanning protocol consists of T_1 , T_2 , FLAIR, and T_2^* -weighted sequences, and a gradient echo white matter suppression (WMS) sequence, all with 0.5–0.8 mm isotropic resolution with 50% overlap of slices; acquisition time was circa 45 minutes. Since 2008 there have been minor adjustments in the protocol, mainly the addition of the WMS sequence and modification of the T_2^* echo times. Parameters of our 1.5, 3, and 7T MRI sequences performed in our center are described in supplementary Table 1. In case of severe motion artefacts, precluding reliable assessment, sequences are repeated to generate at least T_1 , T_2 and/or FLAIR sequences of sufficient quality, at the cost of T_2^* and WMS sequences.

All sequences are routinely reformatted in 0.5 mm (interpolated) multiplanar reconstructions to have axial, coronal, and sagittal images for all sequences, allowing for optimal appreciation of cortical architecture. This protocol covers all sequences recommended in various guidelines for the detection of epileptogenic lesions¹⁰⁰.

MRI assessment

MRIs were routinely reviewed (prior to the ESM) by means of visual analysis on a high-resolution work station, by a dedicated epilepsy radiologist who takes part in the ESMs, and who also revised all scans performed outside the UMCU. During bi-monthly multidisciplinary meetings, patients' full histories, EEGs, and results of other investigations are discussed. MRI scans are shown on a projector screen in the presence of a neuroradiologist and collectively re-assessed by the entire ESM team. The resulting conclusion is taken as final, and used in this study. Scans were sometimes reviewed on more than one occasion. The reported collective assessment of the lower field MRI always took place prior to the meeting during which the 7T MRI was evaluated.

A radiological diagnosis of possible FCD was made in case of presence of distinctive imaging features¹⁰², most notably blurring of the gray-white matter border and transmantle sign. Hippocampal atrophy in combination with signal changes was indicative of mesiotemporal sclerosis¹⁰³. Covert dual pathology was suspected if the clinical presentation could not be explained by mesiotemporal sclerosis alone. MRI scans were considered non-lesional if no possibly epileptogenic lesions were seen. Patients with aspecific, or radiological findings strongly divergent from patient and electrophysiological characteristics, were considered MRI-negative, e.g., arachnoid cysts, age-related or gliotic white matter lesions, and enlarged perivascular spaces. No post-processing techniques were used or evaluated for the purpose of this study.

Surgery

All surgical procedures were carried out in the University Medical Center Utrecht by two neurosurgeons with expertise in epilepsy surgery. All patients in this series were operated either after chronic electrocorticography (ECOG) or with guidance of acute (intra-operative) ECOG. The surgeons strive to resect tissues en-bloc as much as possible, to allow optimal histopathological examination.

Histopathology

Resected brain tissue was fixed in 10% buffered formalin and embedded in paraffin. Paraffin-embedded tissue was sectioned at 5 µm and used for histology and immunohistochemistry. Sections were processed for hematoxylin eosin staining, as well as for immunohistochemical staining for a number of neuronal and glial markers. Surgical samples were evaluated and classified according to the International League Against Epilepsy (ILAE) guidelines^{13,104}.

Results

Patients characteristics, ancillary tests, and surgical procedures

Included in this study were 40 patients in whom 7T MRI was performed on clinical indication and whose scans were assessed in multidisciplinary ESMs. Age at time of 7T scan was 7–48 years (median 18).

Thirty-five (88%) were previously scanned at 3T, four (10%) had at least a 1.5T, and one previously had a 1.0T MRI. Eighteen patients (43%) underwent both 3T and a 1.5/1.0/0.5T MRI. Other ancillary tests performed were FDG-PET (in 38, 95%) and magnetoencephalography (in 29, 73%). So far, single positron CT (SPECT) has been performed in 15 patients, including one failed examination (38%) (ictal subtraction with MRI co-registration: n=12). Three patients were still awaiting SPECT when collecting study data.

Thirteen patients (33%) underwent resective surgery, all guided by ECOG (chronic grid registration n=10, chronic with grid and depth electrodes n=1, acute grid registration n=2). At a follow up ranging 1 month – 5 years (mean 17, median 9 months), 11/12 (91%) patients are seizure free (Engel score 1A, n=10; Engel 1D, n=1). One patient is scored Engel 4B at 6 months (one patient has not yet been evaluated postoperatively).

Seven patients (18%) await invasive EEG monitoring. Twelve (30%) patients were rejected for resective surgery, of whom one underwent multiple subpial transections in the motor cortex and one received a vagal nerve stimulator. Surgery is postponed in one patient with likely FCD because of unexpected and prolonged seizure freedom. In three patients (8%) the pre-surgical evaluation was stopped, because of expected unacceptable motor deficit, or a significant decrease in burden of disease. Four (10%) patients are still under evaluation, and await non-invasive tests: SPECT (n=2) and fMRI-EEG (n=2).

Findings of 7T MRI

Table 1 provides an overview of 7T and lower-field MRI findings in all patients, as well as the consequent treatment strategies and histological diagnoses, if available.

Table 2 summarizes the findings of relevant ancillary tests in patients with new findings at 7T (patients 1–9, table 2), and those patients who underwent surgery following a clinically indicated 7T without newly identified epileptogenic lesions (patients 10–16, Table 2). A table of all 40 patients is provided as supplementary table 2.

Thirty-eight patients underwent 7T MRI because lower field MRI was interpreted as showing no relevant epilepsy-related lesions. In eight of those, a focal abnormality, suspect of FCD could be identified on 7T. Six of them have been operated, with histology confirming FCD type IIa in two, FCD IIb in one, mild malformation of cortical development (mMCD) type 2 in two and mild malformation of cortical development with oligodendroglial hyperplasia in one case. (Exemplary cases in figures 1–3). In patient 5 mMCD type 2 was diagnosed, however close relation to language areas precluded en-bloc resection of the complete MRI abnormality¹⁰⁴. Possibly deeper tissues would have been compatible with FCD I or II types (figure 3). In another patient FCD is also suspected, but surgery with acute ECOG is postponed due to current seizure freedom. One patient is still under evaluation. All patients with new lesions had previous good quality 3T MRI scans.

Two patients underwent 7T MRI because of clinical suspicion of dual pathology in combination with mesiotemporal sclerosis (MTS) visualized at 3T MRI. In one patient (patient 10, Table 2) with left-sided MTS and associated dysplasia in the temporal pole there was a clinical and electrophysiological suspicion of an additional contralateral (right-sided) seizure onset.

Figure 1. Transverse (A) and sagittal 7T T_2 (B), Transverse 3T T_2 (C).

Patient no. 2. ♂, 7yrs, focal impaired awareness seizures. 7T MRI showed blurring of grey-white matter junction suspect for FCD. The lesion was only retrospectively identified on 3T, where thick slices (4 mm +1 mm gap) lead to many false mimics from partial-volume effects of adjacent gyri. Lesionectomy in the medial frontal gyrus was performed. Histology confirmed FCD ILAE type IIa.

Figure 2. Transverse (A) and coronal 7T T_2 (B), 3T FLAIR (C).

Patient no. 6. ♂, 14 yrs., focal impaired awareness seizures. At 7T blurring of grey-white matter junction and cortical thickening compatible with FCD. In retrospect also recognizable on 3T but initially overlooked due to many (similar looking) partial-volume effects and signal variation in adjacent gyri. ECOG registration plus depth electrodes in the MRI lesion was performed followed by lesionectomy of the bottom of a sulcus in the medial frontal gyrus. Histology confirmed FCD type IIb.

Figure 3. Transverse (A) and coronal 7T white matter suppression sequence (B), coronal 3T FLAIR (C).

Patient no. 5. ♂, 21 yrs., focal impaired awareness seizures. On 7T WMS images very subtle white matter signal changes adjoining a deep sulcus (resembling subtle transmantle sign) in a region with a dense gyral pattern, suggestive of subtle cortical dysplasia. No abnormalities had been identified on 3T images. Histology was classified as mMCD type 2, however due to close relation to language areas en-bloc resection of the complete MRI abnormality was not possible and deeper tissues would have been compatible with FCD I or II types.

7T MRI did not indicate the presence of such a lesion and a left-sided anterior temporal lobectomy plus amygdalahipocampectomy was performed. Histopathology confirmed MTS + FCD IIIa.

In the other patient (patient 9, Table 2) 3T MRI suggested MTS, but semiology and electrophysiology indicated temporal neocortical pathology. 7T MRI revealed an abnormal

Table 1. Epilepsy surgery meeting conclusions in patients with non-lesional or unclear lower field MRI.

ESM conclusion based on lower field MRI	ESM conclusion based on 7T	Surgery	Histology
38 Considered non-lesional	8 Lesional	5 Resective surgery (with ECOG)	2 FCD ILAE type IIa 1 FCD ILAE type IIb 1 mMCD type 2 1 Proliferative oligodendroglial hyperplasia with mild malformation cortical development
		1 Resective surgery with ACOG planned 1 ECOG planned	2 Yet unknown (FCD suspected)
		1 Still under evaluation	Yet unknown (FCD suspected)
	30 Non-lesional	6 Resective surgery (ECOG guided)	2 mMCD type 2 1 FCD ILAE type IIa 1 Normal histology 1 Normal histology (sample error suspected) 1 Normal histology (suboptimal assessment due to tissue fragmentation)
		6 ECOG or S-EEG planned	-
		12 Rejected for resective surgery (1 MST, 1 VNS)	-
		3 No surgery (declined / clinical improvement)	-
		3 Still under evaluation	-
	Additional lesion	Resective surgery	mMCD type 2, no MTS
		Resective surgery	MTS + FCD ILAE type IIIa
	No additional lesions	Resective surgery	MTS + FCD ILAE type IIIa

ESM= epilepsy surgery meeting; ECOG = electrocorticography; S-EEG: Stereo EEG; FCD = focal cortical dysplasia; mMCD = mild Malformation of Cortical Development; MTS = Mesiotemporal (hippocampal) sclerosis. MST = multiple subpial transections. VNS = vagal nerve stimulator.

gyration pattern in combination with enhanced venous vasculature on the T_2^* sequence, indicating a possible developmental abnormality in the anterior temporal lobe. Selective left anterior temporal lobectomy with hippocampectomy was performed and the hippocampus was found to have normal histology while in the temporal lobe mild architectural abnormalities compatible with mMCD type 2 were confirmed.

After identification of new lesions at 7 tesla we reviewed lower field strength images once more. Even though these were inconspicuous at first, in hindsight, with knowledge of the 7T MRI findings, lesions could be recognized in 3T images in three of nine patients (33%).

Table 2. Patients with new lesions at 7T or MRI-negative with abnormal histopathology.

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG	MEG	FDG PET	SPECT	1.5T MRI	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
1	♀/44	26	Focal impaired awareness seizures; hyperkinetic seizures	R frontal parasagittal	R frontal parasagittal	Continuous spiking, R frontal parasagittal	Not performed	No abnormalities	Not performed	Not performed	No abnormalities	Subtle blurring, hyperintensity on DIR R frontal parasagittal	R Partial lobectomy frontal parasagittal	FCD IIb	1A+ 5yrs.
2	♂/7	4	Focal impaired awareness seizures, R hand automatisms	R frontal	R frontal parasagittal	Continuous spiking R frontal parasagittal	Not performed	No abnormalities	Not performed	Not performed	No abnormalities	R frontal small area with subtle blurring of G/W boundary, subtle white matter hyperintensity (T2)	R frontal lesionectomy	FCD IIa	1A+ ½yrs.
3	♀/40	24	Focal impaired awareness seizures, dysphasia and alexia; focal to bilateral tonic-clonic seizures	L temporal	L temporal	Interictal diffuse L temporal, ictal neocortical L mid-temporal	Spikes neocortex L medial temporal gyrus	No abnormalities	Not performed	No abnormalities *	No abnormalities	subcortical hyperintensities (FLAIR+ T2), blurring of GW-junction L anterior superior temporal gyrus	Lesionectomy L medial temporal gyrus	mMCD with oligo-dendroglial hyperplasia	1A+ 1yr.
4	♀/22	8	Focal impaired awareness seizures; visual aura; focal to bilateral tonic-clonic seizures	Onset mid-frontal	Mid-frontal	Not performed	Multifocal, at distance from visual cortex	L Occipital hypometabolism	Not performed	No abnormalities	No abnormalities	Subtle white matter signal changes, transmantle sign-like configuration L inferior occipital gyrus.	Under evaluation	n/a	n/a
5	♂/21	15	Focal impaired awareness seizures, stops activity, staring, automatisms	L frontal	L frontal	Continuous spiking L medial frontal gyrus	L frontal epileptic activity	L temporal hypometabolism	L frontal (consistent with 7T MRI) and less pronounced L parietal	Not performed	No abnormalities *	L frontal operculum/ inferior frontal sulcus abnormal gyration and subtle transmantle hyperintensity (WM5), enhanced venous vasculature in sulcus (T2*)	Corticectomy L medial frontal gyrus	mMCD type 2, sample error likely, no examination of center of MRI lesion	1A+ 4 months
6	♂/14	4	Focal impaired awareness seizures, hyperkinetic	L frontal	Ictal onset L frontal	Continuous spiking depth of L superior frontal sulcus (depth electrode)	L frontal spikes	No seizure focus	Not performed	No abnormalities *	No abnormalities	L frontal abnormally deep sulcus with blurring GM-junction, cortical thickening and subtle transmantle sign	Lesionectomy L middle frontal gyrus	FCD IIb	1A+ 1 ½ months

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG	MEG	FDG PET	SPECT	1.5T MRI	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
7	♂/14	10	Focal impaired awareness seizures, nocturnal seizures with automatisms; bilateral tonic-clonic seizures	Not performed	L centro-parietal	Not performed	L parietal epileptic activity	L parietal hypometabolism	Not performed	Not performed	No abnormalities	L parietal radial band of poorly demarcated white matter signal changes	Lesionectomy with ACOG planned	n/a	n/a
8	♀/28	15	Focal impaired awareness seizures, arrest of activity; focal to bilateral tonic-clonic seizures	L temporal	Bilateral fronto-centro-temporal	Planned	Not performed	L temporal, angular gyrus hypometabolism	Not performed	No abnormalities *	No abnormalities *	Abnormal intraparenchymal venous pattern posterior L medial temporal gyrus.	Grid planned	n/a	n/a
9	♂/15	11	Focal impaired awareness seizures, L sided head version, irresponsiveness, dysphasia amnesia; focal to bilateral tonic-clonic seizures	Non-lateralizing	Multifocal	Diffuse L temporo-parietal	L baso-temporal, up to posterior temporal	No abnormalities	Not performed	MTSL + dubious blurring GWM-junction and decreased volume L temporal *	MTSL + dubious blurring GWM-junction and decreased volume L temporal *	MTSL + abnormal gyration L anterior temporal and enhanced venous vasculature (T2*)	NVS after initial grid registration. Ultimately selective L anterior lobectomy + hippocampectomy	mMCD type 2, no MTS	4B+ 1/2yr
10	♀/28	0	Focal impaired awareness seizures, automatisms, expressive dysphasia	L mid-temporal sharp waves	L mid-temporal spikes	L anterior temporal neocortical + hippocampus	Not performed	L temporal hypometabolism	Not performed	Possible MTSL	MTSL + unclear demarcation of GWM-junction on temporal lobe	MTSL + unclear demarcation of GWM-junction temporal lobe. No indication of dual pathology	L anterior temporal lobectomy + amygdalohipocampectomy	MTSL ILAE class 2 + FCD IIIa	1A- 1yr.
11	♀/15	9	Focal aware seizures, R-sided version eyes/head; bilateral tonic-clonic seizures	L fronto-central	No distinct epileptiform activity	Ictal onset L fronto-central	L fronto-central spikes	No abnormalities	Not performed	No abnormalities	No abnormalities	No abnormalities	Lesionectomy L pre-central sulcus	Normal tissue (suspect for sample error. FCD in bottom of sulcus)	1A- 2yrs.
12	♂/14	7	Focal impaired awareness seizures, left tonic; focal to bilateral tonic-clonic seizures	R fronto-central	R posterior temporal	Continuous spiking R baso-temporo-occipital	R temporal spikes	R baso-temporo-occipital hypometabolism	Not performed	No abnormalities *	No abnormalities	No abnormalities (severe signal loss in temporal lobes)	R baso-temporo-occipital lesionectomy	FCD IIIa	1A- 1yr.

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG	MEG	FDG PET	SPECT	1.5T MRI	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
13	♂/18	14	Focal impaired awareness seizures hyperkinetic; focal to bilateral tonic-clonic seizures	No distinct epileptiform activity	No distinct epileptiform activity	No interictal activity, ictal medial frontal gyrus	No abnormalities	R frontal hypometabolism	Not performed	No abnormalities*	Not performed	No abnormalities	Cortical resection R medial frontal gyrus	mMCD type 2	1D+ 5yr
14	♀/22	11	Focal impaired awareness seizures, sensory dysphasia, derealization, sensations in left foot; focal to bilateral tonic-clonic seizures	R posterior temporal	R posterior temporal	Bursts R posterior temporal, sporadic spikes R hippocampus	R hemisphere, no clear localization	Dubious hypometabolism R operculum	Not performed	Not performed	No abnormalities	No abnormalities	Resection posterior baso-temporal, fusiform gyrus	Normal, but suboptimal assessment due to fragmentation	1A+, 1/2yr.
15	♂/33	20	Focal impaired awareness seizures, auditory phenomena, R-sided head version, confusion; focal to bilateral tonic-clonic seizures	L posterior temporal	Aspecific irregularities bilateral fronto-temporal	Sporadic spikes L lateral temporal	Not conclusive	L baso-temporal, parietal, hypometabolism in large area	Not performed	Not performed	No abnormalities	No abnormalities	Cortical resection L superior and middle temporal gyrus	No abnormalities	1A+ 1 month
16	♂/16	12	Focal impaired awareness seizures, myoclonia all extremities, L-sided version of eyes followed by diminished vision; bilateral tonic-clonic seizures	R temporo-occipital	R temporo-occipital	Continuous spikes R lateral temporal, R parietal and lateral temporal	Baso-temporal / lateral and insula	R temporo-occipital hypometabolism	Not performed	No abnormalities*	No abnormalities	No abnormalities	R temporal lobectomy	mMCD type 2	Follows

EEG=electroencephalogram; ECOG= electrocorticography; MEG= Magnetoencephalogram; FDG-PET= Fludeoxyglucose (^{18}F) positron emission tomography.

Engel score: 1A = completely seizure free, 1D = only seizures after discontinuation of anti-epileptic drugs, +: on anti-epileptic drugs, -: anti-epileptic drugs discontinued. L= left-sided; R= right-sided; MTS= Mesiotemporal sclerosis; DIR= double inversion recovery sequence; FLAIR= fluid-attenuated inversion recovery sequence; GW-junction; gray and white matter junction.

*= MRI scan performed outside University Medical Center Utrecht

Six patients were operated even though pre-surgical assessment of 7T MRI did not reveal any relevant lesions. They underwent grid-ECOG-guided corticectomy and in three patients histopathological analysis revealed a dysplastic lesion (2 mMCD type 2, patient 13 and 16; 1 FCD IIa, patient 12, Table 2). With knowledge of the histological diagnosis, even by hind-sight no lesion could be seen at 7T, however temporal signal loss typical to ultra-high field acquisitions with insufficient correction methods, made assessment of the region of resection impossible in one of these patients (patient 12, Table 2). In another patient (patient 11, Table 2) normal histology was seen, even though highly localized continuous spiking patterns in ECOG registration were strongly suggestive of a small bottom of the sulcus FCD. The difficult access to this lesion precluded en-bloc resection and the deeply located lesion was removed with a surgical aspirator, most likely leading to sample error in the pathological diagnosis. Indeed, retrospectively subtle features indicative of FCD were identified on the 7T MRI.

Discussion

Of 40 patients with a clinical pre-surgical indication for 7T MRI because of refractory focal epilepsy and presumed normal or nonexplanatory lower-field MRI, in nine (23%) 7T MRI revealed a lesion, guiding further surgical decision making. Six of these nine patients have been operated upon, with confirmation of FCD ILAE type IIa (n=2), FCD IIb (n=1), mMCD type II (n=2) or mMCD with oligodendroglial hyperplasia (n=1). One patient is planned for surgical treatment under suspicion of FCD, and in one patient with suspected FCD surgery was postponed because of prolonged seizure freedom. One patient is still being evaluated.

A recent study by de Ciantis⁹⁹ evaluated the diagnostic yield of 7T MRI in 21 patients with intractable focal epilepsy and unrevealing 1.5 or 3T MRI. Structural lesions were identified in 6 (29%) of 21 patients, four of whom underwent epilepsy surgery and histopathology confirmed FCD (ILAE type IIa n=2; IIb n=1; IIIa n=1). In that study, however, only half of the six presumed MR-negative patients in whom 7T revealed a new lesion underwent previous MRI at 3T, whereas in our series all nine patients with new lesions underwent 3T MRI before. Our findings therefore truly reflect the added value of 7T over the current standard, 3T MRI, in the pre-surgical evaluation.

Visualization of FCD

Several MRI features characterize FCD¹⁰², but with great variety in conspicuity. Especially in mMCD and FCD type I, changes can be very subtle⁴² and indistinguishable from signal averaging and partial volume effects. The same phenomena can hinder appreciation of blurring of the gray-white matter junction, a tell-tale feature of FCD. Sub-millimeter resolution and slice thickness greatly reduce the detrimental signal averaging and partial

volume effects. Moreover, multi-axial images are needed to be able to optimally assess the highly convoluted cerebral cortex. Subtle signal changes can be obvious in one plane but completely obscured by the cortical anatomy in another plane. In some cases even, the sole identifier can be an abnormal gyration pattern, only recognizable in high quality 3D image sets^{42,102,105}.

With current coil and processing techniques it is not possible to achieve sub-millimeter high resolutions in all essential sequences (T_1 , T_2 , FLAIR, T_2^*)^{100,106} at 3T, while maintaining tissue contrast and staying within acceptable acquisition times. At 7T this is currently possible, and is its most important strength. On the other hand advances in coil design and data processing could possibly also achieve this at lower field strengths in the future¹⁰⁷.

Inability of 7 tesla MRI to visualize dysplasia

In 30 out of 38 (79%) patients with non-lesional lower-field MRI, 7T MRI did not show epilepsy-related lesions either. Possibly there is no structural substrate, but it is known that a portion of these patients do prove to have histological abnormalities eventually⁴². In our series three patients had confirmed FCD or mild malformation of cortical development in the absence of any reported MR abnormalities at 7T, even after retrospective review. In one patient even though histology was normal, probably due to sample error, based on ECOG findings FCD is likely. In retrospect there are signs of a bottom of sulcus FCD on the 7T MRI.

There are several possible reasons why assessment of the high resolution images does not always provide the pivotal information hoped for.

Firstly, experience in 7T MRI assessment is still limited compared to 1.5 and 3T MRI, the clinical standard for many years. The added detail is sometimes a double-edged sword; partial volume effects are greatly reduced and much smaller structures are visible. But as a result the images reveal a great number of structures for which it can be problematic to differentiate between physiological clinically meaningless variation and true pathology.

Secondly, thorough evaluation of 7T examinations is relatively time consuming. With constructed slice thickness of 0.5 mm, a completed scan protocol easily produces up to 4000 slices. Possibly lesions are missed if no extra time is reserved for the clinical evaluation. The number of slices and thus evaluation time could be reduced by increasing slice thickness and reducing reconstruction planes, but this would likely negate the advantage of performing 7T MRI.

Thirdly, with the increase in field strength come more artefacts⁹⁷. Magnetic susceptibility effects and field inhomogeneities cause artefacts and signal loss. Temporal lobes are particularly problematic with varying, but occasionally severe signal loss making assessment impossible. Several techniques to counter these artefacts are already successfully used and more are under development. A rather simple but effective tool we routinely use since 2015 are the dielectric calcium titanate pads with passive RF shimming qualities which

have shown to greatly reduce signal loss in the temporal lobes¹⁰¹. With growing clinical and technical experience, one would expect the diagnostic value of 7T MRI to further increase.

Strengths and weaknesses

We chose to evaluate 7T MRI in daily clinical practice, with the conclusion of the multidisciplinary meetings as final assessment. This design reflects how the added information from 7T MRI affects decision making and how its use may benefit patient care. Naturally this setting does not allow for a formal and perfectly objective comparison of the diagnostic accuracy between 7T and lower field strength, which would require systematic scoring by multiple observers in a controlled environment. For the decision for surgical candidacy, clinical information and ancillary tests are combined for more robust assessments. We are convinced that lesion detection on MRI is more sensitive with fewer false-positives when taking supporting information and other tests in account. Omitting this aspect in evaluating the value of 7T can lead to results that are not fully applicable to clinical practice. However, 7T MRI scans are offered later in the diagnostic work-up, and consequently in 17 of 40 (43%) additional ancillary test were available during the 7T review. This was true for five of nine (55%) patients with new 7T MRI findings. The added information available during multidisciplinary assessment of the 7T scans may have contributed to a better detection rate. Moreover, acquisition time is 45-50 minutes at 7T, compared to 25 minutes for standard protocols at 1.5 or 3T in our center. Hence, the longer acquisition is an additional benefit (leading to either an increase in signal-to-noise, or higher resolution) on top of the increase in field strength.

Conclusion

Our study demonstrates that the use of ultra-high field MRI with an extensive scanning protocol can improve lesion detection, compared to the current clinical standards. It seems worthwhile to further invest in improving MRI techniques for patients with focal refractory epilepsy. In our series of 40 patients with refractory focal epilepsy, multidisciplinary evaluation of 7T MRI identified clinically relevant additional malformation of cortical development lesions in nine patients (23%). 7T MRI delivers on the promise to improve detection of focal cortical dysplasia and mild malformation of cortical development in patients with intractable epilepsy.

Supplementary table 1. Scan Parameters.

		TE ms	TI ms	TR ms	FA degrees	Acquired resolution mm	Matrix (AP x RL) x slices	Acquisition time min:sec
7T	3D T_1w TFE	2.9	1200	9	6	0.81/0.81/0.80	248 x 312 x 475	9:34
	3D T_2w TSE	302	-	3200	90	0.70/0.70/0.70	356 x 357 x 543	10:24
	3D T_2^*w	27	-	88	24	0.50/0.50/0.50	480 x 381 x 600	7:23
	3D MP-FLAIR	300	2200	8000	90	0.80/0.82/1.00	312 x 304 x 380	8:40
	3D WMS	2.0	600	6.73	4.5	0.80/0.80/0.80	320 x 320 x 474	8:42
3T	s T_1 SE	15	-	870	70	0.90/1.12/3.00	256 x 205 x 31	3:00
	t dual TSE SPAIR	10/80	-	2881	90	0.65/0.87/4.00	352 x 210 x 26	4:07
	t DW	68	-	3298	90	0.96/1.21/4.00	240 x 190 x 26	1:09
	c FLAIR	120	2800	11000	90	0.65/0.84/4.00	336 x 210 x 40	6:03
	3D T_1 FFE	3.3		6.6	8	0.60/1.10/1.20	400 x 200 x 300	5:31
	3D T_1 FFE gado	3.3		6.6	8	0.60/1.10/1.20	400 x 200 x 300	5:31
1.5T	s T_1 SE	12	-	604	90	0.98/1.62/4.00	256 x 154 x 27	1:34
	t dual SE	30/100	-	2369	90	0.90/1.28/6.00	256 x 171 x 19	6:47
	t DW SSH	81	-	2576	90	1.80/2.99/6.00	128 x 77 x 19	0:15
	c T_2 FLAIR TSE	140	2600	11000	90	0.90/1.27/4.00	256 x 144 x 40	5:08
	s T_1 FFE	4.6	-	25	30	1.00/1.00/1.00	240 x 240 x 175	4:52
	s T_1 FFE gado	4.6	-	25	30	1.00/1.00/1.00	240 x 240 x 175	4:52

t = transverse; c = coronal; s = sagittal; TFE = Turbo field echo (gradient echo); TSE = Turbo Spin Echo; T_2^* w = T_2^* weighted (susceptibility contrast weighted); FFE = Fast Field Echo (gradient echo); MP-FLAIR = magnetization prepared – FLAIR¹⁰⁸; WMS = White Matter Suppression sequence (gradient echo); SE = Spin Echo; SPAIR = Spectral selection Attenuated Inversion Recovery (fat suppression) DW = diffusion weighted; gado = gadolinium intravenous contrast; dual = dual echo; SSH = single-shot

Supplementary table 2. Characteristics and findings ancillary tests of all 40 patients.

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
1	♀/44	26	Focal impaired awareness seizures, hyperkinetic	R frontal parasagittal	R frontal parasagittal	Continuous spiking, R frontal parasagittal	Not performed	No abnormalities	Not performed	Not performed	No abnormalities	Subtle blurring, hyperintensity on DIR R frontal parasagittal	R Partial lobectomy frontal parasagittal	FCD IIb	1A + 5yrs.
2	♂/7	4	Focal impaired awareness seizures, R hand automatisms	R frontal parasagittal	R frontal parasagittal	Continuous spiking, R frontal parasagittal	Not performed	No abnormalities	Not performed	Not performed	No abnormalities	R frontal small area with subtle blurring of G/W boundary, subtle white matter hyperintensity (T2)	R frontal lesionectomy	FCD IIa	1A+ 1/2yr
3	♀/40	24	Focal impaired awareness seizures, dysphasia and alexia, focal to bilateral tonic-clonic seizures	L temporal	L temporal	Interictal diffuse L temporal, ictal neocortical L mid-temporal	Spikes neocortex L medial temporal gyrus	No abnormalities	Not performed	No abnormalities	No abnormalities	subcortical hyperintensities (FLAIR + T2), blurring of GW-junction L anterior superior temporal gyrus	Lesionectomy L medial temporal gyrus	mMCD with oligo-dendroglial hyperplasia	1A+ 1yr.
4	♀/22	8	Focal impaired awareness seizures, visual aura; focal to bilateral tonic-clonic seizures	Onset mid-frontal	Mid-frontal	Not performed	Multifocal, at distance from visual cortex	L occipital hypermetabolism	Not performed	No abnormalities	No abnormalities	Subtle white matter signal changes, transmantle sign-like configuration L inferior occipital gyrus	Under evaluation	n/a	n/a
5	♂/21	15	Focal impaired awareness seizures, stops activity, staring, L-sided version, automatisms	L frontal	L frontal	Continuous spiking, L medial frontal gyrus	L frontal epileptic activity	L temporal hypometabolism	L frontal (consistent with 7T MRI) and less pronounced L parietal	Not performed	No abnormalities	L frontal operculum/inferior frontal sulcus abnormal gyration and subtle transmantle hyperintensity (WMS), enhanced venous vasculature in sulcus (T2*)	Cortectomy L medial frontal gyrus	mMCD type 2, sample error likely, no examination of center of MRI lesion	1A+ 4 months

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
6	♂/14	4	Focal impaired awareness seizures, hyperkinetic	L frontal	Ictal onset L frontal	Continuous spiking depth of L superior frontal sulcus (depth electrode)	L frontal spikes	No seizure focus	Not performed	No abnormalities *	No abnormalities	L frontal abnormally deep sulcus with blurring GM-junction, cortical thickening and subtle transmantle sign	Lesionectomy L middle frontal gyrus	FCD IIb	1A+ 1 ½ months
7	♂/14	10	Focal impaired awareness seizures, nocturnal seizures with automatisms; bilateral tonic-clonic seizures	Not performed	L centro-parietal	Not performed	L parietal epileptic activity	L parietal hypometabolism	Not performed	Not performed	No abnormalities	L parietal radial band of poorly demarcated white matter signal changes	Lesionectomy with ACOG planned	n/a	n/a
8	♀/28	15	Focal impaired awareness seizures, arrest of activity; focal to bilateral tonic-clonic seizures	L temporal	Bilateral fronto-centro-temporal	Follows	Not performed	L temporal, angular gyrus hypometabolism	Not performed	No abnormalities	No abnormalities *	Abnormal intraparenchymal venous pattern posterior L medial temporal gyrus.	Grid planned	n/a	n/a
9	♂/15	11	Focal impaired awareness seizures, L sided head version, irresponsiveness, dysphasia, amnesia; bilateral tonic-clonic seizures	Non-lateralizing	Multifocal	Diffuse L temporal, parietal	L baso-temporal, up to posterior temporal	No abnormalities	Not performed	MTSL + dubious blurring GWM-junction and decreased volume L temporal	MTSL + dubious blurring GWM-junction and decreased volume L temporal *	MTSL + abnormal gyration L anterior temporal and enhanced venous vasculature (T2*)	NVS after initial grid registration. Ultimately selective L anterior lobectomy + hippocampectomy	mMCD type 2, no MTS	4B+ ½yr
10	♀/28	0	Focal impaired awareness seizures, automatisms, expressive dysphasia	L mid temporal sharp waves	L mid temporal spikes	L anterior temporal neocortical + hippocampus	Not performed	L temporal hypometabolism	Not performed	Possible MTSL	MTSL + unclear demarcation of GWM-junction temporal lobe	MTSL + unclear demarcation of GWM-junction temporal lobe. No indication of dual pathology	L anterior temporal lobectomy + amygdalohippocampectomy	MTSL ILAE class 2 + FCD IIIa	1A- 1yr.
11	♀/15	9	Focal aware seizures, R-sided version eyes/head; bilateral tonic-clonic seizures	L fronto-central	No distinct epileptiform activity	Ictal onset L fronto-central	L fronto-central spikes	No abnormalities	Not performed	No abnormalities	No abnormalities	No abnormalities	Lesionectomy L pre-central sulcus	Normal tissue (suspect for sample error, FCD in bottom of sulcus)	1A- 2yrs.

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
12	♂/14	7	Focal impaired awareness seizures, tonic; focal to bilateral tonic-clonic seizures	R fronto-central	R posterior temporal	Continuous spiking R baso-temporo-occipital	R temporal spikes	R baso-temporo-occipital hypometabolism	Not performed	No abnormalities *	No abnormalities	No abnormalities (severe signal loss in temporal lobes)	R baso-temporo-occipital lesionectomy	FCD IIa	1A- 1Yr.
13	♂/18	14	Focal impaired awareness seizures, hyperkinetic; focal to bilateral tonic-clonic seizures	No distinct epileptiform activity	No distinct epileptiform activity	No interictal activity, ictal medial frontal gyrus	No abnormalities	R frontal hypometabolism	Not performed	No abnormalities *	Not performed	No abnormalities	Cortical resection R medial frontal gyrus	mMCD type 2	1D+ 5Yr.
14	♀/22	11	Focal impaired awareness seizures, sensory dysphasia, derealization, sensations in left foot; focal to bilateral tonic-clonic seizures	R posterior baso-temporal	R posterior temporal	Bursts R posterior temporal, sporadic spikes R hippocampus	R hemisphere, no clear localization	Dubious hypometabolism R operculum	Not performed	Not performed	No abnormalities	No abnormalities	Resection posterior baso-temporal, fusiform gyrus	Normal, but suboptimal assessment due to fragmentation	1A+, 1/2Yr.
15	♂/33	20	Focal impaired awareness seizures, auditory phenomena, R-sided head version, confusion; focal to bilateral tonic-clonic seizures	L posterior temporal	Aspecific irregularities bilateral fronto-temporal	Sporadic spikes L lateral temporal	Not conclusive	L baso-temporo-parietal, hypometabolism in large area	Not performed	Not performed	No abnormalities *	No abnormalities	Cortical resection L superior and middle temporal gyrus	No abnormalities	1A+ 1 month
16	♂/16	12	Focal impaired awareness seizures, myoclonia all extremities, L-sided version of eyes followed by diminished vision; bilateral tonic-clonic seizures	R temporo-occipital	R temporo-occipital	Continuous spikes R lateral temporal, sporadic spikes R parietal and medial temporal	Baso-temporal / lateral and posterior insula	R temporo-occipital hypometabolism	Not performed	No abnormalities *	No abnormalities	No abnormalities	R temporal lobectomy	mMCD type 2	Follows

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
17	♀/11	9	Focal impaired awareness seizures, nightly hyperkinetic seizures, seizures with R-sided head version, head shaking, leg-kicking, drooling	Frontal onset	Frontal L>R	Follows	Not performed	hypometabolism R parieto-frontal and superior temporalis gyrus	Re anterior insula (SISCOM)	Not performed	No abnormalities	Grid + depth electrode follows	n/a	n/a
18	♂/10	7	Focal impaired awareness seizures, nightly seizures with dystonia L arm (and face), screaming, hyperkinesia	Not localizing	Not localizing	Follows	No abnormalities	No abnormalities	Ictal hyperperfusion R superior parietal lobule and one on the L frontal convexity. Ictal, interictal, SISCOM	Not performed	No abnormalities	SEEG follows	n/a	n/a
19	♀/33	22	Focal impaired awareness seizures, lowered consciousness, light-headedness, sensory symptoms L hand, perseveration, L-sided version head and eyes; focal to bilateral tonic-clonic seizures	R centro-parietal/ fronto-central	R centro-parietal and posterior temporal	Follows	R cingulum/ precuneus and parietal just posterior of sensory hand area	Large area with hypometabolism R temporal, parietal and occipital	Ictal hyperperfusion R parietal / cuneus	Not performed	No abnormalities	Grid follows	n/a	n/a
20	♂/13	11	Focal to bilateral tonic-clonic seizures; myoclonia R arm, post-ictal weakness R arm	Mid-parietal, fast spread to centro-parietal	L mid-parietal and parietal parasagittal	Follows	L high parietal, parasagittal	L high parietal	not performed	Not performed	No abnormalities	Grid follows	n/a	n/a

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
21	♀/17	6	Focal impaired awareness seizures, fixed gaze, R-sided automatisms, urinary urgency; bilateral tonic-clonic seizures	Spikes R frontal > L frontal	Possible R temporal onset	Follows	No abnormalities	R temporal	R temporal and R parietal hypoperfusion in subtrac-tion (ictal, interictal, SISCOM)	No abnormalities* (1T)	Not performed	No abnormalities	SEEG follows	n/a	n/a
22	♂/12	6	Focal impaired awareness seizures, hyperkinetic, feeling of strangulation, vomiting, speech arrest, comprehension intact	L fronto-central	L fronto-temporal	Follows	L low fronto-central	L low fronto-central	Follows	No abnormalities* (1T)	No abnormalities	No abnormalities	Possibly SEEG follows after SPECT	n/a	n/a
23	♀/17	12	Focal impaired awareness seizures, epigastric aura, speech arrest, staring; (focal to bilateral tonic-clonic seizures at debut)	L fronto-temporal	Bifrontal	Not performed	No abnormalities	No abnormalities	Follows	No abnormalities	No abnormalities	No abnormalities	Under evaluation	n/a	n/a
24	♂/48	35	Focal impaired awareness seizures, goosebumps, rising epigastric sensation, dysphasia, stops activity, automatisms, amnesia; bilateral tonic-clonic seizures	R fronto-temporal	Fronto-lateral and fronto-temporal	Not performed	Not performed	No abnormalities	Follows	Not performed	No abnormalities*	No abnormalities	Under evaluation	n/a	n/a
25	♂/16	5	Focal to bilateral tonic-clonic seizures, L-sided head and eye version	R Frontal	Generalized, frontal maximum	Not performed	Spikes R fronto-central	Hypometabolism small focus R parietal convexity	No clear focus (ictal, interictal, SISCOM)	Not performed	No abnormalities*	No abnormalities	Under evaluation (EEG-MRI follows)	n/a	n/a

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
26	♂/34	26	Focal aware seizures, epigastric rising sensation, palpitations, goosebumps, depressed mood, sweating, derealization; bilateral tonic-clonic seizures	L temporal	L temporal	Not performed	Not performed	No abnormalities	On hold / Follows	No abnormalities	No abnormalities	No abnormalities	On hold / under evaluation	n/a	n/a
27	♀/18	9	Focal impaired awareness seizures; bilateral tonic-clonic seizures	Mid-frontal, right frontal	Mid-frontal, right frontal	Not performed	Mid-frontal, right frontal frequent discharges	L fronto-parietal	L fronto-temporal interictal hyperperfusion (interictal, ictal failed)	No abnormalities	No abnormalities	No abnormalities	No clinical improvement	n/a	n/a
28	♀/27	8	Focal impaired awareness seizures, nocturnal, hyperkinetic, followed by irresponsiveness, pain R hand	L centro-parietal	L temporal	Not performed (declined because of suspected motor deficit after resection)	Not performed	No abnormalities	Hypoperfusion L sensory hand area, hyperperfusion L orbitofrontal (ictal, SISCOM)	Not performed	No abnormalities	No abnormalities	Declined surgery because of expected motor deficit	n/a	n/a
29	♂/26	14	Focal aware seizures, awareness L hand convulsions L hand and shoulder; focal impaired awareness seizures	Right temporo-parietal	No Abnormalities	Seizure onset zone in hand motor cortex	Inconclusive, diffuse abnormalities	No abnormalities/possible very small abnormality	Not performed	No abnormalities	No abnormalities	No abnormalities	Multiple subpial transections hand motor area	n/a	n/a
30	♀/9	6	Focal impaired awareness seizures, convulsions R arm, L-sided head version, fear, salivation, dysphasia	L seizure onset, incidentally R	Fronto-central L>R	Not performed	R hemisphere	No abnormalities	L perisylvian ictal hyperperfusion (ictal, interictal)	Not performed	No abnormalities	No abnormalities	NVS (no clear localization)	n/a	n/a

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
31	♀/17	14	Focal impaired awareness seizures, loss of vision, hypotonia, post-ictal dysphasia	L fronto-lateral, mid-temporal	L temporo-parietal to occipital	Continuous spikes L parietal, sporadic spikes L inferior temporal, bursts L centro-parietal	Continuous spikes L hemispheric. Seizure onset temporo-occipital junction	No abnormalities	Not performed	No abnormalities* (1T)	No abnormalities	No abnormalities	Rejection after grid registration	n/a	n/a
32	♀/27	11	Focal impaired awareness seizures, hyperkinetic; bilateral tonic-clonic seizures	Frontal onset	Mid-frontal	SEEG: no clear focality	Mid-frontal	L fronto-to-basal hypometabolism	L frontal, singular gyrus in subtraction (ictal, interictal, SISCOM)	No abnormalities*	No abnormalities	No abnormalities	Rejection (after SEEG)	n/a	n/a
33	♂/20	1	Focal aware seizures, fear, tonic clonic movements in all limbs, preserved consciousness	L fronto-central	Frontal parasagittal	Not performed	L fronto-temporal	No abnormalities	Not performed	No abnormalities	Not performed	No abnormalities	Rejection	n/a	n/a
34	♀/16	5	Focal impaired awareness seizures, jerks and R-sided head version, automatisms, perseveration; bilateral tonic-clonic seizures, R sided version	Bifrontal, fast spread to R hemisphere.	R frontal	Not performed	Not performed	Dubious hypometabolism L superior and medial frontal gyri	Failed (ictal)	No abnormalities	Not performed	No abnormalities	Rejection	n/a	n/a
35	♂/15	3	Focal impaired awareness seizures, twitching L mouth corner, L and upward gaze, blinking, salivation, grunting, pain in R tongue; bilateral tonic-clonic seizures	L frontal	Desynchronization	Not performed	L frontal	No abnormalities	Hyperperfusion L pre-central mouth area / frontal (ictal, interictal, SISCOM)	Not performed	No abnormalities*	No abnormalities	Rejection	n/a	n/a

Pat no.	Sex /age	Age at debut	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Histopathology (ILEA type)	Outcome (Engel Score)
36	♀/11	8	Focal impaired awareness seizures, heavy breathing, salivation, feeling of strangulation, vomiting, inability to speak	L central and temporal spikes	Not conclusive	Not performed	L frontal operculum	No abnormalities	Rolandic frontal operculum to insula hyperperfusion (ictal, interictal, SISCOM)	Not performed	No abnormalities*	No abnormalities	Rejection (Suspicion of malignant Rolandic Epilepsy)	n/a	n/a
37	♂/15	6	Focal impaired awareness seizures, R-sided head version, unresponsiveness, sweating, urinary incontinence	R Frontal	R frontal/fronto-temporal	Not performed	R temporal abnormalities	R temporal hypometabolism	Ictal hyperperfusion R hemisphere (SISCOM)	No abnormalities	Not performed	No abnormalities	Rejection	n/a	n/a
38	♂/38	15	Focal to bilateral tonic-clonic seizures, onset with myoclonia without loss of consciousness	bilateral spikes, frontal onset	Bifrontal	Not performed	Not performed	Not performed	Not performed	Not performed	No abnormalities*	No abnormalities	Rejection	n/a	n/a
39	♂/11	6	Focal impaired awareness seizures, jerks of the mouth, speech arrest, intact awareness	R fronto-central, frontal operculum	R fronto-central	Not performed	Not performed	Not performed	Not performed	No abnormalities*	No abnormalities	No abnormalities	Rejection (Suspicion of atypical Rolandic Epilepsy)	n/a	n/a
40	♀/28	12	Focal impaired awareness seizures, nightly seizures, myoclonia R arm and leg	L frontal parasagittal	Frontal parasagittal L>R	Not performed	L hemisphere, no precise localization possible	No abnormalities	Not conclusive (ictal, interictal, SISCOM)	No abnormalities*	No abnormalities	No abnormalities	Rejection	n/a	n/a

EEG=electroencephalogram; ECOG= electrocorticography; MEG= Magnetoencephalogram; FDG-PET= Fluorodeoxyglucose (¹⁸F) positron emission tomography.

Engel score: 1A = completely seizure free, 1D = only seizures after discontinuation of anti-epileptic drugs, +: on anti-epileptic drugs, -: anti-epileptic drugs discontinued. L= left-sided; R= right-sided; MTS= Mesiotemporal sclerosis; DIR= double inversion recovery sequence; FLAIR= fluid-attenuated inversion recovery sequence; GW-junction; gray and white matter junction.

*= MRI scan performed outside University Medical Center Utrecht

7 TESLA T_2^* -WEIGHTED MRI AS A TOOL TO IMPROVE DETECTION OF FOCAL CORTICAL DYSPLASIA - A CASE SERIES

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Focal cortical dysplasia is one of the most common underlying pathologies in patients who undergo surgery for refractory epilepsy. Absence of a MRI-visible lesion necessitates additional diagnostic tests and is a predictor of poor surgical outcome.

We describe a series of six patients with refractory epilepsy due to histopathologically proven focal cortical dysplasia, of whom pre-surgical 7 tesla T_2^* -weighted MRI was acquired. In four out of six patients T_2^* sequences showed areas of marked superficial hypointensity co-localizing with the epileptogenic lesion. 7 tesla T_2^* hypointensities overlying focal cortical dysplasia may represent leptomeningeal venous vascular abnormalities associated with the underlying dysplastic cortex. Adding T_2^* sequences to the MRI protocol may aid in the detection of focal cortical dysplasias.

Introduction

Focal cortical dysplasia (FCD) is one of the most common underlying pathologies in patients who undergo surgery for refractory epilepsy. A substantial percentage of these lesions is not visible with a routine 1.5 tesla (T) or 3T MRI; 37% for FCD type I and 15% for type FCD type II in a review of surgical series from 2000 to 2008; 36% and 2% in a 2000-2007 UCLA series of 97 patients⁷⁰. No visible lesion on MRI is a predictor of poor surgical outcome and necessitates additional diagnostic studies which may include invasive intracranial electrode registrations⁹¹. Advances in imaging techniques are expected to improve the detection of epileptogenic lesions.

Susceptibility-based contrast MRI sequences are sensitive to the susceptibility effect created by the paramagnetic property of deoxyhemoglobin in blood, and thus augment the visibility of the cerebral venous microvasculature¹⁰⁹. Sequences as T_2^* and Susceptibility-Weighted Imaging (SWI) have been proven useful in the detection and characterization of a variety of cerebral vascular abnormalities (e.g. Sturge-Weber Syndrome, cavernous malformations), as well as hemorrhagic, calcified or iron-containing lesions¹¹⁰. Reports on susceptibility-based contrast sequences in epilepsy patients are limited and mostly describe the benefits for detection and characterization of calcified lesions, not for the detection of otherwise inconspicuous lesions, such as FCD¹¹¹. In this series we illustrate the potential of the T_2^* MRI sequence at 7T in assisting the detection of FCD.

Methods

We retrospectively selected six consecutive patients from the Dutch Epilepsy Surgery Program with refractory epilepsy due histopathologically confirmed FCD, of whom T_2^* images at 7T were acquired pre-surgically between November 2008 and November 2014. These patients either had lesions suggestive of FCD on 3T (n=2) or had normal 3T MRIs, with semiology, electroencephalogram and telemetry findings strongly suggesting a structural focal abnormality (n=4). 3T MRI was deemed normal if no indications for structural abnormalities were identified on assessment by expert neuroradiologists and during review by the Dutch Epilepsy Surgery Program board – that includes neurosurgeons, neurologists, and neuroradiologists with expertise in the field of epilepsy. 3T MRI-negative patients underwent additional pre-surgical evaluation, including 7T MRI and PET, SPECT, or MEG in order to localize the epileptogenic zone. In addition 7T MRI was used to further characterize the lesions for the two subjects with lesions on 3T.

The 7T MRI protocol included (conventional) 3D FLAIR, 3D Double Inversion Recovery, 3D T_1 and 3D T_2 -weighted sequences and a previously described T_2^* -weighted sequence¹¹². Parameters used for the gradient echo T_2^* MRI were: isotropic 0.5 mm resolution, echo time 27ms, flip angle 24°, repetition time 57–93 ms (shortest possible), with EPI and flow

compensation. For two patients scans were acquired with 0.6mm resolution (isotropic), echo time 20ms, flip angle 20° and repetition time 25-26ms. Images were acquired on a Phillips 7T system (Philips Healthcare, Cleveland, OH, USA) with a volume transmit and 16- or 32-channel receive head coil (Nova Medical, Wilmington, MA, USA).

7T T_2^* images were reviewed for abnormalities co-localizing with epileptogenic zones. To aid visual detection of hypointense structures, minimum intensity projections of T_2^* sequences were constructed with 5mm slab thickness. Following resective surgery, tissues were histopathologically classified according to ILAE guidelines¹³.

Informed consent was obtained for use of patient data. The report complies with the declaration of Helsinki.

In addition, we reviewed 7T T_2^* images of eight control subjects (four male, four female, age 27 ± 4) (data first published in Neuroimage, 2011¹¹²).

Case Series

Four of six patients had normal 3T MRI findings; in two of these, conventional 7T MRI sequences (T_1 , T_2 , Double Inversion Recovery, and FLAIR) sequences revealed a lesion suggestive of FCD. In two other patients, abnormalities were seen both on 3T and on 7T images. All six patients underwent resective surgery and had histopathological confirmation of FCD (ILAE type Ib in patient II, type IIa in patient V and VI, type IIb in patient I and IV, and mild Malformation of Cortical Development type 2 in Patient III). In four of these patients (patients I-II *Figure 1A*, patients III-IV *Figure 1B*) 7T T_2^* showed areas containing marked hypointensities with a branched, partly tortuous configuration, and a signal compatible with venous blood, suggestive of increased venous vasculature in the sulci neighboring the malformed cortex.

Because the described T_2^* signal changes appeared to be located in the leptomeningeal tissue or subarachnoid space overlying the malformed cortex, we looked for a histopathological correlate in the leptomeninges of the operated patients. Unfortunately, due to the locations of the malformations, respectively in the bottom of a sulcus and interhemispherically, en-bloc resection with intact leptomeningeal structures was only possible in one patient (III). This lesion was classified as mMCD type 2, with fibrotic and thick meninges containing prominent vascular structures. (*supplementary figure 1*).

In summary, hypointensities on T_2^* were identified in four out of six consecutive patients with histologically proven FCD of whom 7T T_2^* images were available. *Table 1* lists clinical and imaging characteristics of all six patients.

Figure 1A. MRI Findings of patients with proven FCD and T_2^* signal changes.

7T T_2 (A-D), T_2^* (E-H) and T_2^* minimum intensity projection (I-L), transverse reconstructions. Lesions depicted in detail in B, D, F, H, J and L.

Patient I; FCD ILAE type IIb (A, B, E, F, I, J). No lesion identified on 3T MRI, subtle gray-white matter junction blurring is seen on 7T T_2 weighted MRI (A, B). On T_2^* (E, F) the neighboring sulcus seems to contain prominent vasculature. T_2^* minimum intensity projection (I, J) aids in the visual detection. Patient II; FCD ILAE type Ib (C, D, G, H, K, L). T_2 -weighted MRI (C, D) shows grey-white matter junction blurring and cortical thickening indicative of FCD. In the same area T_2^* (G, H) shows a wide sulcus with prominent vascular structures. T_2^* minimum intensity projection (K, L) strongly emphasizes the increased vasculature.

On 7T T_2^* images and corresponding minimum intensity projections of eight healthy volunteers, symmetrical venous leptomeningeal vascular structures are clearly visible but the phenomenon described above was not identified. (See *supplementary Figure 2* for 7T T_2^* images of a healthy subject).

Discussion

We describe the observation of 7T T_2^* hypointensities overlying pathologically proven (n=4) epileptogenic malformations of cortical development. We propose that these signal abnormalities reveal a pathological change in the leptomeningeal venous vasculature. The

Figure 1B. MRI Findings of patients with proven FCD and T_2^* signal changes.

7T T_2 (A-D), T_2^* (E-H) and T_2^* minimum intensity projection (I-L), coronal reconstructions. Lesions depicted in detail in B, D, F, H, J and L.

Patient III; mild malformation of cortical development type 2 (A, B, E, F, I, J). On T_2 (A, B), blurring and subcortical hyperintensity represent developmental malformation. T_2^* (E, F) shows a wide Sylvian fissure but no clearly appreciable vascular changes. On T_2^* minimum intensity projection (I, J) there appears to be an increase in vascular signal in the superior temporal pole.

Patient IV (C, D, G, H, K, L); FCD ILAE type IIb. On T_2 (C, D) notable large extracerebral space central parasagittal containing a large vein, but without evident dysplastic characteristics but. T_2^* (G, H) shows the large vein and smaller vasculature that drains from the dysplastic cortex (as proven by histology). Enhanced conspicuity on T_2^* minimum intensity projection (K, L).

location in sulci and on the cortical surface, the configuration, and a T_2^* signal compatible with venous blood all support this hypothesis. The origin of the observed T_2^* hypointensities could be either, or a combination, of the following:

1. Increased blood volume may be the result of increased vascular diameter or density in the context of a developmental anomaly of the leptomeningeal vasculature, parallel to the developmental malformation of the underlying cortex. The leptomeningeal vascular network is formed from a gestational age of 8 weeks, starting as the pial capillary anastomotic plexus which produces penetrating vessels into the cortex¹¹³. It is conceivable that in addition to immaturity and abnormal migration of neurons, the vascular network may show analogous

Table 1. Characteristics of patients with proven or suspected FCD and abnormal findings on T₂*.

Pat no.	Sex /age	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG ictal epileptiform discharges	Electro-cortico-graphy	3T MRI (T1, T2, FLAIR)	7T MRI conventional sequences (T1, T2, FLAIR, DIR/WMS)	T2* T1	SPECT/PET	Surgery	Histopathology	Outcome (Engel Score)
I	♀/45	CPS, hypermotor	R frontal parasagittal	R front parasagittal	Continuous spiking, R frontal parasagittal	No abnormalities	Subtle blurring, hyperintensity on DIR R front parasagittal	Hypointensities R inferior frontal parasagittal	No abnormalities (PET)	R Partial lobectomy frontal parasagittal	ILAE FCD IIb	1A + 1yr
II	♀/22	SPS, sensory mouth/jaw	no epileptiform discharges	no epileptiform discharges	Bursts and continuous spiking R inferior frontal	R inferior frontocentral cortical thickening		Hypointensities R inferior frontal	n/a	R inferior frontocentral lesionectomy	ILAE FCD IIb	IIb + 2yr
III	♀/25	SPS, olfactory, gustatory and experiential auras	L temporal	Bilateral fronto-temporal	Sporadic spiking mesiotemporal and anterotemporal. Continuous spiking baso-temporal	L anterior temporal subcortical T2, DIR		Hypointensities superior anterior temporal pole	n/a	L anterior temporal lobectomy + amygdalohippocampectomy	mMCD type 2 (no hippocampal sclerosis)	1B + 3yr
IV	♀/12	SPS, Left leg sensorimotor	Centroparietal midline	Centroparietal midline	Continuous spiking R medial pre- and post-central	No abnormalities		Hypointensities in wide sulcus R central	No abnormalities (PET)	R pre-central lesionectomy	FCD IIb	1A-1/2yr
V	♂/15	CPS, left motor. sec GS: left sided tonic-clonic seizures	R frontocentral	R posterior temporal	Continuous spiking R baso-temporo-occipital	No abnormalities		No abnormalities	R basotemporo-occipital hypometabolism, (PET)	R basotemporo-occipital lesionectomy	FCD IIa	1A-1yr
VI	♂/7	CPS, R hand automatisms	R frontal	R frontal parasagittal	Continuous spiking R frontal parasagittal	No abnormalities	R frontal small area with subtle blurring of G/W boundary, subtle white matter hyperintensity (T2)	No abnormalities	No abnormalities (PET)	R Frontal lesionectomy	FCD IIa	1A+ 1/4yr

EEG = electroencephalogram; DIR = double inversion recovery; L = left; R = right. CPS = complex partial seizures; SPS = simple partial seizures; TCS = tonic-clonic seizures; GS=generalized seizures; G/W = gray-white matter junction;
HS = hippocampal sclerosis; FCD = focal cortical dysplasia; mMCD = mild Malformation of Cortical Development; N/a = not applicable;
Engel scores 1A+ = Completely seizure-free since surgery, continued use of anti-epileptic drugs; 1A- = Completely seizure-free since surgery, anti-epileptic drugs discontinued; 1B+ = Non-disabling simple partial seizures only, continued use of anti-epileptic drugs; 2B+ = Rare disabling seizures, continued use of anti-epileptic drugs.

abnormalities in dysplastic cortex, resulting in abnormal configuration of superficial cortical vasculature.

2. Paroxysmal increased metabolic demand associated with epileptic activity¹¹⁴ may lead to reactive vascular changes in the form of hypervascularization. An increase in venous vascular diameter or density results in a greater deoxygenated blood volume per voxel and concomitant T_2^* changes.

3. A focal increase in deoxyhemoglobin may be explained by altered hemodynamics. Other studies showed that interictal hypometabolism was associated with decreased perfusion on arterial spin labelling MRI¹¹⁵, while cortical activation is generally linked to positive blood oxygen dependent signal changes caused by an increase in oxygenated blood¹¹⁶. Decreased perfusion in epileptogenic lesions may lead to an interictal increase in venous deoxyhemoglobin, visible as T_2^* hypointensity.

The clinical relevance of the described phenomenon is that it might be indicative of underlying epileptogenic cortex and even distinctive in cases where conventional MRI sequences reveal no abnormalities.

Two additional patients with refractory epilepsy evaluated in our center had clinical and electrophysiological characteristics indicative of a structural seizure focus but no supportive findings on 3 and 7T MRI. Retrospective review of 7T MRI T_2^* images showed hypointensities that might have provided clues for localizing the seizure focus (data not shown). Lack of concordance between other ancillary diagnostic results prevented surgical treatment.

Comparison of T_2^* -weighted sequences acquired at 7T and at lower field strength was not possible since this sequence is not routinely performed on 1.5 or 3T MRI in epilepsy patients in our center. Fine vascular structures should be better detectable on 7T systems because of the higher spatial resolution and the positive relation between field strength and T_2^* effects¹⁰⁹. Because of the nature of the pathology and the still experimental status of 7T MRI it is difficult to obtain T_2^* images in large groups of patients for assessing the presence of the described phenomenon.

An important limitation is that we report a qualitative analysis of the T_2^* signal change. Furthermore histopathological validation of changes in leptomeningeal tissues is limited, because leptomeninges could only be assessed in one surgical specimen. While the impression of increased vasculature on T_2^* images is congruent with the leptomeningeal histological findings (thick and fibrosed meninges containing large caliber vessels) this may be a non-specific finding in patients with epilepsy. To elucidate the histopathological substrate for this potential marker of subtle epileptogenic lesions and assess its clinical

relevance, studies on larger groups of patients are needed, including histopathological analysis of leptomeningeal vascular structures.

Despite its limitations, this case series suggests that it may be worthwhile further exploring the possibilities of using T_2^* sequences at 7T – and possibly also at lower field strength – to improve the detection of FCD in patients with refractory epilepsy.

Conclusion

We report a novel, 7T MR T_2^* finding co-localizing with FCD. This hypointense signal may indicate an increase in vascular prominence in the leptomeningeal vascular network overlying the dysplastic cortex. Adding T_2^* -weighted sequences to the 7T MRI protocol may aid in the detection of FCDs and guiding effective epilepsy surgery, while obviating the need for additional (invasive) diagnostic tests.

Supplementary figure 1. Histology.

A-B: Normal neocortex (HE, hematoxylin and eosin). B-C (HE) and D (CD34): neocortex from patient III with thick (focally fibrotic) leptomeninges and prominent vascular structures. E-F (NeuN): neocortex from patient III without cortical dyslamination, but with microscopic neuronal clusters (arrow; insert in F) and excess of neurons of normal morphology in the in deep white matter (isolated mild malformations of cortical development, mMCD Type 2). Scale bar: A, B, E, F: 320 μm ; C, D: 160 μm .

Supplementary figure 2. Transverse 7T T_2^* images of a healthy subject.

T_2^* -weighted images, transverse slices parallel to the anterior commissure–posterior commissure line at the level of the foramen of Monroe (A) and at the level of the cingulate sulcus (B). Minimum intensity projections with 5mm slab thickness of the level of A (C) and B (D). These images show the normal appearance of venous cerebral vasculature in a healthy subject.

CHANGES IN VASCULAR DENSITY IN RESECTED TISSUE OF 97 PATIENTS WITH FOCAL CORTICAL DYSPLASIA OR TSC-RELATED CORTICAL TUBERS

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Recent studies suggested a possible association between malformations of cortical development and microvascular density. In this study we aimed to further elucidate the relation between microvascular density and cortical developmental abnormalities in a cohort of 97 patients with epilepsy and histologically proven mild malformation of cortical development (mMCD), focal cortical dysplasia (FCD) or tuberous sclerosis complex (TSC). Surgical tissue samples were analyzed with quantitative measures of vessel density, T-cell response, microglial activation and myelin content. Subsequently, the results were compared to an age- and localization matched control group. We observed an increase in microvasculature in white matter of TSC cortical tubers, which is linked to inflammatory response. No increase was seen in mMCD or FCD subtypes compared to controls. In mMCD/FCD and tubers, lesional cortex and white matter showed increased vascular density compared to perilesional tissues. Moreover, cortical vessel density increased with longer epilepsy duration and older age at surgery while in controls it decreased with age. Our findings suggest for that the increase in white matter vascular density might be pathology-specific rather than a consequence of ongoing epileptic activity. Increased cortical vessel density with age and with longer epilepsy duration in mMCD/FCD's and tubers, however, could be a consequence of seizures.

Introduction

Epilepsy in young people is often caused by malformations of cortical development¹⁰, some of which are focal in nature and therefore termed focal cortical dysplasia (FCD). The International League Against Epilepsy (ILAE) published a new classification system for FCD which acknowledges three subtypes of FCD¹³. Type I is characterized by abnormal cortical layering, either by persistent radial micro-columnar architecture (Ia) or by a disruption of the horizontal lamination (Ib) or both (Ic). Type II is characterized by severe disorganization of the cortex and dysmorphic neurons (IIa), and balloon cells in addition (IIb). FCD type III refers to FCD in combination with other pathologies, such as hippocampal sclerosis (FCD type IIIa), glial or glioneuronal tumors (FCD type IIIb), vascular malformations (FCD type IIIc) or any other principal lesion acquired during early life (FCD type IIId).

The most subtle architectural abnormalities in the FCD spectrum are classified as mild malformations of cortical development (mMCD), characterized by excessive number of neurons in the molecular layer (type 1) or white matter (type 2) without the presence of any other structural abnormalities – in contrast to FCD I types and II types^{13,14}. mMCD as separate entity is subject to debate, but was included in the FCD ILAE classification, albeit as addendum¹³. Our group's recent surgical cohort study demonstrated differences between patients with mMCD and FCD histological diagnoses in clinical presentation and surgical outcome, in support of this clinical differentiation¹¹⁷.

A syndrome associated with refractory epilepsy that begins often in early childhood is Tuberous Sclerosis Complex (TSC). TSC is a genetic disorder caused by mutations in either the *TSC1* or *TSC2* gene expressed as hamartomas or benign tumors in several organ systems^{17,118}. In the brain TSC can cause subependymal nodules, subependymal giant cell astrocytomas and cortical tubers which are typically associated with seizures. Tubers found in TSC are histologically often indistinguishable from FCD type IIb¹³. Calcifications may differentiate tubers from FCD, but since over two-thirds do not exhibit this stigma, it is not a reliable distinguishing feature¹¹⁹.

In both isolated mMCD/FCD of all types, and in TSC, surgical removal of the epileptogenic lesion can result in seizure freedom^{10,120}. Because many of these lesions are difficult to spot or outline on conventional MRI, or multiple in nature, it is often difficult to determine in which patients surgery is indicated. Therefore, it is of clinical importance to investigate associations between radiological features of FCD, histological characteristics and clinical outcomes. Recently 7 tesla MR imaging suggested a possible association between FCD and microvascular density^{121,122}, which could possibly be used as diagnostic marker for dysplastic lesions.

Evidence for increased vasculature in FCD lesions was found by Wintermark et al. using quantitative histological techniques and Arterial spin labelling (ASL)-MRI¹²³. In contrast, Blauwblomme et al. found disorganized vascular structure but no increased density, and in their study PET-CT and ASL-MRI suggested lesional hypo- rather than hyperperfusion¹²⁴. ASL MRI showed hypo and hyperfused tubers in patients with tuberous sclerosis, and while it was uncertain if hyperperfusion reflected epileptogenicity, a larger number of hyperperfused tubers in a patient was associated with increased seizure frequency¹²⁵.

Uncertainty remains whether there is true increased vascular density in FCD or tubers, and if these changes would be an intrinsic etiological factor of epileptogenesis, part of the underlying malformation, or a result of aberrant neuronal activity. In this study we aimed to further elucidate the relation between microvascular density and cortical developmental abnormalities in a cohort of patients with histologically proven mMCD/FCD or TSC, and with possible etiopathological factors.

Material & Methods

All patients who underwent epilepsy surgery between 2000 and 2012 at the University Medical Center Utrecht and had an initial histopathological report compatible with current mMCD/FCD subtypes were included. The pathological diagnosis was reviewed and established according to the currently available classification schemes for lesions associated with therapy-refractory epilepsy^{13,14}. Patients with complex malformations (e.g. polymicrogyria, hemimegalencephaly) and associated cortical dysplasia (FCD III types) were omitted from the study.

Cortical tuber samples were added to the cohort by selecting patients from the database operated between 2002 and 2013 with confirmation of TSC through genetic testing and of whom tissues samples were available after resective epilepsy surgery.

In addition to TSC gene defects, (combinations of) multiple cortical or subependymal abnormalities, cutaneous stigmata or typical abnormalities in kidneys, heart or lung lead to a clinical TSC diagnosis, following the standard diagnostic criteria for TSC¹²⁶.

The age- and localization-matched control group consisted of 14 autopsy cases. None of these patients had a history of seizures or other neurological diseases. All control samples were collected at the Department of (Neuro)Pathology, AMC, Amsterdam, The Netherlands.

Tissue was obtained and used in accordance with the Declaration of Helsinki and the AMC Research Code provided by the Medical Ethics Committee and approved by the science committee of the UMC Utrecht Biobank.

Tissue preparation and immunohistochemistry

The tissue was carefully oriented, cut perpendicular to the pial surface, fixed overnight in 4 % formaldehyde and routinely processed into liquid paraffin. Sections were cut at 4-6 μm with a microtome (Microm, Heidelberg, Germany), and mounted on positively charged slides (Superfrost + Menzel, Germany). Each specimen was histopathologically examined using hematoxylin & eosin (H & E). The revised diagnosis was based on following commonly used immunohistochemical stainings: NeuN, SMI32, Vimentin, GFAP, and MAP2. The tissue samples were reclassified according to the 2011 ILAE classification system. Additional stainings were performed when necessary.

The immunohistochemical examinations for the quantitative analysis of all surgical specimens was performed using the following antibodies: CD34 (cluster of differentiation 34, 1:600, Qbend, Immunotech) endothelial staining for vascular density, CD3 (cluster of differentiation 3, 1:200, clone F7.2.38, DAKO) for T-cell presence, Cr3/43 (HLA-DP, DQ, DR antigen, DAKO) for microglia activation, GFAP (glial fibrillary acid protein, 1:4000, Dako, Glostrup, Denmark) for gliosis, MBP (myelin binding protein, 1:400 DAKO) for myelination, NeuN (neuronal nuclei 1:100, clone A60, Chemicon, Billerica, MA, USA) for neurons, non-phosphorylated neurofilament H (SMI32) (1:1000, clone SMI32, Sternberger, Lutherville). The semi-quantitative analysis of blood brain barrier disruption was performed using albumin staining (Albumin Rabbit polyclonal 1:20,000 DAKO, Glostrup, Denmark). The slides were air dried overnight at 37°C. All immunohistochemical stainings were performed with a Ventana semi-automated staining machine (Benchmark ULTRA; Ventana, Illkirch, France) and the Ventana DAB staining system according to the manufacturer's protocol.

Quantitative measurements

The CD34 stained slides were digitized using an IntelliSite Ultra Fast Scanner (Phillips healthcare Best, the Netherlands). Other stainings were digitized with an Olympus dotSlide system (vs 2.5, Olympus, Tokyo, Japan). In both scanners the magnification size was set on 100x and pixels size was 0,64 μm^2 . The slides were converted to Tagged Image File Format (TIFF) files. Every file was subsequently divided into evenly spread tiles. In Photoshop (Version: 2015.1.2) regions of interest (ROI's) with a median size of 0.83 mm^2 (range: 0.23 – 6.5 mm^2) were selected for the analysis. The selected ROI's were evenly spread within cortex, subcortical white matter (white matter was defined as being >500 μm away from the grey white matter border), lesion and, when available, perilesional tissue. Perilesional tissue was defined by the absence of aberrant cell forms such as dysmorphic neurons and balloon/giant cells (for FCD II or TSC) and by normal cortical architecture (FCD subtypes and TSC). The lesional border in mMCD is less obvious, due to the subtle characteristics; perilesional tissue shows no excessive neurons in layer 1 or white matter.

The available images were color deconvoluted according to the following RGB values: red: 0.26814753, green: 0.57031375 and blue: 0.77642715. The resulting 8bit image was converted to a black and white mask (black: positively stained area, white: no staining). To discard background noise, a grey intensity value (GI) for each individual stain was selected.

Quantitative analysis of cells and microvasculature

To improve the accuracy and to avoid counting vessels or cells multiple times, a size range in μm^2 was selected for each staining and no watershed filter was used. Microvessel count was performed using CD34 (GI: 210, Pixel: 30-unlimited μm^2), including capillaries and parenchymal arterioles. For the count of inflammatory cells, CD3 was used (GI: 160, Pixel: 10-unlimited μm^2). Neuronal cell bodies were counted with NeuN (GI: 180, Pixel: 30-unlimited μm^2). To analyze the amount of dysmorphic neuron, SMI32 was used (GI: 50, Pixel: 100-unlimited). All images were automatically processed via FIJI (ImageJ2, v64, open source) with a specifically developed macro based on Java programming language. Semi-automated quantification methods have been validated in our lab and described previously¹²⁷.

Initial analysis revealed differences in vascular densities between tubers and controls that prompted further analysis of potential additional and explanatory contrasts or relationships regarding neuroinflammation, blood brain barrier dysfunction, and myelin content in tubers.

Quantitative analysis of area size

For area size analysis the total area of the ROI was determined using Image J. Subsequently the GI was set to remove background noise. Finally, field fraction of positively stained area was calculated. CR3/43 was used to measure microglial activation. To determine the number of reactive astrocytes, GFAP was used. Myelin content was examined using MBP. The GI for all area size stainings was set on 180. The images were processed with a specific macro-based field fraction Java programming language in FIJI.

Semi-quantitative scoring of balloon/giant cells and blood brain barrier damage

The number of balloon/giant cells was scored on high power fields using an Olympus BX 50 microscope (each representing 1.081mm^2). The area of highest density was taken into account. We assessed the total coverage of abnormal cells within the grid area resulting in the following scores: I: 0-25%, II: 25-50%, III: 50-75% and IV: 75-100%. Blood brain barrier damage was also analyzed according to the same technique as used in earlier studies¹²⁸⁻¹³⁰. The intensity of the albumin staining was scored using a semi-quantitative scale ranging from 0-3 (0: negative, 1: weak, 2: moderate, 3: strong reactivity).

Quantitative and automated methods were not suitable for scoring balloon/giant cells or albumin extravasation due to non-specific staining patterns.

Clinical data

Clinical patient data was collected for age at surgery, seizure duration in years and location of resected tissue (frontal, temporal, parietal or occipital).

Statistical analysis

Statistical analysis was performed with SPSS version 22 and 25 (IBM, PASW Statistics, USA). Descriptive statistics were used for the primary analysis: counts, mean, standard deviation. Vascular density counts were tested for normal distribution using Shapiro-Wilk test and for homogeneity of variances (scatterplot predicted versus observed). Test for normality (Shapiro-Wilk, $p < 0.001$) and homogeneity of variances (scatterplot predicted versus observed for vascular density and age) – failed for white matter vascular density. Therefore, we performed a natural logarithmic transformation for white matter vascular density. Grey matter and the log-transformed white matter vascular density showed normal distribution and homoscedasticity. T-test was used to assess differences in vascular densities between sex. We used multivariate analysis of covariance (MANCOVA) to test dependence of grey and white matter vascular density on age, region, epilepsy duration and consequently for differences between pathologies and for groupwise comparison of lesional versus perilesional densities. Multiple testing in post-hoc analysis was corrected for with Hochberg or Dunnett's test because of differences in sample sizes. Correlation was tested with Pearson's test, Kendell-Tau for correlation with semi-quantified balloon cell density and Spearman's rho for semi-quantifications of albumin leakage.

Paired-T test was performed to assess lesional versus perilesional vessel densities within the same patients.

Results

Patient characteristics are summarized in table 1. In total 97 patients with either mMCD, FCD or TSC were included (31 mMCD, 52 FCD, 13 TSC). Median age at surgery was 14 years (range 3 months – 56 years). Three patients with FCD underwent a second surgical resection because of recurrent seizures.

Control tissue vessel density

As control group we included 14 cortical post-mortem samples (median age 15 years, range 2-42), sex: 9 male; location: 4 frontal, 8 temporal, 2 occipital). For 7 patients perilesional samples were also available (1 FCD Ib, 1 FCD IIa, 2 FCD IIb and 3 TSC). "Perilesional" was defined as cortex surrounding a main FCD II or TSC lesion without presence of giant cells or dysmorphic neurons. Subtle cortical abnormalities may be present. The group size of all pathologies included was highly variable, therefore we fused the groups into mMCD (n=31), FCD I (n=10), FCD IIa (n=18), FCD IIb (n=28) and TSC (n=13). Quantifications of vascular density are provided in table 2.

Table 1. Patient characteristics.

Diagnosis	Number of patients	Gender ♂ / ♀	median age at surgery yrs. (range)	Localization tissue samples F T P O				Lesional ^a	Perilesional ^a	median epilepsy duration yrs. (range)
mMCD	31	13 / 18	23 (1-48)	9	17	4	1	32	0	10 (1-32)
mMCD type 1	7	3 / 4	32 (19-48)	4	3	0	0	8	0	16 (7-32)
mMCD type 2	24	10 / 14	19 (1-46)	5	14	4	1	24	0	7 (1-30)
FCD I	9	3 / 6	13 (7 months – 56 yrs.)	3	5	1	0	9	1	5 (1-42)
FCD Ia	4	2 / 2	2 (7 months – 56 yrs.)	0	3	1	0	4	0	2 (1-42)
FCD Ib	4	1 / 3	14 (5- 40)	2	2	0	0	4	1	7.5 (2-23)
FCD Ic	1	0 / 1	32	1	0	0	0	1	0	23
FCD II	44	25 / 19	13 (3 months – 46)	32	7	4	1	46	3	7.5 (0-37)
FCD IIa	17	11 / 6	13 (3 months-45 yrs.)	12	4	1	0	18	1	6 (0-37)
FCD IIb	27	14 / 13	13 (8 months-41 yrs.)	20	3	3	1	28	2	9 (1-36)
TSC	13	7 / 6	9 (10 months-47	9	4	0	0	14	3	5 (1-35)
total	97	48 / 49	14 (3months-56 yrs.	53	33	9	2	101	7	8 (0-42)

Overview of the patients, gender distribution (♂ = male, ♀ = female) lesion type and location, epilepsy duration in years. The different pathology types are mild malformation of cortical development (mMCD), Focal cortical dysplasia (FCD) and Tuberous Sclerosis Complex (TSC). The different brain regions are Frontal (F), Temporal (T), Parietal (P), Occipital (O).

^a Note, for certain patients only lesional, perilesional tissue or both were available for analysis. The sum of lesional and perilesional samples exceeds the number of patients due to three patients that underwent surgery twice and seven patients with both lesional and perilesional samples available for analyses.

In post-mortem control tissue we measured a mean vascular density of $154.3 \pm 40.8/\text{mm}^2$ in cortex and in white matter a median density of $65.2/\text{mm}^2$ (range 44.6-118.3). To further characterize vascular density in controls, we first compared vascular density between men and women and found no significant differences in cortex ($t(12) = 1.026$, $p = 0.325$), or white matter ($t(12) = 1.346$, $p = 0.195$).

White matter and cortical vascular density were correlated ($r = 0.551$; $p = 0.041$).

In multivariate regression analysis we found a significant association between vascular densities and age ($F(2,9) = 5.252$; $p = 0.031$; Wilk's $\lambda = 0.461$) and brain region ($F(4,18) = 3.988$; $p = 0.017$; Wilk's $\lambda = 0.280$). Corrected for region, there was a negative association between age and cortical vessel density ($F(1) = 11.512$; $p = 0.007$; $r(14) = -.704$, $p = 0.005$) (figure 1 and figure 2a) but not between age and white matter vessel density ($F(1) = 2.289$; $p = 0.161$) (figure 1 and figure 2c). There was a significant effect of region on cortical vascular density for cortex ($F(2) = 11.024$, $p = 0.03$), correcting for age. Occipital regions ($221.0 \pm 23.0/\text{mm}^2$) had highest cortical vascular density followed by frontal ($168.9 \pm 16.8/\text{mm}^2$) and temporal regions ($130.4 \pm 29.2/\text{mm}^2$) (no parietal control samples).

Patient tissue vessel density

In the analysis of vascular density in lesional tissue of 97 patients no sex difference was detected either (cortex T-test $p = 0.771$; white matter T-test $p = 0.917$).

Corrected for age, there were significant regional differences in cortex ($F(3) = 8.021$, $p < 0.0005$) but not in white matter ($F(3) = 1.482$, $p = 0.224$) vascular density. Highest cortical vascular density was found occipitally (mean 184.57 ± 57.02 vessels per mm^2), followed by frontal lobe (mean 162.24 ± 40.36), parietal lobe (mean 147.41 ± 20.53) and temporal lobe cortex (mean 122.53 ± 43.60). In post-hoc analysis – omitting age as co-variate – there were significant differences between frontal and temporal (LSD, $p < 0.0005$, Hochberg $p < 0.0005$) and between temporal and occipital lobe cortical vascular densities (LSD, $p = 0.041$, Hochberg = 0.220).

Corrected for region, vessel density in lesional samples was positively associated with age at surgery ($F(2,93) = 7.163$ $p = 0.001$) and with epilepsy duration ($F(2,93) = 5.400$, $p = 0.006$) in

Figure 1. Cortical vascular density in healthy controls declines with age, $p = 0.007$. The temporal region of a 2-year-old (a), 17-year-old (b) and 42-year-old subject (c) scale bar in d = 100 μm .

Figure 2. Vascular density in cortex (**a,b**) and white matter (**c,d**) in controls, mMCD, FCD and TSC samples in relation to age at surgery (**a,c**) and epilepsy duration (**b,d**). There is no relation between age and epilepsy duration and vascular density in white matter (**c,d**), while in cortex density declined with age in controls (**a**) and increased with age and epilepsy duration in lesions, most pronounced in TSC (**b,d**).

Table 2. Vascular densities in cortex and white matter.

	vascular density in cortex, count/mm ²				vascular density in white matter, count/mm ²			
	mean ± SD	median	range	p	mean ± SD	median	range	p
controls	154.3 ±40.8	154.1	73.7- 237.2	0.057 ^a	69.1 ±17.4	65.2	44.6-118.3	0.866 ^a
Lesional	147.2 ±44.4	142.3	5.51 – 277.4		76.3 ±51.1	62.8	32.2-348.7	
perilesional	116.7 ±39.4	99.0	73.7- 192.0		69.0 ±23.3	65.8	41.4-99.2	
frontal (controls)	168.9 ±16.8	164.7	154.3- 191.8	0.03 ^b	65.6 ±10.5	66.8	51.7-76.9	0.579 ^b
temporal (controls)	130.4 ±29.2	133.1	73.7- 166.3		66.0 ±11.7	65.1	44.6-80.5	
parietal (controls)	-	-	-		-	-	-	
occipital (controls)	221.0 ±23.0	204.7	204.7- 237.2		88.6 ±42.0	88.6	58.9-118.3	
frontal (lesional)	162.2 ±40.4	167.4	71.0- 233.1	<0.0005 ^b	84.2 ±56.9	67.7	32.2-348.7	0.224 ^b
temporal (lesional)	124.5 ±43.8	123.2	5.3- 277.4		67.2 ±48.6	56.3	32.4-325.3	
parietal (lesional)	140.8 ±28.5	142.3	81.2- 181.7		66.9 ±16.7	62.1	48.5-106.5	
occipital (lesional)	184.6 ±57.0	184.6	144.3- 224.9		54.0 ±5.0	54.0	50.5-57.6	
mMCD (lesional)	145.1 ±39.6	139.0	55.3- 233.1	0.495 ^a	57.0 ±12.7	56.2	32.2-81.8	<0.0005 ^a
FCD I (lesional)	143.2 ±41.7	135.2	89.0- 220.6		59.5 ±8.3	61.8	44.8-71.1	
FCD IIa (lesional)	153.3 ±58.3	168.2	5.3- 231.0		60.7 ±11.3	62.4	35.6-77.2	
FCD IIb (lesional)	149.5 ±30.9	151.4	96.3- 221.7		73.6 ±11.3	62.4	35.6-77.2	
TSC	143.0 ±59.1	131.3	52.5- 277.4		146.2 ±98.2	106.8	41.3-348.7	

^a Mancova, corrected for age and region^b Mancova, corrected for age

mMCD: malformation of cortical development. FCD: focal cortical dysplasia. TSC: tuberous sclerosis complex

cortex (figure 2a,b), but not in white matter (figure 2c,d). The positive relation between age and cortical vascular density was more pronounced in TSC (figure 2b).

Corrected for age and region we found no significant vascular density differences in groupwise comparison between lesional samples and controls ($F(2,104)=0.982$, $p=0.399$, Wilk's $\lambda=0.928$).

Groupwise comparison between lesional (147.2 ± 44.4 /mm²) and perilesional (116.7 ± 39.4 /mm²) cortical density showed a trend for higher density of lesional tissues, regardless of age and region ($F(1)=4.025$, $p=0.048$). No difference was found in white matter vascular densities ($F(1)=0.152$, $p=0.698$).

In a pairwise analysis of the seven patients of whom both lesional and perilesional samples were available we found significantly higher vascular densities in the lesional cortex ($t(6)=3.156$, $p=0.02$) (figure 3a) and in the lesional white matter ($t(6)=2.733$, $p=0.034$) (figure 3d) compared to perilesional tissue (lesional cortex 160.2 ± 30.4 , perilesional cortex 116.7 ± 39.4 /mm²; lesional white matter median 96.7, range 52.9-206.0, perilesional white matter median 65.8 range 41.4-99.2 /mm²). In all individual cases lesional cortex had a higher vascular density than the neighboring perilesional tissue. In five of seven white matter pairs, vascular density was higher in the lesional tissue.

We compared vessel densities between the different pathologies with grouping of mMCD (type 1 and 2), FCD I (a, b and c), FCD IIa, FCD IIb and cortical tubers in TSC. Corrected for age and region there were significant differences between pathologies in lesional vascular density ($F(10,184)=5.478$, $p<0.005$, Wilk's $\lambda=0.594$). This is due to significant differences in white matter vessel densities across pathologies ($F(5)=9.569$, $p<0.0005$). No differences were found in cortical density ($F(5)=0.884$, $p=0.495$). Post-hoc testing showed that only TSC samples (median 106.8, range 41.3-348.7 mm²) had a significantly higher white matter vascular density compared to control samples (median 65.2, range 44.6-118.3 mm², Dunnett, $p<0.0005$) (figure 4a). Perilesional samples were excluded from these analyses.

There was no correlation between balloon cell density and vessels in FCD IIb and TSC (Kendall-tau, grey matter $p=0.679$, white matter $p=0.196$).

FCD IIb and cortical tubers have identical histological features. However, we observed a difference in white matter vascular density between FCD IIb and tubers. Corrected for age, white matter vascular density was significantly different ($F(2,36)=9.683$, $p<0.0005$, Wilk's $\lambda=0.650$), with higher densities in TSC (median 106.8 range 41.3-348.7 mm²) compared to FCD IIb (median 62.4, range 35.6-77.2) ($F(1)=16.481$ $p<0.0005$). Cortical vessel density did not differ between FCD IIb and tubers.

Figure 3. Lesional vs perilesional vascular densities in cortex (**a**) and white matter (**d**). Significant difference in vessel density (per mm²) in pairwise analysis between lesional and perilesional grey matter ($p=0.02$) (**a**, mean and 1 std for bar and whiskers), and white matter ($p=0.034$) (**d**, median for bar, non-parametric data). A characteristic feature of type IIb focal cortical dysplasia is the presence of dysmorphic neurons (**b**) (as shown by NeuN staining, in grey matter). Perilesional grey matter (**c**) does not contain dysmorphic neurons. Lesional vessel density in FCD IIb (**e**) (as shown by CD34 staining) compared to perilesional vessel density (**f**) in grey matter. Scale bar in **f** = 100 μ m and also applies to **b**, **c**, **e**, **f**.

Because cortical tubers of the TSC cohort showed a significantly higher number of vessels in white matter compared to controls, we continued to examine the tuber samples with different stainings which were mainly focused on the role of inflammation and the damage of the blood brain barrier (BBB). Because of the lack of differences between the FCD and control groups, we did not examine more stainings within FCD or mMCD groups.

TSC samples showed a positive correlation between vascular density in white matter and microglial activation (Cr3/43 positivity, $r(10)=0.716$, $p=0.02$) (figure 4e).

White matter vascular density tended to correlate with inflammatory cell response (CD3 activity, $r(10)=0.563$, $p=0.09$).

Inflammatory cell response significantly correlated with microglial activation in white matter ($r(10)=0.823$, $p=0.003$). We did not find a correlation between vascular density and myelin content.

Figure 4. Histological subgroups, vascular density and microglia activation. **a** Increased white matter vessel density in TSC compared to control in white matter, $p < 0.001$ (box shows median and 25th and 75th percentile, whiskers min-max). Vessel density in white matter in a healthy control (**b**) and a cortical tuber in a patient with TSC (**c**). micro-vasculature is clearly visible with CD34 endothelial staining (**d**). increased vessel density is accompanied with increased microglia activation in a tuber (**e**), $p < 0.05$. Microglial activation in control (**f**) vs TSC (**g**). Microglial activation can clearly be seen with Cr3/43 staining (**h**). Scale bar in g = 100µm and also applies to b-c and f-g.

We then performed a semi-quantitative analysis of the BBB leakage, with albumin as marker. Albumin deposits were observed in grey and white matter in all TSC samples (figure 5), but there was no significant correlation between quantities of extracellular albumin and vascular density.

Discussion

This study provides the first description of relationship between microvascular density and cortical developmental abnormalities in a large cohort of patients with either cortical dysplasia or cortical tubers in TSC.

We found that cortical and white matter vascular density is increased in FCD and tubers compared to the surrounding perilesional cortex, irrespective of age. While in healthy controls cortical vascular density decreased with age, in epilepsy patients with FCD or tubers density appeared to increase with age and with longer epilepsy duration. No time-relation was seen in white matter vascular density. Tubers had an increased microvascular

Figure 5. Albumin deposits in TSC. **a** Deposits of albumin in grey matter indicating damage to the blood brain barrier. **b** similar deposits can be seen in white matter *Scale bar in b* = 100µm and also applies to **a**.

density in white matter compared to age-matched controls. The latter was associated with microglial activation and an increased number of inflammatory cells.

In both healthy subjects and in patients there were significant regional differences in cortical vascular density. The highest densities were found in the occipital region, followed by frontal, parietal and temporal locations. This is likely linked to the regional differences in brain metabolism^{131,132}. Moreover, several studies have indicated that the same area consists of a higher numbers of neurons which are necessary for the visual processing^{133–136}. However, the high level of variance amongst the existing studies does not allow clear-cut conclusions concerning topography of vascular density. As described by Riddle et al., a more robust analysis of the different regions in the brain, with a standardized method is needed, to see if this finding is consistent in the general population¹³⁷.

Furthermore, our data suggests not only differences in vascular density between brain regions, but also variation with age due to constant remodeling of the brain, a process thought to be mainly based on the functional needs of the brain^{113,138}.

Although there is not yet a clear consensus on the effect of age on vascular density, a decline has been observed in several studies¹³⁷. Correspondingly, we found a decrease in cortical vascular density with advancing age in controls.

In contrast to normal cortex, in resected malformations of cortical development – most pronounced in cortical tuber – we observed an increase in vascular density with ageing. This could suggest that certain processes in lesional or epileptogenic cortex drive ongoing

angiogenesis and increasing vascularity, rather than that the higher vessel density is static and reflects an intrinsic part of the developmental abnormality. Moreover, in contrast to cortical vascular density, vessel density in white matter was related to pathology specific processes. Compared to controls, increased density was only observed in tubers and not in FCD subtypes.

We did not perform rater variability analysis for the mMCD/FCD reclassification, but prior research has shown good intra-observer reliability, good interobserver agreement for FCD II subtypes and moderate agreement for non-FCD and FCD Ia en Ib types in the evaluation according to the 2011 ILAE classification¹³⁹. However, we acknowledge that no such attempt has been made for the mMCD so far and therefore this group remains rather heterogeneous, albeit with distinct clinical features¹¹⁷.

During brain development, early migration in particular, neuronal progenitor cells associate with blood vessels of the vascular plexus in the (sub)ventricular zone—which develops relatively early in embryogenesis—and migrate abluminally to their target location in the cortex. This vasophilic migration mechanism is one of several involved in cortex formation^{140,141}. Analogously, neuroblast association with microvessels is a phenomenon that is nowadays widely accepted as one of the main drivers of the massive infiltration in human gliomas, proving that vessel sprouting and the mode of neuronal migration can be re-activated any time¹⁴².

Interestingly it is however that severe cortical disorganization is not invariably associated with aberrant vessel architecture or density, as shown in studies investigating the development of cortical microvasculature in *reeler* mice, exhibiting normal vasculature patterns and abnormal, inverted, laminar position of cortical neurons¹⁴¹. Nevertheless the observation that newly formed neurons and glia cells strongly associate with the vascular plexus and are involved in migration processes suggest there is possible interaction between developing vasculature and cortex formation, which could have pathomechanistic significance¹⁴¹.

In tubers, TSC1 or TSC2 gene dysfunction causes disinhibition of mTOR activity, which leads to upregulation of HIF-1 α expression, increased vascular endothelial growth factor (VEGF) production and angiogenesis¹⁴³. Tubers are dynamic processes and serum VEGF levels in TSC patients are related to proliferation of lesions. VEGF production appears to be secondary to mTOR activation and is rapidly reversed by rapamycin, a mTOR inhibitor^{118,143}. This is also compatible with the observation of increasingly high vascular densities at older age.

BBB dysfunction is known to occur as a result of epileptic seizures and reciprocally plays a role in epileptogenesis and can sustain or aggravate epilepsy¹⁴⁴. Almost all TSC samples showed evidence of BBB dysfunction, with extravasation of albumin. In addition, there

was an increase of T-cells that are not able to infiltrate the cortex and white matter without leakage of the BBB¹⁴⁵. However, we did not find a correlation between quantifications of microvascular density and BBB leakage.

Tubers exhibit complex activation of pro-inflammatory pathways, including chemokines, interleukin-1 β , HMGB1 (high-mobility-group-box-1), TLR (toll-like-receptor), RAGE (receptors for advanced glycation end products) and complement mediated pathways¹¹⁸. Local seizure activity triggers the release of a multitude of vasoactive substances which alter neurovascular coupling and can cause oxidative stress which both contribute to BBB dysfunction. In addition to promoting angiogenesis, VEGF increases BBB permeability through direct induction of inflammation¹⁴⁶ and activation of matrix metalloproteases which break vessel walls and degrade tight junctions¹⁴⁷.

Alternatively, extravasated serum proteins are phagocytized by activated microglia and astrocytes¹⁴⁸, elicit an inflammatory response¹⁴⁹ and in turn possibly drive angiogenesis. In TSC samples we found a correlation between white matter vascular density and microglial activation, which co-occurred with T-cell activation. This supports a relation between inflammation and the observed angiogenesis.

Even though angiogenic processes and BBB dysfunction in epilepsy are generally considered detrimental for brain tissue and progression of disease, these could also be seen as protective mechanisms. Fast VEGF release, through FLK-1/AKT pathways, and activation of nitrous oxide-synthase, mediates vasodilation through nitrous oxide release^{150,151}. In combination with increased vasculature these processes help to ensure adequate perfusion in tissue with increased metabolic demand due to epileptic activity.

We had hypothesized vascular density would be higher in lesional tissue compared to both control and perilesional samples due to processes related to epileptic activity and the underlying structural abnormality, and have no explanation for why we instead found no overall difference in vascular density between lesions and control samples, and lower perilesional vascular density.

Recent 7 tesla MRI studies showed an increased prominence of vascular structures co-localizing with cortical malformations in polymicrogyria¹²² and focal cortical dysplasia¹²¹. These vascular structures appeared to be mostly localized in the leptomeninges and sulci bordering the lesions. This was apparent on susceptibility-weighted contrast series, and therefore it could not be determined if this prominence was due to hemodynamic or structural changes, or a combination of both. Unfortunately, it was not possible to examine leptomeningeal vasculature in the current study, since meninges were not sufficiently preserved in the surgical specimens.

Although in our study a significant increase vascular density in FCD lesions compared to normal controls could not be found, we did see an increase in vessel density in the FCD lesion compared to peri-lesional tissues.

Our findings do not provide conclusive support there are vascular structural changes that could be the substrate of possible identifying or localizing imaging features of FCD. Hemodynamic and consequent blood oxygen level variations possibly better explain vascular prominence on T_2^* MR images.

ASL-MRI would have added valuable information regarding hemodynamic changes and possible correlation with vascular density and cytoarchitectural abnormalities, especially since prior studies showed conflicting results^{123–125}. Unfortunately, ASL-MRI was not part of the standard pre-surgical diagnostic work-up and the retrospective nature of this study precluded collection of this data.

Sun et al.¹⁵² observed fine vascular structures in white matter of tubers on 7 tesla susceptibility weighted images. This imaging feature is compatible with the findings of our study, and potentially could be of use in characterizing tubers. It remains to be clarified if this conveys information on epileptogenicity of individual tubers.

Conclusion

Cortical vascular density decreases with age in healthy controls, while it increases with age and with epilepsy duration in patients with epilepsy due to FCD or tubers in TSC. White matter vascular density was not correlated with age or epilepsy duration. In dysplasia and tubers lesional brain tissue showed higher vascular density compared to perilesional tissue. Compared to controls, we observed an increase in microvasculature in white matter of TSC cortical tubers, which was linked to an inflammatory response.

The increased white matter vascular density of tubers compared to FCD suggests that it is the underlying pathology, rather than seizure activity itself, that explains differences in vascular index. The lesional cortical vascular density increase with age and duration of epilepsy, however, appears to suggest a direct effect of ongoing epileptic activity.

CORRELATION BETWEEN IN VIVO AND EX VIVO MULTIMODAL HIGH FIELD MRI AND HISTOPATHOLOGICAL PARAMETERS IN CORTICAL DYSPLASIAS

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Introduction

In patients with refractory and focal lesional epilepsy, surgical resection can be considered. Presurgical evaluation of epilepsy has the main objective of defining the epileptogenic zone, including the location and extent of a brain structural abnormality¹⁵³. MRI is an important diagnostic tool for identification of structural abnormalities. When cortical structural abnormalities are suspected current best clinical practice is to use epilepsy specific protocols at 3T that include 3D T1 and FLAIR sequences with isotropic resolution of 1 mm², with assessment by dedicated neuroradiologist and incorporating electroclinical information. Post-processing techniques should be preferably used to support visual evaluation¹⁵⁴. Unfortunately not all lesions are detected with current MRI imaging techniques, particularly so in patients suspected of minimal malformation of cortical development (mMCD) or with some subtypes of focal cortical dysplasia (FCD)^{42,70,92}. In so-called MRI negative patients, ultra high-field MRI (7 tesla) can lead to an additional lesion detection in approximately a fifth of the patients^{99,155,156}. Improving MRI lesion detection and delineation could lead to more refractory epilepsy patients becoming eligible for surgery and could improve surgical outcome.

The increased field strength of 7 tesla (T) systems translates into improving spatial resolution and contrast in clinical practice¹⁵⁷. The increased signal-to-noise ratio is generally not used to shorten the acquisition time. It remains unclear what is required to approach 100% detection, if ever feasible: should the focus be to improve spatial resolution or improve tissue contrast? Ideally, improved scanning techniques optimize specific tissue contrast based on histological changes in the targeted lesion type.

We initiated a comparative in vivo 7T MRI, ex vivo 7T MRI and histology project, in order to correlate histological characteristics of mMCD/FCD lesions with specific MRI signal changes. With ex vivo 7T MRI, extremely lengthy scanning protocols are allowed, not restricted by Specific absorption rate (SAR) levels or affected by motion artefacts.

Previous quantitative neuroimaging and histology correlative studies have shown correlations between ex-vivo MRI signal changes and neuronal density, demyelination and balloon cells in FCD II^{158,159}. In subtle dyslamination there is absence of the normal signal dip in cortical layer IV caused by the horizontal plexus and variation in cellularity and axonal density¹⁶⁰. Analyses of ex-vivo 9.4T MRI revealed subtle white matter changes related to mMCD, while these lesions escape MRI detection in vivo¹⁵⁹. These studies did not incorporate in vivo MRI in the analyses. However, Goubran et al.¹⁶¹ and Zucca et al.¹⁶² have demonstrated successful co-registration of in vivo whole brain MRI and ex vivo MRI of surgical specimen. Comparing in vivo with ex vivo MRI will demonstrate potential benefits of further increasing resolution and guide future technical developments. The eventual goal of this project is

to improve the accuracy of MRI, both in increasing sensitivity for cortical dysplasia and in defining the borders with surrounding normal brain tissue. Moreover, better recognition of specific histological characteristics on MRI should aid in predicting histological MCD subtypes based on preoperative ultra high-field (UHF) MRI.

As a first step in this project, we report on the methodological framework that was constructed to allow analyses of image data of multiple modalities, which differ considerably in resolution and contrast, and which have to be reformatted into one space.

For optimal comparative analyses it is necessary to correct for deformations that occur at several different processing steps¹⁶³. Deformations between in vivo and ex vivo MRI are caused by resection of the specimen and loss of coherence with connected structures, mechanical distortion and inhomogeneous shrinkage during fixation. Following the ex-vivo MRI the specimen is processed into histological slides during which stretching and shearing cause further deformation. As opposed to whole brain, co-registration of surgical resections poses an additional challenge since this requires part-to-whole registration¹⁶¹.

Methods

This is a prospective single centre cohort study, performed in line with the Declaration of Helsinki and Local Ethical committee approval was granted (protocol number 14-623/M). Prior to 7T MRI written informed consent was acquired from all patients or legal custodians. Surgical resection was performed exclusively for therapeutic reasons and the extent of excision was planned preoperatively and on the basis of the epileptogenic zone.

Subjects

Patients eligible for study inclusion were identified during regular multidisciplinary epilepsy surgery meetings. Patients were included if prior clinical 1.5 or 3T MRI had been performed using epilepsy dedicated scanning protocols and mMCD/FCD was suspected as underlying cause, with or without the presence of another, possibly associated primary lesion (such as mesiotemporal sclerosis, LEAT, vascular malformation or traumatic brain injury at young age, all potentially related to FCD subtype III). Patients were also eligible when standard MRI did not reveal clear abnormalities; so-called “MRI-negative” patients.

Excluded were patients with contra-indications for 7T MRI, those who were unable to undergo unsedated imaging and all children under the age of six years.

Study participants underwent additional 7T MRI imaging during their pre-surgical work-up. Patients were also invited for the study if 7T MRI had already been requested by the treating physician in the context of a suspected developmental abnormality but with normal 1.5 or 3T MRI. These “clinical” 7T scans were acquired using the same scanning protocol and the

work-flow after surgery (ex vivo MRI and histopathology) was identical to that described below.

MRI Acquisition

In vivo 7T MRI

All in vivo scans were performed on a whole-body 7 tesla MRI system (Philips Healthcare, best, Netherlands) with a volume transmit coil and 32-channel receive head coil (Nova Medical, Wilmington, Massachusetts, USA). Dielectric pads, containing calcium titanate (Leiden University Medical Center, Leiden, the Netherlands), were placed bilateral to the head to reduce artefacts and signal loss in the temporal regions¹⁰¹.

The 7T scanning protocol consists of T_1 , T_2 , FLAIR, and T_2^* -weighted sequences, and a gradient echo white matter suppression (WMS) sequence, all with 0.5–0.8 mm acquired resolution; acquisition time was circa 45 minutes. All sequences are routinely reformatted in 0.5 mm (interpolated) multiplanar reconstructions to have axial, coronal and sagittal images for all sequences, allowing for optimal appreciation of cortical architecture. This protocol covers all sequences recommended in various guidelines for the detection of epileptogenic lesions¹⁰⁰. Parameters of the in vivo 7T MRI sequences performed in our centre are described in Table 1.

Ex vivo 7T MRI

Tissue samples were scanned using the same hardware as the in vivo 7T MRI acquisition. Tissue fixation with formalin reduces tissue T_1 and T_2 relaxation times¹⁶⁴, and accordingly scanning parameters were chosen to produce images that resemble tissue contrast in vivo. Long acquisition times and less stringent SAR limitations allowed 0.18–0.40 mm isotropic resolution in combination with high tissue contrast. Image based B0 shimming was performed, and manual power optimization was used to obtain correct flip angles. Homogeneity correction based on pre-scan sensitivity maps was applied to all sequences except WMS. No SENSE acceleration was used. The used sequences and main parameters are provided in table 2.

While the T_1 , T_2 , T_2^* and WMS sequences yielded consistent high quality images, the FLAIR sequence was of variable and often mediocre quality, even after increasing the TI and TR from an initial 1150 ms and 5000 ms to 1600 and 8000 ms, respectively, and thereby drastically increasing acquisition time.

Directly after scanning the ex vivo MRI was visually assessed and the optimal orientation for image reconstruction was chosen to obtain orthogonal cutting angles through the cortical layers and underlying white matter, needed for accurate evaluation of histomorphological abnormalities. Multiplanar reconstructions (MPR) were made with 0.2 mm slice thickness.

Table 1. In vivo and ex vivo 7TMRI protocol and parameters.

		TE ms	TI ms	TR ms	FA degrees	Acquired resolution mm	Matrix (AP x RL) x slices	SEN- SE R	Acquisition time min:sec
in vivo	3D T_1 w TFE	2.9	1200	9/3500 ^b	6	0.81/0.81/0.80	248 x 312 x 475	2.6	9:34
	3D T_2 w TSE	302	-	3200	90	0.70/0.70/0.70	356 x 357 x 543	5.6	10:24
	3D T_2^*w	27	-	88	24	0.50/0.50/0.50	480 x 381 x 600	2.3	7:23
	3D MP- FLAIR	300	2200	8000	90	0.80/0.82/1.00	312 x 304 x 380	7.5	8:40
	3D WMS	2.0	600	6.73/10000 ^c	4.5	0.80/0.80/0.80	320 x 320 x 474	4	8:42
ex vivo	3D T_1 FFE	3.6	700	7.8/2000 ^d	6	0.40/0.40/0.40	348 x 384 x 100	1	1:09:37
	3D T_2 TSE	163	-	2000	90	0.40/0.40/0.40	500 x 210 x 221	1	0:44:30
	3D T_2^*w FFE 0.22m ^a	30	-	75	30	0.22/0.22/0.22	680 x 365 x 181	1	1:37:11
	3D T_2^*w FFE 0.18mm	25		75	35	0.18/0.18/0.18	823 x 445 x 222	1	1:36:43
	3D FLAIR	164	1600	8000	90	0.40/0.40/0.40	500 x 210 x 221	1	3:31:20
	3D WMS FFE	4.3	300	9.2/3000 ^e	6	0.40/0.40/0.40	348 x 199 x 111	1	2:02:33

TE = echo time; TI = inversion time; TR = repetition time; FA = flip angle; SENSE R = SENSitivity Encoding acceleration factor;

TFE = Turbo Field Echo (gradient echo); TSE = Turbo Spin Echo; $T_2^*w = T_2^*$ weighted (susceptibility weighted contrast); FFE = Fast Field Echo (gradient echo); MP-FLAIR = magnetization prepared – Fluid-attenuated Inversion Recovery ¹⁰⁸; WMS = White Matter Suppression sequence (gradient echo); SE = Spin Echo; SPAIR = Spectral selection Attenuated Inversion Recovery (fat suppression) DW = Diffusion Weighted; gado = gadolinium intravenous contrast; dual = dual echo; SSH = Single-Shot.

^a 3D T_2^*w FFE 0.22m uses 1.5 factor oversampling for improved SNR.

^b TR between excitation and inversion pulses respectively, TFE factor 245.

^c TR between excitation and inversion pulses respectively, TFE factor 233.

^d TR between excitation and inversion pulses respectively, TFE factor 60.

^e TR between excitation and inversion pulses respectively, TFE factor 36.

Sample preparation for ex-vivo 7T MRI and immunohistochemistry

Tissue samples of 17 patients were of sufficient size for further analyses and were processed according to the study protocol. One author (TV) was present during each surgical procedure to observe and register orientation of the resected specimen. Neurosurgeons in our centre strive for en-bloc resection of the lesion to aid histopathological examination. When needed, colour markings were applied. The fresh tissue was directly transported to the pathology lab and immersed in 4% buffered formaldehyde for at least 48 hours, considering a diffusion rate of 1mm per hour within the tissues. Complete fixation is essential for homogeneous MRI signal throughout the specimen¹⁶⁰ and for tissue preservation for histopathological diagnosis. The fully fixated specimen was then embedded in Agar (5%) in a rectangular mould. After cooling, the block is stable and can be handled safely. One corner of the agar block was marked in the X, Y and Z axes for later spatial reference. Barrel cactus needles were used for these purposes, which proved optimal due to their rigidity and lack of magnetic properties and susceptibility effects. The tissue and agar block was fixated in a plastic tray and submersed in Perfluoropolyether fluorinated lubricant, a highly stable protein-free substance with no MRI signal, (Fomblin®, Solvay) to further reduce imaging artefacts. The tray was then placed in the head coil for scanning. Using a spinning blade slicing machine, 4 mm slabs were cut from the Agar-tissue block. The sectioning plane was identical to the ex-vivo multiplanar reconstructions.

Resected tissue was included integrally in histology cassettes. Tissue slices were processed into liquid paraffin and sectioned at 5 µm with a microtome (Leica Biosystems, Nußloch, Germany), and mounted on positively charged slides. All tissue samples were histologically examined using hematoxylin & eosin (HE) and NeuN (NEUronal Nuclei). These examinations were essential for clinical pathological diagnosis. Based on these slides, for more extensive immunohistological characterization, 2-3 tissues blocks were selected that provided optimal tangential sectioning of the dysplastic lesion. HE and NeuN stainings were complemented with eight immunohistological stainings commonly used to diagnose and further specify focal developmental abnormalities in epilepsy patients (MAP-2, Vimentin, SMI32, SMI31, CD34, GFAP, MBP, Olig2).

For the detection of the inflammatory cells and proinflammatory pathways the following antibodies were used: NeuN (1:100, clone A60, Chemicon, Billerica, MA, USA) stains neuronal nuclei; MAP-2 (microtubule associated protein 2; 1:500 polyclonal rabbit, AB5622, Merck, Amsterdam, the Netherlands) a neuronal marker preferentially labelling dendrites. Vimentin (1:500, clone V9, Dako, Glostrup, Denmark) stains type III intermediate filament (IF) protein and is a marker for balloon cells, the hallmark feature of type IIb dysplasia; SMI32 antibody (non-phosphorylated neurofilament H, 1:1000 dilution, clone SMI32, Sternberger, Lutherville) is a marker for immature or dysmorphic neurons as present in FCD type IIa; SMI31 antibody (neurofilament H 1:1000, clone SMI31, Sternberger, Lutherville) stains axons. CD34 (cluster

of differentiation 34, 1:600, Qbend, Immunotech) used as a marker of vascular endothelial cells to determine vascular density; GFAP (glial fibrillary acid protein, 1:4000, Dako, Glostrup, Denmark) as astrocytic marker; MBP (myelin binding protein, 1:400 DAKO) as major myelin constituent; Olig2 (Oligodendrocyte lineage transcription factor 2, 1:100 dilution, IBL, Minneapolis, USA) as oligodendroglial lineage marker.

All immunohistochemical stainings were performed with a Ventana semi-automated staining machine (Benchmark ULTRA; Ventana, Illkirch, France) and the Ventana DAB staining system according to the manufacturer's protocol. Slides were air-dried overnight at 37 °C. Histopathological classification was performed in accordance with the 2011 ILAE guidelines for mMCD and FCD¹³. Histological slides were digitalized at 10x magnification at 648,58 nm/pixel resolution (Olympus life sciences, Waltham, Massachusetts, USA) and saved in BigTiff format for further analyses.

ROI's for lesional and perilesional areas in grey and white matter were drawn based on visual inspection of MAP-2 stained digital histology slices. Tissue was considered white matter if > 500 µm away from the grey-white matter border. Perilesional cortex was only available in FCD type II lesions and defined by the absence of cortical dyslamination, dysmorphic neurons and balloon cells.

To develop the methodological pipeline of this multimodality study, a subset of cases were selected first. In order to achieve optimal co-registration, these were patients with the largest resected tissue samples, and in whom ex vivo MRI artefacts were minimal.

Co-registration

Histology

Firstly, because of its high resolution, all histological sample image files were resized to enable co-registration and histological analysis, resulting in a spatial resolution of 25 DPI. Different histological samples were aligned using an in-house developed software tool¹⁶⁵. MAP-2 images were used as a reference to which all other stainings were registered. All stainings were aligned interactively after which an affine transformation algorithm (including translation, rotation, scaling and sheering) was performed. Results were checked manually and a non-rigid registration was performed subsequently using Elastix (Image Sciences Institute, UMC Utrecht, Utrecht, the Netherlands) (see diagram 1).

Histology to ex vivo 7T MRI

Histological samples were sectioned in the same orthogonal angle as the optimal ex vivo 7T MRI multiplanar reconstruction (as described above). Since histological spatial resolution was much higher, ex vivo 7T MRI sequences were rescaled in the X and Y direction matching histological slides. Subsequently these T_1 , T_2^* and WMS sequences were registered to T_2 sequences using affine and non-rigid (Elastix) registration algorithms. MRI slices corresponding

Diagram 1: Diagram showing different registration steps.

to histological slides were manually allocated. The allocated T_2 slices were used as a reference in the next registration step. MAP-2 shares the clear differentiation between white and grey matter with its radiological counterpart, the T2 sequence. Therefore, these slides were registered using the same steps as described above in co-registration histology section. The obtained transformation matrix is applied to the spatially corresponding slides (see diagram 1).

Ex vivo 7T MRI to in vivo 7T MRI

All in vivo 7T MRI scans were registered to the T_2 sequence using an affine and non-rigid (Elastix) registration algorithm. Subsequently, in-house developed software (java navigation, described previously by Noordmans et al.¹⁶⁶) was used to construct a 3D rendering of the cortex using the registered Flair in vivo sequence and of the resected tissue using the T_2 ex

vivo sequence. Photographs obtained during surgery and orientation markers were used to find the resected area on the in vivo scan. Cortical landmarks were placed on both in vivo and ex vivo renderings. These were used to manually overlay the ex vivo Flair image over the T_2 ex vivo image. Results were checked in all three planes. Subsequently the ex vivo T_2 image was used as a reference and the FLAIR image is registered using both affine and non-rigid transformation. The obtained transformation matrix was applied to all other in vivo sequences and the in vivo scans are resliced in the same orientation as the ex vivo images (see diagram 1).

Histology to in vivo 7T MRI

Since the histological images were already registered to the corresponding slices in the ex vivo images, we were able to construct a voxel image in which on the Z-axis histology images were put corresponding to the allocated slices in the ex vivo image. All images in the Z-direction without a corresponding histology image remained empty. The histological voxel images correspond to the registered and resliced in vivo 7T MRI images (see diagram 1).

ROI selection

Whole slide images (WSI) are used to define the type of lesion according to the most recent ILAE¹³ and WHO¹⁶⁷ classifications. Subsequently, lesion centre, lesion border and normal tissue regions were manually defined based on MAP2 immunohistochemistry and HE staining. All ROI were delineated in FIJI (ImageJ2). In the corresponding slices of both 7T in vivo and ex vivo imaging, the tissue visible in histology samples was also defined as centre, border, and normal tissue, and manually delineated as such.

Results

33 subjects underwent in vivo 7T MRI, of which 21 on clinical indication (i.e. suspected mMCD/FCD but normal previous imaging on lower-field MRI) and 12 for research purposes only, in whom previous imaging at lower-field had already revealed a lesion. In one patient 7T MRI failed due to patient non-compliance. In 7 of these 33 patients there was no indication for resective surgery. Five underwent surgery outside the study period, precluding ex vivo MRI analyses. Twenty-one patients underwent resective epilepsy surgery in the study period. The resected specimen was not suited for ex vivo imaging in four of those. In 17 subjects sufficient tissue was resected to allow ex vivo MRI imaging (figure 1). Frequency of ILAE histopathological diagnoses was: mMCD type 2 n=8; FCD IIa n=1; FCD IIb n=5; FCD IIId n=1, Ganglioglioma n=1; glioneuronal tumour–not otherwise specified=1. Seven tissue samples were selected for preliminary analyses; mMCD n=3; FCD IIa n=1; FCD IIb n=3.

Figure 1. Example of resected brain tissue (patient number S03). *Left* resected area visible during surgery; *Middle* resected tissue; *Right* 3D render of 7T ex vivo MRI image of resected tissue.

Clinical information of the seven patients that were selected for this preliminary study to construct and optimize the methodological pipeline for the proposed multimodality study, as described above, is supplied in table 2.

In each of the seven resected brain tissue samples, two or three sections were used to obtain 10 different stainings. These were all registered towards the MAP-2 slides. Corresponding ex vivo MRI images were selected and histology samples were registered towards ex vivo imaging (see figure 2).

3D renders of both in vivo and ex vivo 7T MRI images were constructed. These were used together with photographs obtained during surgery to manually align the seven resected brain samples to the in vivo 7T imaging, after which an affine en non-rigid registration was performed, resulting in registered 7T in vivo and ex vivo images (see figure 3).

Voxel information of the 7T ex vivo scans was used to create a voxel image of all stainings. These combined registration steps resulted in registered image data of all 10 stainings, 7T ex vivo T_1 , T_2 and WMS MRI imaging and 7T in-vivo T_1 , T_2 , T_2^* and FLAIR imaging (example of co-registered ex vivo T2 MRI and MAP2 staining is given in figure 4).

ROI selection was performed on 7T in vivo MRI imaging, 7T ex vivo MRI imaging and histological samples. Lesion centre, lesion border and normal tissue regions were delineated manually.

Discussion

In the comparative analysis of different imaging modalities accurate registration is key. Although challenging, registration of histological stainings, ex vivo acquired UHF imaging of resected brain tissue and pre-operative acquired UHF brain imaging can be performed.

Figure 2. Example of results, histology registration process (patient number S03).

A 3D rendering of the 7T ex vivo MRI image is shown with corresponding two locations of histology samples (A and B). On the right, results of different registration steps are shown for both location A and B. First row shows original histology stainings, second row histology samples registered towards MAP-2 staining. The third row shows corresponding T_2 , T_1 and WMS MRI images and results of histology samples registered towards 7T ex vivo images.

Table 2. Clinical information of included seven patients.

Sex /age Pat no.	Age at debut (surg)	Semiology	Surface EEG ictal epileptiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Visual assessment ex vivo 7T MRI	Histopathology (ILEA type)	Histological features	Outcome (Engel Score)
S03	♂/17	12	Focal impaired awareness seizures, myoclonia all extremities, L-sided version of eyes followed by diminished vision; bilateral tonic-clonic seizures	R temporal; poro-occipital	R temporal; spikes R lateral temporal, spikes R parietal and lateral medial temporal	Baso-temporal / lateral and R posterior insula	R temporal; poro-occipital hypometabolism	Not performed	No abnormalities*	No abnormalities	No abnormalities	R temporal lobectomy	Intact cortical layering, Normal CX-WM interface and normal signal intensities.	mMCD type 2	Excess neurons in WM and molecular layer. Normal cortical architecture (some columnar arrangement, normal for temporal lobe) Gliosis.	1D 2yr
S04	♂/34	20	Focal impaired awareness seizures, auditory phenomena, R-sided head version, confusion; focal to bilateral tonic-clonic seizures	L posterior temporal	Aspecific irregularities bilateral fronto-temporal	Sporadic spikes L lateral temporal	Not conclusive	L baso-temporal; parietal, hypometabolism in large area	Not performed	No abnormalities*	No abnormalities	Cortectomy L superior and middle temporal gyrus	Subtle signal change in deeper cortical layers. Normal CX-WM interface	FCD IIa	Dysmorphic neurons, not orientated. Excess neurons in WM and molecular layer. Gliosis.	1A+ 6 month
S13	♂/22	10	Focal impaired awareness seizures, facial flushing, facial automatisms	L temporal and central	L temporal	Sporadic spikes L temporal	L temporal focus	L posterior temporal hypometabolism	not performed	suspected FCD: trans-mantle sign, CX and WM strong signal changes (1T)	suspected FCD: trans-mantle sign, CX and WM strong signal changes	lesionectomy L temporal ACOG	Bottom of sulcus pronounced signal changes in CX and WM, cortical thickening and blurring. In centre lesion no cortical layering visible. Transmantle sign. Outside lesion cortical layering preserved	FCD IIb	In depth of sulcus disorganized cortex, dysmorphic neurons, balloon cells in cortex en WM, demyelination	1A- 2yr

Sex /age Pat no.	Age at de- but	Semiology	Surface EEG ictal epilep- tiform discharges	Surface EEG interictal epileptiform discharges	ECOG/SEEG	MEG	FDG PET	SPECT	1.5T MRI (or lower)	3T MRI	7T MRI	Surgery	Visual assess- ment ex vivo 7T MRI	Histopa- thology (ILEA type)	Histological features	Outcome (Engel Score)
S17	♂/16	13 Secondary generalized, automatisms progressing to tonic clonic seizures	R posterior temporal	R posterior temporal	sporadic spikes and bursts R temporo-oc- cipital	not perfor- med	not perfor- med	not perfor- med	Suspected FCD, CX and WM signal change (1T)	trans- mantle sign, blurring, CX and WM signal changes	trans- mantle sign, blurring, CX and WM signal changes	Lesion- ectomy R parie- to-occipital with ACOG	Blurring of CX- WM interface. Moderate WM and CX signal change. Pre- served cortical layering	FCD IIb	Intact cortical layering. Dysmorphic neurons, only a few balloon cells. Gliosis.	1A+, 1yr
S24	♀/58	14 Focal impar- red awareness seizures, epi- gastric rising sensation, automatisms. Incidental secondary ge- neralisation	R temporal	R temporal	Sporadic spikes R temporal	not perfor- med	R tempo- ral diffuse hypome- tabolism	not perfor- med	Not per- formed	No abnor- malities except chronic vascular ischemic changes	No abnor- malities except chronic vascular ischemic changes	R temporal lobecto- my with ACOG	Intact cortical layering. Normal CX-WM interface and normal signal intensities.	mMCD type 2	excess neu- rons in WM and molecular layer, normal cortical layering and cytoarchitec- ture	1A+ 0.5yr
S26	♀/61	16 Focal impar- red awareness seizures, automatisms, staring, unres- ponsive, inad- equate speech, amnesia	L temporal	L temporal anterior to midtemporal	Burst L hippocam- pus and anterotem- porobasal neocortex	Not per- formed	L tempo- ral hypo- metabolism	not perfor- med	Suspected FCD, CX-WM interface, cortical thic- kening, mild signal changes CX and WM	Suspected FCD L temporal. Blurring of CX-WM interface, cortical thic- kening, mild CX changes signal CX and WM	L temporal extensive blurring of CX-WM interface, increased cortical thickness. mild CX and WM signal changes. WM	L anterior temporal resection and amygdala- hippocam- pectomy	Intact cortical layering. Signal change at CX- WM interface and in deeper WM	FCD IIb (no MTS)	Large number of dysmorphic neurons and some balloon cells. Relatively intact cortical layering.	1A+ 0.5yr
S32	♀/38	19 focal impaired awareness seizures, automatisms, staring	R fronto- lateral to temporal	R more than L frontotem- poral	Sporadic spikes R temporal	not perfor- med	R tempo- ral hypo- metabolism	not perfor- med	Not per- formed	No abnor- malities	No abnor- malities	R temporal lobecto- my	Intact cortical layering. Normal CX-WM interface and normal signal intensities.	mMCD type 2.	excess neu- rons in white matter and molecular layer. Gliosis in white matter	1A 3month

L = left; R = right; CX = cortex; WM = white matter; ACOG = acute (intra-operative) electrocorticography

Figure 3. Example of registration result of in vivo 7T and ex vivo 7T imaging for histology location A and B (patient number S03).

However, extensive documentation during surgery, meticulous tissue preparation and scanning is necessary to obtain samples usable for further analysis. Moreover, sample size needs to be of sufficient in order to register accurately.

The described registration pipeline currently requires numerous registration steps, making registration time consuming. In-house developed software was used and further development of this software is needed to allow for more automated registration. This will result in the possibility to register bigger volumes of data. Moreover, the vast amount of data requires meticulous data handling. The development of a data management system could greatly reduce time and human errors.

In our ongoing research, we will assess differences of lesion type and allocated area of lesion centre, lesion border and normal tissue between histology samples, in vivo MRI and ex vivo MRI imaging. Furthermore, we will perform line profile analyses and histogram comparison to quantitatively correlate MRI and histology, resulting in a novel comparison between 7T in vivo MRI imaging and histology samples.

Figure 4. Example of registration result of ex vivo 7T imaging and MAP-2 staining of three patients. First two columns show results of two locations (A and B) of T_2 ex vivo 7T imaging and corresponding MAP-2 histology samples of patient number S03, subsequently two locations (A and B) of patient number S04 and S17 are shown.

Conclusion

This study demonstrates the feasibility to register histological samples of resected brain tissue in epilepsy patients with both ex vivo UHF MRI imaging of resected brain tissue and pre-surgical (in vivo) UHF MRI imaging. We show a pipeline in which all different steps are described and display registration results. Registration of these modalities makes it possible to perform further comparative analysis. Furthermore, comparison of histology and MRI imaging data can provide new insights in the development of novel post-processing techniques, with potential to improve the detection of subtle cortical dyslamination in vivo. This could both improve accuracy of presurgical planning and hence seizure outcome.

8

SUMMARY AND GENERAL DISCUSSION

Summary

Patients with refractory epilepsy who undergo surgical treatment frequently have mMCD of FCD as the underlying cause. In this thesis we aimed to expand understanding of how histological differences (as defined in the most recent ILAE classification) relate to patient characteristics and successful surgical therapy. Moreover, we explored ways to improve diagnostic imaging for subtle lesions that escape detection by current techniques fail to identify. More accurate identification of the presence and delineation of mMCD and FCD lesions will eventually lead to more patients benefitting from surgical therapy and improving surgical outcome.

The first step was to evaluate the histopathological diagnoses and clinical characteristics in patients operated before. We retrospectively studied a consecutive cohort of all patients who underwent epilepsy surgery in the UMC Utrecht over a period of 12 years (2000-2012) with a histopathological of mMCD or FCD according to the 2011 ILAE guidelines. In the past decades there has been considerable inconsistency in the used definitions for these specific entities. The 2011 guidelines are the most recent attempt to harmonize histopathological classification. For the research described in this thesis we revised the histopathological diagnosis in the majority of tissue samples in accordance with the new classification.

In **chapter 2** we described this cohort of 88 patients of whom during surgery tissues had been obtained for histology and an unequivocal diagnosis could be made. We found a strikingly high number of patients with mMCD (31/88, 35%) in our cohort, while FCD I made up 9% (8/88) and FCD II 56% (49/88) of the study population. Considerably less patients were diagnosed with mMCD in previous studies, and more with FCD I¹⁰. We observed a predilection of FCD II types for the frontal lobe, while mMCD and FCD I were most often localised in the temporal lobes. Patients with mMCD had onset of epilepsy later in life and consequently underwent surgery at older age compared to those with FCD.

Epilepsy surgery resulted in lasting seizure freedom rate of 49% at a median follow-up of 7 years; 36% had also discontinued anti-epileptic medication. Patients with mMCD were less likely to reach seizure freedom. Patients with FCD IIb had a markedly increased chance of achieving seizure freedom.

In a subset of this cohort (42 children), we studied cognitive ability prior to, and after surgery. These findings are described in **Chapter 3**. Among the 42 children who underwent cognitive testing, 51% had intellectual disability prior to surgery. Preoperative cognitive functioning was lower in patients with onset at younger age and patients with severe delay (IQ/DQ<55) were more often operated at very young age, leading to a spurious correlation low cognitive function, and surgery at young age and short epilepsy duration. In patients with IQ/DQ>55, shorter epilepsy duration was associated with better cognitive functioning.

All FCD I patients had a cognitive disability and their mean IQ/DQ was significantly lower than that in children with mMCD or FCD II.

Following surgery, relevant improvement, defined as ≥ 7 IQ/DQ points, was seen in 43% of the children, while cognitive functioning was stable in 46% and deteriorated in 11%. Post-surgical cognitive functioning was strongly related to pre-surgical functioning. Only a diagnosis of epileptic encephalopathy predicted cognitive improvement after surgery. We did not observe an association between postoperative cognitive outcome and seizure status.

In **Chapter 4** we studied a series of 40 patients who underwent additional 7 tesla MRI in the presurgical diagnostic trajectory. 7 tesla MRI was indicated for patients with refractory and presumed lesional focal epilepsy, and the absence of a lesion on one or more previous lower field strength of sufficient quality, or when concealed pathology was suspected in combination with MR-visible hippocampal sclerosis, so-called dual pathology. Multidisciplinary evaluation of 7 tesla MRI identified additional lesions not seen on lower-field MRI in nine patients (23%), which guided further surgical decision making. Six of these patients had been operated at time of the study, with confirmation of FCD ILAE type IIA (n=2), FCD IIB (n=1), mMCD type II (n=2) or mMCD with oligodendroglial hyperplasia (MOGHE) (n=1).

During assessment of 7T MRI scans that had been performed on clinical indication, we noticed an emerging pattern of a specific signal change co-localizing with confirmed mMCD/FCD lesions. These observations in a series of six patients are described in **Chapter 5**. In four of six patients in our series, 7T T_2^* sequences showed areas containing marked hypointensities with a branched, partly tortuous configuration, and a signal compatible with venous blood, suggestive of increased venous vasculature in the sulci neighbouring the malformed cortex and to a lesser extent in brain parenchyma. In two of these patients 3T MRI was without abnormalities and in one also 7T MRI did not reveal a lesion.

We reviewed 7T images of eight healthy subjects, and found that symmetrical venous leptomeningeal vascular structures were clearly visible but the phenomenon described above was not identified.

In **Chapter 6** we set out to elucidate the relation between micro-vascular density and cortical developmental abnormalities, in order to determine whether there is a pathological substrate for imaging findings compatible with vascular changes. We performed quantitative immunohistochemical analysis of microvascular density and inflammation in 97 patients with either mMCD, FCD or TSC and an age- and localization-matched control group of 14 autopsy cases. We found that cortical and white matter vascular density is increased in FCD and tubers, compared to the surrounding perilesional cortex, irrespective of age. As opposed to normal cortex, in resected malformations of cortical development – most pronounced in cortical tubers – we observed an increase in vascular density with ageing. No time-relation was seen in white matter vascular density.

Moreover, vessel density in white matter was related to pathology specific processes. Compared to controls, there was increased density in TSC tubers. Hypervascularity in tubers was associated with microglial activation, and an increased number of inflammatory cells. Vascular density was not significantly different between FCD subtypes and controls, neither did we find an association between cortical vascular density and pathology subtypes.

The first clinical experiences of 7T MRI were promising, however it was clear that in the majority of patients with normal 3T MRI, neither did 7T MRI lead to identification of a lesion. The preceding chapters underline the importance of pre-surgical lesion identification and delineation and accurate histopathological diagnosis. Further progress requires analysis tools that correlate 7T image and histological characteristics. In the course of this PhD project the prospective CODE 7 study was initiated and data collection performed. Candidates for epilepsy surgery with suspected mMCD/FCD were included and underwent additional pre-surgical 7T MRI. The resected brain specimens were studied with ex vivo 7T MRI, in isotropic resolutions up to 0.2 mm. The in vivo and ex vivo MR images were co-registered with digitized histology slides with the use of in-house developed software tools. The development of this methodological framework is described in **Chapter 7**. These preliminary results appear promising, and we in ongoing research we pursue to elucidate how specific MR imaging features relate to histological characteristics in an extensive set of stainings.

General Discussion

The first two chapters of this thesis describe how clinical characteristics and both seizure and cognitive outcome after epilepsy surgery relate to histopathological diagnoses as defined by the 2011 ILAE classification for mMCD/FCD¹³. The work bundled in the second half of this thesis focuses on our experiences with 7T MRI in this patient group and the pursuit in improving lesion detection and delineation.

Outcome differences between histological subtypes

There is substantial disparity in mMCD/FCD literature with regards to the frequency of occurrence of the histological subtypes and their relation with several clinical parameters, also in recent publications that applied identical FCD classifications. A possible factor is that observer variability remains despite efforts to harmonize histopathological classification. Discrepancy in utilization of immunohistochemical methods may cause variability in subclassification.

Especially the milder pathologies can be debatable. There was no formal inclusion of mMCD in the 2011 ILAE classification system, which might persuade the pathologist to classify very subtle dysplastic lesions as FCD I, when mMCD would be more suitable, leading to less awareness of, or attention for mMCD as an entity. Consequently in the last years mMCD has

been rarely reported on, where it used to be a common diagnosis after epilepsy surgery, particularly in children ¹⁰.

In recent literature, there is inconsistency in the reported correlation between ILAE FCD subtypes and clinical presentation and surgical outcome. Multiple studies did not find differences in clinical presentation^{44,168,169} or surgical outcome^{35–37,41,168–170} between FCD subtypes. Studies that did report different clinical characteristics found more MRI negative lesions and later onset of epilepsy in patients with FCD I compared to those with FCD II diagnoses^{35,40,171}. FCD I lesions are more frequently multilobar while in FCD II there was a predilection for frontal localization^{35,171}. Several studies found better surgical outcome in the FCD II subtypes, with highest chance of seizure freedom after a FCD IIb diagnosis^{28,40,44,171–173}.

We studied a large cohort of children operated from 2000 to 2012 for whom histology was revised according the ILAE classification, with isolated mMCD or FCD. In both cognitive and seizure outcome we observed differences between histological subtypes.

Seizure freedom following surgery is less likely in mMCD, probably due to difficulties in determining location and margins on imaging and during surgery, thus complicating accurate lesion identification, delineation, and complete removal of the epileptogenic zone. In our study we did not observe differences between histological subtypes, in occurrence of suspected incomplete resection based on intra-operative observations by the surgeon, ECOG or post-surgical imaging. Nonetheless residual lesions are most likely the best explanation for unfavourable seizure outcome in patients with mMCD or FCD. Complete resection is the most reported predictor of seizure-freedom in literature, along with factors that relate to completeness of resection: a temporal focus, severe pathological features, and an MRI-defined lesion⁴³. Accordingly, in our multivariate analyses, mMCD, extra-temporal surgery and an indication for incomplete resection were all predictors of unfavourable seizure outcome. Consistent with existing literature, mMCD and FCD I remained more frequently undetected on MRI than FCD II^{35,37,42,70,75}. However, in our cohort absence of an MRI lesion did not appear to negatively influence surgical outcome. Over half of the studied patients underwent long-term invasive monitoring, needed to localize and delineate of the epileptic zone in patients without identified structural lesion and facilitate complete removal.

While seizure freedom rates are lower compared to FCD II subtypes, surgery is also worthwhile for patients suspected with the more subtle mMCD or FCD I subtypes. Over the last decades we have witnessed an increasing trend to operate on MRI-negative cases⁶⁴. Nevertheless we should strive to improve localisation and delineation of especially mMCD and FCD I to improve surgical outcome. There is still debate concerning the definitive identifying contrasting histological and clinical features of mMCD versus FCD ¹⁷⁴. Our findings do suggest that clinical characteristics differentiate patients with mMCD subtypes and FCD.

Future studies should continue to include mMCD pathology to improve understanding of clinical distinctions. Well-coordinated long-term prospective multicentre studies seem necessary to definitely establish predictors of seizure outcome and build predictive models in patients with mMCD/FCD. A prerequisite is high inter-rater reliability of histological evaluation and classification. Future studies could potentially further differentiate mMCD and FCD subtypes through genetic and molecular fingerprinting⁷⁴. For example, mTOR pathway pathogenic variants appear to be reserved for FCD II subtypes, while brain-only loss-of-function SLC35A2 mutations were exclusively found in mMCD/FCD in a single study¹⁷⁴. It is not unlikely that genetic information will further change the classification of focal developmental abnormalities.

Previous research showed that developmental delay is common among patients with epilepsy due to FCD, with 53–68% having an IQ below 70^{27,28}. Most studies reported overall cognitive improvement following epilepsy surgery^{45–48}, and factors related to post-surgical cognitive outcome were seizure freedom^{28,48–51}, parental education⁵², anti-epileptic drug (AED) policies^{51,53}, preoperative seizure frequency²⁷, and aetiology^{53,54}. More severe cognitive impairment was predicted by earlier onset^{27,46,55–62} and longer duration^{27,63} of epilepsy. Previous studies did not find differences in cognitive outcome between patients with different FCD subtypes^{27,42}. In our cohort, cognitive disability was slightly more prevalent (74%) than expected. We confirmed the association of early onset of epilepsy with cognitive impairment in patients with mMCD/FCD. Also longer epilepsy duration negatively affected cognition. Cognitive outcome was strongly related to pre-surgical functioning.

Epileptic activity, even when associated with strictly focal semiology, causes disruption of cognitive development and memory functions. In these patients there is evidence of loss of optimal, small-world, network configuration and of structural white matter abnormalities, wide spread and distant to the epileptogenic focus^{30,31,80,81}. In addition, many anti-epileptic drugs are associated with cognitive problems, due to a generalized suppressive effect with alteration of neuronal excitability^{80,82}. Consequently, longer exposure to epileptic activity and AEDs would reasonably increase the gap in cognitive function with normal developing peers. Another factor that might contribute to a more pronounced cognitive delay with earlier epilepsy onset, is that there is evidence of a critical period in cognitive development during the first years of life, when the foundation is laid for development of higher-order skills later in life⁶⁰. Alternatively, both a lower IQ and earlier onset epilepsy could be the consequence of a more severe or more widespread underlying epileptogenic pathology, rather than being causally related⁶¹. FCD I is typically more diffuse than other FCD subtypes and in our study patients with FCD I had more severe cognitive delay, although they did not present with earlier seizure onset.

We expected to find more cognitive improvement in seizure – and medication–free patients but could not confirm this association in our study, in contrast to several previous studies^{49–51}. These studies had a markedly longer follow-up duration. We observed overall improvement in cognition, but it is likely that follow-up exceeding two years is required to appreciate post-surgical long-term positive effects of seizure freedom and cessation of anti-epileptic drugs on cognitive development.

There is a shift towards epilepsy surgery at younger age, especially for patients with focal developmental abnormalities. In the pediatric population improvement of cognitive functioning should be regarded as a significant aim of surgical treatment. Preservation of cognitive abilities is a strong argument for streamlining the pre-surgical diagnostic trajectory. We should strive to minimize delay between onset of epilepsy and surgery in surgical candidates, while safeguarding the quality of the diagnostic process. Disease duration has significantly decreased over the last decades, but as an European survey points out there are still massive gains to be made, with a mean epilepsy duration of 7.7 years in children and 24.4 years in adults in the last decade⁶⁴.

Ultra-High field MR imaging

Improving lesion detection and delineation is one of the ways to improve quality of care for epilepsy surgery patients. One technical development that has potential is 7 tesla (T) MRI. Current standard of care is the use of an epilepsy-dedicated scanning protocol on 1.5 or 3 tesla. Higher field strength increases the signal to noise ratio, which allows higher image resolution, or faster acquisition. Such theoretical advantage will not unquestionably translate to clinical benefits.

In 2017 the first 7T MRI system received clearance for clinical use. Meanwhile clinical systems with 9.4T, 10.5T and 11.7 T magnets have been developed and await approval for clinical use. These systems make scanning at isotropic resolutions under half a millimetre possible. There will be a limit to further increasing field strength as more severe physical sensations, or unacceptable high SAR-levels form unsurmountable obstacles at some point. Animal studies found no evidence of short or long term irreversible effects with exposure to 11.1T, 17.6T and 21.1T magnetic fields for over one hour. However, possible detrimental effects of 10T fields and beyond on replicating cells and growing tissues require further investigation¹⁷⁵.

Increasing field strength is not the only way to improve resolving power of MRI. Other groups have used anaesthesia to be able to increase acquisition times to over an hour, increasing maximal resolution and attenuating motion artefacts on conventional MRI systems. General anaesthesia to undergo imaging is not without risks and requires additional staff, incurring high costs. Rather technical improvements should be sought for increasing resolution. Besides increasing field strength, there are potential gains by for instance using increasingly

sophisticated multi-channel RF coils, parallel scanning techniques allowing faster acquisition and more efficient pulse designs^{112,176,177}. Further improvements in spatial resolutions can be expected to result in an increase in sensitivity for subtle cortical abnormalities. However, epilepsy surgery has seen a trend towards epilepsy surgery in increasingly complex cases, which also include more cryptogenic epilepsies. Increasing sensitivity carries a risk of decreasing specificity; limiting false positive MRI findings will be a challenge. Integration of diagnostic modalities is quintessential to address this potential pitfall.

In our 7T MRI cohort (chapter 4) no lesion was identified prior to surgery using standard epilepsy dedicated (1-3T) MRI protocols in 9/57 (16%) of patients with FCD and 14/31 (45%) of patients mMCD. This is in line with previous studies^{37,42,70}. Multidisciplinary evaluation of 7T MRI led to identification of a structural lesion which had not been found with standard epilepsy MRI studies in 23% of patients. These findings were guiding in further clinical decision making. Other groups have now reported similar yield of 7T MRI^{99,156,178}. However, in 77% of the studied cohort, even 7T MRI did not reveal a lesion. Several patients have already undergone surgery at time of this study, and in some a mMCD/FCD was demonstrated with histopathological investigation. It is clear 7T MRI is not a panacea. Experience with 7T MRI is still relatively limited. The biggest advantage, added anatomical detail, can paradoxically also be an extra challenge in differentiating between meaningless variation and true pathology. Moreover, 7T examinations can produce datasets of thousands of slices, incurring proportionately longer visual assessment time. It is to be expected that with growing experience accuracy of visual assessment by radiologist will improve.

In the last decade there has been a swiftly growing interest in using post-processing techniques as an adjunct to conventional visual analyses. Numerous techniques have been described of which most are based on voxel-based morphometry (VBM) or surface anatomy analyses. Several studies have shown that post-processing can lead to identification of subtle abnormalities even after reading by specialised neuroradiologists and multidisciplinary review failed to identify a lesion¹⁷⁹⁻¹⁸⁵. Moreover, studies found an association between complete resection of retrospectively flagged regions and favourable seizure outcome^{181,183}. Scrutiny is essential concerning the results of these analyses since cut-off values that are able to detect subtle abnormalities will inherently have low specificity and thus carry a risk of false positivity¹⁸⁶. Nevertheless, also in a recent prospective study morphometric analyses were found to impact on management and aid in detection of subtle FCD's¹⁸⁷. To date, these computer-aided analyses have been restricted to 1.5 and 3 tesla acquisitions. In general, a sufficiently large reference data set is required with ideally identical image specifications, and performance is also dependent on proper age-matching. These analyses are also hampered by the challenges related to image inhomogeneity at 7T. However, In the near future there could be a place for morphometric analyses and Artificial Intelligence

algorithms in flagging potential lesions in the extensive 7T MRI datasets, hereby shortening reading times and increasing clinical yield.

Even though the brain is a relatively stationary organ, at submillimetre resolution even the slightest motion produces artefacts, for instance those that are due to arterial pulsation. Inherent to incremental field strength is an increase in artefacts due to magnetic susceptibility and field inhomogeneities. Especially signal drop-out in the temporal lobes has hampered clinical evaluation of epilepsy patients. Several techniques have been developed that successfully counter artefacts due to field inhomogeneity and susceptibility effects¹⁸⁸. Motion artefacts can be reduced by implementation of specific pulse sequences that track movement during image acquisition¹⁸⁹. These developments greatly improved temporal lobe visualisation at 7T MRI. Concludingly, accuracy of 7T MRI is expected to improve with growing experience and technological advancement.

What is the current place of 7T MRI in the diagnostic work-up for epilepsy surgery in patients suspected of subtle cortical developmental anomalies? In patients with focal epilepsy and normal MRI findings on standard MRI investigations, mMCD and FCD I are frequently the underlying cause. If available 7 tesla MRI should be considered. We argue there is clinical benefit in applying scanning protocols with isotropic resolutions of 0.5 to 1 mm for T_1 , T_2 and FLAIR sequences. Careful consideration concerning the indication is warranted since availability of 7T MRI will remain relatively limited and its operational costs higher compared to standard 1.5 or 3T MRI. There should be a convincing hypothesis for focal onset of seizures, based on semiology and EEG, and either 1.5 or 3T MRI should have been performed using an epilepsy dedicated protocol and discussed in multidisciplinary meetings, and not have revealed an explanatory lesion or have ambiguous findings. Non-structural causes of focal refractory epilepsy – such as certain genetic etiologies – should have been excluded and a high index of suspicion for a structural underlying cause is required. If available, computerized post-processing MRI analysis methods should have been applied. Still, it is to be expected that in a majority of these cases 7T MRI will not provide information that contributes to a stronger hypothesis.

Our findings described in **chapter 5** are a product of the pursuit for alternative imaging characteristics to localise subtle cortical dysplasias. We hypothesized that these T_2^* signal changes at 7T MRI could be a reflection of structural vascular changes, either developmental or acquired and related to epileptic activity. Alternatively, hemodynamic alterations and changes in blood oxygen levels could play a role.

The histological study that followed did not provide conclusive support for vascular structural changes as a substrate of T_2^* findings. Importantly, however, leptomeningeal vasculature – which could be related to the observed T_2^* changes – was not studied histologically since this part of the removed tissue is rarely available for histopathological review. FCD did not

appear to be hypervascular compared to controls, even though there was an increase in the vascular density in the lesion compared to perilesional tissue. Hemodynamic and consequent blood oxygen level variations, perhaps in meningeal vascular structures, possibly better explain vascular prominence on T_2^* MR images. Both positive and negative BOLD responses have been shown to be concordant with proven FCD lesions and also seemed to correspond with epileptogenic tissue in MRI negative patients¹⁹⁰. Future research is needed to ascertain the value of BOLD fMRI in detection of otherwise MRI negative dysplasia.

It remains unclear what is required to approach 100% lesion detection on MRI, if ever feasible. Should the focus be to improve spatial resolution or improve tissue contrast? Or should scanning techniques be altered to optimize specific tissue contrast based on histological changes in the targeted lesion type? These are questions that led to the start of our study in which we performed ex-vivo/histology co-registered analyses, to allow direct comparison of MR signal and histological characteristics. Analyses of the findings of this study – currently work in progress – hopefully aids in improving lesion identification and delineation.

Structural MRI is still the keystone imaging modality for patients with epilepsy, however other techniques could potentially gain importance in the future. Nuclear imaging for epilepsy in the form of PET-CT and SPECT are established and commonly used techniques to provide information on tissue involved in epileptogenesis or propagation through visualisation of respectively metabolic activity or perfusion differences. Few epilepsy centres employ MR spectroscopy or MR spectroscopic imaging which can also reveal metabolic changes in patients with epilepsy¹⁰⁰. These techniques are frequently used to study brain tumours and neurodegenerative disorders, but so far relatively little research has focused on epilepsy. Single- and multi-voxels studies have proven able to lateralise epileptic – in particular hippocampal – foci and demonstrate epileptogenicity in known structural abnormalities, based on abnormal metabolite concentrations – N-acetyl aspartate, choline and creatine especially. However, voxel-based MRS is of little value in MRI-negative patients with a suspected neocortical focus¹⁹¹. MR spectroscopic imaging (MRSI) can potentially provide full brain coverage, but its use is currently limited by technical limitations in e.g. spatial resolution, acquisition times, detector sensitivity and artefacts related to field inhomogeneity. Recent developments in acquisition techniques in combination with higher field strength bring within reach MRSI that could be clinically useful in detection of epileptogenic, but MRI-occult, dysplastic lesions¹⁹². Future research in this field demands special attention.

Final Remarks

Although patients with most subtle abnormalities in the spectrum of mMCD/FCD have a significantly lower chance of achieving seizure freedom, still one third of these patients are expected to reach complete and ongoing seizure freedom. Suspected mMCD should not deter physicians to offer surgery as option. We found that patients with mMCD clinically

differ from those with ILAE FCD I and FCD II subtypes and advocate to maintain mMCD as a separate entity and continue to study this patient group in future research. An iterative histopathological agreement trial to identify areas of diagnostic challenges within the present classification scheme is ongoing (FCD classification Task Force of ILAE). Developmental delay forms a significant burden of disease in patients with refractory epilepsy. Cognitive functioning can significantly improve after resective epilepsy surgery in patients with mMCD/FCD. Moreover, cognitive delay builds up with the duration a patient suffers from epilepsy. This is one of the reasons to be swift in determining candidacy for surgical therapy. Surgical care for epilepsy patients can be further improved by progress in imaging of potential underlying structural abnormalities, such as the often highly subtle lesions associated with mMCD/FCD. Our work showed that the clinical use of 7T MRI increases identification of subtle lesions and can be a valuable tool in the pre-surgical work-up. However, this technique as it is, still has far from perfect diagnostic accuracy. Increasing imaging resolution could improve lesion detection, facilitated by technical advance in field strength, coil technique and image reconstruction. Research should also focus on additional localising structural and possibly functional MR image features. Optimal care will not only come from these technical innovations but also the integration of data of the different ancillary tests, and from the multidisciplinary approach with neurologists, electrophysiologists, neurosurgeons, technical physicians, neuropsychologists and radiologists closely working together. Patients with refractory epilepsy, relatives and caregivers often experience tremendous burden of disease. Epilepsy surgery is arguably one of the most rewarding therapies in modern neurology, and fortunately we can expect continuing improvement of the standard of care, due to worldwide intense efforts by scientists and healthcare professionals.

APPENDICES

References
Nederlandse samenvatting
Acknowledgements
Persoonlijk dankwoord
Curriculum Vitae
List of publications

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Nederlandse samenvatting

Achtergrond

Een epileptische aanval wordt gedefinieerd als het tijdelijk optreden van symptomen ten gevolge van abnormale, overmatige of gelijktijdige activiteit van neuronen in de hersenen. Een epileptische aanval kan verschillende vormen aannemen. Dit is mede afhankelijk van de locatie in de hersenen waar de aanval begint en hoe de activiteit zich verspreid naar andere delen van de hersenen. Er kunnen veranderingen optreden in waarneming, bewegen, gedrag, denken en autonome functies. Er is sprake van de aandoening 'epilepsie' wanneer er een blijvende neiging is om epileptische aanvallen te ontwikkelen zonder aanwijsbare externe uitlokkende factor (zoals medicatie, drugs, hersenschudding etc.). Epilepsie kan focaal zijn, waarbij de aanvallen in één of meerdere afgebakende gebieden ontstaan, of generaliseerd, waarbij aanvallen in het gehele brein ontstaan. Bij de meeste patiënten met epilepsie wordt in de eerste plaatst gestart met medicatie, desondanks houdt tot ongeveer één derde van de patiënten epileptische aanvallen. Deze patiënten hebben medicatie-resistente epilepsie ofwel refractaire epilepsie.

Dit proefschrift gaat over patiënten met epilepsie ten gevolge van zogenaamde Focale Corticale Dysplasie, afgekort als FCD. Dit is één van de meest voorkomende oorzaken van focale epilepsie. De grote hersenen zijn opgebouwd uit een diepere laag witte stof waarin alle lange zenuwbanen lopen met daaroverheen als buitenste laag de hersenschors. De hersenschors (ook wel grijze stof of cortex) bestaat uit zes te onderscheiden lagen van zenuwcellen (neuronen). Per laag verschillen de neuronen van vorm en in functie. Bij FCD is de opbouw van deze lagen verstoord en kunnen er ook afwijkende neuronen voorkomen. In dit gedeelte van de hersenen is de functie verstoord, waardoor er makkelijk epileptische aanvallen kunnen ontstaan.

In 2011 is er een nieuw systeem opgesteld door de International League Against Epilepsy (ILAE) om de verschillende soorten FCD te classificeren op basis van het histologisch (microscopie) onderzoek. Het betreft de volgende classificaties:

FCD Type I: alleen afwijkende gelaagdheid van de hersenschors. Te onderscheiden in subtype Ia met gestoorde radiaire gelaagdheid en Ib met gestoorde tangentele (horizontale) gelaagdheid. In Ic is er een combinatie van gestoorde radiaire én tangentele gelaagdheid. FCD Type II: afwijkende gelaagdheid van de hersenschors én afwijkende neuronen. Bij IIa zijn er afwijkende, onrijpe neuronen. In type IIb zijn er typische balloncellen: grote ongedifferentieerde hersencellen.

FCD Type III: FCD in combinatie met andere hersenafwijkingen: hippocampale sclerose, tumoren, vasculaire malformaties of afwijkingen ten gevolge van hersenschade vroeg in het leven (bijvoorbeeld littekens na een bloeding of infectie rond de geboorte). Hippocampale sclerose is op zichzelf een van de meest voorkomende diagnoses bij patiënten met epilepsie. Dit wordt gekenmerkt door verlies van neuronen en verlittekening van de hippocampus (dit is een gepaarde structuur aan de binnenzijde van de temporaalkwabben met een belangrijke rol in het geheugen)

Er is ook een variant genaamd mild Malformation of Cortical Development (mMCD). Hierbij is de cortex normaal maar zijn er abnormaal veel neuronen in de meest oppervlakkige cortexlaag (mMCD type 1) en/of de witte stof (mMCD type 2). mMCD wordt gezien als de mildste vorm van dysplasie in het FCD-spectrum wat betreft de histologische afwijkingen. Zie ook figuur 1 voor voorbeelden van histologische kenmerken van mMCD en FCD.

We weten nog niet precies waarom deze dysplasieën ontstaan, maar er zijn steeds meer aanwijzingen dat er fouten ontstaan in bepaalde fasen van de hersenontwikkeling. Hierbij zijn meerdere eiwitten betrokken die door genetische afwijkingen niet normaal functioneren.

Patiënten met FCD hebben doorgaans al voor het 10^e levensjaar een eerste aanval. Deze aanvallen zijn bij veel patiënten frequent en niet goed te behandelen met medicatie. Patiënten ervaren vaak ook andere problemen die verband hebben met de onderliggende hersenafwijking, de epileptische aanvallen of van de anti-epileptische medicatie. Voorbeelden hiervan zijn migraine, vaatlijden en traumatische verwondingen. Patiënten met focale epilepsie hebben een hoger risico op psychiatrische problemen zoals stemmings-, angst-, en persoonlijkheidsstoornissen en psychoses. Verder hebben meer dan de helft van de jonge patiënten met FCD cognitieve problemen, gedefinieerd als een IQ-score lager dan 70. Dit is het gevolg van de lokaal verstoorde architectuur en daardoor ook verstoorde functie van de hersenen, maar ook door een ontregeling van het globaal functioneren van de hersenen door het optreden van de epileptische aanvallen. Bovendien heeft anti-epileptische medicatie een negatief effect doordat ook de normale functies van de hersenen worden beïnvloed.

Voor patiënten met medicatie-resistente epilepsie kan chirurgische behandeling een optie zijn, met vaak goede kansen op vermindering van epileptische aanvallen tot zelfs volledige aanvalsvrijheid. Het mogelijke vooruitzicht om te kunnen stoppen met de medicatie is een ander argument voor opereren, in verband met de eerdergenoemde bijwerkingen en negatieve effecten op de cognitie.

Afhankelijk van het ziektebeeld en de hersenafwijkingen wordt het afwijkend stuk hersenen of een volledige hersenkwab verwijderd. Soms wordt een gebied met afwijkende functie losgekoppeld van de rest van de hersenen, zodat de epileptische aanvallen zich niet verder kunnen verspreiden naar de rest van de hersenen.

Figuur 1. Voorbeelden van histologische kenmerken van mMCD en FCD.

A: mMCD type 2. Normale opbouw van de cortex, overmaat van neuronen in moleculaire laag (boven in figuur) en witte stof (onder in figuur) (NeuN kleuring).

B: FCD Ia. Abnormale radiaire gelaagdheid, met typische columnaire organisatie (NeuN kleuring).

C: FCD IIb. In FCD II subtypes is er vaak volledige verstoring van de corticale gelaagdheid en verlies van de grens tussen grijze en witte stof.

D: FCD IIa. Immature, dysmorfe neuronen met abnormale oriëntatie en positieve kleuring door SMI32, kenmerkend voor FCD IIa (SMI32 kleuring).

E: FCD IIb. Typische balloncellen, grote ongedifferentieerde cellen zonder Nissl-substantie en met positieve aankleuring voor vimentine (vimentine kleuring).

Eerder onderzoek heeft laten zien dat de helft van de geopereerde patiënten met FCD geen enkele epileptische aanval meer heeft. De belangrijkste voorspeller van aanvalsvrijheid is compleetheit van de resectie, dus of het afwijkende weefsel volledig is weggehaald. Andere belangrijke voorspellers hebben te maken met factoren die complete resectie vergemakkelijken, zoals een afwijking die zichtbaar is op de MRI, een afwijking in de temporaalkwab en afwijkingen die zich slechts in één kwab bevinden. Ook een kortere epilepsieduur en chirurgie op kinderleeftijd geven een hogere kans op aanvalsvrijheid. Er is echter nog relatief weinig bekend over de relatie tussen kans op aanvalsvrijheid en het type FCD, al zijn er wel enkele studies uitgevoerd die betere resultaten vonden voor FCD type II.

De meeste studies laten een verbetering zien in cognitief functioneren na epilepsiechirurgie wat samenhangt met aanvalsvrijheid, opleidingsniveau van de ouders, aanvalsfrequentie voor de operatie, de onderliggende oorzaak van epilepsie en anti-epileptische medicatie. Kinderen met een vroeg begin van epilepsie en reeds langer bestaande epilepsie hebben ernstigere cognitieve problemen. Er zijn nog niet verschillen gerapporteerd in cognitieve vermogens tussen de verschillende histologische mMCD/FCD types.

De laatste jaren is chirurgie een steeds belangrijkere behandeling van epilepsie geworden. Dit is het gevolg van de goede uitkomsten van operatieve behandeling en een lage kans op complicaties, en de grote technische ontwikkeling. Op dit moment is chirurgie de enige kans op aanvalsvrijheid bij medicatie-resistente epilepsie. Nauwkeurige lokalisatie van een afwijking is essentieel voor succesvolle chirurgie. Er gaat hier dan ook een uitgebreid diagnostisch traject vooraf. In eerste plaats wordt een elektro-encefalografie (EEG) en een MRI-scan gemaakt. Het EEG registreert hersenactiviteit met elektrodes op de hoofdhuid. Specifieke patronen tijdens en tussen aanvallen geven de locatie aan waar de epileptische activiteit ontstaat. De MRI (magnetic resonance imaging), ook wel magneetscan genoemd, levert beelden van de anatomie van de hersenen. Dit is mogelijk door magnetische excitatie van atoomkernen en verschillen in de relaxatie tussen weefsels te meten. Atoomkernen gedragen zich door de sterke magneet als kleine tolletjes. Door elektromagnetische golven van de MRI-scanner worden deze tolletjes in een bepaalde richting gedwongen (de excitatie). De gelijke bewegingen van atoomkernen zorgen voor een meetbaar signaal. Vervolgens gaan de atoomkernen weer terug naar de neutrale stand en verdwijnt de synchroniciteit van de bewegingen en daarmee het signaal (relaxatie). De MRI is zeer veelzijdig doordat verschillende soorten opnamen kunnen worden gebruikt, die weer specifieke weefseleigenschappen uitvergroten. Deze verschillende soorten opnamen worden sequenties genoemd, zoals T_1 en T_2 gewogen sequenties (niet te verwarren met tesla of T als maat van magneetveldsterkte). Naast anatomische informatie is MRI ook in staat informatie te geven over activiteit van de hersenen op basis van het zuurstofgehalte in hersenen en bloedvaten. Bloed heeft meetbare verschillen in MRI-signaal afhankelijk van

hoeveel zuurstof het bevat. Dit is het zogenaamde Blood Oxygen Level Dependent (BOLD) effect wat wordt benut in functionele MRI (fMRI). fMRI wordt ook gebruikt om aan te tonen waar bijvoorbeeld taalfuncties zitten, wat weer invloed heeft op de gekozen behandeling.

Naast het EEG en de MRI worden nog verschillende andere technieken ingezet, zoals magneto-encefalografie (MEG). Dit is een zeer gevoelig apparaat wat subtiele magnetische velden meet die ontstaan door de actiepotentialen in de hersenen. De nucleair geneeskundige onderzoeken Positronemissietomografie (PET) en Single Photon Emission Computed Tomography (SPECT) geven informatie over hoe actief regio's binnen de hersenen zijn en daarmee ook of er sprake kan zijn van epileptische activiteit. Er wordt een licht radioactieve stof in de bloedbaan gespoten, welke afhankelijk van het gekozen middel verzamelt in bepaalde weefsels en daar radioactief verval ondergaat, wat de scanner vervolgens registreert. Als beeldvorming en de niet-invasieve EEG-onderzoeken geen zekere locatie van de bron van de epileptische aanvallen heeft kunnen aantonen, dan bestaat nog de optie om een invasief EEG te maken. Hierbij wordt de patiënt geopereerd om de elektrodes als een matje tussen de hersenen en schedel te plaatsen, of als naalden in de hersenen. Doordat er een veel kleinere afstand is tussen de elektrode en de neuronen is het invasieve EEG gevoeliger voor het registreren van lokale afwijkende hersenactiviteit.

De MRI speelt een steeds grotere rol in het preoperatieve traject. Een structurele afwijking is een sterke en betrouwbare aanwijzing voor de aanwezigheid van een bron van de epilepsie. Echter is er niet altijd een exacte overeenkomst tussen het afwijkend gebied op de MRI en het weefsel dat de epileptische aanvallen veroorzaakt. Toch maakt een duidelijke en goed begrensde afwijking aanvullende tests vaak onnodig en vormt het een goed doelwit voor een operatie of invasief EEG-onderzoek. Desondanks kan ook zonder afwijking op MRI een operatie plaatsvinden op basis van andere onderzoeken, maar de kans op aanvalsvrijheid wordt daarmee kleiner. Het is bekend dat bij 15-40% van de patiënten die geopereerd zijn de FCD niet zichtbaar was op MRI. Voor mMCD kan dit zelfs meer dan 50% zijn.

Er zijn een aantal typische kenmerken van mMCD en FCD op de MRI. Zo is er een onscherpe grens tussen de hersenschors en onderliggende witte stof en zijn er lokale signaalveranderingen. Ook kan de hersenschors verdikt zijn of andere vormen van de windingen hebben. FCD type II wordt gekenmerkt door het zogenaamde 'transmantle sign', een wigvormige band van signaalveranderingen richting het midden van de hersenen. De overige types laten zich minder makkelijk onderscheiden op basis van MRI. Zie ook figuur 2 voor voorbeelden van MRI-afwijkingen bij FCD.

Idealiter levert de MRI precies de informatie aan het behandelteam wat voor soort afwijking het betreft en wat de grenzen zijn met normaal hersenweefsel en wat voor soort afwijking het betreft.

Figuur 2. Typische MRI-bevindingen bij FCD. Coronale reconstructies van 3D T_2 beelden op 7T MRI. Twee patiënten met FCD IIb.

A: Dysplasie in de bodem van groeve rechts met verdikking van de cortex, vervanging van de grijs-witte stof overgang en signaalveranderingen in cortex en witte stof.

B: Uitgesproken FCD laesie achter in de temporaalkwab met ook typische 'transmantle sign'.

Ondanks de grote ontwikkelingen van de laatste decennia in MRI en beeldverwerkings-technieken, is het te verwachten dat verdere ontwikkelingen een belangrijke verbetering kunnen brengen in de chirurgische behandeling van epilepsie. In de afgelopen tien jaar zijn 7 tesla (T) MRI-systemen voor menselijk gebruik beschikbaar gekomen. Zeven tesla staat voor de sterkte van de magneet, en in vergelijking met de huidige standaard – 1,5 en 3 tesla MRI – zorgt de sterkere magneet voor een betere signaal-ruisverhouding. Deze betere signaal-ruisverhouding kan worden gebruikt om beelden met hogere resolutie en meer contrast te maken. Daar tegenover staat dat bij een sterkere magneet er grotere technische uitdagingen zijn om verstoringen in het beeld te onderdrukken. Ook is het 7T MRI-systeem vele malen duurder. Daarom is het noodzakelijk om doormiddel van wetenschappelijk onderzoek aan te tonen dat de theoretische voordelen van 7T MRI ook werkelijk leiden tot betere uitkomsten.

Sinds het begin van de epilepsiechirurgie in het eind van de 19^e eeuw hebben er grote veranderingen plaatsgevonden in de zorg voor mensen met medicatie-resistente epilepsie. Ons begrip over hoe epileptische aanvallen ontstaan, de relaties met structurele hersenafwijkingen en de functionele anatomie is sterk toegenomen. Chirurgische technieken zijn verfijnd en nieuwe methodes zijn ontwikkeld. Patiënten met aanlegstoornissen als MCD/FCD maken een steeds groter deel uit van de totale patiëntpopulatie en er gaat veel onderzoek uit naar uit om de zorg verder te verbeteren. Er blijven belangrijke uitdagingen: onder andere het vaststellen van de oorzaak van de epilepsie, met name

bij afwezigheid van grove structurele afwijkingen en de identificatie van de grens tussen epileptogeen en normaal weefsel, te meer omdat verschillende modaliteiten vaak niet perfect overeenkomende informatie geven. De keuze voor de beste behandeling vereist een sterke a priori diagnose, een goed begrip van de kans van slagen en van de risico's van de verschillende behandelopties.

Doel van het onderzoek beschreven in dit proefschrift was om meer begrip te krijgen over hoe bij mMCD/FCD histologische verschillen gerelateerd zijn aan patiënteigenschappen en aan de kans op een succesvolle chirurgische behandeling. Bovendien werd onderzocht hoe de diagnostische beeldvorming kan worden verbeterd met betrekking tot subtiele afwijkingen die met huidige technieken niet worden gezien. Meer accurate identificatie van de aanwezigheid en begrenzing van mMCD en FCD-laesies zal er uiteindelijk voor zorgen dat meer patiënten kunnen profiteren van chirurgische behandelingen met betere uitkomsten.

Bevindingen in dit proefschrift

De eerste studie evalueerde de histopathologische diagnoses en klinische kenmerken in eerder geopereerde patiënten. We hebben retrospectief een groep bestudeerd van alle patiënten geopereerd in het UMC Utrecht in de periode 2000-2012 met een histologische diagnose passend bij mMCD of FCD. Voor deze studie hebben we de histologische classificaties gereviseerd volgens de 2011 ILAE richtlijnen. In **hoofdstuk 2** beschrijven we dit cohort van 88 patiënten. 9% (8/88) had een FCD I diagnose en 56% (49/88) FCD II. Het grote aantal met mMCD (31/88, 35%) in vergelijking met voorgaande studies is opvallend. FCD II kwam relatief vaker voor in de frontaalkwab, mMCD en FCD I vaker in de temporaalkwab. Patiënten met mMCD hadden een later epilepsiedebuut en ondergingen ook op latere leeftijd chirurgie in vergelijking met patiënten met FCD. In de totale groep leidde epilepsiechirurgie tot langdurige aanvalsvrijheid bij 49%, bij een mediane follow-up duur van 7 jaar; 36% was ook volledig gestopt met anti-epileptische medicatie. Patiënten met FCD IIb werden het vaakst aanvalsvrij, bij mMCD was de kans op aanvalsvrijheid juist kleiner.

We bestudeerden een groep van 42 kinderen, die onderdeel waren van de hierboven beschreven groep, wat betreft hun cognitieve vaardigheden voor en na epilepsiechirurgie. Deze bevindingen zijn beschreven in **hoofdstuk 3**. Van deze groep kinderen had 51% een cognitieve beperking, gedefinieerd als een intelligentiequotiënt (IQ) lager dan 70. Preoperatief cognitief functioneren was lager in patiënten met een vroeg epilepsiedebuut. Er is sprake van een ernstige cognitieve beperking bij een IQ of ontwikkelingsindex (OI, ontwikkelingsniveau in jaren gedeeld door kalenderleeftijd) onder de 55. Patiënten met een ernstige cognitieve beperking worden vaak geopereerd op zeer jonge leeftijd vanwege optreden van epileptische encefalopathie, wat leidde tot een schijnbare correlatie tussen

laag cognitief functioneren en operatie op jonge leeftijd en daarbij korte epilepsieduur. In de patiënten met preoperatief IQ/OI >55 was een korte epilepsieduur geassocieerd met betere cognitief functioneren. Alle kinderen met FCD I hadden een cognitieve beperking, en het gemiddeld IQ/ontwikkelingsindex was significant lager dan bij kinderen met mMCD of FCD type II. Na chirurgie was er bij 43% klinisch relevante verbetering in de cognitie, gedefinieerd als ≥ 7 punt stijging in IQ/OI. Bij 46% was cognitief functioneren stabiel en bij 11% was er verslechtering van 7 IQ/OI punten of meer. Alleen een diagnose van epileptische encefalopathie voorspelde een cognitieve verbetering na chirurgie. We vonden geen associatie tussen postoperatieve cognitieve uitkomst en aanvalsvrijheid.

In **hoofdstuk 4** onderzochten we een groep van 40 patiënten bij wie er een aanvullende 7T MRI was verricht in het preoperatieve diagnostische traject. Deze 7T MRI was geïndiceerd voor patiënten met medicatie-resistente epilepsie en veronderstelde focale epilepsie, zonder dat er een afwijking was aangetoond op één of meerdere MRI-scans op lagere veldsterkte. Deze eerdere MRI moest wel van adequate kwaliteit zijn en gemaakt met epilepsie-specifieke protocollen. Ook werd een 7T MRI gemaakt wanneer een verborgen afwijking werd verdacht in combinatie met MRI-zichtbare hippocampale sclerose. Bij multidisciplinaire beoordeling van het 7T MRI-onderzoek werden nieuwe afwijkingen geïdentificeerd bij negen patiënten (23%), wat invloed had op verdere medische besluitvorming. Ten tijde van de studie waren zes van deze patiënten reeds geopereerd, waarbij er bevestiging was van FCD ILAE type IIA (n=2), FCD IIB (n=1), mMCD type II (n=2) en mMCD met oligodendrogliale hyperplasie (MOGHE) (n=1).

Gedurende de herevaluatie van de klinisch geïndiceerde 7T MRI-scans ontdekten we een patroon wat samenviel met histologisch bevestigde mMCD/FCD laesies van specifieke signaalveranderingen op de T_2^* sequentie. Deze T_2^* sequentie is zeer gevoelig voor de aanwezigheid van onder andere bloedaafbraakproducten, kalk en ijzerdeposities. Onze observaties in een groep van zes patiënten hebben we beschreven in **hoofdstuk 5**. In vier van de zes patiënten, waren er gebieden met hypointensiteiten (laag signaal) met een vertakkende, deels tortueuze configuratie, en een signaal passend bij veneus bloed, suggestief voor toegenomen veneuze vaatstructuren in de groeven aangrenzend aan afwijkende hersenschors, en in mindere mate ook in het hersenparenchym. In twee van deze patiënten waren er geen afwijkingen gezien op eerdere 3T MRI en bij één patiënt liet ook de 7T MRI geen structurele afwijking zien. We vergeleken de beelden vervolgens met 7T MRI-beelden van acht gezonde proefpersonen, waarbij symmetrische veneuze vasculaire structuren in de hersenvliezen duidelijk zichtbaar waren, maar het hierboven beschreven fenomeen niet werd gezien.

Vervolgens onderzochten we in de studie in **hoofdstuk 6** de hypothese dat de MRI-bevindingen beschreven in hoofdstuk 5 betekenen dat er een relatie is tussen de

dichtheid van microvasculatuur en corticale dysplasie. We voerden een kwantitatieve immunohistochemische analyse uit, van microvasculaire densiteit en ontsteking in hersenweefsel van 97 patiënten met mMCD, FCD of tubereuze sclerose complex (TSC) en van 14 personen zonder epilepsie, gematched op leeftijd en lokalisatie in het brein als controlegroep.

TSC is een genetische aandoening waarbij er hamartomen (goedaardige gezwellen) kunnen ontstaan in verschillende organen, onder andere in hersenen, nieren, hart, lever, ogen en huid. In de hersenen worden deze hamartomen tubers genoemd, en is er microscopische grote gelijkenis met FCD IIb. Patiënten met TSC kunnen ook epilepsie en cognitieve problemen hebben en klachten gerelateerd aan de orgaanafwijkingen.

We vonden een hogere vaatdichtheid in de hersenschors en witte stof in FCD en tubers ten opzichte van het omliggende weefsel, onafhankelijk van de leeftijd. In tubers, en in mindere mate ook bij FCD, was er een toename in vaatdichtheid in de hersenschors op hogere leeftijd, wat niet het geval was in de controlegroep. In vergelijking met weefsel van de controlegroep was er in de tubers bij TSC een verhoogde vaatdichtheid in de witte stof, die geassocieerd kon worden met activatie van microglia en een toename van ontstekingscellen. Deze relatie werd niet gezien in de hersenschors. Er was geen significant verschil in vaatdichtheid tussen FCD en de controlegroep zonder epilepsie.

De eerste klinische ervaringen met 7T MRI bij patiënten met epilepsie zijn veelbelovend, hoewel het ook duidelijk werd dat in de meerderheid van de patiënten met een normale 3T MRI, ook de 7T MRI geen afwijking laat zien. Eerder werd al benadrukt wat het belang is van preoperatieve identificatie en bepaling van de begrenzing van een afwijking en van een accurate histopathologische diagnose. Verdere vooruitgang en aanvullende kennis kan worden verworven door analysehulpmiddelen die het mogelijk maken de 7T MRI-beelden met histologische bevindingen te associëren. Tijdens dit promotietraject is de prospectieve CODE 7 studie opgestart en dataverzameling volbracht. Kandidaten voor epilepsiechirurgie met een verdenking van mMCD of FCD werden geïnccludeerd en ondergingen een aanvullende preoperatieve 7T MRI-scan. Het weefsel dat tijdens een operatie werd verkregen, werd ex vivo gescand op de 7T MRI, met driedimensionale resoluties tot 0,2 mm. De in vivo en ex vivo MRI-beelden zijn gecoregistreerd met gedigitaliseerde histologische coupes door middel van software ontwikkeld door leden van het onderzoeksteam. Dit betekent dat verschillende beelddata in één zelfde driedimensionale ruimte zijn geplaatst, ten behoeve van één-op-één vergelijkingen. De ontwikkeling van de methodologische opzet is beschreven in **hoofdstuk 7**. De eerste resultaten van de CODE 7 studie zijn veelbelovend en in het nog lopende onderzoek streven we ernaar om verder te verhelderen hoe specifieke MRI-kenmerken gerelateerd zijn aan verschillende histologische kenmerken.

Algemene Discussie

Chirurgische behandeling van medicatie-resistente epilepsie bij patiënten met mMCD of FCD is de enige therapie met kans op aanvalsvrijheid en kent een hoge succeskans en lage risico's. Ondanks de relatief lagere succeskans bij de subtielere afwijkingen in mMCD en FCD type I dient chirurgie overwogen te worden. De kans op daadwerkelijk bereiken van aanvalsvrijheid na een operatie wordt bepaald door volledige verwijdering van de epileptogene zone, en voorspellende factoren die daarmee samenhangen. Wij onderzochten de relatie tussen patiënteigenschappen, histologische typering van mMCD/FCD en de kans op een succesvolle chirurgische behandeling. Volledige aanvalsvrijheid op lange termijn werd bereikt in 58% met FCD en 32% met mMCD. Onze bevindingen ondersteunen het onderscheid tussen mMCD, FCD I en FCD II subtypes vanwege verschillen in klinische uitingvormen.

In de toekomst zijn er meer grote studies met een lange follow-up nodig om met volledige zekerheid predictiemodellen te maken voor aanvalsvrijheid in relatie tot histologische subtypes. Een vereiste is een goede reproduceerbaarheid van de histologische classificaties door verschillende beoordelaars. Er zijn nog verschillen in rapportage van de verschillende mMCD/FCD subtypes, mogelijk door verschil in gebruik van immunohistochemische technieken. Er is met name over mMCD als separate entiteit nog een debat gaande. Nieuwe inzichten en bijvoorbeeld inzet van genetische en moleculaire typering zouden de classificatie verder kunnen doen ontwikkelen.

Veel patiënten met epilepsie ten gevolge van mMCD of FCD hebben cognitieve beperkingen. In overeenkomst met eerdere studies vonden we een associatie tussen een vroeg begin van epilepsie en de ernstigere cognitieve beperkingen. Ook had een langere epilepsieduur een negatief effect op de cognitie. Het bleek dat patiënten met FCD type I ernstigere cognitieve problemen hadden, vermoedelijk door de meer diffuse aard van de afwijkingen. We vonden tegen onze verwachtingen in geen groter herstel in cognitie bij patiënten met aanvalsvrijheid en medicatievrijheid na chirurgie. Dat we dit in onze studie niet konden bevestigen is mogelijk een gevolg van de relatief korte follow-up duur van 2 jaar. Langer vervolgen van patiënten zal een beter beeld geven van de postoperatieve effecten op cognitie en samenhang met histologische subtypes.

De laatste decennia is er een verschuiving naar opereren op steeds jongere leeftijd bij patiënten met medicatie-resistente epilepsie, met name bij focale ontwikkelingsstoornissen. De hoop op preservatie van cognitieve functies is een sterk argument hiervoor. Er dient gestreefd te worden naar zo korte mogelijke tijd tussen het ontstaan van epilepsie en de chirurgische behandeling, waarbij uiteraard de kwaliteit en grondigheid van het diagnostische proces optimaal moet zijn.

Verbetering van laesiedetectie en -begrenzing is één van de manieren om kwaliteit van de zorg voor deze patiëntgroep te verbeteren. Een technische ontwikkeling met potentie is 7T MRI, waarmee beeldvorming met hogere resolutie en contrast mogelijk is, ten opzichte van de huidige standaard – 3T MRI. Wij vonden dat bij multidisciplinaire beoordeling van 7T MRI, er bij 23% van de patiënten afwijkingen werden gevonden die niet waren gedetecteerd op de eerdere beeldvorming op lagere veldsterkte. Deze bevindingen hadden invloed op de klinische besluitvorming. Echter, bij de meerderheid van de patiënten leidde 7T MRI niet tot identificatie van een eerder verborgen afwijking. Dit is deels te verklaren doordat 7T MRI nog vroeg in de ontwikkeling is. Het optreden van diverse soorten artefacten beperken de beoordeelbaarheid van de scan. Daarnaast is de klinische ervaring van klinici nog relatief beperkt. De verwachting is dat de voortdurende technologische ontwikkeling de accuratesse van de beoordeling nog verder zal verbeteren. De inzet van 7T MRI lijkt zinvol te zijn bij een geselecteerde groep patiënten zonder aantoonbare structurele afwijkingen met een sterke klinische verdenking op locatie-gebonden epilepsie. Niet-structurele oorzaken moeten al zijn uitgesloten en, indien beschikbaar, dienen computergestuurde beeldanalyses te zijn uitgevoerd op de eerder gemaakte beeldvorming.

De T^{2*} sequentie op 7T MRI suggereerde dat afwijkende vaatstructuren als mogelijke nieuwe marker voor subtiele mMCD/FCD kunnen dienen. Onze vervolgstudie toonde echter geen toegenomen vaatdichtheid in mMCD of FCD ten opzichte van normaal hersenweefsel. Wel zagen we bij mMCD, FCD en tubers een hogere vaatdichtheid in de laesie ten opzichte van omliggende weefsel. Een verklaring voor het MRI-vaatpatroon kan mogelijk worden gezocht in hemodynamische variatie en daarbij horende veranderingen in bloedzuurstofspiegel. Verder onderzoek is nodig om te bepalen of BOLD fMRI (dynamisch MRI-onderzoek gebaseerd op visualisatie van variaties in bloedzuurstofspiegel) misschien van waarde kan zijn in de detectie van anderszins MRI-negatieve dysplasie.

De vraag wat er nodig is voor 100% detectie, als dat al ooit mogelijk gaat zijn, is nog niet opgelost. Moeten we ons concentreren op spatiale resolutie of op het verbeteren van het weefselcontrast? Of moeten scantechnieken worden aangepast om weefselcontrast te optimaliseren op basis van specifieke histologische veranderingen in de verwachte afwijking? Deze vragen waren de aanleiding voor het opstarten van de CODE 7 studie. Dit proefschrift beschrijft de eerste bevindingen wat betreft histologische verschillen en de kans op een succesvolle chirurgische behandeling, gebruik makend van data verzameld in de CODE 7 studie. Onze toekomstige analyses dragen hopelijk bij aan verdere verbetering van de identificatie en begrenzing van subtiele afwijkingen.

Optimale epilepsiezorg zal niet alleen moeten komen van technische innovatie maar ook de integratie van data van de verschillende aanvullende onderzoeken en van een multidisciplinaire benadering waarbij er een nauwe samenwerking is tussen neurologen,

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Curriculum Vitae

Tim Veersema was born on 1 July 1987 in Amsterdam, the Netherlands. After completing a bilingual trajectory (tweetalig VWO) at the Anne van Rijn College secondary school in Nieuwegein and graduating with honours, he started his Medicine study at the University of Maastricht in 2005. During his studies Tim had the opportunity to do medical internships in Kubang Kerian in northern Malaysia, Brussels and Capetown. It was during this neurology rotation in South Africa where Tim developed interest in the nervous system and brain in particular. After graduating in 2011, Tim travelled five months through South America with his girlfriend Caroline, after which he started working as resident at the department of Neurosurgery in the University Medical Center Utrecht. Consecutively he started his PhD under supervision of Kees Braun (neurology), Eleonora Aronica (neuropathology, Amsterdam UMC, location AMC), Cyrille Ferrier (clinical neurophysiology) and Pieter van Eijdsen (neurosurgery). During his years as researcher Tim did weekend, evening and night shifts for neurosurgery at the UMCU, was a night-doctor at the Alexander Monro Breast Cancer Hospital, Bilthoven and worked as a resident at the department of neurosurgery of the Amsterdam AMC, location VU Medical Center. January 2019 he started his specialist training in radiology and nuclear medicine in the Meander Medical Center in Amersfoort and University Medical Center Utrecht. Caroline and Tim live in Utrecht, together with their daughter Coco, born January 2020.

List of publications

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in preparation

* contributed equally as first authors

shared senior authorship

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